

S. Marie · J.R. Piggott (eds.)

Handbook of Sweeteners

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Preface

The study of sweetness and sweeteners has recently been an area well-served by books at all levels, but this volume was planned to fill what we perceived as a gap in the coverage. There appeared to be no book which attempted to combine a study of sweetness with a thorough but concise coverage of all aspects of sweeteners. We set out to include all the important classes of sweeteners, including materials which do not yet have regulatory approval, so that clear comparisons could be made between them and their technological advantages and disadvantages. To achieve our first aim, of sufficient depth of coverage, the accounts within this volume are comprehensive enough to satisfy the requirements of a demanding readership, but cannot be exhaustive in a single volume of moderate proportions. The second aim, of breadth and conciseness, is satisfied by careful selection of the most pertinent material.

For the purposes of this book, a sweetener is assumed to be any substance whose primary effect is to sweeten a food or beverage to be consumed, thus including both the nutritive and non-nutritive varieties, from the ubiquitous sucrose to the lesser known, newer developments in alternative sweeteners.

The volume has its contents structured in a logical manner to enable it to be used in an ordered study of the complete subject area or as a convenient reference source. The book opens with a study of sweetness and its influence on food selection, to set the scene for the following chapters discussing how this demand for sweetness is satisfied, and its implications. The following five chapters discuss the major classes of sweeteners, including their sources and technological applications. The following chapter discusses the marketing of sweeteners and their uses in product development. No study of sweeteners can be complete without a consideration of the health implications of what may constitute a significant proportion of the diet, and this is included in the next three chapters covering dental health, metabolic disorders and body weight. The final chapter considers the legislative framework providing for control of sweeteners as food ingredients.

It is anticipated that this volume will be read by all those seeking an authoritative review of the whole subject of sweeteners in a single volume. Its appeal should thus be wide, attracting readers such as food scientists and technologists, and developers of food, beverage and pharmaceutical products. Selective reading is also expected by workers concerned with dental health, metabolic disorders such as diabetes mellitus, and weight control, as well as those, such as psychologists interested in sweetness as a perceptual phenomenon, seeking background information.

The reader must judge whether we have succeeded in our aim of producing a complete but concise review of sweetness and sweeteners. If so, it is due to the help of all those concerned: the many contributors who have worked hard to turn our ideas into print; and the publisher's staff, for their encouragement, patience, and skilled work.

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1 Sweetness and food selection

M.T. CONNER

1.1 Introduction

The sweetness of a food or drink is an important influence on its selection. However, sweetness is merely one attribute out of the whole complex which forms the eating or drinking situation. Within the food itself there is a whole range of attributes likely to affect selection, e.g. bitterness, texture, aroma, etc. and these attributes are likely to interact in various ways. In addition, a whole range of factors not intrinsic to the food are also likely to affect selection, e.g. bodily state, attitude towards the food, the appropriateness of the food to the eating context, etc. The relationship between sweetness and food selection and the interaction between sweeteners and the other aspects of the eating situation are considered in this chapter.

The conjunction of particular levels of each of the different factors influencing selection in an eating situation is learned from early life onwards and familiar levels come to be preferred (Section 1.2.4). Any deviation from this learned complex is likely to be less preferred, whether there is an excess or a deficit of the particular characteristic (Booth *et al.*, 1987). This will be equally the case for characteristics which are perceived as major attributes of foods (e.g. sweetness in confectionery) as for characteristics that are little noticed (e.g. sweetness in soup). The only difference in attributes will be in the size of the effect a minor deficit or excess of the characteristic has on overall preference for the food or drink. Such a model of the influences on food selection provides the basis for a procedure for optimising the level of a particular food attribute such as sweetness (Section 1.3).

In the case of sweetness, there is considerable evidence that there is an innate response that may contribute to preference in addition to the learned responses (Section 1.2). The evidence is, however, that this innate aspect of sweetness preference can eventually become totally overlaid by the learned preference (Section 2.2.4). The innate sweetness preference is, thus, likely to have important effects on food selection in eating contexts where learning is incomplete, as for example in the cases of novel or unfamiliar foods (which all foods are, early enough in childhood). In such contexts, individual differences in the learned preferred level of particular characteristics across related foods are nevertheless also likely to influence food selection. A net

preferred level of sweetness across foods is commonly supposed to be a personal characteristic known as a 'sweet tooth' (Section 1.4).

The different factors influencing selection within a particular eating context will interact in a variety of ways. Within the food, levels of the different constituents may influence the perceived level of a particular attribute (Section 1.5); for instance, the colour of a drink may affect its perceived sweetness. Aspects of the person will also influence food selection (Section 1.6). For instance, the bodily state of the person eating or drinking will influence what and how much they choose to consume. Such decisions, like the most preferred levels of attributes of foods and drinks, are learned.

1.2 Innate versus learned preference for sweetness

Interest in the relative contributions of innate and learned preferences for sweetness has generated a great deal of research. One way to study the relative importance of innate versus learned influences on food preferences is to examine the development of responses to foods. Presumably, the responses of newborns and infants should be little affected by experiential factors which are likely to have a marked influence on adult preferences. This has led to much study of the newborn human infant to determine whether responses to sweetness are innate.

1.2.1 Studies with newborn infants

Studies have typically measured intake over short periods of time (usually up to 3 min) of one or more concentrations of a tastant (usually sucrose) in solution and the solvent alone (usually water) with the solutions presented in a counterbalanced order either within or across infants. The infants are allowed to consume as much of the solution as they like during the period and the amount of tastant solution consumed is compared with the consumption of the solvent alone. Using such a paradigm, Desor *et al.* (1973) found that 1–3-day-old infants consumed more of an aqueous sugar solution than of the water alone. It was also found that infants consumed more of the sweeter sugars (sucrose and fructose) than of the less sweet sugars (glucose and lactose) and consumed more of the more concentrated solutions. Desor *et al.* (1977) reported that aqueous sugar solution was preferred to equicaloric unsweetened milk formula in newborn infants. This consumption of larger amounts of sweetened than of unsweetened stimuli in newborn infants has been shown in a number of studies (e.g. Maller and Desor, 1973; Crooke, 1978; Beauchamp and Moran, 1982), even when the sugar is the very first food that the infants have ever tasted (Blass *et al.*, 1984).

It is less clear whether sweet sugars elicit the same reaction of pleasure in infants as they do in adults (Beauchamp and Cowart, 1987), although pleasurable experience has been inferred from several kinds of measures including facial expressions. Steiner and his co-workers (Steiner, 1973, 1974, 1977, 1979; Ganchrow *et al.*, 1983) have captured photographically the reflex action elicited by sucrose solutions in newborn infants. This reaction is characterised by a marked relaxation of many of the facial muscles, a slight smile and often licking and sucking of the tongue. This is in marked contrast to bitter and sour stimuli which produce gaping or expulsive reactions in newborns, if they are strong enough to produce any reaction. Steiner reports no marked differences in response between normal newborns of 3–7 days of age and less than 20 h of age (tested before their first feed). Hence, it is likely that sweetness is perceived as pleasant from birth and that higher levels are perceived as more pleasant.

A wide variety of substances elicit the sensation of sweetness in man. It remains to be tested in human newborns whether the congenital preference for sweetness extends to non-nutritive (intense) sweeteners. Lindley (1987) suggested that there would be no innate preference for intense sweeteners such as saccharin that have aversive side- and after-tastes which simple carbohydrate (bulk) sweeteners do not exhibit.

1.2.2 Studies with older infants

These innate sweetness preferences observed in newborn infants seem to persist in older infants and preschool children. Thus, Desor *et al.* (1977) found that infants between 5 and 28 weeks of age consumed significantly more sweetened than unsweetened water, while Filer (1978) found 2–6-year-olds consumed significantly more sweetened than unsweetened spaghetti. Note that both of these sweetened materials are unlikely to have been familiar drinks or foods.

The results of Desor *et al.* (1977) suggest the ingestive response to sweetened water remains constant in early life. They found no differences between infants aged 5–28 weeks in their relative consumption of two glucose and fructose solutions. However, the relationship of test intakes with dietary exposure to sugar was not reported and hence this does not provide good evidence that ingestive responses to sweet stimuli are unaltered by age or postnatal dietary experience.

Together this work would appear to indicate an innate preference for sweetness present from birth. Beauchamp and Cowart (1987) argue that this innate responsiveness to sweetness reflects an evolutionary pressure to ensure detection and recognition of food sources likely to be high in calories. Booth (1990) has criticised this common assumption and proposed that a sweet receptor and preference is needed by omnivorous mammals to

stop the aversion to the taste of nitrogenous alkaloids causing newborns to reject protein and amino acid rich milk.

1.2.3 The role of dietary experience

The work discussed so far does not allow us to examine the extent to which postnatal experience may serve to alter or modulate the expression of the unlearned preference for sweetness. There has been much speculation on the role of experience in preference for sweetness, but there are few human data available (Beauchamp and Cowart, 1987). Several studies have examined the relationship between children's preferences and those of their parents and siblings (e.g. Greene *et al.*, 1975; Ritchey and Olson, 1983; Rozin and Vollmecke, 1986). However, at best only a weak relationship has been found. This is perhaps not surprising given that even young children are likely to have a number of dietary experiences outside the family and parents are unlikely to feed young children exactly the same food as they would choose for themselves.

Beauchamp and Moran (1982, 1984) report the results of a longitudinal study aimed at assessing the effect of different levels of dietary exposure to sweetness on subsequent individual differences in sweet preference. Relative intake measures were used to assess sweet preference in groups of children at birth, 6 months of age and 2 years of age. In all cases the relative intakes of water and 0.2 M and 0.6 M sucrose solutions were measured. Newborn infants ingested more of the 0.2 M sucrose than of the water alone and more 0.6 M than 0.2 M sucrose. At 6 months of age the infants were split into two groups based upon their mothers' reports of whether they were regularly fed sweetened water or not (Beauchamp and Moran, 1982). Although these two groups had not differed in their consumption of sucrose solutions at birth, by 6 months the infants regularly fed sweetened water consumed more sweetened water than did the infants not regularly given sweetened water by their mothers. These differences between groups appeared to be attributable to a fall in the acceptability of sweetened water in the group not fed sweetened water.

A further follow-up was reported when the same children were 2 years old (Beauchamp and Moran, 1984). Here three groups of children were distinguished: those never fed sweetened water, those fed sweetened water for fewer than 6 months and those fed sweetened water for more than 6 months. There were no observed differences between these three groups at birth, while at 6 months and 2 years only those groups who had been fed sweetened water showed a preference for sweetened over unsweetened water. No differences between the groups fed sweetened water for more or less than 6 months were observed at 2 years of age. No differences were observed between the three groups at 2 years in their preferences

for sweetened over unsweetened Kool-Aid: all ingested more sweetened Kool-Aid, indicating that the effects of learning with sweetened water did not generalise to other sweet drinks. Beauchamp and Moran (1984) also report that there was no evidence of the reverse relationship, i.e. experience with sweet foods being related to test ingestion of sweetened water.

1.2.4 The mature response to sweetness

These studies suggest that the innate sweetness preference may be subject to modification quite early in life (Beauchamp and Cowart, 1987) and that the innate sweetness preference becomes overlaid by preferences based upon experience with sweetness. The learned basis of preference for other attributes of foods and drinks (Galef and Sherry, 1972; Booth *et al.*, 1974) is now widely accepted. For instance, a slight innate preference for weakly salted food is replaced by a preference for a salt level closely related to dietary experience (Harris and Booth, 1987; Harris *et al.*, in press). Such learning probably takes place from early life onwards. The above evidence indicates that mature preferences for sweetness are no different, except that there is an innate preference to be replaced by the learned preference (as it is for salt). The learning of such preferences takes place through processes such as familiarisation (Birch and Marlin, 1982; Pliner, 1982; Harris and Booth, 1987), socio-affective reinforcement (Galef and Sherry, 1972; Birch, 1980; Birch *et al.*, 1980) and association with nutritional benefits (Booth *et al.*, 1972, 1974; Booth, 1985). Mere exposure removes any dislike of novelty and also induces some genuine attraction to that particular substance. For instance, Birch and Marlin (1982) showed that 2-year-old children's preferences for novel cheeses and fruits was directly influenced by their frequency of exposure to the novel foods. The effect of socio-affective reinforcement is nicely illustrated in a study by Birch (1980). She found that children shifted from choosing their most preferred vegetable to choosing the vegetable preferred by their peers over a 4-day period. The nutritional benefits associated with the consumption of foods containing required nutrients has been shown to have a marked effect on subsequent choice of such foods. For instance, Booth and Toase (1983) found that eating disguised carbohydrate induced a flavour preference in hungry people and Booth and Gibson (1988) claim that subjects deprived of protein will consume more of a food they had previously learned to be rich in protein.

This learning of food preferences is often contextualised, i.e. the stimulus eliciting selection is a complex not just of food characteristics but also of other aspects of the eating situation such as bodily state (Booth *et al.*, 1982; Booth and Toase, 1983; Baker *et al.*, 1987) or the appropriateness of the food to the time of day (Birch *et al.*, 1984). Any deviation from the

acquired complex of distinctive characteristics of a food or perhaps of the context in which it is often eaten is likely to reduce the motivating power of that situation. Therefore, if a particular level of a characteristic has become liked in a particular context, then any deviation from that level, whether excess or deficit, should weaken that motivation and reduce the preference.

If this model is correct, both an excess and a deficit of a particular characteristic should produce equal declines in acceptability. Such equal declines in acceptability of a food with excess and deficiency of a characteristic have been demonstrated for cases such as milkiness of white coffee (Booth *et al.*, 1987; Marie, 1987), the redness of strawberry jelly (Conner *et al.*, 1988b), the saltiness of bread (Conner *et al.*, 1988a), the saltiness of soup (Booth *et al.*, 1983) and levels of flavouring, salt, creamer and starch in chicken soup (Conner, 1988).

If the learned response to sweetness is similar to other attributes of foods, it should also show equal declines in acceptability above and below a most preferred level. However, in adults sweetened water usually produces a monotonically rising or asymptotic response with tastant concentration (Cabanac, 1971; Thompson *et al.*, 1976; Desor *et al.*, 1977) (Figure 1.1) or sometimes even a monotonic decline in pleasantness rating (Witherly *et al.*, 1980). A monotonic rise of pleasantness with sweetener concentration would contrast with their acquired effect, if sweeteners were like any other food constituent in becoming liked most at the particular level which is usual in a familiar food. This liking of sweetness more, the more there is of it, looks like the innate reflex coming through in the adult ratings of the unfamiliar drink of plain sweet water (Figure 1.1, dashed line). An asymptotic hedonic function (Figure 1.1, crosses) is perhaps a compromise between the infantile unlimited preference (Figure 1.1, dashed line) and a learned preference for no more than the sweetness of lemonade or other simply flavoured sweet drinks (Figure 1.1, solid line). (A decline in rating is presumably a learned aversion to all sweetness, perhaps from habits of sugar avoidance for health reasons.) The acceptability of a variety of foods and drinks (lime drink, chocolate and tomato soup) has indeed been found to decline above as well as below the sweetness that is most acceptable to each subject and equally with discriminable differences in sucrose concentration from ideal (Conner *et al.*, 1986, 1988c).

Thus, it seems reasonable to hypothesise that the sweetness of a familiar food or drink has the same sort of effects as any other salient characteristic; experience of a sweet food in an eating situation induces an attraction to a particular ideal level of sweetness in that item which completely overwhelms the congenital monotone (Booth *et al.*, 1987). On this theory, the lack of a peak in the acceptability ratings of pure aqueous sugar solutions is likely to be due to unfamiliarity with sugar water as a food

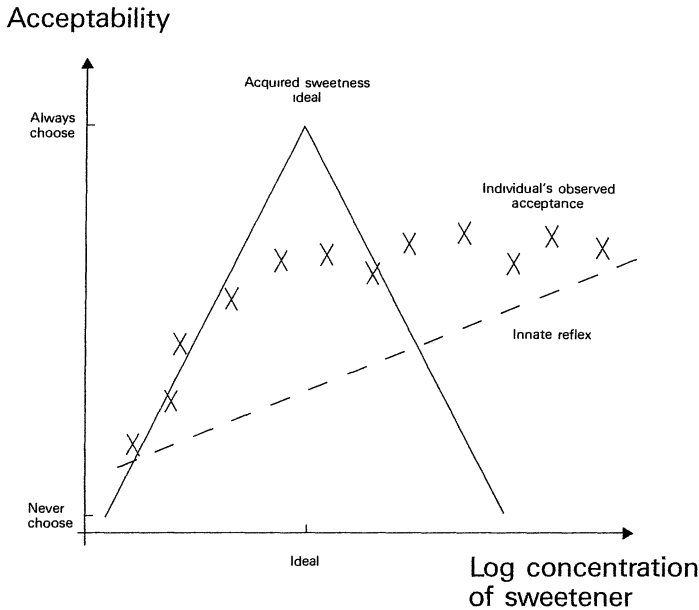


Figure 1.1. Possible relationships between sweetener concentration and acceptability. The solid line indicates the form of an acquired sweetness preference (the acceptance triangle), the dashed line indicates the form of the innate sweetness reflex (congenital monotone) and the crosses indicate a possible compromise acceptance response between these two functions (asymptote).

or drink. As a result, many subjects can make little sense of the task being set them in rating the acceptability of sugar water and revert to an infantile response (Booth *et al.*, 1987). Such an interpretation casts doubt on the usefulness of studies reporting preferences for sweetened water to the understanding of the role of sweetness in food selection. The role of sweetness in food selection can be effectively investigated only by methods using test foods and situations that are familiar to the subject.

In contrast, it has been widely assumed that it is the innate preference for sweetness that is expressed in children's and the 'sweet-toothed' adult's liking for sweet foods and drinks. This assumption can be tested against the above theory by means of their differential predictions about the shape of the psychophysical functions of sweetness preference in familiar foods. On the theory that preferences are learned, the decline of acceptability on either side of the ideal point for a food characteristic should normally be symmetrical: excess sweetness should be as intolerable as insufficient

Table 1.1. The slope for linear regression lines of preference ratings onto log concentrations for sucrose and whitener above and below each individual's most preferred level ($N = 9$) for instant coffee drinks (Booth *et al.*, unpublished).^a

Sugar				Whitener			
Below		Above		Below		Above	
Mean	SD	Mean	SD	Mean	SD	Mean	SD
0.2	0.2	0.8	0.7	0.2	0.3	0.4	0.4

^aNo significant differences between above and below ideal were observed for either sucrose or whitener.

sweetness. On the other hand, the innate preference should produce a continuing increase in preference to any upper limit tested. Thus, if the conditions of test (such as novelty of the food or drink) release the innate reflex to some extent, a reduction in slope of the upper limb of the observed sweetener preference function should be diagnosed as expression of the innate monotonic sweetness preference function. Even an increase in variance in supra-ideal choice behaviour would be a symptom of the innate and learned tendencies competing for expression. In other words, if the innate reflex ‘breaks through’, the food-sweetness preference peak could be reduced to an asymptote, or even overwhelmed in a rising monotone. This also means that consumer tests of optimum sweetness are liable to give incorrectly high ideal points if some panellists find the test samples unfamiliar or poor in quality in some respect.

Whenever individual's preference functions have been measured in an unbiased way, evidence has been for entirely learned preferences in familiar foods, including the case of sweetness. The strengths of aversion to excess and to insufficiency in familiar foods and drinks are as similar in the case of sugar as in the case of other constituents which can have just as much influence on liking for the food or drink but for which no innate preference (or aversion) is known (Booth *et al.*, unpublished). Table 1.1 presents some data for levels of whitener and sugar in coffee in the same subjects. Absence of lower slope above ideal supports the hypothesis that the preference for sweetness in a familiar food or drink is entirely acquired. This view that the liking for sweetness in foods and drinks becomes entirely contextualised by adulthood is currently not universally accepted (Beauchamp and Cowart, 1987).

It should be granted therefore, that the mature response to sweetness is based upon learning to prefer the normally experienced level, as it is for any other element of the particular eating context. Thus the indiscriminate

adding of a sweetener to a food or drink will not increase its acceptability if the added sweetness causes a deviation from the level that the consumer already prefers. However, the innate preference for sweetness is likely to have an effect on the initial response to unfamiliar foods, possibly making the sweeter variant preferred initially to less sweet variants. However, even in this case, learned sweetness preferences in similar foods are also likely to influence the preferred level of sweetness.

1.3 The linear food acceptance function

The most preferred level of an attribute of a food or drink is usually determined by consumer popularity polls amongst samples varying in the attribute(s) of interest. The procedures usually involve some form of preference ranking or rating. For instance, consumers are asked to choose the most preferred of pairs of samples or to rank the variants in order of preference. Of the rating techniques, the nine hedonic categories of like and dislike (Peryam and Girardot, 1957) are the most widely used (Meiselman, 1984), while the use of 'magnitude estimation' techniques (scoring under ratio instructions) for acceptability has been advocated (Moskowitz, 1982, 1983). Those rating techniques could be used for individualised analysis (especially with bias free sample selection). However, the results of these procedures are usually combined across the panel of consumers tested before analysing the data to draw conclusions. This approach can give only the relative popularities of the tested variants or perhaps a product-attribute preference space extracted from the panel's data as a whole. As they do not consider how the varied attribute might determine acceptability within each individual, they cannot relate attribute level to acceptability in any causal way, no matter how complex the statistical analysis employed (Booth, 1988).

The above theory of acquired food preferences provides the basis of a procedure that can be used to relate the level of sweetness (intensity) in a particular food to its acceptability within an individual in a fashion that actually measures the strength of influence of sweetness on acceptance. On this theory, any individual faced with a food or drink will have a personally familiar formulation that they like most. Differences from the most preferred level in any readily perceived attribute are liable to reduce acceptability. The stronger the influence of that constituent on that person's attraction to that type of food, the more likely is a barely detectable deficit or excess to have an effect on selection among variants. As a result, the form of the relationship between the degree of acceptability and the levels of a perceived attribute is described by an isosceles psychophysical function, called the acceptance triangle (Booth *et al.*, 1987).

1.3.1 The acceptance triangle

The acceptance triangle represents a mental mechanism relating acceptability ratings to determinants of acceptance within an individual consumer. This is a quantitative scientific theory of the mind, stating that decreases in acceptability from its maximum are directly proportional to the number of discriminable differences the sample stimulus level is from the maximally acceptable level. Hence, unbiased acceptability ratings should plot against the measure of the varied factor scaled in equally discriminable differences to give an inverted V, with equal slope(s) above and below maximum acceptability (Figure 1.2).

A crucial proviso is that various sources of bias in ratings, demonstrated by experimental psychologists (Poulton, 1968, 1979, 1987, 1989), are avoided in rating the acceptability of samples of a food or drink. Procedures for minimising such biases have been developed and applied in food research (Riskey *et al.*, 1979; Booth *et al.*, 1983; McBride, 1985; Conner *et al.*, 1987). The bunching of responses in any part of the response dimension

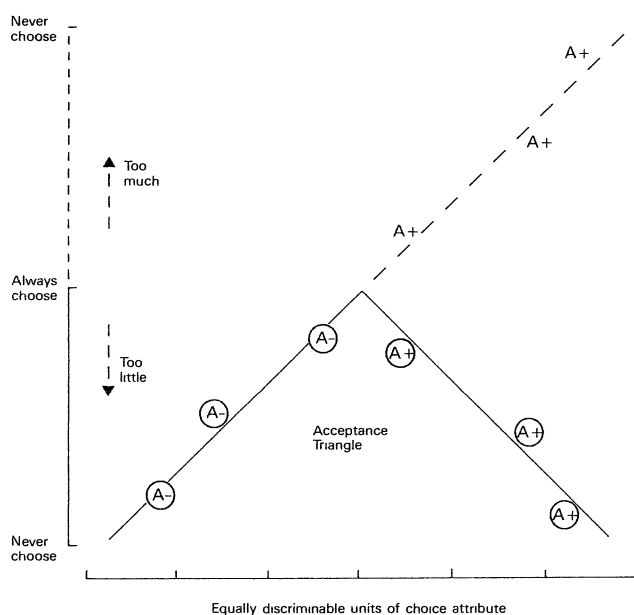


Figure 1.2. The acceptance triangle, showing the predicted relationship between measures of a varied factor scaled in equally discriminable differences and acceptance ratings. The dashed line indicates how the acceptance triangle can be unfolded to give a linear food acceptance function. The circles show ratings of acceptability for insufficient (A-) and excess (A+) of the varied attribute.

will distort a psychophysical function and so successive samples should be selected in order to spread responses evenly over the response range (Booth *et al.*, 1983). Also a balance of responses between excess and insufficiency of an attribute is necessary in order to avoid the mis-estimation of the most preferred level which commonly occurs in sensory optimisation (Conner *et al.*, 1987). Perhaps most important of all, responses near and therefore potentially beyond the end(s) of the response dimension must be avoided, i.e. in this context, samples that would be rated unacceptable by an individual consumer.

On this causal theory, the appropriate scaling of the varied influence on acceptability is in those units which give equally discriminable differences in the factor. For aqueous solutions of sucrose in the ranges commonly used in foods, ratios of concentration units give equally discriminable differences (Schutz and Pilgrim, 1957). In such a case, acceptability ratings will plot linearly against a logarithmic scale of concentration. For other sweeteners it may be first necessary to determine those units which give equally discriminable differences. The correct units will be those giving the tightest linear relationship between acceptability ratings and levels of the factor influencing acceptability.

According to this theory, the inverted U often observed or even the asymptotic curve (Moskowitz *et al.*, 1974) obtained from hedonic ratings of sweet stimuli are artefacts of a poorly designed test situation or scoring procedure or of analysis based upon premature aggregation of each individual assessor's data. Optimisation procedures have to resort to polynomial regression (Moskowitz, 1983) to describe such indeterminate curves and even then the distortion will remain. When such data are appropriately collected and analysed, on the other hand, the strength of an influence on acceptability lies in the characteristics of a mathematically determinate inverted V (Booth *et al.*, 1987).

1.3.1.1 Linear acceptance functions. As long as there is a peak preference, intensity increases monotonically with physical amount from too weak to too strong. That is to say, the acceptance triangle (Figure 1.2, solid line) 'unfolds' into a straight line, because it has the same slope value above and below ideal concentration (Figure 1.2, dashed line; Figure 1.3). In the example in Figure 1.3, the ratings were obtained by getting the assessor to mark a position on a line anchored with 'sweetness just right' in the middle, 'so little sweetness I'd never choose it' at the left-hand end and 'so much sweetness I'd never choose it' at the right-hand end. However, it is possible to use simply a bipolar response format (e.g. 'at this sweetness I always choose it', 'at this sweetness I'd never choose it') and to unfold the ratings afterwards by the criterion of best linear fit. This has the theoretical advantage of leaving the rater completely free to determine the slope of both limbs of the triangle or unfolded line.

Note that this is to say that it is not necessary even to characterise the

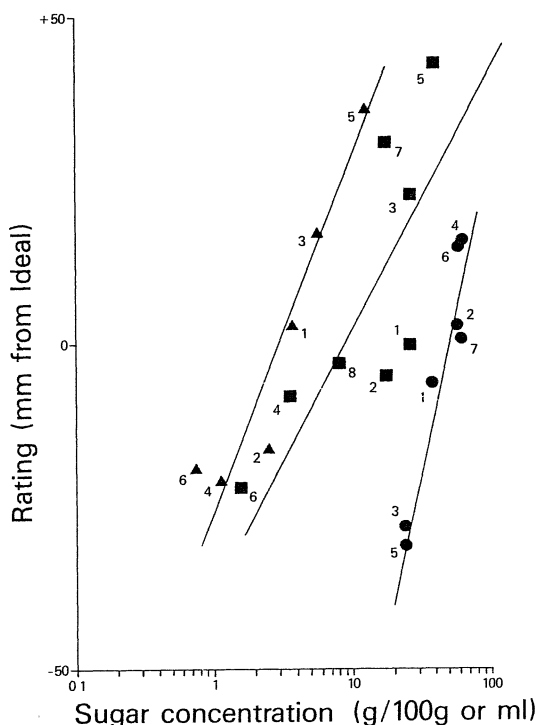


Figure 1.3. An individual assessor's ratings of the acceptability of the sweetness of each of three foods (●, chocolate, $r = 0.94$; ▲, lime drink, $r = 0.94$; ■, tomato soup, $r = 0.77$). The numbers beside each data point indicate the order of presentation of samples to illustrate the stimulus-selection algorithm designed to minimise bias in the ratings (Conner *et al.*, 1988c).

responses given by the assessor. Therefore, it is possible to obtain ratings on a response format labelled with descriptors of overall acceptability such as 'always choose' and 'never choose' or 'always buy' and 'never buy'. The only requirements are that pairs of descriptors are easily usable by the assessor for placing responses and that an assessor's uncharacterised acceptability ratings are substantially enough affected by sweetener concentrations.

Individuals' bias-minimised ratings of normal variations of sucrose (or salt, whitener, etc.) in familiar foods and drinks do indeed fall rather precisely on a straight line against the logarithmic concentration of the perceived constituent (Booth *et al.*, 1983, 1986, 1987; Griffiths *et al.*, 1985; Conner *et al.*, 1986, 1987, 1988a, 1988b, 1988c). Thus, for several perceptually simple tastants (including sweetness), aroma substances and colourings of common foods and drinks, there appears to be discrete sensory influences on preferences that theoretically are entirely acquired and in practice are very readily measured in strength.

1.3.2 Psychophysical acceptance parameters

Like any calibration line, the unfolded triangle has three independent parameters, i.e. slope, intercept and residual variance. Converted into preference measures, these parameters can be recognised as the ideal point, the rejections ratio and the tolerance discriminations ratio. These derived parameters are theoretically meaningful aspects of the individual assessor's motivation. An individual's acceptance triangle provides measures of the strength of influence of the factor on acceptability of that food in that situation. Once these measures of one influence on personal preference have been calculated, they can be combined with other influences to predict overall acceptability. Also, individuals' acceptability characteristics can be aggregated into estimates of market response.

1.3.2.1 The ideal point (IP). The maximally preferred level of the varied factor is that concentration corresponding by interpolation to the ideal response category ('always choose' or whatever). McBride and Booth (1986) found the internal standard of the ideal for strength of taste to be at least as precise as an externally presented standard. Hence, having ideal as a response category is likely to improve the precision of ratings of taste acceptability, in addition to providing a readily understandable and potentially useful parameter of acceptance.

In studies so far with sweetness in a variety of foods, it is usually found that the ideal points are log-normally distributed across assessors, with the mean ideal level varying greatly between different foods (Table 1.2).

1.3.2.2 The rejections ratio (RR). The ratio between rejection points is a measure of slope (like the exponent of a magnitude estimation power function) and tolerance of deviations from the ideal point. It is the ratio of that level of the varied factor extrapolated to be at the 'too much', excess rejection category (RP_{XS}) to that level of the varied factor extrapolated to be at the 'too little', deficit rejection category (RP_{def}). The rejections ratio gives a measure of the range of the varied factor that is tolerable to the individual. This measure is solely dependent on the slope of the regression line for the unfolded acceptance triangle which in turn entirely depends on the assessor's interpretation of the exact wording of the rejection response categories. In that sense, RRs are rather subjective. They may also be rather unreliable, since extreme levels of the acceptance factor may be unfamiliar.

In studies with sweetness, the RR has been found to be log-normally distributed, like the IP (Table 1.2).

1.3.2.3 The tolerance discriminations ratio (TDR). Another measure of tolerance of deviations from the ideal level is the tolerance discriminations

Table 1.2 The derived parameters of the acceptance triangle for sucrose in various studies.

Food	Ideal point ^a		Range ratio ^a		Tolerance discriminations ratio	
	Mean	SD	Mean	SD	Median	Range
Lime drink ^b	5.40	1.82	3.16	3.80	1.00	0.50–0.37
Lime drink ^c	6.20	1.74	22.8	2.90	0.47	0.25–1.56
Chocolate ^c	41.4	1.24	18.3	5.67	0.68	0.21–2.94
Tomato soup ^c	11.6	1.91	52.8	5.25	2.00	0.28–4.75
Coffee ^d	5.40	1.82	3.16	3.80	0.94	0.41–4.78

^aMean and SD based upon log-normal distribution.

^bConner and Booth (1988).

^cConner *et al.* (1988c).

^dConner and Booth, unpublished data.

ratio (TDR). This is a more objective measure of the slope of any psychophysical function (Booth *et al.*, 1989) as it is unaffected by an individual's idiosyncratic use of an exact level for the rejection response category or categories. The TDR is a measure of the assessor's discriminative performance in producing variations in acceptance responses with differing levels of the determining attribute. It is based on the relationship between the slope of the unfolded acceptability triangle and the residual variance around that regression line. When response variances are constant over the tested range of the varied attribute, the change in the varied attribute that is just discriminable in the acceptance ratings can be estimated by a version of the traditional just-noticeable-difference (JND) or signal discriminability calculation (Booth *et al.*, 1987). However, since it is calculated from acceptance ratings not intensity ratings, the difference is in what is tolerable not in what is noticeable. This just-tolerable-difference (JTD, a JND of acceptability instead of intensity), when expressed as a ratio to the stimulus factor level at which it is calculated (TDR) is given by:

$$\text{TDR} = \text{antilog}(2 \times 0.675 \times \text{SD/slope}) - 1,$$

where 0.675 is the z-score for 25% overlap (the overlap of the response distributions for half-perfect discrimination), SD is the standard deviation of the acceptance responses around the regression line and slope is the ratio of response difference to difference in stimulus units that give a linear psychophysical acceptance function (e.g. log concentration). Derivation of this formula can be found in Conner *et al.* (1988a, 1988c).

In studies with sucrose in foods and drinks, we find that the TDR often is much greater than the perceptual limit of the Weber's ratio of discrimination for stimulus descriptions (approximately 0.12 for sweetness of sucrose in aqueous solution; Schutz and Pilgrim, 1957). This indicates

that many people are not pitting their ratings against the perceptual limit; that is, motivational factors are flattening the slope of the psychophysical function. Our data so far on sweetness indicate that a majority of people are sufficiently intolerant of non-preferred magnitudes of food sweetness to give a distribution of TDRs across assessors which is heavily skewed to a sharp low limit, at about double the two-alternative forced-choice Weber ratio for sweetness (Table 1.2). Possibly other tastes and texture constituents of these foods and drinks are masking sweetness.

The acceptance parameters can be used to give meaningful predictions of the response of individuals to changes in the varied factor(s). Two predictors that we have found particularly useful are the individual's ideal range and acceptable range. The ideal range is represented by those levels of the varied factor that the assessor's ratings do not reliably distinguish from the ideal level (i.e. between IP minus one JTD and IP plus one JTD). The acceptable range is represented by those levels of the varied factor that the assessor's ratings distinguish from those levels which would be rejected (i.e. between RP_{def} plus one JTD and RP_{XS} minus one JTD).

1.3.3 The aggregation of individual acceptance responses

Once each individual's psychophysical acceptance parameters have been determined, it is a simple matter to aggregate these across individuals sampled from whatever population is of interest. This individualised approach avoids the common assumption in most sensory and consumer research that the qualitative character of what is going on is the same in every head (Booth, 1988). No amount of cross-tabulation of frequency data can avoid this fallacy of common individual structure which has long been recognised in the social sciences (Robinson, 1950). The fallacy is equally universal in tests using preference scores or ranks and in sensory panel averaging and all analyses of grouped data aimed at diagnosis of psychological phenomena. The individualised procedure advocated here avoids this problem by examining causal structures within individuals and obtaining parameters of these individual causal structures. The predicted responses of individuals can then be quite simply summated across the panel of respondents, for instance by using individual ideal or acceptable ranges. In estimating the responses of a particular segment of the market, the problem then becomes one faced by all consumer research, that of representative sampling of the population groups of interest.

Levels of the varied factor of interest can be selected and the number of assessors out of the sample for whom the concentration is within their ideal and acceptable range simply summated (Table 1.3). Such summaries of the data take into account variations across the panel both in individuals' most preferred levels (IPs) and their tolerances of deviations from their ideal level

of the varied attribute (TDRs). These calculations have been programmed for the single-factor case (Conner, 1989), considerably speeding these individualised regression analyses for data to be grouped into market response distributions. As with all consumer data, such predicted market responses need calibrating against real market data. Our experience so far, however, is that action can be taken on a one-to-one interpretation of predicted against actual market behaviour.

Table 1.3. Percentage of assessors ($N = 105$) for whom the level of sucrose is within their ideal range (IP minus one JTD to IP plus one JTD) and acceptable range (RP_{def} plus one JTD to RP_{XS} minus one JTD) for a beverage (Conner and Booth, unpublished data).

Percentage of marketed sucrose level	Percentage of assessors for whom the sucrose level is in	
	Ideal range	Acceptable range
25	21.9	6.7
38	31.4	11.4
50	57.1	19.0
63	84.8	30.5
75	96.2	35.2
88	95.2	44.8
100	94.3	38.1
113	92.4	33.3
125	80.0	32.4
138	76.2	22.9
150	66.6	21.9

1.3.4 Combined action of several determinants of acceptance

This methodology can be extended to the situation where several factors in a food are varying at once. The acceptance triangle represents a single discrete cause-effect relationship between a unitary determinant or set of determinants and the acceptance ratings. That is, accounting for overall acceptance may require several orthogonal (statistically independent) acceptance triangles, to enable the effect of each determinant to be measured. Once the linear relationship between each discrete determinant and the acceptance ratings has been identified, it is then a matter of determining how each person combines the different determinants' parameters in order to decide overall acceptance. This combination rule will include any sensory interactions between determinants (Booth, 1987a) and additive or interactive combination of sensory with conceptual marketing factors should also be allowed for in any integrative formula predicting overall acceptance in the market situation (Booth and Blair, 1988). Booth (1987a, 1987b, 1988) discusses various plausible decision models used widely elsewhere that can be tested on the multiple triangle

data to provide evidence of how the effects of more than one determinant of acceptance are combined.

Marie (1987) illustrates the testing of such combination models on the effects of sucrose, whitener and coffee solids levels on the acceptability of coffee, while Conner (1988) used the methodology to study the combined effects of salt, creamer, whitener and flavouring on chicken cup-a-soup acceptability. Booth and Blair (1988) illustrate how this methodology can be extended to the assessment of the combined influence of a health concern (high or low calorie labels) and palatability on preferred levels of sweetener in coffee.

1.4 Sweetness and food selection

The theory of acquired acceptability also implies that we may learn to prefer sweet to non-sweet foods in particular contexts, e.g. between meals and at the end of meals. It is the habitual use of sweet foods and drinks in particular contexts that induces a liking for these foods in such situations. Indeed, the theory also is that, some individuals may, as a result of habitual choice or addition of sugar, prefer higher levels of sweetness relative to other people in all the foods and drinks they have in certain situations. These same individuals may indeed always choose sweet over non-sweet alternatives in those situations. These would be two aspects of the individual characteristic of a sweet tooth, as this has been commonly conceived.

1.4.1 The sweet tooth defined The full construct of a sweet tooth can be defined as the combination of a greater preference for sweet food relative to non-sweet foods and also a greater preference for higher than for lower levels of sweetness intensity in foods (Pangborn and Simone, 1958; Frijters and Rasmussen-Conrad, 1982; Booth *et al.*, 1987; Conner *et al.*, 1988c). Such an individual characteristic has been thought to be a factor in causing obesity and dental caries (see Chapters 10 and 8). Much of the work that has examined this individual characteristic has used simple sugar solutions and so, on the basis of considerations presented above has questionable relevance to acceptance of foods in everyday life and hence their impact on health (Booth *et al.*, 1987).

1.4.2 Partial assessment of the sweet tooth construct The measurement of the full construct of a sweet tooth would involve relating preference for higher levels of sweetness in foods to a preference for sweet compared to non-sweet foods. Very few studies have done this. Olson and Gemmill (1981) got 31 4–5-year-olds to rank order four sugar-water solutions for preference and to choose among apple juice drinks and peanut-butter snacks with 0%, 5% or 10% added sugar by weight. A significant positive

correlation was observed between the level of sugar in the most preferred sugar water and the most preferred apple juice. They reported a similar but non-significant relationship between the most preferred level of sugar in sugar water and peanut-butter. It was noted that children who preferred the sweetest sugar water tended to have sweet foods among their favourite foods, at least as reported by their parents in a dietary questionnaire. They also reported a positive correlation between a sucrose taste preference index, calculated from the rank-order preference of the sucrose solutions (Greene *et al.*, 1975), and a greater number of preferred sweet items reported on the questionnaire. However, Olson and Gemmill (1981) failed to state the correlation between the choices of the two sweet foods (the apple juice and peanut-butter) which would allow examination of the extent to which the children generally preferred sweeter versions of the foods. Also, they failed to report any correlation between the sweet food choices and the preference for sweet foods as assessed by the questionnaire, which would be evidence for the full construct of the sweet tooth.

Pangborn and Giovanni (1984) obtained ratings of liking for lemonade with varying levels of added sugar in 51 adults. In the same assessors, they assessed by questionnaire the intake of various sweet and non-sweet foods and the preferences for high-sweet over low-sweet food alternatives. A positive correlation was found between the intake of sweet foods and questionnaire preferences for sweet food alternatives. The most preferred level of sweetness in the lemonade also correlated with preferences for high- over low-sweet foods, but not with intake of sweet foods.

Finally, Mattes and Mela (1986) found positive correlations between diary reports of the frequency of consumption of selected sweet foods and questionnaire assessment of the choice of sweet over non-sweet foods. They also found that the choice of sweet foods over non-sweet foods was positively correlated with the most preferred level of sweetness in oatmeal, but not with the most preferred sweetness of coffee.

These studies have thus provided only fragmentary evidence that people's food and drink sweetness preferences do indeed fall in patterns that match the full construct of the sweet tooth. In several recent studies, we have attempted to assess both aspects of the sweet tooth (Conner *et al.*, 1988c; Conner and Booth, 1988; Booth *et al.*, unpublished).

1.4.3 Full assessment of the sweet tooth construct

The first aspect of the sweet tooth, that of preference for sweet over non-sweet foods, was assessed by questionnaire responses to choices of sweet over non-sweet foods in situations where such a choice was likely. The second aspect, that of preference for higher over lower levels of

sweetness, was assessed by the psychophysical determination of the most preferred level of sweetness by the methods outlined above.

In a first study (Conner *et al.*, 1988c), the ideal points for sucrose in a lime drink, a chocolate and tomato soup were assessed in two separate sessions (between 1 and 4 days apart) in 18 assessors, using the psychophysical procedures based on the acceptance triangle. In each session, a bias-minimised selection (Booth *et al.*, 1983) of between 6 and 10 samples of a particular food varying in added sucrose were presented to an assessor, who rated each sample relative to the most preferred level of sweetness. This provided an estimate of the most preferred concentration of sucrose for each food in each assessor. These estimates of the sucrose ideal point within individuals were reasonably stable across sessions up to several days apart (r values from 0.67 to 0.86, $p < 0.01$) and no appreciable shift in group means. The individual preference for higher over lower levels of sweetness was investigated by three-way interaction as the error estimate (Table 1.4). This analysis revealed no significant effect of sessions but a significant effect of both foods and subjects. The subjects effect confirms one aspect of the sweet tooth—that there is a personal characteristic of higher or lower average peak sucrose preference. The foods effect indicates that the peak preference varies substantially among foods. Of the two-way interactions only foods \times subjects was significant. This reflects the fact that while all assessors preferred their chocolate the sweetest, some assessors preferred their lime drink sweeter than their tomato soup while for others the reverse was true (Figure 1.4).

Table 1.4. Analysis of variance of the ideal point (IP) measured in two separate sessions for 18 subjects for three foods (chocolate, lime drink and tomato soup) (Conner *et al.*, 1988c).

Source of variation	df	<i>F</i> ratio	<i>p</i>
Sessions	1	0.44	n.s.
Foods	2	11.1	0.01
Subjects	17	14.2	0.01
Sessions \times foods	2	0.43	n.s.
Sessions \times subjects	17	1.52	n.s.
Foods \times subjects	34	8.35	0.01

In the same assessors (Conner *et al.*, 1988c) the choice of sweet over non-sweet food alternatives was assessed by a questionnaire based upon an examination of the common eating or drinking situations currently available in England. Only six common situations were identified where there was a choice between an item containing little or no sweetener and one or more items in the same category of food or beverage containing substantial amounts of sweetener. These were: for a light uncooked lunch, young celery or carrot sticks; for hors d'oeuvres, tomato juice or orange juice; for dessert, cheeseboard or cake trolley; in a café, ice-cold milk or

Sugar content (%)

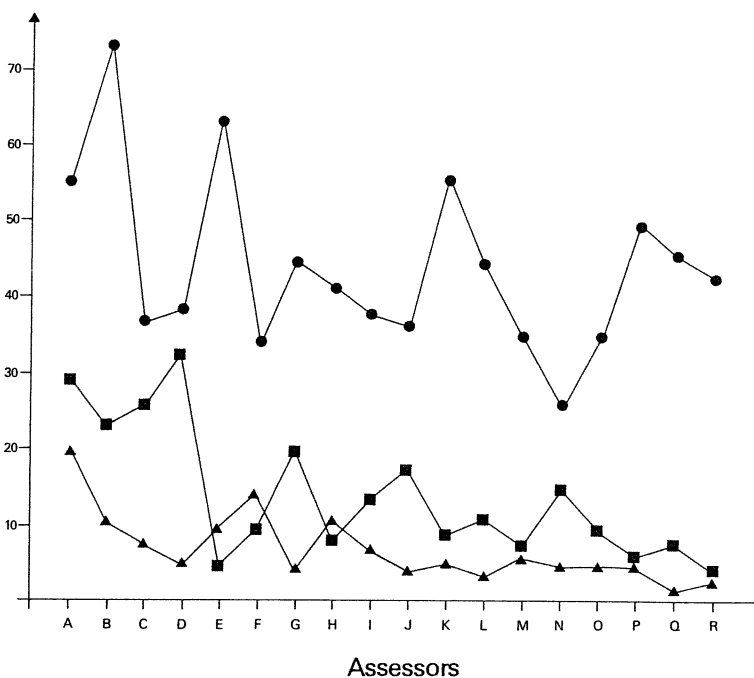


Figure 1.4. The most preferred level of sucrose for three different foods (●, chocolate; ▲, lime drink; ■, tomato soup) in 18 individual assessors ordered by decreasing size of the geometric mean of the three ideal points (Conner *et al.*, 1987c).

flavoured milk shake; when mildly thirsty, soda water, tonic water or lemonade; and with a piece of bread and margarine, no other spread, honey or chocolate spread. In each case, a preference ratio was calculated as the rated likelihood of choice of the sweet item over the sum of the rated choice for both the sweet item and unsweet item. Principal components analysis of these six preference ratios revealed several separate factors. The first factor, which was interpreted as a preference for sweet snacks had high loadings from choices of cake, flavoured milk shake and bread with honey or chocolate spread. Carrot sticks and tomato juice loaded on the second factor, which was interpreted as a preference for sweet vegetables. Finally, the choice of tonic water or lemonade loaded highly on a third factor. The loading of several preference ratios on the same factor is evidence for the other aspect of the sweet tooth—the choice of sweet over non-sweet alternatives. However, the separate sweet snack and sweet vegetable factors are evidence against the existence of the generally assumed unitary form of the sweet tooth.

The full construct of the sweet tooth would be supported to the extent that these sweet preference factors correlated with the ideal sucrose levels in the

three sweet foods. However, again evidence for only a differentiated form of the sweet tooth was found: there was no significant correlation between any of the factor scores and the geometric mean of ideal levels across the three psychophysically tested foods. However, the sweet snacking factor scores were correlated with the chocolate sucrose ideas ($r = 0.51$, $p < 0.01$) and the sweet vegetable factor scores correlated with the soup sugar ideals ($r = 0.43$, $p < 0.05$).

It thus appeared that choice between sweet and non-sweet alternatives was somewhat differentiated between situations. Therefore, the mean ideal sugar level was compared in turn with each instance of sweet and non-sweet choice. The geometric mean ideal sugar level correlated with the preference ratios for choice of carrots over celery ($r = 0.30$), for tonic water or lemonade over soda water ($r = 0.41$, $p < 0.05$), and for bread and margarine, with honey or chocolate spread, over plain bread with margarine ($r = 0.60$, $p < 0.01$). This is further evidence for a differentiated form of the sweet tooth: some people like foods highly sweetened but prefer sweet foods to non-sweet foods only in some situations.

A second study (Conner and Booth, 1988) aimed to replicate part of these results in a substantially larger sample. Choices of sweet over non-sweet foods were assessed using the same questionnaire. However, in order to test a large number of assessors, each very briefly, the most preferred level of sucrose was assessed in only one food, the lime drink and usually only three samples of the lime drink varying in added sucrose level were presented to an assessor, as this procedure had been shown to be very similar to a longer session in values estimated for the most preferred sucrose concentration (Conner *et al.*, 1986). Principal components analysis of the six sweetness preference ratios revealed two factors. The first factor, which was similar to the sweet snacking factor in the Conner *et al.* (1988c) study, loaded highly on the choices of cake trolley, flavoured milkshake, tonic water or lemonade and bread with honey or chocolate spread. The second factor, which may represent a sweet tooth for vegetables and fruit, loaded highly on the choice of carrot sticks and orange juice. Evidence for at least a differentiated part of the full construct of a sweet tooth in this study was provided by a significant positive correlation between the sweet snacking factor and the lime drink ideal sucrose levels ($r = 0.26$, $N = 302$, $p < 0.0005$). In contrast, the sweet vegetable and fruit factor failed to correlate with the lime drink ideal ($r = -0.01$), again indicating no unitary sweet tooth across foods or situations.

Hence, these two studies provide evidence for the full construct of the sweet tooth: some people both liking foods generally highly sweetened and preferring sweet foods to non-sweet alternatives. However, the concept of a food-universal sweet tooth was not supported in either study. Indeed both studies seem to point to the idea of a snacking sweet tooth: some people preferring sweet to non-sweet foods and higher to lower levels of sweetness

in snacking situations. The sweet vegetable/fruit factor was also evident in both studies.

There were also small but reliable group differences between males and females and between older and younger respondents in the sweet tooth measures in the Conner and Booth (1988) sample. This replicated an earlier finding (Conner *et al.*, 1986) with the lime drink, that the young and males tended to show slightly greater sweetness preferences on average. However, these differences were rather small and not consistent across foods. Tuorila-Ollikainen and Mahlamaki-Kultanen (1985) suggest that females are more concerned with the problems associated with sweet foods because they are traditionally more exposed to and more interested in nutritional information. Conner *et al.* (in preparation) found females to hold significantly more negative attitudes towards sweet foods than do males. Similar explanations may account for differences between older and younger groups (Conner, in preparation).

The evidence for a sweet snacking tooth in these two studies has been taken to imply that habits of taking sugar in drinks and foods between meals may induce a liking for sweetness in those contexts (Booth *et al.*, 1987). The traditional interpretation that the adult sweet tooth reflects a strong innate sweetness preference is rejected because of the evidence that adult sweet preferences are learned, as discussed earlier. Further work is necessary to determine whether the young and males are less susceptible as a group to current warnings about sugar or whether other factors are operative to produce their slightly greater average sweetness preferences. Drewnowski and colleagues (Drewnowski and Greenwood, 1983; Drewnowski *et al.*, 1985; Drewnowski, 1987) have suggested that the individual preference for high levels of sweetness is in fact a preference for high levels of both sweetness and fat (the 'sweet-fat tooth') in particular foods (e.g. ice-cream, cream cakes, etc). The studies presented here found no evidence that the individual preference for sweetness depended on the co-occurrence of high levels of fat with the sugar, e.g. as in chocolate; fat-free lime and tomato sugar preferences were no more clearly related than chocolate sugar preferences with either. The relationship between the individual differences in a sweet tooth, a fatty tooth and sweet-creamy tooth requires further investigation.

The data presented here provide evidence for individual differences in the choice of sweet over non-sweet foods and in preferred levels of sweetness in particular foods and drinks in certain contexts. The distribution of these individual differences in the population and their relationships with food selection require further study. The measures of characteristics of individuals' sweetness preferences in particular foods used in these studies can begin to elucidate the effects of sweetness preference on food choices and sugar consumption at the mechanistic level, rather than just at the level of social and market statistics. Such information on determinants of food

consumption patterns in individuals will also elucidate the roles of sugars in obesity (Chapter 10) and dental caries.

1.5 Other factors affecting sweetener perception

The sweetness of a compound can vary with the medium in which the sweetener is dispersed, with other constituents mixed in the medium or even with the labelling of the food or drink. The procedures detailed above can be applied to the investigation of the interaction between sweetness and other factors (e.g. Booth and Blair, 1988). Bartoshuk (1978) deals with interactions between sweeteners and different tastants. This section briefly considers data on interactions between sweeteners and other factors in foods and drinks.

Effects of colour, aromas, viscosity, solution temperature and flavours amongst others on sweetness perception have been examined, but in general the results are equivocal. In the interests of simplicity and experimental control, most studies in this area have used simple aqueous solutions and so any generalisation to real foods can only be tentative. In addition, a wide variety of different psychophysical methods have been applied, with divergent results (Pangborn, 1987).

The colour of a food can have a marked effect on the rated sweetness of the food, presumably through effects on expectation. Pangborn (1960) reported that the colour of aqueous samples of sugar and peppermint flavouring affected ratings of sweetness and flavour strength: orange, red and cherry-coloured solutions produced higher sweetness and flavour intensities while green had no effect compared to uncoloured solutions. Johnson and colleagues (Johnson and Clydesdale, 1982; Johnson, *et al.*, 1982) reported that the concentration of colouring also affected the rated intensity of sweetness, with more intense red aqueous solutions being judged up to 10% sweeter.

Similar effects have also been seen with real foods. For instance, colouring dry white wine to simulate sauterne, sherry, rose, claret or burgundy wines produced no effect in naive judges but produced a marked effect with experienced wine tasters (Pangborn *et al.*, 1963): the rose-coloured wines were judged the sweetest. Rose wines do indeed tend to have a higher level of sweetness. The appearance of the effect only in experienced judges is good evidence that the effect of colour on sweetness perception is based upon expectation.

Viscosity also influences sweeteners perception, but by affecting the rate at which the sweetener reaches the gustatory receptors, not by a sensory mechanism. For example, in an early study by Mackey and Valassi (1956), sucrose was more easily detected in pure aqueous solution than in tomato juice. Marshall and Vaisey (1972) reported that those gels which took

more effort to disintegrate reduced the sweetness of sodium sacaryl. Several studies have attempted to develop mathematical relationships of the effects of viscosity on sweetness intensity, with some degree of success in certain cases (e.g. Izutsu *et al.*, 1981). Unfortunately many of the studies have used aqueous solutions and it is not clear that the relationships found apply to more complex food systems (Pangborn, 1987).

The effect of temperature on sweetness perception has generally only been investigated in solutions. In general, the results indicate a maximum perceived sweetness in solutions in the range 20–40°C with drops in perceived sweetness on either side of this range (Pangborn, 1987). Again this may or may not be some combination of sensory and physical effects.

There is clearly a need for studies to examine taste interactions in real foods and drinks. The procedures based upon the acceptance triangle described above provide one way in which this can be done. The fundamental issue is the discriminativeness among levels of tastants shown by an integrative judgment such as acceptance (Booth, 1990).

1.6 Satiety and sweetness preference

Satiety refers to an eating induced decrease in eating motivation (Booth *et al.*, 1987) or the graded termination of eating (Rolls, 1987). These changes can involve both a temporary reduction in the palatability of some foods and the relief of bodily sensations labelled hunger (Booth, 1990). Like the individually preferred composition of familiar foods and drinks, all individual decisions to consume and to cease consuming on the basis of bodily sensations, remains on the food plate or social cues are learned (Booth, 1972b; Booth *et al.*, 1974, 1976, and unpublished). This learning sets up cognitive processes cued by sensory characteristics of the food or drink, physiological changes following ingestion of food, and the environmental factors of time of day, servings, others' behaviour, etc. Of the metabolic changes, energy production in the liver is likely to have a fundamental controlling influence on satiety (Booth, 1972a; Booth and Mather, 1978; Langhans *et al.*, 1983; Tordoff and Friedman, 1986).

Various studies have examined the effects of consumption or preloads of sweet solutions on the subsequent acceptability of sweet solutions (e.g. Cabanac, 1971; Wooley *et al.*, 1972; Cabanac *et al.*, 1973). However, many of the early observations have not been confirmed (Thompson *et al.*, 1976; Drewnowski *et al.*, 1982). In addition, as noted earlier, the relevance of ratings of the acceptability of plain sweet solutions to normal eating is questionable. Studies on satiety using real foods have however been carried out and it is these studies that will be considered here. Some links between sweetness preference, satiety and body weight are considered in Chapter 10.

1.6.1 *Sensory-specific satiety*

In one study (Rolls *et al.*, 1981), subjects rated the pleasantness of the taste of eight foods and then consumed as much of one of the foods as they liked. Two minutes after the end of this meal the subjects re-rated the taste of the eight foods. The pleasantness of these foods consumed in the meal decreased significantly more than the foods not eaten in the meal. Thus satiety appeared to be specific to the food that has been consumed. It was also found that this 'sensory-specific' (i.e. food stimulus-specific or cognitive) satiety caused less of the same food to be consumed in a subsequent meal while having no effect on the consumption of very different foods.

Rolls and colleagues (Rolls *et al.*, 1984, 1986) have found that, in addition to reducing the pleasantness of the food consumed, there is also an interaction between foods, so that similar foods show decreases in pleasantness. For instance, Rolls *et al.* (1984) found that, after the consumption of a sweet food, other sweet foods declined in pleasantness while savoury foods were unaffected, whereas the consumption of savoury food decreased the pleasantness of other savoury foods but not of the sweet foods. Thus stimulus-specific satiety will limit the consumption of one type of food within a meal and may promote the consumption of a nutritionally varied diet (Rolls, 1986), unless many powerful sensory differences are introduced into basically the same food type.

1.6.2 *Effects of intense sweeteners*

Intense sweeteners provide a useful way of separating the calorie and sensory effects of sweetness on satiety. Rolls *et al.* (1986) had subjects rate both their own hunger and the pleasantness of an orange gelatin dessert. They then consumed a meal of as much as they liked of an aspartame-sweetened or a sucrose-sweetened gelatin dessert. Then 2, 20, 40 and 60 min after the completion of the meal they re-rated both their own hunger and the pleasantness of the taste of the dessert. In both sets of ratings a similar pattern of responses was observed for high-calorie and for low-calorie desserts: a large drop in hunger and pleasantness at 2 min which had not recovered by 60 min. In addition, there was no difference in the amount of cheese and biscuits eaten 1 h later by the high-calorie and low-calorie groups.

These results have been interpreted as evidence that food intake is determined by preconceived notions about the calorie content of food (Rolls, 1987), shown to be influential by Wooley *et al.* (1973) and Booth *et al.* (1982) for example (cognitive satiety). These expectations and habits are presumably based on learning about what are appropriate portions of particular foods and drinks in particular contexts, probably

based largely upon considerable experience of the post-ingestive effects of consumption of particular foods and drinks (such as satiety). Birch and Deysher (1986) obtained similar results to Rolls *et al.* (1986) in studies comparing children's and adult's consumption of puddings sweetened with either fructose or aspartame. They found that 2–5-year-old children tended to consume more of the aspartame sweetened pudding than the fructose sweetened pudding, while adults tended to consume similar amounts of each. This was interpreted as suggesting that children may be better at compensating for missed calories because they rely more on bodily signals and less on learning what sizes of portions are appropriate.

In contrast, a study by Rogers and Blundell (1989) suggests that adults may be quite good at such caloric compensation. They gave subjects either a plain yoghurt or a yoghurt supplemented with starch or sweetened to an equal intensity with either saccharin or glucose and then 1 h later a sandwich lunch. Food intake at the meal was significantly greater following the saccharin preload compared to the other (caloric) preloads. This effect can be attributed to interaction of cognitive with physiological influences on satiety, with the subjects who had the saccharin-sweetened yoghurt presumably feeling less full at the sandwich lunch than they expected. Such an effect could be attributable to learning about post-ingestional satiety based upon previous experience with yoghurt.

Other studies (cf. Rolls, 1987) suggest that intense sweeteners are as satiating as bulk sweeteners within a meal but that in the longer term there is caloric compensation for the missing carbohydrate. However, in some studies this caloric compensation has been thought to be incomplete (e.g. Porikos *et al.*, 1982). The intense sweeteners obviously do not affect satiety by providing energy but the foods and drinks they are part of do provide some similar physiological reactions to foods and drinks sweetened with bulk sweeteners (Rolls, 1987). Booth (Chapter 10) discusses the efficacy of foods and drinks sweetened with intense sweeteners in weight control.

1.6.3 Overriding satiety

The question of the quantitative relationship between the amount of sweetness added to a food or drink and the amount consumed appears not to have been addressed (H.R. Kissileff, personal communication, 1989), although the relationship between palatability and consumption has received attention (e.g. Kissileff, *in press*). Within a meal, sensory-specific satiety would suggest that more of a sweet course than of a savoury course would be consumed following the consumption of a savoury course. In addition, the learned basis of sweetness preferences would suggest that a food with a personally most preferred level of sweetness

would be more likely to be consumed than a food with more or less sweetness. Thus, the precise relationship between level of sweetness and amount consumed is likely to be highly contingent on past and present circumstances. It is only in the case of a novel food where the simple addition of sweetness might be expected to increase consumption due to a breakthrough of the innate sweetness preference (Booth *et al.*, unpublished).

1.7 Conclusions

Food selection has complex determinants. Sweetness is merely one attribute that affects selection. Other attributes both of the food and of the eating context are likely to have effects on food selection just as much as sweetness, and indeed these influences may interact. All these interacting influences on food selection, including sweetness, are learned, even though there is an innate sweetness preference. In the adult who is thoroughly familiar with eating the food or drink being presented, the sweetness preference is likely to be entirely acquired. Only in the case of unfamiliar foods is the innate preference likely to have any substantial effect.

The learned nature of food preferences provides the basis for a procedure for optimising the level of a factor such as sweetness in a food for its acceptability to an individual, at least in a given context of consumption. Data obtained by such procedures have begun to elucidate patterns of individual difference in sweetness preferences among foods. These studies have revealed evidence for the individual characteristic of a sweet tooth, but in a more fragmentary form than traditionally assumed. The role of sweetness in the starting and finishing of meals has also begun to be investigated. Intense sweeteners can be valuable tools in dissociating the effects of calories from sweetness in such research on control of intake, so long as expectations as well as physiological effects are taken into account.

Moreover, the replacement of bulk sweeteners with intense sweeteners in ordinary products is problematic. Intense sweeteners have differing qualities of sweetness among themselves and from each other (Lindley, 1987) and the bulk sweeteners often serve several purposes in the food or drink (e.g. texture) in addition to providing sweetness. Lindley (1987) suggests that, rather than attempting to match an existing product using an intense sweetener, it may be simpler to formulate a new product with quite different sensory properties; otherwise, consumers may base their judgements on similar products which are sweetened with other sweeteners. The principles of optimisation based upon learned preferences would apply equally to the use of intense sweeteners in foods and drinks. Furthermore,

it must never be forgotten that sweetness is merely one of many influential attributes of a food or drink, for all of which each consumer has her or his own preferred intensity, quality and context.

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2 The carbohydrate—sucrose

W.M. NICOL

2.1 Source

Carbohydrates are a family of naturally occurring substances of varying sweetness comprising hydrogen, carbon and oxygen. Sugars, starches and celluloses are sub-sections of the family but in this chapter the focus is on sugars and in particular on sucrose, the most important member of the family. Sucrose, or sugar as it is commonly known, occurs widely in nature. It is present in the juice of most fruit and some root vegetables notably the sugar beet *Beta vulgaris* which is a temperate climatic zone plant. It occurs in the sap of many trees such as the American sugar maple *Acer saccharum*, the carob *Ceratonia siliqua*, and certain palm trees. It is also found in the stem and roots of grasses, particularly the sub-tropical continental grass, the sugar cane *Saccharum officinarum*.

Sugar is generated in the leaves of plants by the photosynthetic process whereby carbon dioxide from the air is combined with water drawn through the roots, with the aid of biological catalysts in the leaves, to form sugars. These are stored in the stalk of sugar cane or the root of sugar beet. Both are high yielding crops of world importance. Sugar cane outproduces, in terms of dry matter per unit area, any other tropical plant including rice and cassava, while sugar beet is equally efficient against temperate crops. For example under comparable conditions, barley yields a human metabolisable energy per hectare of 43 GJ in comparison to sugar beet's 87 GJ in the form of refined sugar. In addition, sugar beet provides animal feed of 34 GJ from the green tops and 35 GJ from the spent pulp and molasses (Shore, 1978). Austin *et al.* (1978) have calculated very carefully the energy balance of beet grown in the United Kingdom with cane grown in one of the most efficient cane areas in the world, Queensland. Per ton of sugar, the usable energy from sugar and the by-products is 28 GJ for beet and 29 GJ for cane, while the energy required to produce them is 26 GJ for beet and 14 GJ for cane. Taken together, both sugar crops are undoubtedly the most efficient means of producing energy for human nutrition.

For more than ten years now a major part of the cane crop in Brazil has been used for fermentation to alcohol. Motor vehicles have been converted to run on the alcohol fuel. In absolute cost terms, the alcohol is more expensive than petroleum fuel but politically it is expedient to minimise importation of oil.

Traditionally sugar beet has been grown, processed and consumed largely in the countries or regions of origin, where it is a very important part of the crop rotation cycle. On the other hand, cane sugar is extensively grown in developing countries both for local consumption and for export around the world. Of the six largest cane sugar producing countries (Brazil, United States, India, Cuba, China and Australia) only China is not a major exporter. The volume of sugar produced in the world today (1990) exceeds 100 million tons, an order of magnitude less than the availability of starch from grain and roots. Around 60% of the sugar is extracted from cane and the rest from beet. Half of the cane sugar comes from the Americas. In the European Community, most sugar is beet based but 10% of the demand is met by cane sugar imported under the Lome Convention from several developing countries which at one time were administered by European states. Sucrose from other plant sources, mainly sugar maple and carob, is relatively insignificant in commercial terms and tends to be sold in syrup form as concentrated natural extracts with attractive associated flavours providing the main selling point.

2.2 Development and processing

Sucrose has been consumed wittingly or not by humans for thousands of years and has become, not by chance but for a number of important technical reasons, the principal sugar of commerce. Its importance to the food supply has made it a very political commodity. There are very few countries in the world where it is traded at its real market price, the so-called world price. The reasons are economic and protectionist since in many countries both the economy and the population rely on the agricultural crop for income and employment.

In general, sugar by the nature of its colour and inorganic impurities requires a double purification before standard, white sugar can be realised. The reasons for purifying to the white state are first to achieve stability in storage, and secondly to produce a versatile ingredient without colour and flavour, except sweetness. Both cane and beet are first processed to yield raw sugar which, as it is customarily traded, is an article of commerce and is *not* a food ingredient. Thus raw sugar is subsequently purified in a refinery to render it fit for human consumption. Processing is carried out on site in the growing area to generate raw

sugar which is then either shipped in bulk to a refinery or processed further to meet the needs of the local market. The principle reason for not completing the processing of cane raw sugar at source where there is cheap power has been mainly political and based on tariffs, but there are other reasons such as the need for adequate quality control and cost of special sealed transport for a food ingredient in bulk.

Over the last 100 years the trend has been one of increasing purity of the raw sugar traded between countries, reflecting market needs, improvements in technology and a breakdown of the political tariff barriers (Bennett, 1982). For example in 1950, the average purity (polarisation) was 97.33, whereas by 1980 it had risen to 98.6, and now it can be effectively 99%. This achievement is possible by transferring the first stage of the refinery operation to the end of the extraction process.

The role of refineries for sugar used to be for purifying and sanitising the sugar but now it is more accurately described as the conversion of sugar to the required shapes and forms for use as a food ingredient. Raw cane sugar has a high microbiological count by virtue of the conditions obtained in the producing countries; it is hot and humid and the impurities associated with the raw sugar raise its moisture level favouring microbial growth. Thus to import direct consumption 'raw' sugar requires considerable quality control and incurs increased freight costs. Notwithstanding these difficulties, it is now technically possible to transport in sealed containers consumption grade sugar from the cane growing areas.

To ensure fairness to both buyers and sellers in the international market, standardised methods of analysis have been agreed as a basis for trading sugar through ICUMSA, the International Commission for the Uniform Methods of Sugar Analysis.

The basic principles of sugar processing have not changed since the last century but the equipment and methodology, of course, have. The process is capital intensive and economies of scale are important factors in the industry.

2.2.1 Beet processing

The beet roots are first sliced into thin slivers and added to a diffusion vessel which is normally a large cylindrical drum rotating about a horizontal axis. Here the beet cossettes meet a countercurrent of hot water allowing a diffusion of the sweet juice from the beet cells into the water. The very dilute juice tapped from the diffuser is immediately limed and in a subsequent large tank aerated with carbon dioxide, a

process known as carbonatation. Calcium carbonate is precipitated and this is separated by filtration which has the effect of removing both the solid and high molecular weight impurities from the beet juice. At this stage the juice is of a fairly dark colour resulting from the oxidation of plant substances (through the action of polyphenol oxidase). The level of colour in the thin juice is reduced by the addition of sulphur dioxide. This sulphitation also helps to reduce the generation of colour in the subsequent high temperature processing conditions, for now the juice with a sugar content of only a few percent has to be concentrated to a supersaturated state (75–80% solids) to enable the sugar to be crystallised. It is important to realise that sugar is heat labile and to minimise the destruction of sugar, evaporation and crystallisation are effected under vacuum (0.3 atm). Although continuous crystallisation is being introduced very slowly—it is very difficult for a number of reasons—most of the sugar produced today is still by batch crystallisation in a vacuum pan. The crystals are separated from the mother liquor in high speed basket centrifugals with perforations small enough to retain the sugar crystals but sufficient to allow the passage of the sugar syrup. Finally the crystals are dried in a falling-cascade, hot-air drum drier or in a fluidised bed.

To ensure that the mixture of crystals and syrup can flow from the vacuum pan to the centrifuge, not much more than 50% of the available sugar can be crystallised in each cycle in the batch process. Thus, the residual solution has to be recycled several times for further crystallisation to extract as much sugar as commercially possible. Normally three or four sequential crystallisations are possible before the level of impurities makes further extraction uneconomic. Thus nearly 90% of the original sugar can be separated as crystals. The residual solution is termed molasses. Beet molasses is typically rather bitter and unpleasant to the human palate but is acceptable to animals and provides a good substrate (source of sugar) for fermentation processes as in citric acid production.

The most common crystallisation practice is the double einwurf system whereby the crystals grown in the least pure syrup are fed back into the next highest purity crystallisation while the separated syrup moves to the next lowest purity crystallisation. The final crystals are therefore separated from the highest purity syrup to maximise overall purity. In beet sugar extraction, the penultimate crystals are dissolved and added to the concentrated virgin beet juice. In this way it is possible to obtain a white sugar of high enough purity for the market, but more often this is treated as raw sugar and reprocessed in a refinery. The beet industry is geared because of the nature of its raw material, towards a range of high purity white sugar of different crystal size.

2.2.2 *Cane processing*

Cane grows only in frost free areas roughly between 35°N and 30°S. Basically it is easy to grow; sections of fresh cane each with a growth node are strewn in furrows and covered. The nodes quickly sprout and grow, but competition with weeds in tropical areas is a big problem requiring regular cultivation until a leaf canopy is formed. The major requirements are sun and water. Generally cane is an annual crop. Once cut it has to be transported rapidly to the factory. Microbial contamination at the cut ends causes such a rapid sugar loss that good coordination between the factory and the farmers is necessary to minimise delay between harvesting and milling. At the factory the cane is cleaned and macerated to break open the woody stalk. Water is added to facilitate extraction by milling. In the last 20 years, a cane diffusion system has been developed in South Africa, but this requires very fine pre-shredding.

In the milling operation the macerated cane is passed through up to five sets of heavy duty, three-roller mills. Around 96% of the sugar is extracted in this way. The few cane factories that practise diffusion can achieve extractions of 98%, but because proportionately more impurities are also extracted, the difficulty of crystallising the sugar is increased. The residual fibre, bagasse, is used as fuel to generate power and there is usually enough to make the factory self-sufficient in energy. The juice at this stage is brown-green in colour and contains suspended solids, starch, chlorophyll and other plant impurities. Lime is added, and sometimes sulphite to reduce and prevent colour generation. The juice is superheated to coagulate impurities and is clarified by settling. By now the juice is straw coloured and very flavoursome, although dilute. The energy conscious cane industry has developed the use of multiple-effect evaporators to a high degree of efficiency to concentrate the juice to saturation. Almost without exception the double einwurf system, as described above, is practised for crystallisation to produce a consistent raw sugar from the virgin juice.

The spent cane syrup (molasses) is the most pleasantly flavoured of all molasses and is used widely as a food ingredient.

In the past, a wide range of types of native sugars was available according to the location of production and procedures used. Names like Muscuvado, Demerara and Barbados identified particular sugars which were shipped in the old way in hessian sacks. After 1950, however, sugar from the tropical producer countries began to be shipped in the holds of bulk carriers and it is rare now to be able to use these indigenous sugars commercially. Sugar suppliers, however, go out of their way to simulate the characteristics of the particularly sought after brown sugars.

2.2.3 Refining

The operations in the refinery are similar to those of raw production but here the objective is to produce sugar as a food ingredient in the right shape and size, and with characteristics most appropriate for the purpose for which it is sold.

Raw sugar from the warehouse is added to saturated syrup from earlier processed crystals at about 40°C and stirred to soften the molasses layer on the crystals. The mixture is centrifuged at a high speed (generating an acceleration of about 300×g) to separate the syrup from the crystals which are subsequently dissolved in hot water, subjected to carbonatation and filtered. The resulting clear coloured liquid is decolorised, traditionally by bone charcoal but carbon and ion-exchange resins are now also used. Ion-exchange resins are particularly effective for achieving the very high purity required for some applications but are easily poisoned unless care is exercised. A bone charcoal system, although very expensive to run, is undoubtedly the most effective all-round purification agent. For white sugar crystallisation, the einwurf system is not generally practised since the first, second and third crops of crystals are usually of high enough purity to meet the white sugar specification. The fourth crop, however, is decidedly yellow and finds a ready market with some manufacturers who do not require pure white crystals. Run off from the fourth crop is usually further processed into a range of syrups golden in colour and mildly flavoured. The basic refining process is geared primarily to white sugar production.

The basic grade of crystal is granulated sugar which has a mean crystal size of around 500 µm. Larger crystals can be grown but the technical difficulties are greater. Caster sugar with a mean crystal size of around 250 µm is the smallest that can be economically crystallised. Finer grains are produced by milling. Brown sugars to meet consistent specifications are most easily produced by coating white crystals with specially prepared saturated solutions having the appropriate colour and flavour characteristics. In summary the refiner can offer, through mixing, separation and processing, a range of products more or less tailored to the needs of the manufacturer and the consumer. For the latter, there is granulated sugar (the basic and most economic grade), caster (whose fine crystals make it particularly suited to baking) icing (a milled sugar for confectionery decoration) and brown sugars with a wide range of flavour, colour and crystal size. For the manufacturer, the range is even more extensive including liquid sugars which are blends often with invert^{1*} and corn syrups.

^{1*}Sucrose hydrolyses readily in the presence of acid or invertase enzymes to the monosaccharides glucose and fructose. This mixture of monosaccharides is known commercially as invert sugar.

The crystallisation of sugar is exothermic and this fact is used effectively in primitive countries for the production of a basic microcrystalline sugar. There are numerous names for the product around the world reflecting the localised nature of the process. Areado and gur, however, are quite common. The juice is concentrated in large open pans until it is supersaturated, at which point it is nucleated and stirred rapidly. As crystallisation takes place, the heat generated is sufficient to dry off the sugar completely. This simple process, called transformation, has been modernised both in the United States by Amstar, who produce a range of products in this way including a free-flowing brown sugar, and in the United Kingdom by Tate & Lyle. Sugars of this type have the advantage of being microcrystalline and so have a large surface area which enables them to dissolve very quickly and to enable them to be used as carriers for flavours and colours. In addition, both companies have developed the process further to allow co-crystallisation of sugar with other ingredients like fibre, chocolate, nuts and other combinations of interest to the confectioner. Because they are only partially crystallised, the moisture of these sugars lies between that of crystal sugar and the liquidus isotherm as will be described later.

With skill and experience, it is possible to spray dry sugar although it is not easy. There are a number of patents which teach this practice but essentially it is necessary to recirculate a portion of the crystals as nucleation centres for further growth. Its relatively high cost rules it out for normal large scale production but for smaller volume products of higher added value it has advantages. It has been reported that cane juice dried by this method is being marketed.

There has recently been an interesting development for the extraction of juice from cane. Most of the juice is in the pith, the soft internal core of the cane, but lack of effective separation from the hard, outer sheath has so far prevented selective extraction. A machine has now been developed which slices the cane longitudinally and scrapes out the pith. At this stage in its development, however, it is much too expensive and delicate a mechanism for routine operations in a factory. Additionally, since the juice in the rind is not extracted, the economic use of this juice for other purposes would need to be developed. However, the great advantage is that the juice from the pith is of a higher purity and could be readily solidified by either of the two methods just listed to provide a natural and stable granular sugar.

2.3 Properties

The pre-eminence of sucrose is due to its universal availability and versatility. It is the most abundant, free sugar in plants and this has

been, and is still being, expanded by increased production in the developing world. Sucrose has a unique set of properties (Table 2.1) which has gained it this degree of importance in the food supply. McKay (1979) emphasised the other functionalities of sucrose by claiming that, even if sucrose was not sweet, it would still be a major food ingredient.

Table 2.1. Major properties of sucrose.

-
- High quality sweetness
 - Forms flavours and colours on heating
 - High and easy solubility
 - Bulking agent, carrier and diluent
 - High osmotic pressure
 - Cheap, translucent, pure chemically and microbiologically
 - Reacts with proteins and starches to form structure in food
 - Remains dry and unreactive so stores well
 - Rapidly and totally fermentable
-

2.3.1 Sensory properties

‘A spoonful of sugar makes the medicine go down’ encapsulates one of the principal roles of sugar if ‘medicine’ is taken in a broader sense to include unpleasant or tasteless food. It is, in short, a major aid to palatability. The human being appears to have an inborn desire for sweetness which he has used since the early days of his evolution to select safe and wholesome foods in contrast to the bitter taste which is frequently associated with toxic plants. Thus sweetness is a pleasurable sensation as evidenced by the use of honey in the past, and nowadays by the widespread use of sugar to increase the desirability of food. Its low cost and convenience has made it the generally used vehicle of sweetness in the world today. Even with the inroads of starch-based sweeteners (high fructose corn syrup in North America and isoglucose in Europe) and intense sweeteners, it still commands around 90% of the world sweetness market. For that reason, also, it is generally accepted as the standard for sweetness quality having a clean, smooth and shortlived profile in the mouth. Humans perceive it as having only sweetness almost by definition. Other sweeteners are judged with reference to sucrose and their profiles show that each one has different characteristics.

Unfortunately there is no standard instrument to measure sweetness or its quality, and experimental investigations have to rely on the use of the human tongue whose response suffers from normal person-to-person biological variation. Van der Wel *et al.* (1978) showed that the tip of the tongue has the greatest sensitivity to sucrose, whereas other sweeteners

demonstrate different areas of maximum sensitivity. The subjects of sweetness and sensory evaluation have received a great deal of attention in recent decades. A current review of sweetness by Dobbing (1987) indicates the diversity of study of the subject. Although the science of sensory evaluation has developed considerably and there are now many more sophisticated techniques available to analyse oral perception (Amerine *et al.*, 1965), great care has to be exercised in the interpretation of taste panel studies.

The difficulties are great enough in establishing the characteristics of even the commonly accepted standard of sweetness, sucrose. Its intensity is dependent on the conditions of test, particularly on temperature, concentration and acidity. Hyvonen *et al.* (1977) reported that at 5°C sucrose sweetness was reduced by about 10% and at 50°C it was equally enhanced compared to the measurement at 22°C. The effect of acidity is less clearly defined but overall the perception of sweetness is reduced as acidity increases (Pangborn, 1965).

Apart from contributing sweetness, sucrose enhances the flavour of food and drinks by creating a better balance between acidity, bitterness and saltiness. For example consumer tests have shown that the adjustment of sucrose and acid levels in food are critical in optimising acceptability in terms of sweetness, flavour and texture. A recent development has been the introduction by Amstar in the United States of a flavour system for use with sucrose which contributes to flavour balance by reducing the perception of sweetness. It is expected that it will find applications in jams, icings and confectionery.

Heating pure sugar leads to browning as it degrades and gives rise to the distinctive caramel odour and flavour. Further heating causes the formation of bitter compounds and eventually to total carbonisation. Heating in the presence of amino acids (proteins) leads also to attractive Maillard reaction flavours developed in the production of toffee.

Sucrose has natural flavours associated with it: flavours which are co-extracted with the sucrose from the plant and are carried through the initial stages of processing. Flavours are also developed during heating in the extraction and purification process, and are concentrated in the molasses. Unfortunately because of the presence of bitter impurities like betaine, the sugar beet flavour is not very pleasant in contrast to the desirability of the flavour emanating from the sugar cane and its process. Thus cane juice is an attractive drink in the producer countries. Canned juice and dehydrated cane juice are available as specialised products in other countries. Cane molasses from a raw sugar factory is widely used on account of its more attractive yet complex flavour resulting from the concentration of salts, and the thermal decomposition of sugar during the evaporation and crystallisation stages of production.

2.3.2 *Physical properties*

2.3.2.1 Solubility. Sucrose is readily soluble up to the saturation concentration which is 2 g/g water at 20°C rising to 4 g/g water at 86°C. However, solution from the crystal state is not instantaneous but varies directly with temperature, rate of agitation, degree of undersaturation and inversely with crystal size. Of all the commercially available sugars, caster generally dissolves the fastest for although icing is finer, it tends to clump and behave as if it were large crystals.

Addition of mixtures of sugars to water leads to higher dissolved solids. The inclusion of invert sugar in the optimal ratio increases the solubility at 20°C from 67.7% for sucrose alone to 75.1% for the mixture. The effect is used to advantage commercially in the manufacture of golden syrup in which partial inversion of sucrose reduces the potential of the syrup to crystallise.

2.3.2.2 Bulk. Sucrose, apart from being the ideal sweetener, also has other important functionalities in the food supply; it acts as a bulking agent (filler), a diluent and a carrier for trace ingredients. Its large particle size, relative to other ingredients, provides adequate surface area to carry trace ingredients like colours and flavours and thereby improve their dispersion. The sugar crystals also improve the particulate flow characteristics of mixtures, an important feature in a highly mechanised food industry. Its particle size aids wetting and dispersion when water is added. When mixed with fats it enables the incorporation of air into the mixture, so important in generating the lightness of cakes.

Through its viscous solution in water, it provides mouthfeel in soft drinks at relatively low concentrations while at the other end of the spectrum its immobility as a glass when the water content is reduced to a few percent gives the characteristic boiled sweet. Although the solution is highly supersaturated, crystallisation at that concentration is inhibited by virtue of the very high viscosity. Gradual crystallisation of boiled sweets is due to the absorption of moisture from the air diluting the glass sufficiently to allow crystallisation to proceed.

To meet the changing needs of the market, the sugar industry is adapting its technology. The transformation process described above has been developed to produce, efficiently and continuously, the novel microcrystalline agglomerates which retain all the handling advantages of normal crystal sugar but provide a greater surface area to carry other ingredients. Its rate of solution is enhanced and its bulk density can be markedly reduced making it an important ingredient in powdered drinks for example. Sucrose is difficult to compress satisfactorily into tablets (confectionery or pharmaceutical) but this is facilitated when formulated to include transformation sugar.

By co-crystallisation with sugar, non-granular solids like fats can be made free-flowing. The process opens up the possibility of novel texture forms to increase the range of choice for the consumer.

Niedick and Babernics (1979) found that freeze-dried sucrose in its amorphous state had a greater absorptive capacity for aroma compounds than the normal sucrose crystal. For high value materials, the higher cost of production can be justified but the amorphous form of sucrose is much more deliquescent than the crystal.

2.3.2.3 Osmotic pressure. The very high osmotic pressure generated by sugar solutions is a major factor in the use of sugars as preservatives. The practical significance is that sucrose binds water and so makes it unavailable to support spoilage organisms. Few fruits contain sufficient sugar to ensure the preservation of their flavours and textures. Addition of sugar to increase the dissolved sugars above 65% is the customary means of fruit preservation. Although this form of preservation is declining now in the face of increasing availability and use of freezing, the addition of sucrose prior to quick freezing inhibits oxidative degradation in the fruit. In canning too, sucrose diffuses into the fruit cells to preserve the integrity of the fruit and reduces the oxidative browning when ultimately exposed to the air. It is thought that a recent outbreak of botulism poisoning in the United Kingdom might have been prevented had the sugar in the hazelnut purée not been replaced by an intense sweetener.

Sucrose is also an important preservation agent in meat curing. In fermented sausages, the fermentation of sugar to acids inhibits the formation of toxins by food poisoning bacteria. Clearly it has to be used in much smaller amounts, usually below the threshold of sweetness at about 1%, but it does contribute to moisture retention in the final product.

2.3.2.4 Purity. Sucrose is one of the purest foods in the world today. The standard product, granulated sugar, is freely available with an impurity level of less than 0.1%, mainly plant residues. When it leaves the refinery, it has a tight microbiological specification which ensures that it is substantially free of any organisms or toxins that would generate a health hazard. In solution it has a clarity resulting from a very low colour and turbidity which matches the rigorous specification for the manufacture of soft drinks.

In relative terms sucrose is a cheap food ingredient. It is difficult to be specific because ingredient prices change with supply and demand, from place to place and with the almost universal political intervention.

2.3.2.5 Structure. Sucrose is particularly important in baking, regulating the chemical and physical reactions especially in crumb formation whose structure improvement, softness, is related directly to the amount of sugar.

The structure of a baked product is determined by its state when the starch present gelatinises and protein denatures. Since these phenomena take place at higher temperatures when sugar is present, the baked product has a longer time to expand (rise) before it is set by gelatinisation and denaturation. Wootton and Bamunuarachchi (1980) found that the temperatures of onset and termination of gelatinisation were not affected by the presence of sucrose but the temperature at which it reached its greatest activity was greater in the presence of sucrose. The effect is nearly proportional to the concentration of sucrose in solution, suggesting that it is related to competitive water binding. Before that, Bean *et al.* (1978) determined the gelatinisation temperature of wheat starch in the presence of concentrations of sucrose up to 60%. The most effective gelatinisation temperature for cakes is 90°C which corresponds to a sucrose/water ratio of 1.3 in the batter. At a fixed concentration, sucrose generated higher gelatinisation temperatures than glucose or fructose.

The interaction between sugar and proteins has also been studied in detail (Back *et al.*, 1979). As with starch the role of sugar is related to its water binding capacity. Razanajatovo *et al.* (1978) studied the gel formation resulting from heating mixtures of whey protein and sugar.

The structure of meringue is heavily dependent on sucrose both in solution and in its crystalline state. Fine grain sugar with its large surface area provides structural stabilisation of the egg white foam. The particle size of the sugar is critical in determining the mouthfeel of the product.

2.3.2.6 Water affinity. The pre-eminence of sucrose over all other sugars is the ease with which it can be produced in the dry crystalline form and its tolerance to a wide range of humidities. However it does have its limitations, if not in the factory at least in the home, in its tendency to caking or solidification in its storage container.

Relative humidity changes affect the moisture content of most foods. For example, bread and cakes become stale in a dry atmosphere. In addition to its high solubility in water (a saturated solution at 20°C has a concentration of 66% w/w), one of the other most interesting features about pure white crystalline sugar is that over the relative humidity (RH) range 20–70% the equilibrium moisture content is effectively constant. Thus sugar remains free flowing under normal European climatic conditions. The humidity isotherm for sugar, however, rises steeply around 80% RH and joins the liquidus isotherm around 85% RH (Figure 2.1). Therefore if the ambient RH rises above 85%, water is absorbed (slowly!) by the sugar and forms a thin saturated layer of syrup on the crystal surface. When the RH drops below 70% this syrup film crystallises and binds the crystals together. This type of RH cycle can occur diurnally and can lead to caking of sugar. The severity of the effect increases as the crystal size decreases because of the greater surface area of crystal contact. Carelessly stored confectioners' (icing) sugar

for example can solidify in just a few days. Bulk sugar in silos can cause trouble in this and many other ways. One of those is the effect of a thermal gradient across the sugar. Moisture is driven from the warmer to the colder areas where it can condense causing stickiness, caking or in extreme cases the formation of syrup.

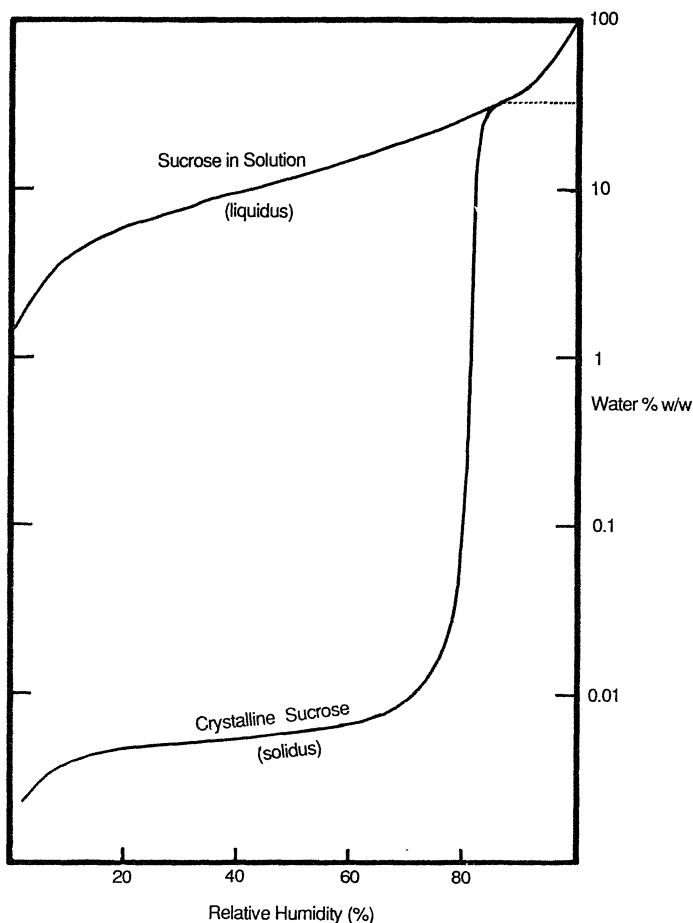


Figure 2.1. Sucrose-water equilibria.

In the manufacture of crystalline sugar, when the crystals are separated from the mother syrup and rapidly dried, there is a residual thin layer of uncrystallised glass on the surface of each crystal. In the course of a few hours this crystallises releasing moisture which has to be removed. Because of the rapidity of crystallisation there is also a small amount of

water trapped in the crystal lattice and this takes about 14 days to diffuse to the surface. While this seems to be a trivial issue, it does lead to problems if the sugar is not properly ventilated. Freshly crystallised sugar sealed in a plastic bag will generate sufficient free moisture to make the sugar 'creepy' and susceptible to spoilage. Sucrose for bulk delivery is therefore carefully conditioned by flushing it with air for many hours to carry away the slowly released moisture and so minimise subsequent problems.

Brown or raw sugar, because it has a higher inherent moisture content, poses greater handling problems as it is even more susceptible to RH changes, quite apart from its poor granular flow characteristics due to the syrup film on the crystal surface.

2.3.2.7 Fermentability. Sucrose is an excellent substrate for many organisms some of which can be harnessed while others create spoilage as evidenced in raw sugar production. The most important fermentation for which sucrose is used is the production of alcohol. The purity of the sugar source is not critical and so molasses with about 50% fermentable sugars is the foundation of the rum industry. Industrial alcohol is also produced from molasses. In the process of fermentation, sucrose is inverted to glucose and fructose so the fermentable solids are actually 105.26% of the sucrose solids. This is an important factor to be taken into account when considering the economics of substrates for fermentation.

Liquid sugars are used in brewing because they ferment totally, quickly eliminating any possibility of slow secondary fermentation in storage. In winemaking too sucrose is sometimes added when the natural sugar content of the grape juice is low.

In North America in particular, sucrose is added to bread-dough to reinforce the natural sugars and so increase the raising effect of the yeast.

2.4 Health and nutrition

2.4.1 Nutrition

Sucrose is an easily assimilable pure source of energy whose nutrient energy value is 16 kJ/g (expressed as monosaccharide) in contrast to that of protein (17 kJ/g) and fat (37 kJ/g) (McCance and Widdowson, 1978). Thus while it is fashionable to eliminate sugar from the diet for weight reduction, fat would be a better target. Whether slimming or not, a balanced diet is essential and so to reduce caloric intake, individual items should be restricted not removed. Booth (1988) argues that it is not only what is eaten but the pattern of eating that affects body weight. Indiscriminate replacement of sugar will not necessarily achieve weight reduction. In the United Kingdom

and United States, sugars contribute on average between 15 and 20% of a person's energy intake.

Sucrose in the latter half of the twentieth century became a fashionable target for certain people as an alleged cause of numerous diseases. Their hypotheses have led to a vast number of research studies and reports addressing the issues culminating in major reviews by some of the most eminent scientific organisations in the world. Four of the more recent reports have come from the FAO/WHO (1980), the US FDA's Sugars Task Force (Glinsmann *et al.*, 1986), the British Nutrition Foundation Task Force on Sugars and Syrups (1987), and the UK COMA Report (1989). The general conclusion reached was that sugar at the present level of consumption posed no hazard to health except for being a risk factor in the incidence of dental caries. The most comprehensive review of recent research work in the field of nutritional and safety aspects of sucrose has been published in the ILSI Human Nutrition Reviews series (Macdonald and Vettorazzi, 1988).

Sucrose in admixture with salt (Chatterjee *et al.*, 1977) is now widely used in developing countries as the most cost effective and convenient means of controlling dehydration in children suffering from loose bowels. Recent pioneering work in South America (Hersage and Montenegro, 1984) has shown that the application of granular sucrose to deep wounds accelerates the healing process and obviates the disfiguring scars left by normal suturing.

2.4.2 Cariogenic potential

Dental caries is a multifactorial disease requiring the coincidence of several critical factors for its initiation and progression. All fermentable carbohydrates (which include sucrose) constitute a risk of generating dental caries but are not the only cause. A much better understanding of caries is leading to more constructive means of countering it. In the developed world, there has been a marked fall in the incidence of caries although there has not been a corresponding fall in sugar consumption. The widespread use of fluoride in toothpaste and water is believed to be the major mediating factor, but the composition and quantity of saliva appears also to be important. One of the most significant recent meetings was the consensus conference on methods for the assessment of the cariogenic potential of foods in San Antonio in Texas in 1985 (Hefferren *et al.*, 1986).

It is apparent that since carbohydrates form an important part of human nutrition, yet pose a risk of caries, a priority for dental research is the discovery of means of neutralising the cariogenic potential of food that we need and desire as part of a well-balanced diet.

2.5 Strengths and limitations

The diversity of properties of sucrose makes it one of the most versatile food ingredients in the food supply. It is cheap and easy to produce the world over either from beet or cane. It is easily handled and stored and can be eaten without cooking. Its limitations are few but are the result of its versatility. Its sweetness can be overpowering in those applications where high concentrations are encountered, in jam for instance, although flavour modification can now deal with that problem. Dilute solutions are susceptible to microbial spoilage and require aseptic handling or the use of preservatives. To sum up its strengths, it is available, adaptable, acceptable and palatable.

2.6 Applications

The applications of sucrose are legion and not restricted to food. In this section a few of its principal applications will be expanded to illustrate its multifunctional role in food.

2.6.1 Confectionery

Chocolate is a suspension of finely divided sugar crystals and cocoa solids in cocoa butter, the technical term for the fat constituent of the cocoa bean. Sucrose serves two very important roles: one is to make more acceptable the mouthfeel of the fat and the other is to balance with sweetness the bitterness of the cocoa. The ratio between the two ingredients is limited by viscosity and sweetness both of which increase directly with sucrose concentration. Although sucrose is present as dry crystals, they are ground so finely, of the order of 20 μm , that they are imperceptible to the tongue. Lecithin is added to lower viscosity to ease moulding in manufacturing. The presence of moisture would adversely affect the viscosity and reduce consumer appeal and so must be excluded.

Milk chocolate is frequently manufactured from milk crumb. In this process, granulated sugar is dissolved in milk after it has been evaporated to around 40% solids. The mixture is further evaporated to about 90% solids and then kneaded with a quantity of cocoa liquor all under vacuum until all of the water is removed. The kneading ensures that there is adequate nucleation of sucrose crystals and ensures that the crystal size remains small.

Sucrose solutions can withstand a high degree of supersaturation for long periods. Boiled sweets where the water concentration is around 2% are an example of this. The very high viscosity of the solution effectively

prevents nucleation and crystallisation. As the sweet equilibrates with a humid atmosphere it will in time form a dilute layer on the surface which is more susceptible to crystallisation. To counter this, other sugars like glucose or invert are added. There is a threshold moisture level below which no crystallisation takes place. This threshold level for a sucrose/glucose mixture is equivalent to adding glucose syrup containing 14% water to sucrose.

Between chocolate and boiled sweets there is the intermediate state in which crystal and solution co-exist. Such products are called grained goods and range from fondant to fudge. The suspension of minute crystals is nucleated by the input of energy of agitation (beating) following a calculated evaporation of water. Ideally the crystals should not exceed 15 µm in size otherwise the product appears gritty in the mouth.

2.6.2 *Baking*

When sucrose is used as an ingredient in breadmaking it plays an important part in the structure of the loaf. Of course maltose is present naturally and other sugars such as glucose and lactose are often added as well. The presence of sugars provides the food for the yeast to grow and generate carbon dioxide to expand the structure. Sucrose is inverted by the yeast to glucose and fructose the latter of which imparts the darker rich crust colour while all of the sugars contribute to the characteristic bread flavour, aroma and sweetness quite apart from extending the keeping quality by binding free water. Sugars are added extensively in North America but not generally in the United Kingdom where the Chorleywood Baking Process (CBP) is used predominantly. However even in that process, sucrose reduces the proof time and the energy input. An addition of 8% sucrose on flour prolongs the softness of bread and delays the onset of mould which is one of the disadvantages of the CBP compared to the earlier bulk fermentation method. The production of alcohol when sucrose is added boosts the water binding created by any residual sugar in prolonging shelf-life.

Sucrose is a major determinant in the structure of cakes. It opens the texture and increases tenderness. As explained before, sugar delays the gelatinisation of starch and denaturation of protein by increasing the temperature at which they occur, thus allowing the mixture to rise more before being fixed. Sucrose influences the structure of the baked product by controlling competitively the hydration of proteins and starches which if allowed to hydrate too much cause a weak structure.

The particle size of the sucrose crystals is also important. When fat and sugar are creamed initially, air is trapped as the fat coated crystals are sheared. The trapped air expands on cooking to generate the light texture associated with sponge cakes. Caster sugar has been found to be the optimal crystal size for this application. In biscuit making, fine grain sucrose causes

a greater spread during baking than coarse grain and affects the surface appearance.

It is desirable to preserve the eating quality of a baked product by retaining moisture yet simultaneously reducing its water activity to prevent spoilage. Some of the so-called humectants which do this are shown in order of their effectiveness in Table 2.2. Sugars although less effective than salt are certainly more palatable.

Table 2.2. Humectancy factors of cake ingredients (after Seiler, 1969).

Salt	11
Glycerol	4
Baking powder	3
Glucose, fructose	1.4
Sucrose	1
Sultanas, currants	0.9
Flour	0.2
Butter, margarine	0.2

2.6.3 Soft drinks

Sucrose contributes sweetness, mouthfeel and flavour enhancement in soft drinks. Without carbohydrates, drinks tend to be thin and watery although this has been overcome to some extent by careful formulation in the fast growing low calorie drinks sector where intense sweeteners replace sugars. Mixtures of fructose and glucose derived from starch often referred to as high fructose corn syrup (US) or isoglucose (Europe) are serious competitors to sucrose for they can match sucrose in almost every respect. The choice between them is based on cost (US) or political intervention (Europe). For either ingredient, the important factors are microbiological sterility, freedom from floc-forming impurities and low levels of colour and inorganic impurities.

2.6.4 Preserves

The high osmotic pressure exerted by sugar solutions is a major factor in preventing spoilage by moulds and other organisms in the storage of food. Jams for this reason contain between 65 and 70% dissolved sugars. To many, sweetness at this high concentration is a disadvantage but unless the preserve is to be frozen or refrigerated, reduction of the sugar level would quickly lead to spoilage. The acidity of the fruit is sufficient in most cases to ensure the inversion of the sucrose to glucose and fructose whose combined osmotic pressure and solubility are both greater than sucrose. Since acidity is important in the setting of the pectin in the jam, the higher buffering capacity

of lower grade sugars precludes their use in favour of the regular high quality granulated sugar.

2.7 Conclusion

Sucrose as a highly desirable food ingredient has been known for thousands of years, attaining an importance and versatility in today's food supply which is remarkable. Hugill (1979) described it as the Royal Carbohydrate, and with good reason.

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3 Non-sucrose carbohydrates

M.A. CLARKE

3.1 Introduction

The chapter title, 'non-sucrose carbohydrates', is correct, in that sucrose in the form of cane or beet sugars will not be discussed. Many of the sweeteners included, however, do contain sucrose, in combination with other carbohydrates, generally monosaccharides and other disaccharides. The oldest sweetener known to man, honey, is a liquid mixture of mono- and di-saccharides. Honey, maple syrup (probably the first sweetener made by man in America), and their twentieth-century companions, corn sweeteners, are major subjects of this chapter. Molasses and syrups made from sugar cane or sugar beet and containing sucrose among other sugars, are the other major subjects. Each group of sweeteners will be described from the point of view of origin and manufacture, composition, physical, chemical and sensory properties, applications in the food, beverage and pharmaceutical industries, and range of products available.

Honey, maple syrup, corn sweeteners and other sucrose-containing products described are subject to few regulatory legislative restrictions, and in the United States are on the Generally Recognized as Safe (GRAS) list.

Most carbohydrate sweeteners described here have effects on dental caries, as described in Chapter 8, since most contain glucose and fructose and some contain sucrose. Little work has been carried out on the relative cariogenic properties of these sweeteners, and so that area will not be further discussed in this chapter. Several oligosaccharide sweeteners whose non-cariogenicity is their major selling point are described.

3.2 Honey

Honey has been defined by the US Food and Drug Administration as 'the nectar and saccharide exudation of plants gathered, modified and stored in the comb by honeybees (*Apis mellifera* and *Apis dorsata*)'. Modification procedures performed by the bee after the nectar is carried to the hive

include inversion of sucrose in the nectar, and concentration to over 80% solids. Annual world-wide production is over 1 million tonnes.

3.2.1 Composition and properties

The composition, color and flavor of any honey depend in great measure on the flowers from which nectar has been gathered, with color ranging from almost colorless sweet clover honey, to almost black buckwheat honey (White and Underwood, 1974). Raw honey from the comb requires no processing to be ready for consumption, but is generally heated to kill yeasts and microorganisms, and strained or filtered. Typical compositions of honeys are shown in Table 3.1.

Table 3.1. Composition of honey.

Component	Range (weight %)	Average (weight %)
Fructose	25–45	38.5
Glucose	25–37	31.0
Maltose	2–12	7.2
Sucrose	0.5–3	1.5
Water	12–23	17.1
Proteins		0.3
Minerals and vitamins		0.2

Source: National Honey Board, P.O. Box 281525, San Francisco, CA 94128–1525, U.S.A.

Table 3.2. Chemical and physical properties of honey.

pH	Range 3.4–6.1; Average 3.9
Isoelectric point	4.3
Specific heat	0.54–0.60 cal/g per °C (liquid)
	0.73 cal/g per °C (granulated)
Thermal conductivity	118×10^{-5} to 143×10^{-5} cal/(cm sec °C)
Freezing point depressions	For 15% honey, –1.42 to 1.53°C

Source: White and Underwood (1974); Crane (1979); National Honey Board, P.O. Box 281525, San Francisco, CA 94128–1525, U.S.A.

Some chemical and physical characteristics of honeys are outlined in Table 3.2.

Like specific heat and thermal conductivity, other physical properties vary with water (or total solids) content. Viscosity and rheological properties also vary with the floral source. Rheological behavior is generally Newtonian, but high protein honeys, e.g. heather and manuka, display thixotropic behavior. Typical viscosities are shown in Table 3.3, along with specific gravity and refractive index.

Table 3.3. Viscosity, specific gravity and refractive index of honeys.

Water content (%)	Viscosity (P) at 25°C
15.5	138.0
18.2	48.1
20.2	20.4
Temperature (°C)	Viscosity (P) 16.1% H ₂ O
13.7	600.0
20.6	189.0
39.4	21.4
Floral source (examples)	Viscosity (P) at 25°C, 16.5% H ₂ O
Sage	115.0
Sweet clover	87.5
White clover	94.0
Water content (%)	Refractive index (20°C)
16	1.4966
17.5	1.4927
18.6	1.4900
Water content (%)	Specific gravity (20°C)
15	1.4350
18	1.4171

3.2.2 Applications

Applications depend on color and flavor properties, and therefore on the floral source: most light colored honeys, such as clover honey, have light, delicate flavor and darker colored honeys, such as buckwheat or mint, have stronger flavors. Like all carbohydrate sweeteners, honey will darken on heating as the result of a variety of chemical reactions, including Maillard reactions, caramelization and hydroxymethylfurfural production (and subsequent decomposition). Honey is widely used in baked goods and confectionery where its humectancy properties can contribute extended product life, as well as sweetness, flavor and color. Honey's hygroscopicity finds use in the curing of hams and meats. Honey can be included in dairy products, sauces, spreads and dressings, dessert mixes, chilled and frozen desserts, cereals and dried fruits, often for reasons of flavor, color and the 'nature's sweetener' aspects; the functional properties of viscosity, humectancy, freezing point depression, fermentability and hygroscopicity are similar to those of invert syrups and starch-based syrups. Honey can

be used to replace sugars or starch-based syrups in most food formulations, with pH adjustment where required.

Dry, granulated forms of honey are available, usually mixed with a carrier (starch) to inhibit moisture pick-up, for use in dry mixes.

3.3 Maple syrup and sugar

Maple sweeteners, made by concentration of the sap of the sugar maple tree (*Acer saccharum*), are produced in Eastern Canada and the North-eastern United States. Heating of the sap causes flavor and color development, as well as concentration, and the characteristic maple flavor of the syrup and sugar (sold in granulated or in block form) is a major attraction. The pure product is more expensive, by an order of magnitude, than sucrose or starch-based sweeteners, and so many blends of maple syrup, with other syrups, or of other syrups with maple flavoring, are available, and nowadays are generally labelled as such; false labelling of maple products was common some years ago.

The composition of maple syrup is shown in Table 3.4.

Table 3.4. Composition of maple syrup.

Component	Amount (%)	Component	Amount (%)
Water	34.0	Soluble ash	0.30–0.81
Sucrose	58.2–65.5	Insoluble ash	0.08–0.67
Hexoses	0.0–7.9	Calcium	0.07
Malic acid	0.093	Silica	0.02
Citric acid	0.010	Manganese	0.005
Succinic acid	0.008	Sodium	0.003
Fumaric acid	0.004		

The carbohydrate make-up of fresh maple syrup is almost entirely sucrose. This will invert with time, but provides a means of detecting adulteration or replacement of maple syrup with cheaper starch-based syrups; the genuine maple product does not contain maltose while starch-based products do.

The physical properties of maple syrup are similar to those of sucrose syrups, making the product suitable for baking and confectionery, but because of its cost, most maple syrup is sold directly, and used as pancake syrup or in home baking. Maple sugar is sold in granular form for use on cereals and in home cooking. In slab or cake form, it is usually eaten as candy.

The characteristic maple flavor has been the source of many investigations and has been shown to be a mixture of many components, some from plant phenolics (vanillin, syringaldehyde, guaiacyl acetone)

and some from thermal degradation of carbohydrates (isomaltol, acetol, methylcyclopentenolone and α -furanone).

3.4 Molasses and cane syrups

Molasses is the general term for concentrated juice from sugar cane or sugar beet after varying amounts of sucrose have been removed. Because this book is about sweeteners in foods, this chapter will discuss sugar cane molasses only in that it is the major food molasses. Food grade molasses is known as treacle in the United Kingdom. Both beet and cane molasses are used for animal feed and as fermentation sources for ethyl alcohol and other chemicals, but these uses are amply described in the literature and are not discussed here (United Molasses, 1976, 1986; Paturau, 1982; Morano, 1984; Meade and Chen, 1985; McGinnis, 1987). Recent developments in technology have made possible some sugar beet molasses food products, but these are available in very small quantity at this time.

3.4.1 *Composition and properties*

Several common terms for molasses are defined as follows. Blackstrap molasses is the by-product from a sugar cane factory or raw sugar refinery; it is the heavy, dark viscous liquid remaining after the final stage of sugar crystallization from which no further sugar can be crystallized economically by the usual methods.

Types of blackstrap are further defined by the US Department of Agriculture as superior, normal or utility (Meade and Chen, 1985) but these are really definitions for feed grade material.

High-test molasses is the product obtained by concentrating clarified cane juice to approximately 85° Brix, partially inverted with either acid or invertase enzyme. High-test molasses is produced from cane juice instead of sugar, not as a by-product of sugar production.

High-test molasses, also known as cane invert syrup, cane juice molasses or fancy molasses, is a premium product, higher in sugars content and of a more aromatic flavor than blackstrap. It has been subjected to less heat than blackstrap, and so contains relatively fewer sugar decomposition products, which can add bitter flavour.

‘Sulfured’ molasses refers to the by-product of sugar manufacture where sulfur dioxide has been used to bleach color. Sulfured molasses may be lighter in color, but is higher in ash of the insoluble sulfate type. The term ‘unsulfured’ is more common. The approximate composition of cane molasses (blackstrap) is given in Table 3.5.

Table 3.5. Approximate composition of cane molasses.

Main constituents	Components	Normal percentage range
Water		17–25
Sugars	Sucrose	30–40
	Glucose (dextrose)	4–9
	Fructose (levulose)	5–12
	Other reducing substances (as invert)	1–4
	Total reducing substances (as invert)	10–25
Other carbohydrates	Gums, starch, pentosans, also traces of hexitols; myoinositol, D-mannitol, and uronic acids (MeO, 2.0–3.0)	2–5
Ash	As Carbonates ^a	7–15
	Bases: K ₂ O (30–50)	
	CaO (7–15)	
	MgO (2–14)	
	Na ₂ O (0.3–9)	
	R ₂ O ₃ (Fe) (0.4–2.7)	
	Acids: SO ₃ (7–27)	
	Cl (12–20)	
	P ₂ O ₅ (0.5–2.5)	
	SiO ₂ and insol.	
Nitrogenous compounds	'Crude protein' (as N × 6.25)	2.5–4.5
	True protein	0.5–1.5
	Amino acids, principally aspartic and glutamic acids, including some pyrrolidine carboxylic acid	0.3–0.5
	Unidentified nitrogenous compounds	1.5–3.0
Non-nitrogenous acids	Aconitic acid (1–5%), citric, malic, oxalic, glycolic	1.5–6.0
	Mesaconic, succinic, fumaric, tartaric	0.5–1.5
Wax, sterols, and phosphatides		0.1–1.0
Vitamins	Thiamine (B1)	2–10 ppm
	Riboflavin (B2)	1– 6 ppm
	Pyridoxin (B6)	1–10 ppm
	Niacinamide	1–25 ppm
	Pantothenic acid	2–25 ppm
	Folic acid	10–50 ppm
	Biotin	0.1– 2 ppm

Source: United Molasses (1976, 1986); Meade and Chen (1985).

^a Percent of ash given in parentheses.

Most commercially available molasses for food industry use is made by blending various factory and refinery molasses with cane syrup to provide a range of products of constant quality. These are discussed in Section 3.4.2.

The physical properties of molasses depend on composition. Viscosity,

in particular, can vary over several orders of magnitude depending on the inorganic composition (also polysaccharide) and temperature of the molasses. Specific heat and thermal conductivity values are, because of the above variations, usually those for an equivalent weight percent of sucrose (Meade and Chen, 1985). Cane molasses has an acid pH, usually between 5 and 7. The salts content (2–8%) can contribute buffering capacity, to stabilize flavors and prevent hydrolysis.

Color and flavor are the major properties that molasses contribute to food processing. Molasses always contributes some sweetness, in a degree usually decreasing as color darkens. The range of flavors is broad and complex, ranging from caramel, 'cane' flavor in light high-test molasses, to heavy, bitter notes, sometimes with licorice flavor. Extensive research has shown many, many compounds that contribute to molasses flavor. Because of the range of flavors, molasses can be used to mask or disguise other, less pleasant flavors, as for example the bitterness of bran in whole-wheat products. Molasses flavor can also be used as an enhancer in some sauces and in licorice products. The chocolate or coffee notes in some molasses can enhance, or substitute for, these flavors. Molasses can also be used as a coloring agent, for golden-brown to dark brown colors, especially in baked goods, and as a color enhancer, or masking agent, to disguise grey or grey-brown tones. Molasses displays the humectancy, colligative and water absorption (lowering of water activity) properties that would be expected of a sucrose syrup of similar solids composition (see Chapter 2); these properties lead to wide application in intermediate-moisture foods, and baked goods with extended shelf-life. However, non-sugars in molasses apparently have some effect on humectancy: molasses has been shown to be more effective in retaining moisture than either sucrose or corn syrups (Morano, 1984). These same non-sugars also exhibit an antioxidant effect, shown in Table 3.6, which is significant when this fraction is used at some 3% of the fat level in the food product. Inclusion in whole grain products is one potential role for this fraction.

Table 3.6. Antioxidant effect of molasses extract.^a

Prime steam lard		Days at 60°C			
Additive	Amount	0	2	4	10
Control	None	0.7	4.0	8.0	50.0
BHA	200 ppm	0.7	2.2	5.0	12.6
Molasses extract	200 ppm	0.7	1.8	3.7	23.3

^aGiven as peroxide numbers.

Molasses is a food product that has often been claimed to be a secret of youthful behavior and appearance (e.g. to inhibit hair greying).

Technological investigations, such as the isolation of the antioxidant fraction, may provide a scientific basis for what appeared to be myths.

A dried molasses product, usually mixed with corn syrup solids to absorb water, is also available for use in dry mixes.

3.4.2 Applications

Potential applications resulting from humectancy and antioxidant products are discussed above. Molasses products sold to the food industry represent the result of blending various molasses and syrups to produce products of consistent color, flavor and functional properties.

Table 3.7 lists several typical blends, their composition, flavor characteristics and potential applications. These blends are typical of material sold as molasses in the United States, and treacles, syrups or blends in other countries.

3.4.3 Cane syrup

The term cane syrup as applied to consumer products has a regional orientation to the southern United States, where cane syrups and blends are sold for use on pancakes, biscuits, cereals and in cooking. In general, for food industry use, cane syrups are produced at sugar cane factories (see high-test molasses, above) or at cane sugar refineries, where a blend of brown and golden colored streams are combined to produce a syrup. There are still several producers of direct cane syrups in Louisiana and Hawaii, but most other cane syrups are made from blends. The product is not well known outside the United States. Cane syrups are dark golden-brown in color, with medium flavor intensity (caramel, butterscotch, 'cane' and 'green' flavors; no heavy molasses flavor), and are partially inverted. Often a factory evaporator syrup is completely inverted, and mixed with uninverted syrup, to give a product of approximately 85° Brix, 25–30% sucrose and 50–52% invert. This is a relatively clear and low ash material.

Cane sugar refineries make 'liquid sucrose', a water white solution of 67–70° Brix sucrose, generally prepared by redissolving granulated sugar, but in special refineries, e.g. in Brazil, by extensive ion-exchange resin decolorization and carbon treatments. This product is often sold to beverage companies and canners because the color and clarity are immediately apparent, and there may be a saving on time and dissolution equipment.

Liquid invert sugar, a range of liquid products of sucrose, glucose and fructose of 75–77° Brix was, until the late 1970s, a major product (15% of market) in the United States. It was replaced by the cheaper starch-based

Table 3.7. Molasses blends.

Characteristic description	Unsulfered molasses (%)	Bakery molasses (%)	Confectionery and all purpose molasses (%)	Condiment molasses (%)	Robust molasses (%)
Composition					
Sucrose	35	32-36	33-37	30-36	33-37
Invert	37	36-40	28-32	21-27	16-20
Total sugars	72	70-74	63-67	54-60	51-55
Ash	2.5	1.2-2.5	4.5-5.5	6.5-8.5	8.0-9.0
Color	Golden-brown	Light brown	Medium brown	Dark brown	Dark brown
Flavor	Sweet, mild aroma, syrup flavor	Sweet, mild, distinctive	Moderate sweet, strong aroma	Strong, pungent flavor, good background	Strong flavor, heat resistant
Humectancy	Some	Good	Good	Some	Some
Buffer effect			Yes	Yes	Yes
Applications	Table syrups, toppings, peanut butter, fruit purées, confectionery and alcohol products	Fruit cakes, brownies, muffins, spiced baked goods	Barbecue sauce, extended products, candies (hard and caramel), toasted foods, gingerbread	Fermented products, condiments and sauces	Leavened and fermented goods, soy sauce, tobacco, licorice, baked beans, caramel, snacks

syrups, most notably high fructose corn syrup. Liquid products are still made in the United Kingdom, Europe, South America and other areas. Their properties and uses are covered in Chapter 2, and in later sections in this chapter on fruit syrups and starch-based syrups.

3.4.4 Golden syrup

Golden syrup, a product most popular in the United Kingdom, Canada, South Africa and Australia (countries in which it is manufactured), is a high Brix (77–82°), partially inverted syrup, filtered several times over bone char to give it a special golden color, very mild flavor and high clarity. The syrup is used directly on cereals, breads and baked goods, and in home baking. It tends to crystallize on storage and so is usually sold in cans. Composition and details of manufacture are usually proprietary information.

3.4.5 Sorghum syrup

Sweet sorghum (*Sorghum bicolor*) is a giant grass, somewhat similar in appearance to sugar cane, which can withstand cooler climates than cane. In the mid-west and parts of the southern United States, the juice of sweet sorghum is heated, clarified by skimming and concentrated into a syrup. Sorghum juice tends to be higher in invert sugars than is cane; therefore, it is difficult to crystallize sweet sorghum sugar, and syrup is the product of choice. Sorghum syrup is produced by small plants in Tennessee, and northern Mississippi and Alabama (information is available from the National Sweet Sorghum Producers Association, P.O. Box 1071, Knoxville, TN 37901–1071, U.S.A.)

The light-brown colored syrup has a distinctive pungent odor and flavor, in addition to its sweetness and molasses-like flavor. It is often blended with other (sugar or starch-based) syrups, but is seldom sold industrially.

3.4.6 Fruit syrups

A new product has entered the sweetener syrup field in the United States, Europe and South America: fruit syrup, or fruit juice concentrate. Excess fruit crops, or damaged fruit unsuitable for its original destination, is prepared in juice form. The juice is decolorized and the flavors removed, through proprietary processes, and the remaining material—usually a solution of invert sugar, because fruit sucrose is hydrolyzed to invert in the process—is concentrated to about 75° Brix at pH 4, and sold as a ‘natural fruit sweetener’. The product is designed for the ‘natural’ foods

market, so that the terms 'fruit sweetener' and 'no sugar added' can be displayed on the label, although the latter statement is questionable. These fruit products, made in different places from apple, peach, pear, citrus and grape juices, sell, on solids basis, at five to six times the price of sucrose.

3.5 Disaccharides other than sucrose

3.5.1 Lactose and lactulose

Lactose is a disaccharide, found only in milk, composed of a glucose and a galactose residue combined as 4-(β -D-galactosido)-D-glucose. It has low water solubility, and, upon hydrolysis to its component sugars, it becomes more soluble and sweeter. Lactose itself is sold in various dry solid forms, as crystalline or spray-dried α -lactose and α -lactose monohydrate. It is only really about 30% as sweet as sucrose, and is used not as a sweetener but as a bulking agent (most notably as a carrier for non-nutritive sweeteners such as saccharin and aspartame), in pharmaceuticals and in baked goods. Lactose hydrolysate syrups have been proposed as possible sweeteners for dairy products and ice-creams.

Lactulose, 4-O- β -D-galactopyranosyl-D-fructose, is an epimer of lactose, which can be prepared by alkaline isomerization (with lime or with basic ion-exchange resins) of lactose. Solutions of lactulose over the concentration range 5–35% (w/v) are about twice as sweet as lactose, or 48–62% of the sweetness of sucrose (Parrish *et al.*, 1979).

Lactulose can be manufactured from whey, a cheap and readily available by-product of dairies, and has been recognized as a safe food additive in the United States and Japan (Mizota *et al.*, 1987). It is utilized by bifidobacteria in the gut and is therefore used in infant formulae and milk-based health foods. It does not elicit the intolerance that many people, especially those of South-East Asian extraction, display to lactose. It can be tolerated by diabetics, and is used to treat chronic constipation and control portal systemic encephalopathy. It is also used as a non-cariogenic, low-energy sweetener in some processed foods and candies.

3.5.2 Palatinose

Palatinose, 5-O- α -D-glucopyranosyl-D-fructofuranose, also called isomaltulose, is made from the action of α -glucosyltransferase on sucrose (Amylase Research Society of Japan, 1988). It is sweet and non-cariogenic, but mainly of interest for its hydrogenated product, Palatinit, which is discussed in Chapter 4.

3.5.3 Leucrose

Leucrose, 5- α -D-glucopyranosyl-D-fructopyranose, has the same components (glucose and fructose) as sucrose, but linked α -1,5 instead of α -1,2. Leucrose is produced by the action of the bacteria *Leuconostoc mesenteroides* on sucrose in the presence of fructose. A glucosyl transferase component of the bacteria's dextransucrase complex adds a glucose unit to the available fructose molecule. Leucrose was first observed as a by-product of dextran synthesis, but is now in pilot production as a sweetener (Schwengers *et al.*, 1988). It is quite soluble (64% at 30°C), has about half the sweetness of sucrose and is non-cariogenic. Unlike some other disaccharides, it does not have a laxative effect, because it is hydrolyzed in the small intestine. Leucrose, a reducing sugar, crystallizes readily as a monohydrate. It has been tried, sometimes in combination with intense sweeteners, in the manufacture of candies, jams and chocolate, and shown to have a taste and mouthfeel similar to sucrose, but without cariogenicity. Toxicity tests have shown no difficulties, but the regulatory status of leucrose has not yet been determined.

3.5.4 Maltose

Maltose syrups are discussed in the section on starch-based syrups. However, crystalline maltose is sold as a sweetener and bulking agent (Johnson, 1976). Maltose has 33% of the sweetness of sucrose, but has similar physical properties and can inhibit retrogradation of starch and so prolong the shelf-life of baked goods; it stabilizes icings and glazes without increasing sweetness as much as would sucrose.

The higher analogues of maltose, maltodextrins, also starch hydrolysis products, are decreasingly sweet as molecular weight increases, and the tetramer is barely sweet. These products are used as fillers, bulking agents and water absorbing agents in dry mixes.

Maltodextrins is the term used for the smaller oligosaccharides formed by acid or acid-enzyme hydrolysis in dry form. Corn syrup solids is the term for the dried version (spray or drum dried) of refined corn syrup. Corn syrup solids have higher dextrose equivalent (DE) value (over DE20), and are sweeter than maltodextrins, because they have a higher degree of starch conversion, i.e. more glucose.

It may be mentioned at this point that malt and malt syrup are sometimes sold as sweeteners. Malt products are defined as follows: 'Malt is the product of barley germinated under controlled conditions. Malt syrup and malt extract are interchangeable terms for a viscous concentrate of a water extract of germinated barley grain. Malt syrup is usually a brown, sweet, and viscous liquid containing varying amounts of amylolytic enzymes.

Barley is first softened after cleaning by steeping operations and then allowed to germinate under controlled conditions. The germinated grain then undergoes processing, such as drying, grinding, extracting, filtering, and evaporating, to produce malt extract with 78 to 80% solids content.'

Malt products have a distinctive flavor in addition to some degree of sweetness. Dried malt syrup is sold as a tabletop sweetener, but generally with saccharin or other non-nutritive sweeteners added to increase the sweetness sufficiently for tabletop purposes. They will not be further considered here.

3.6 Oligosaccharides

In addition to the maltodextrins mentioned above, which contribute so little sweetness that they cannot be considered as sweeteners, there are several tri- and tetra-saccharides that are sweet to some degree, and are suggested as non-cariogenic sucrose substitutes. The caloric value of these compounds is not certain, as there is some debate about the degree of their absorption in the large intestine (Grenby, 1983; Amylase Research Society of Japan, 1988).

3.6.1 *Coupling sugar*

Coupling sugar is produced by the action of cyclomaltodextrin glucanotransferase enzyme on a mixture of starch and sucrose. The product has the following composition (Amylase Research Society of Japan, 1988):

Fructose	0.5–1.5%
Glucose	5–7%
Sucrose	11–15%
Maltose	10–12%
Glucosylsucrose	11–15%
Maltotriose	7–9%
Maltosylsucrose	7–11%
Higher oligosaccharides	35–41%

The sweetness is said to be about 60% that of sucrose (note that 10–15% of the mixture is sucrose), but cariogenic potential is low. Coupling sugar is sold as a syrup and used in confectionery, but only within Japan.

3.6.2 *Neosugar*

The term 'neosugar' refers to a mixture of varying quantities of fructofuranosyl sucrose oligomers: 1-kestose (GF₂), nystose (GF₃), all of which oligosaccharides occur naturally in some vegetables (onion, wheat, Jer-

usalem artichoke). They are now produced by the action of fungal α -fructofuranosidase, from *Aspergillus niger*, on sucrose (Amylase Research Society of Japan, 1988). The composition of two commercially available mixtures is shown in Table 3.8.

Neosugar is claimed to be non-cariogenic and non-nutritive, although the latter property is in question because the product seems likely to be degraded in the large intestine, if not in the small intestine. The sweetness of Neosugar G is about 60% that of sucrose, and of Neosugar P, about 30%. Both are stable at neutral pH and at temperatures up to 140°C.

Table 3.8. Composition of commercially available neosugars.

Product	Fructo-oligosaccharides					
	G(F)	GF	GF ₂	GF ₃	GF ₄	(GF ₂₋₄)
Neosugar G	<33	<12	25	25	11	>56
Neosugar P		<5	35	50	11	>95

Source: Amylase Research Society of Japan (1988).

Neosugars are claimed to be utilized by bifidobacteria in the large intestine, so that their consumption improves the level of bacterial flora in the gut, decreases constipation, lowers blood lipid levels and serum levels of triglycerides and cholesterol. Toxicity studies on animals indicate a lack of toxicity, cariogenicity and genotoxic effects (Fishbein *et al.*, 1988). The product is sold as a syrup, and used in confectionery in Japan. Neosugar is also sold as an animal feed additive at 0.2–0.5%. Preliminary results (Fishbein *et al.*, 1988) indicate that the addition of neosugar to the diet of broiler chickens and piglets increases feed efficiency, and leads to weight gain. The correlation of weight gain in animals with the claim for neosugar as a non-nutritive non-caloric sweetener for human use has not been observed.

3.7 Starch-based sweeteners

Starch is a polymer of glucose and upon hydrolysis by acid or by amylase enzyme yields glucose and a variety of maltooligosaccharides. The process has been employed in the United States to produce sweetener syrups since the 1840s, at first on potato starch and some 20 years later on corn, or maize, starch. Crystalline glucose, more often called dextrose in the corn refinery industry, and glucose syrup remained the major starch-based sweetener products until the 1950s when maltodextrins were developed; more significantly for the sweetener industry, in the 1960s, enzyme-catalysed isomerization of glucose to fructose, a sweeter sugar, made

possible high fructose syrups, with equivalent sweetening power/calorie ratio to sucrose. The corn refining industry in the United States now makes available a wide range of sweeteners, with crystalline fructose as the newest addition. Corn-based starch hydrolysis products are also manufactured in Argentina, Canada, Japan and Eastern Europe. In the European Community, the starch base for these sweeteners is usually potato, increasingly wheat, and, in at least one factory in Finland, barley. Products are similar in composition, properties and use to those made from corn, and so, in this chapter, corn starch-based products are used as examples (Howling, 1979; Pancoast and Junk, 1980; Corn Refiners Association, 1989).

In general, corn-based sweeteners are classified into the following seven categories (Corn Refiners Association, 1989).

- (1) *Corn syrup (glucose syrup)* is the purified concentrated aqueous solution of nutritive saccharides obtained from edible starch and having a dextrose equivalent of 20 or more.
- (2) *Dried corn syrup (dried glucose syrup)* is a corn syrup from which the water has been partially removed.
- (3) *Dextrose monohydrate* is purified and crystallized D-glucose containing one molecule of water of crystallization with each molecule of D-glucose.
- (4) *Dextrose anhydrous* is purified and crystallized D-glucose without water of crystallization.
- (5) *Maltodextrin* is a purified concentrated aqueous solution of nutritive saccharides obtained from edible starch, or the dried product derived from the solution and having a dextrose equivalent less than 20.
- (6) *High fructose corn syrup* is a purified concentrated aqueous solution of nutritive saccharides obtained from edible starch in which a portion of the dextrose has been isomerized to fructose.
- (7) *Crystalline fructose* is a purified and concentrated D-fructose obtained from edible starch in either dry crystal or remelted liquid form.

Corn syrup, dried corn syrup, dextrose monohydrate and dextrose anhydrous are all the subject of Food and Drug Administration (FDA) food standards codified in 21 CFR 168—Sweeteners and Table Syrups. These definitions parallel those issued by the International Codex Alimentarius Commission. Maltodextrins and high fructose corn syrup are products considered to be Generally Recognized as Safe (GRAS) by the FDA. Specifications for crystalline fructose may be found in the *Food Chemicals Codex* published by the National Academy of Sciences.

Composition of the typical range of syrups is outlined in Table 3.9 (Corn Refiners Association, 1989), where 'DE' means dextrose equivalent, or total reducing sugars reported as dextrose percent solids and 'DP' means degree of polymerization.

Table 3.9. Composition of corn syrups.

Designation	Ash	Saccharides, carbohydrate basis ^a			
		DP ₁	DP ₂	DP ₃	DP ₄₊
28 DE	0.3	8	8	11	73
36 DE	0.3	14	11	10	65
34 HM	0.3	9	34	24	33
(high maltose)					
43 HM	0.3	9	43	18	30
(high maltose)					
43 DE	0.3	19	14	12	55
43 DE	0.03	19	14	12	55
(ion exchanged)					
53 DE	0.3	28	18	13	41
63 DE	0.3	36	31	13	20
63 DE	0.03	36	31	13	20
(ion exchanged)					
66 DE	0.3	40	35	8	17
95 DE	0.3	95	3	0.5	1.5
95 DE	0.03	95	3	0.5	1.5
(ion exchanged)					
HFCS 42	0.03	95	3	0.7	1.3
HFCS 55	0.05	95.7	3	0.4	0.9
Crystalline fructose	0.05	100.0			

^aDP, degree of polymerization; DP₁, dextrose (dextrose + fructose for HFCS, fructose for crystalline fructose); DP₂, maltose; DP₃, maltotriose; DP₄₊, sum of saccharides DP₄ and above.

3.7.1 Manufacturing process

The most common methods employed in the commercial production of corn syrups are the acid process, the acid-enzyme process and multiple enzyme process (Pancoast and Junk, 1980; Fishbein *et al.*, 1988; Corn Refiners Association, 1989). In the acid conversion process, a starch slurry of the appropriate dry substance is acidified to attain a pH of about 2 and pumped to the converter. After neutralization, liquor is clarified and concentrated by evaporation to an intermediate density. The intermediate syrup is further clarified, decolorized, and finally concentrated in evaporators to finish density. Some syrups are treated with ion-exchange resins for further refinement.

The acid-enzyme process is similar except that the starch slurry is only partially converted by acid to a given DE, then treated with an appropriate enzyme or combination of enzymes to complete the conversion.

In multiple enzyme processes, starch granules are gelatinized and the preliminary starch splitting or depolymerization is brought about by an α -amylase enzyme rather than by means of acid.

Various intermediate syrups of differing composition may be further converted with enzymes having specific modes of action or provide particular types of end products, such as high maltose syrups, high fermentable syrups and others.

3.8 High fructose corn syrups

Dextrose or glucose solutions or high DE substrates produced by acid-enzyme or dual enzyme processes are refined by carbon and ion-exchange systems and treated enzymatically with isomerase, on an immobilized enzyme system. Isomerization is usually carried to a point where the substrate contains 42% fructose. Following this step, the product is refined again through carbon and ion-exchange systems and is evaporated to a dry substance level of 71%.

In the production of syrups with a fructose level above 50%, the original 42% fructose feedstock is passed through separation columns of cationic ion exchange resins which retain fructose and dextrose. The fructose is flushed from the system, while dextrose is recirculated for further isomerization. Continuous systems rely on a standard moving bed system. The fructose fraction is generally recovered at an 80–90% concentration, and blended with 42% fructose feedstock to produce 55% fructose content. After blending, the syrup is refined again with both carbon and ion-exchange systems and is evaporated to a dry substance level of 77%. The high-level feedstock may also be refined and evaporated to produce 90% high fructose syrup.

3.8.1 Crystalline fructose

Crystalline fructose is produced by concentrating the fructose portion of high fructose corn syrups, and then seeding, crystallizing and purifying the product. The dry version of the product is generally supplied at a maximum moisture content of 0.2%. A liquid fructose may also be produced by re-melting crystalline fructose to yield a clear, colorless liquid product at approximately 77% dry solids.

3.8.2 Dextrose syrups

Starch slurry is gelatinized as in the manufacture of corn syrup, and partially converted by acid or α -amylase, and a purified glucoamylase enzyme is added to effect the conversion to dextrose.

The resulting liquor is concentrated, cooled and pumped to crystallizers and the temperature is lowered on a fixed schedule. Crystallization is controlled by the amount of seed crystals used. Dextrose monohydrate crystallizes, is separated centrifugally, washed and dried to about 8.5% moisture. The mother liquor can be recycled and crystallized to produce a second crop of dextrose hydrate.

3.8.3 *Dextrose anhydrous*

Anhydrous dextrose is usually produced by re-dissolving dextrose hydrate, and evaporating the solution to a very high solids content. Anhydrous α -D-glucose is crystallized on induced or added seed crystals at elevated temperatures, centrifuged, washed and dried.

3.8.4 *High maltose syrups*

Enzyme technology has been developed to produce syrups that are higher in maltose than an acid converted syrup of similar DE. These syrups are used for fermentation (brewing) and for applications in ice-cream and confectionery where physical properties are required more than sweetness. The low dextrose concentration allows control of color development in a heated product. The 65% maltose syrup is a fine material for catalytic reduction.

3.8.5 *Chemical and physical properties*

Corn syrups all have pH values on the acid side, minimum from 3.5–5.5, to minimize color and flavor development. Ash levels in corn syrups are low; ash is mainly sodium chloride. Although sulfur dioxide is used extensively in the preparation of starch from corn, processing, especially those processes using ion-exchange resins, can remove residual SO₂ so that products contain acceptably low levels of sulfite and its oxidation product, sulfate. Fermentability of corn syrups is another useful chemical property: fermentable extract is defined as the percentage of carbohydrates, on a dry weight basis, fermented by bakers' yeast under controlled conditions. The higher the DE, the higher the fermentability.

The reducing sugars characteristics of glucose, fructose and maltose allow participation in Maillard, or browning reactions, and make corn sweeteners valuable for brown crust formation in baking (color and flavor) and production of caramel color.

The density of corn syrups is usually measured in degrees Baumé instead of Brix. Syrups content (e.g. 55% high fructose corn syrup) is always expressed on a solids basis. Detailed tables on relationships between Baumé, specific gravity, temperature, refractive index and dry substance are available in the literature (Howling, 1979; Pancoast and Junk, 1980; Corn Refiners Association, 1989).

Corn syrups, because of their solids content, have colligative properties similar to those of sucrose invert syrups (Chapter 2) as regards boiling point elution and freezing point depression, the latter an important factor in ice-cream hardness and melting. Osmotic pressure increase is a measure of control against spoilage in jams and preserves. The syrups' very high

viscosities are an important property in food processing, controlling handling characteristics and air retention.

The humectant, or hygroscopic, properties of corn syrups vary with their carbohydrate composition. Lower DE syrups are less hygroscopic, while maltotriose and maltotetraose may be the most hygroscopic sugars (Howling, 1979; Pancoast and Junk, 1980). Variation in this property may be used to control the texture and keeping quality of baked goods, confectionery and intermediate moisture foods, where corn syrups are employed as moisture conditioners, food plasticizers, crystallization inhibitors and stabilizers.

Crystalline fructose has somewhat different properties from other starch-based sweeteners. It has poor sweetness in hot drinks (its sweetness of 140 relative to sucrose is only in the cold, solid state), it is rather hygroscopic and develops color quickly in storage, and so must be stored cool and dry (at less than 50% relative humidity) (Righelato, 1987).

3.8.6 Applications

Among the myriad of applications and uses for starch-based sweeteners are the following (Howling, 1979; Pancoast and Junk, 1980; Corn Refiners Association, 1989).

- (1) *Corn syrup and dextrose.* Baby and geriatric foods; bakery products; beverages, brewed, alcoholic, carbonated and still; breakfast foods; cheese spreads and foods; coffee whiteners; condensed sweetened milk; confectionery; eggs (frozen or dried); extracts and flavors; frosting and icings; fruits and vegetables; ice-creams; industrial products (adhesives, chemicals, dyes and inks, explosives, paper, textiles, tobacco); jams, jellies, marmalades and preserves; meat products (sausages, etc.); peanut butter; pharmaceutical and medical; pickles and pickle products; pork and beans; prepared mixes; seafood (frozen); syrups (table, chocolate, cocoa, fruit, medicinal, soda fountain, cordials, etc.); soups; toppings.
- (2) *High fructose corn syrup.* Bakery products; beverages (colas and other carbonated soft drinks, and still drinks); canned juices; canned fruits; condiments; confectionery products; frozen desserts; jams, jellies and preserves; pickles, wine.
- (3) *Crystalline and liquid fructose.* Baked goods; confections; cereal coatings; dairy products; frozen foods; gelatins and pie fillings; intermediate moisture foods; powdered beverages; reduced calorie foods and beverages; sports drinks; table syrups; wine coolers.
- (4) *Maltodextrins.* Bakery mixes; beverage powders; condiments; dehydrated foods; dry soup mixes; gum confections; icings and glazes; infant foods; instant tea; instant breakfast foods; low calorie sweeteners;

marshmallows; nougats; pan coatings; sauce and gravy mixes; snack foods.

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4 Sugar alcohols

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B. MESSING

Polyols are distinguished from other saccharides by the reduction of the aldehyde or ketone functions. Some polyols are present in nature, particularly in the vegetable kingdom, but as their extraction is scarcely a viable proposition, they are manufactured industrially by catalytic hydrogenation of the corresponding saccharides. The substitution in a sugar of an alcohol function instead of an aldehyde or ketone group transforms a cyclical form into a linear form, and also has the following consequences:

- higher chemical stability
- higher affinity for water
- lower capacity to crystallise
- absence of the Maillard reaction

The chemical structure of polyols enables their classification as:

- (1) Hydrogenated monosaccharides:
 - sorbitol
 - mannitol
 - xylitol
- (2) Hydrogenated disaccharides:
 - isomalt
 - maltitol
 - lactitol
- (3) A mixture of hydrogenated saccharides and polysaccharides:
 - hydrogenated glucose syrup

4.1 Sources, development and processing (see Figure 4.8)

4.1.1 *Sorbitol*

Sorbitol was isolated from the berries of the mountain ash for the first time in 1872 by Joseph Boussingault. This natural substance is present in many

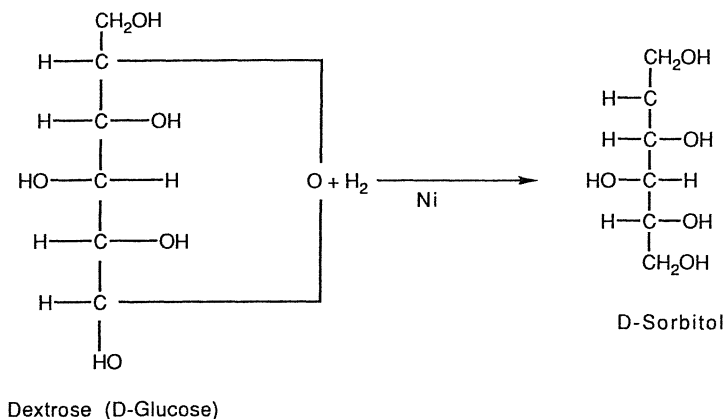


Figure 4.1 Principle of sorbitol production.

fruits, sometimes in high concentrations, especially in the cherry and pear, and in some fermented beverages such as cider (5–6 g/l).

The most commonly used production process is catalytic hydrogenation of glucose which has been derived from starch or from invert sugar (Figure 4.1). Glucose is hydrogenated under pressure in the presence of a catalyst (Raney nickel) at 120°C. The solution obtained is de-mineralised on ion-exchange resins, purified and concentrated. The result of hydrogenation then leads to a 70% sorbitol, 30% mannitol mixture. Other methods under study are based on the capacity of some yeasts to biosynthesise sorbitol (*Candida boidinii*) from glucose (Tani and Vogosuvanlert, 1987) or (*Zymomonas mobilis*) from sucrose (Viikari, 1984).

4.1.2 Mannitol

An isomer of sorbitol, mannitol is present in nature in the manna of the flowering ash, in olives, figs and in some seaweeds such as *Laminaria* spp.

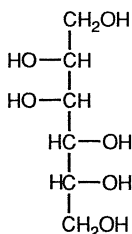


Figure 4.2. Structural formula of mannitol.

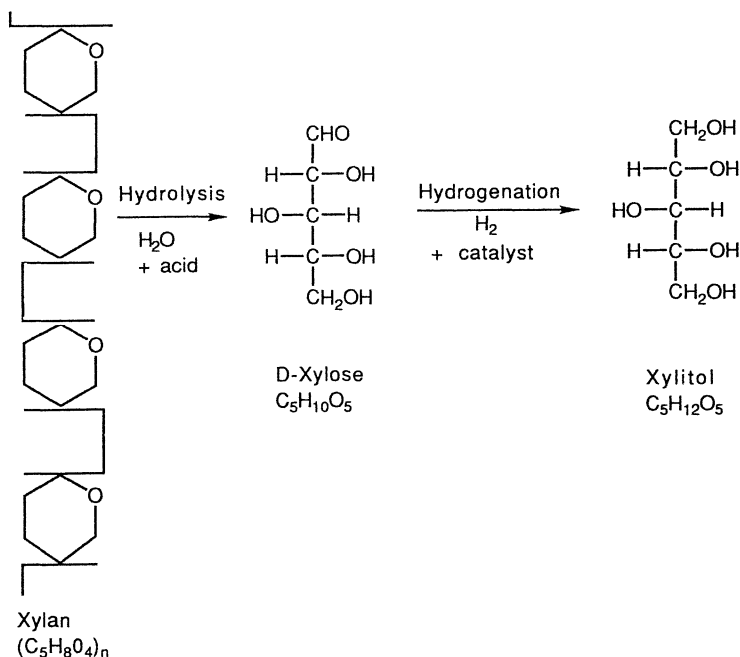


Figure 4.3. Principle of xylitol production.

(Figure 4.2). It can be prepared by catalytic hydrogenation of mannose, a widely dispersed component of mannans and hemicelluloses. Industrial manufacture is by catalytic hydrogenation of fructose, obtained from invert sugar or glucose syrup. There are two production processes: epimerisation of glucose; isomerisation of glucose. These operations lead to a mixture of sorbitol and mannitol, the proportions of which vary depending on the process used (Makkee *et al.*, 1985). Mannitol and sorbitol are separated by fractional crystallisation, sorbitol being far more soluble than mannitol.

4.1.3 Xylitol

Xylitol is present in nature in fruits (raspberry), vegetables, mushrooms, algae, lichens and yeasts. It is a hydrogenated monosaccharide with a five carbon atom chain. Xylitol is obtained by catalytic hydrogenation of xylose which in its turn is obtained by hydrolysis of wood xylans (hemicellulose) or maize pericarps and cobs (Voirol, 1985) (Figure 4.3). Two methods of manufacture by biosynthesis are at present under study: fermentation of xylose by the yeast *Mycobacterium smegmatis* (Izumori and Tuzaki, 1988) and conversion of xylose into xylitol by some strains of *Candida* (Gong *et al.*, 1981).

4.1.4 Isomalt

Isomalt is a sweetener manufactured from sucrose. It is an equimolar mixture of α -D-glycopyranosyl 1,6 mannitol and α -D-glycopyranosyl 1,6 sorbitol.

The synthesis is carried out in two steps (Figure 4.4). Sucrose, the raw material, is first transformed by an enzymatic reaction (*Protamino bacter rubrum*) into another disaccharide, isomaltulose. The 1–2 type glucose-fructose linkage in sucrose becomes a 1–6 type linkage in isomaltulose. Then catalytic hydrogenation of isomaltulose leads to this equimolar mixture of two stereoisomeric sugar alcohols.

4.1.5 Maltitol

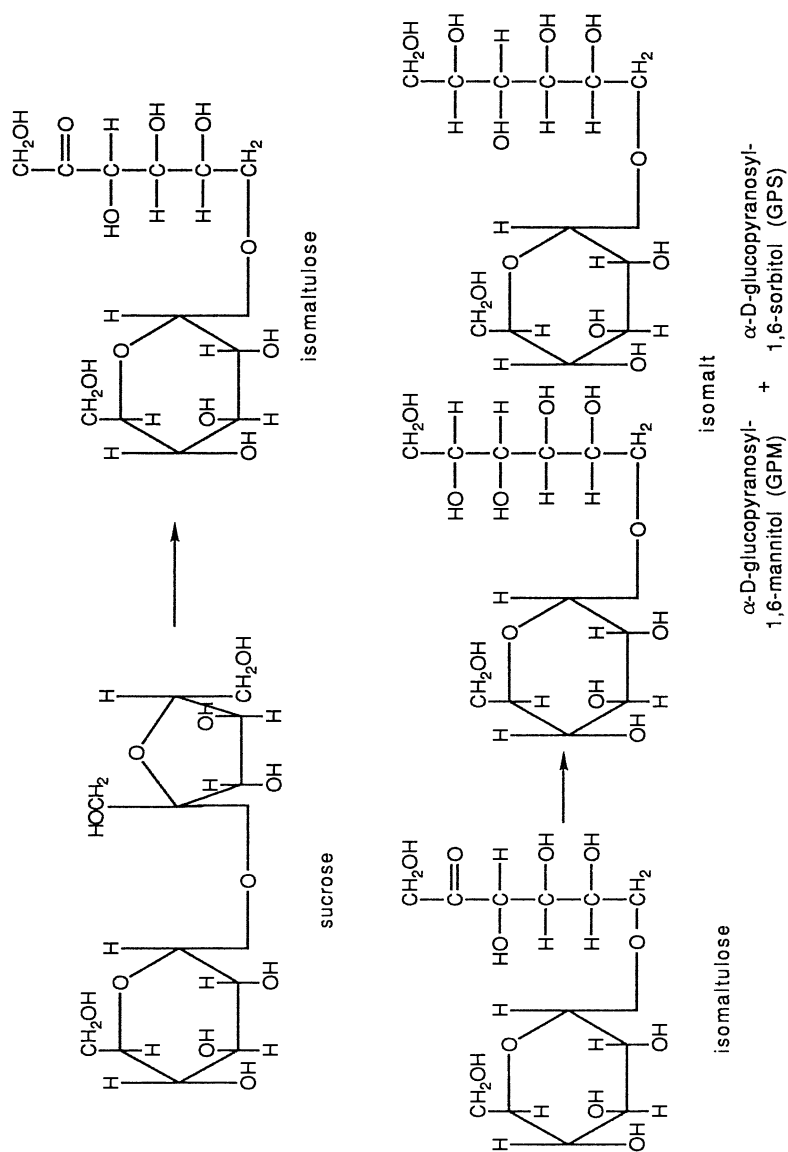
Maltitol does not occur in nature. Industrial manufacture is based on catalytic hydrogenation of maltose, produced by the hydrolysis of starch (Imfeld and Lutz, 1984; Celia, 1985) (Figure 4.5). The present process, patented in 1960 by the Japanese company Hayashibara, consists of the liquefaction of precooked starch by α -amylase, then cleavage of the amylopectin contained in starch by means of β -1-6-glucosidase. This cleaving leads to linear chains which are then cut by β -amylase into maltose, some maltotriose and traces of oligosaccharides. Maltitol syrup (74%) is obtained by catalytic hydrogenation of this mixture.

4.1.6 Lactitol

Lactitol is manufactured by catalytic hydrogenation from the glucose part of lactose (Van Velthuisen, 1979) (Figure 4.6). Control of the reaction conditions is very important as temperatures or pressures which are too high greatly diminish the lactitol yield owing to epimerisation of lactose into lactulose and hydrolysis of lactose into galactose and glucose (Guidini, *et al.*, 1983).

4.1.7 Hydrogenated glucose syrups

Hydrogenated glucose syrups are a range of products containing, in variable proportions, a hydrogenated monosaccharide (sorbitol), a hydrogenated disaccharide (maltitol), a hydrogenated trisaccharide (maltotriitol) and many hydrogenated oligo- and polysaccharides (Figure 4.7). They are manufactured from a hydrolysed starch syrup, then hydrogenated by catalytic hydrogenation. All the glucose extremities are reduced into sorbitol units (Weber, 1988).

Figure 4.4. Principle of isomalt production.

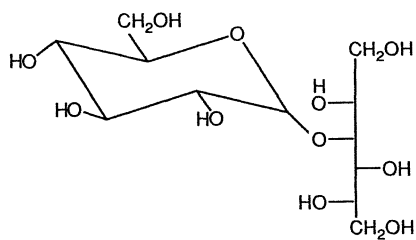


Figure 4.5. Structural formula of maltitol.

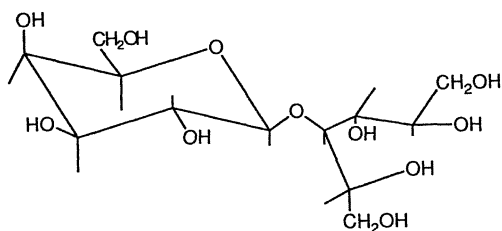


Figure 4.6. Structural formula of lactitol.

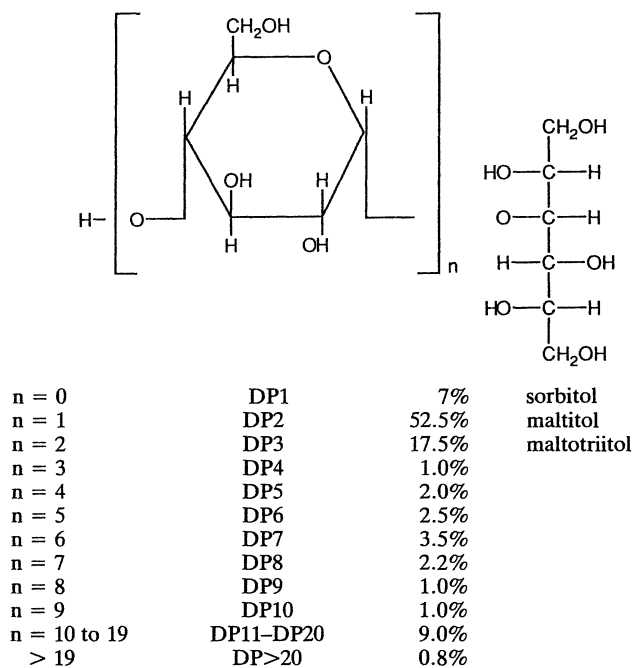


Figure 4.7. Structural formula of Lycasin®.

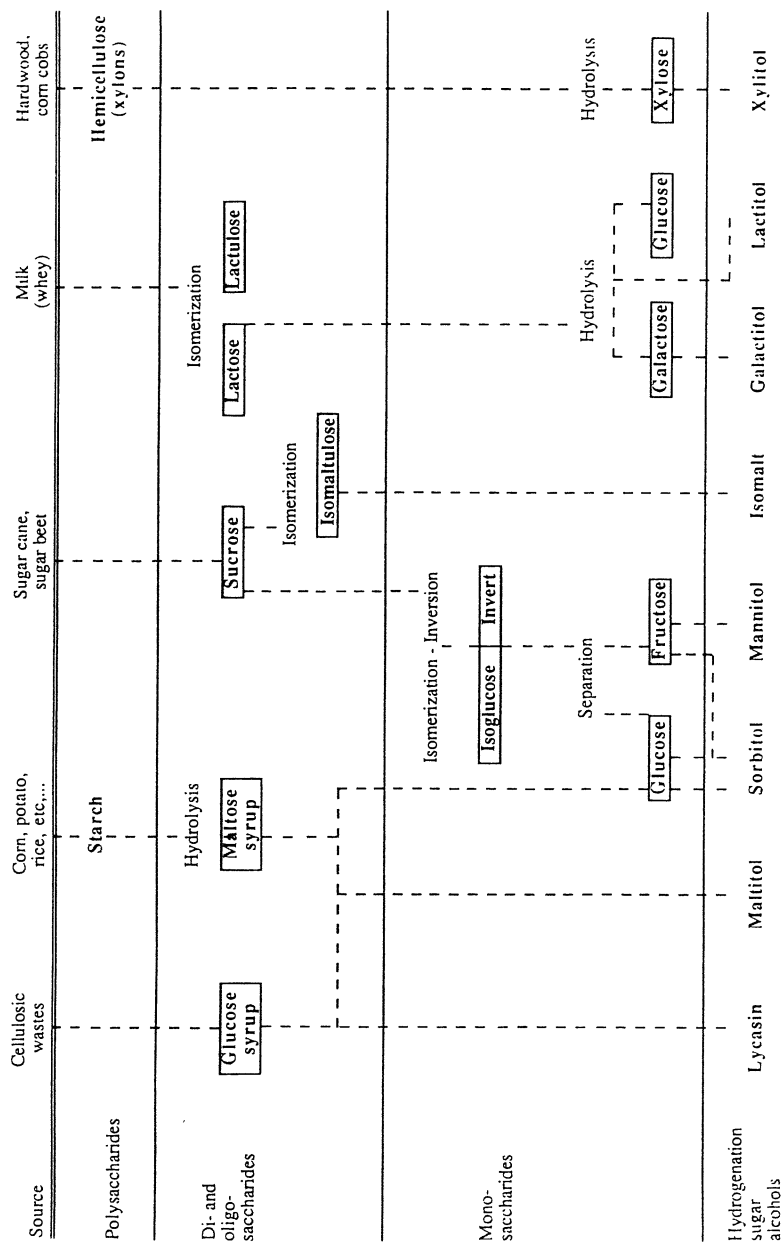


Figure 4.8. Scheme of industrial production of polyols.

Table 4.1. Purity criteria of polyols.

	Sorbitol	Mannitol	Xylitol	Maltitol	Isomalt	Lactitol
Polyalcohol content (%)						
Liquid	> 91	–	–	–	–	–
Crystalline	> 97	> 98	> 98.5	> 50	> 47	> 96
Water content (%)						
Liquid	–	–	–	< 30	–	< 49
Crystalline	–	–	–	< 1	< 7	< 5.5
Loss in drying (%)	–	–	< 0.5	–	–	–
Reducing sugars (% solid content)	< 0.3	< 0.05	< 0.2	< 0.3	< 1.5	< 0.1
Sulphated ash (% solid content)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.05	< 0.1
Sulphates (% solid content)	< 0.01	< 0.01	–	< 0.01	–	< 0.005
Chlorides (% solid content in Cl)	< 0.005	< 0.005	–	< 0.005	–	< 0.005
Nickel (mg/kg in Ni)	< 2	< 2	< 2	< 2	< 2	< 2

Source: Fabry (1987).

4.2 Physical and chemical properties

4.2.1 Molecular weight

Sorbitol	182	Lactitol	344
Mannitol	182	Palatinit*	360
Xylitol	152	Lycasin* 80/55	630
Maltitol	346		

4.2.2 Density

In the case of powder polyols, density depends on the granulometry. The density of polyol solutions varies with the concentration (Hyvonen and Koivistoinen, 1982; Celia, 1985; Roquette plaquette technique, 1988) (Figure 4.9).

Table 4.2. Densities of polyols.

	D20
Crystal sorbitol	0.6 to 0.7
Sorbitol (70% solution)	1.2879
Maltitol (70% solution)	1.30000
Crystal maltitol	0.65
Xylitol (40% solution)	1.1407
Xylitol (70% solution)	1.2667
Lycasin 80/55	1.3600

*Palatinit and Lycasin are registered trademarks

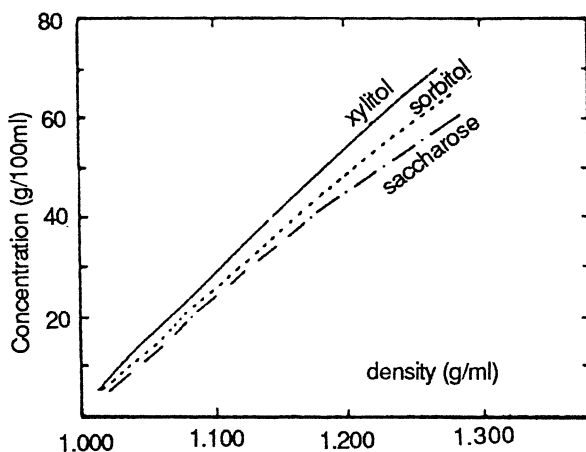


Figure 4.9. Densities of xylitol, sorbitol and sucrose solutions as a function of concentration (from Hyvonen and Koivistoinen, 1982).

4.2.3 Solubility

Solubility varies greatly depending on the polyols. Some, such as mannitol and isomalt, are barely soluble in water whereas others, such as sorbitol and lactitol, are excessively soluble. Solubility varies with temperature (Van Velthuisen, 1979; Celia, 1985; Den Uyl, 1985). The solubility of sorbitol in water increases greatly with temperature as shown in Figure 4.10. At 20°C, the saturated solution of sorbitol contains 220 g/100 cm³ water, i.e. 68.7% dry matter. The solubility of mannitol is far lower (Table 4.3). At 20°C it is 17 g/100 cm³ water, i.e. 14.5% dry matter. Hydrogenated glucose syrups cannot crystallise and act as anti-crystallising agents in many products. They control the crystallisation of sorbitol just as glucose syrups control that of sucrose.

Table 4.3. Solubility of polyols.

	g/100 g H ₂ O						
	0°C	10°C	20°C	25°C	30°C	40°C	50°C
Sorbitol	147	180	220	235	270	360	500
Mannitol	10	14	17	22	25	34	45
Xylitol			170	200	215	285	400
Palatinit	9	17	25	29	32	40	48
Maltitol				165	180	210	240
Lactitol			170	200	220	280	330
Saccharose	179	190	195	200	218	238	260

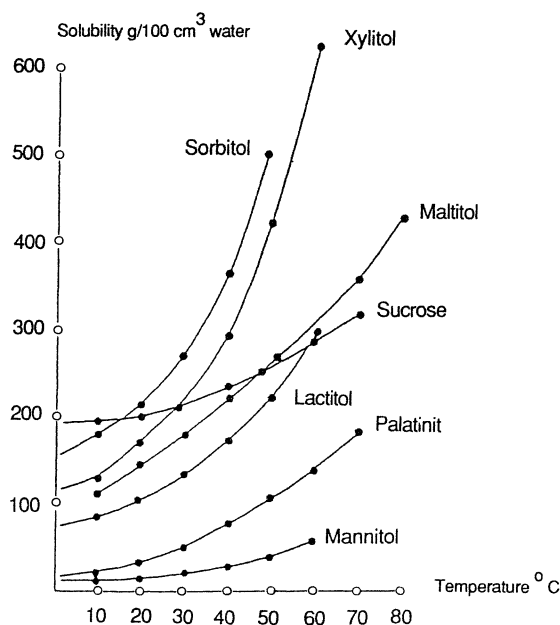


Figure 4.10. Solubility of polyols as a function of temperature (from Le Bot, 1983).

4.2.4 Viscosity

The viscosity of polyol solutions is a function of their molecular weights. It varies with the temperature and the concentration of the solution (Van Velthuisen, 1979; Den Uyl, 1985; Booy, 1987). The viscosity of sorbitol is lower than that of sucrose solutions. The viscosity of hydrogenated glucose syrups is slightly higher than that of the corresponding glucose syrups (Table 4.4).

Table 4.4 The viscosity of polyol solutions as a function of temperature.

	Viscosity (cP)			
	20°C	25°C	30°C	40°C
Sorbitol 70% DS	200	120	90	50
Xylitol 70% DS		45		20
Xylitol 63% DS		0.3		
Lycasin 74% DS	2000		1100	
Lycasin 70% DS	700	500	350	200
Lactitol 60% DS	90	70	47	45
Palatinit 60% DS	80	60	50	34
Sucrose 70% DS	420	300	250	100

4.2.5 Refractive index

The refractive index gives the degree of purity of a liquid or the dry matter content in a solution (Withmore, 1985).

10% solution of sorbitol	$n = 1.347$ at 25°C
70% solution of sorbitol	$n = 1.458$ at 25°C
Lycasin 80/55	$1.476 < n < 1.481$ at 25°
10% xylitol solution	$n = 1.347$ at 25°
70% xylitol solution	$n = 1.413$ at 25°

4.2.6 Rotatory power

Measurement of the rotatory power is used to determine the specific constituents of a solution. Aqueous solutions of sorbitol and mannitol turn the plane of polarisation towards the left. Lycasin 80/55 turns it towards the right. Xylitol has no effect.

4.2.7 Hygroscopicity

Hygroscopicity characterises the capacity of a product to retain or absorb water. It is expressed by the notion of water activity (a_w). The water activity of a product is the ratio of the water vapour pressure of the product (P) over the vapour pressure of pure water (P_0) in the same conditions, thus $a_w = P/P_0$.

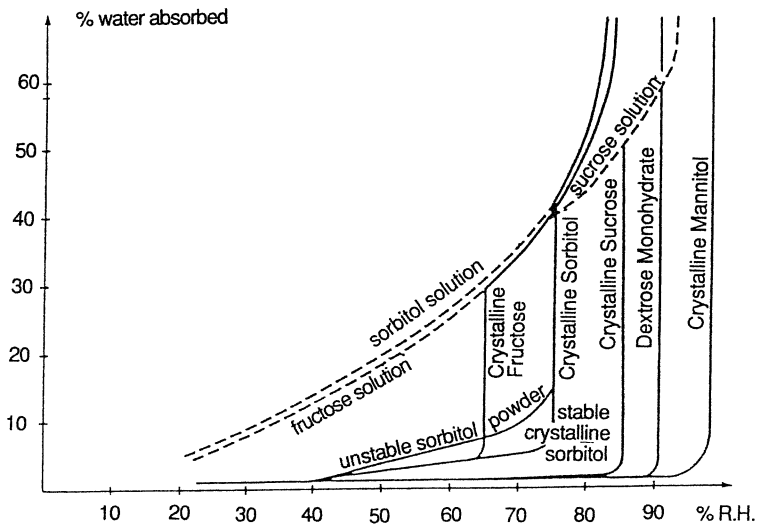
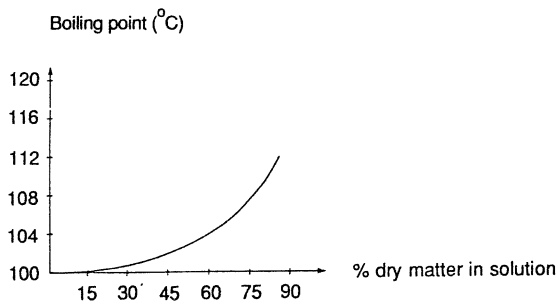
There is no relation between the hygroscopicity of a product in solution and that of the same product in the crystalline state. Consequently, although sorbitol is an excellent humectant in solution, the crystalline form is not very hygroscopic, as shown in Table 4.5. The hygroscopicity increases (and the a_w decreases) when the molecular weight diminishes. Xylitol is very hygroscopic for a relative humidity lower than 80%. Above this value, moisture uptake by xylitol greatly increases. In its crystalline form, maltitol has a low hygroscopicity. Mannitol and Palatinit have a very low hygroscopicity (Withmore, 1985; Serpelloni, 1988a) (Figure 4.11).

4.2.8 Boiling temperature

As a general rule, sugars whose molecular weight is lower than that of sucrose have a solution boiling point higher than that of sucrose solutions, and vice versa. The higher the concentration of the solution, the higher its boiling point (Figure 4.12).

Table 4.5. Value of the a_w of polyols at 20°C.

Polyols (20°C)	a_w
Mannitol	0.95
Lactitol	0.85
Maltitol	0.80
Xylitol	0.85
Lycasin 80/55	0.78
Sorbitol	0.70
Fructose	0.70

**Figure 4.11.** Sorption isotherms of amorphous and crystalline powders.**Figure 4.12.** Boiling temperature of solutions of sorbitol.

4.2.9 Freezing temperature

The freezing temperature of aqueous solutions of polyols is a function of their concentration. The same applies to freezing point depression. This physical property is a function of the molecular weight of the polyols concerned according to Raoult's law (Figure 4.13).

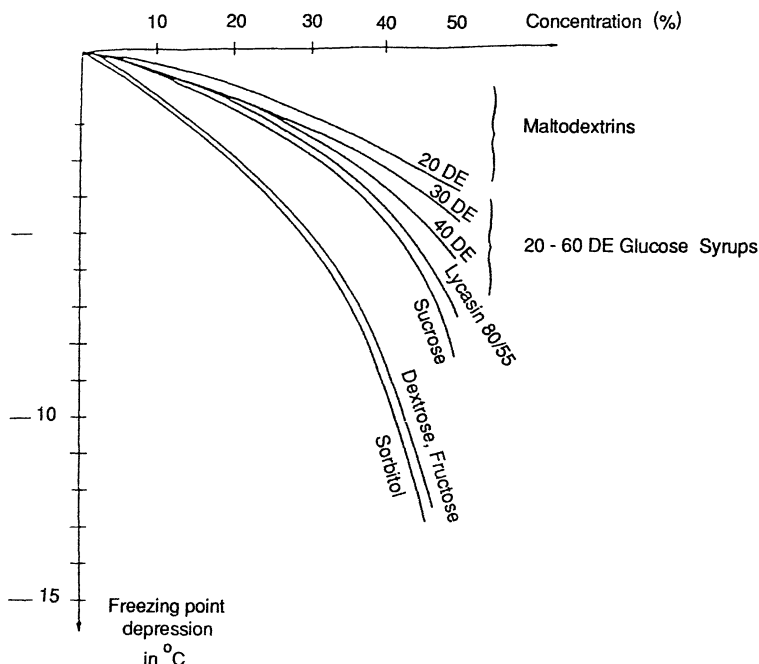


Figure 4.13. Freezing point depression of solutions of various sweeteners as a function of their concentration (from Serpelloni, 1985).

4.2.10 Heat of solution

The solution of crystals of any polyol is accompanied by thermal phenomena. In the crystalline form, polyols are characterised by a more or less high negative heat of solution; this phenomenon is better known as the 'cooling effect' (Emodi, 1982; Van Velthuisen, 1979; Hyvonen and Koivistoinen, 1982) (Table 4.6).

4.2.11 Reducing properties

Owing to their hydrogenation, polyols no longer have any free reducing groups (Serpelloni, 1988a). The disappearance of the reducing power

explains why some very well known reactions of sugars are practically non-existent with polyols: i.e. no formation of Maillard compounds with amino acids (absence of browning) and no yellowing on heating up to around 170°C.

Table 4.6. Heat of solution of polyols at 25°C.

	cal/g (25°C)
Sucrose	5.52
Fructose	12.0
Anyhydrous dextrose	14.5
Monohydrate dextrose	25.2
Palatinit	9.5
Lactitol	12.7
Sorbitol	26.5
Mannitol	30.0
Xylitol	34.8

4.2.12 Bacteriological properties

Polyols are not used by yeasts and by many bacteria. Their presence limits the development of moulds and yeasts in foodstuffs (Emodi, 1982; Van Velthuijsen, 1979; Hyvonen and Koivistoinen, 1982; Celia, 1985).

4.2.13 Sensory properties

Generally speaking, the sweetening power of polyols is lower than or equivalent to that of sucrose (Emodi, 1982; Van Velthuijsen, 1979; Hyvonen and Koivistoinen, 1982; Celia, 1985). Like sucrose or glucose, polyols provide a bulking effect, which means they can be substituted for traditional sugars. For this reason, polyols are often called 'bulk sweeteners'.

4.2.14 Synergistic effect

There is a synergistic effect when the sweetening power (SP) of a mixture is higher than the theoretical sweetening power provided by each of the constituents (25 mg aspartame + 1 g xylitol; SP = 6.6). In a 60% Palatinit, 40% Lycasin solution, Lycasin raises the sweetening power, ensures a suitable hygroscopicity, improves the solubility and lowers the cost (Figure 4.14).

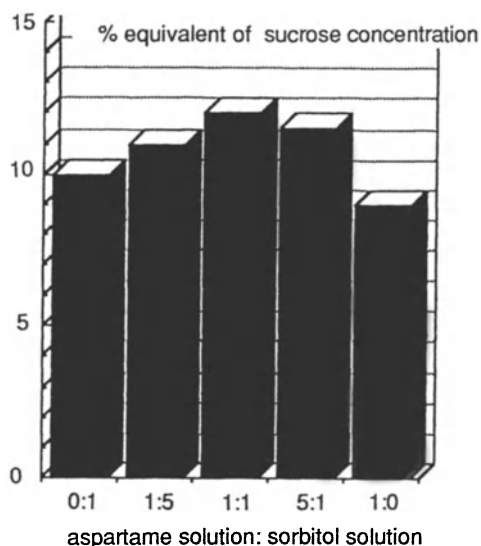


Figure 4.14. Synergistic effect of aspartame-sorbitol mixture.

4.3 Applications of polyols

4.3.1 Biscuit-making and bread-making

The roles and actions of sweetening products in this field are as follows:

- they affect the rheological properties of doughs
- they act on the browning of baked products
- they supply sugars which can be fermented by yeasts
- they provide a sweet taste
- they influence the preservation properties of baked products

Polyols are mainly used to texturise, to supply dry matter and, possibly, to provide colouring (Table 4.7).

4.3.1.1 Sorbitol. Sorbitol can totally replace sugar (Friedman, 1978). It delays browning and minimises water exchange with the atmosphere. It is less sensitive to microbial development. It can be used in combination with sucrose, invert sugar or glucose to forestall recrystallisation.

4.3.1.2 Xylitol. Xylitol can be used without changing customary formulae (Emodi, 1982). However, in order to obtain caramelisation, or non-enzymatic browning, then fructose must be added. The physical volume of products containing xylitol is very slightly lower than that of the same products containing sucrose.

Table 4.7. Use of polyols in biscuit-making and bread-making (adapted from Verel, 1989).

Polyols	Sweetness	Advantages	Drawbacks
Sorbitol	0.5	Cooling effect Retains water Good stability	
Mannitol	0.5	Pleasant taste Good stability	Light solubility
Xylitol	1	Cooling effect	Expensive cost
Maltitol	0.9	No Maillard reaction	Hygroscopic
Lycasin	0.75	Can prevent the crystallisation No Maillard reaction at high temperature	Hygroscopic
Isomalt	0.45	No Maillard reaction Stable	
Lactitol	0.35	No Maillard reaction Very stable	Hygroscopic

4.3.1.3 Palatinit. The replacement of a weight of sucrose by the same weight of Palatinit does not lead to any notable change of volume, consistency, or colour of dough provided the hydration of the dough is slightly increased (Strater, 1988). The colouring is more pronounced, the taste slightly less intense and the texture is crisper after a few weeks. Sucrose can be replaced by a 50/50 mix of fructose/Palatinit to influence the browning and the sweet taste. The fermentation time is then lower than with sucrose, whereas total substitution by Palatinit entails a 20% longer fermentation time. The sweet taste can be enhanced by using Palatinit in association with cyclamate (1.5%) or saccharin (0.15%).

4.3.2 Non-alcoholic beverages

Sweeteners are used in soft drinks for several reasons (Strater, 1988):

- to obtain the desired sweet taste
- to intensify the aroma and reinforce the taste
- to provide the necessary 'body'
- to improve microbiological stability.

Polyols can be used alone or in association with intense sweeteners. They lend great stability to products and a pleasant mouth sensation. When used alone, a very high quantity of polyol must be employed to obtain the equivalent sweet taste.

4.3.3 Ice-creams

Ice-creams contain between 12 and 20% sucrose. An ice-cream containing less than 14% sucrose does not have a sufficiently sweet taste. When the

quantity of sucrose exceeds 16%, the freezing point is lowered greatly and the ice-cream melts easily. Sucrose has an effect not only on the palatability of ice-cream but also on its textural properties (viscosity, dry matter concentration), raising the viscosity and the solids concentration of the mixture, and lowering the freezing point. The lowering is inversely proportional to the molecular weight of the sugar; sugars with a high molecular weight lead to the smallest freezing point depression.

Polyols can replace sugars in ice-creams. Abril *et al.* (1982) have studied the characteristics of frozen desserts sweetened with xylitol and fructose. Sorbitol lowers the freezing point and prevents crystallisation of the other sugars present.

4.3.4 Confectionery (Table 4.8)

Confectionery technology is based on the art of obtaining special textures with sugar. The various types of confectionery products encountered can be classified into a few main families on the basis of their structure. A further subdivision can then be made in terms of the size of the crystals and product consistency. Confections are formed by two phases:

- (a) the solid phase, i.e. micro-crystals of sucrose and other solid products depending on the formulation;
- (b) the liquid phase, i.e. water (residual moisture), the recipe anti-crystallising agents (invert sugar, glucose syrups, sorbitol).

The numerous technological characteristics of the mixture depend on the characteristics of these two phases:

- the solid/liquid ratio; the greater the solid (crystallised) phase, the more the mixture is 'dry', firm and mat
- the size of the micro-crystals; this determines the impression of smoothness or roughness in the mouth
- the water content of the liquid phase
- the quantity, nature and physico-chemical properties of the constituents in solution in the liquid phase.

A variation of the temperature, pressure or humidity can affect this balance and therefore the stability of the product during its storage. An increase in temperature allows an increase in the fraction of sugar solubilised in the liquid phase and therefore a decrease in the solid/liquid ratio, whence softening of the product occurs. Repeated variations of temperature lead to disappearance of the micro-crystals in favour of larger crystals, whence hardening of the product occurs. The Equilibrium Relative Humidity (ERH) is an important notion influencing product stability:

- stability of the texture (softening or hardening)
- microbial stability

Table 4.8. Use of polyols in confectionery.

	Sorbitol/mannitol	Xylitol	Lycasin® 80/55
Chocolate	Lack of sweetening power but can be compensated by addition of intense sweeteners Cooling effect Hygroscopic, precautions to be taken to limit the absorption of humidity Maximal temperature 43°C	Same sweetening power as sucrose Maximal temperature 54°C	Replaces glucose syrups Cannot be used in chocolates
Hard-boiled candies	Gives a transparent, shiny but non-crystalline product Better results with a sorbitol-mannitol mixture Requirement of a high boiling temperature	Does not produce transparent and shiny confectionery like sorbitol	Gives a transparent product which is hard Poor conveyor of flavours
Fondants	Produces a crystalline phase when used alone, but imparts a very viscous texture	Produces a good crystalline phase Better results in combination with sorbitol or fructose	No crystalline phase Must be used with xylitol as a crystalline phase
Jellies Toffees	Forms a gel but with a short texture	Tends to crystallise Does not brown Can be used for fudges if combined with fructose	Acceptable in the presence of fructose to favour the Maillard reaction

This value can be determined by various methods, either direct measurement (electrical hygrometry) or calculation according to various formulae. If the ERH rises, the drying tendency and the microbial risk increase and vice versa. At a given residual humidity, an increase in the percentage of anti-crystallising agent will lead to an increase in the percentage of dry matter in the liquid phase, and therefore a decrease in the ERH. With a constant sugar/anti-crystallising agent ratio, an increase in the residual moisture will lead to a decrease in the percentage of dry matter in the liquid phase and therefore an increase in the ERH (Table 4.9).

Table 4.9. Mean values of the ERH for some confectionery products.

Confectionery	ERH (%)
Fondants	75–85
Fudges	65–75
Caramels, nougats	40–55
Hard-boiled candies	10–20

4.3.4.1 Chocolates. Sorbitol must be incorporated in the dry form (Pepper and Olinger, 1988). With hygroscopic polyols, the water content of the ingredients must be low in order to avoid dissolution of the latter which could well lead to an increase in viscosity. The use of sorbitol limits the conching temperature to 50°C; that of xylitol, more hygroscopic, to 40–45°C; that of Palatinit, to 45°C. For scarcely hygroscopic polyols such as maltitol and mannitol, the classical process is retained. Owing to the maximum conching temperature recommended for very many polyols, the elimination of water is not optimal. Additional fatty material must therefore be employed to obtain the desired viscosity characteristics. The negative heat of solution of polyols produces an impression of freshness in the mouth (cooling effect) and this may be perceived as unpleasant as regards chocolates.

4.3.4.2 Hard-boiled candies. Hard-boiled candies can be manufactured with sorbitol or Lycasin (Caliari, 1983; Le Bot, 1983; Serpelloni, 1988b), while keeping not only the appearance but also the taste properties of traditional products. Lycasin presents a similar composition to glucose syrup and can totally replace sugar and glucose syrup in hard-boiled candies. The boiling temperature must then be taken to 160°C under a vacuum so as to obtain a moisture lower than 1%. Mannitol can be added to favour a superficial crystallisation and thereby limit the absorption of water.

4.3.4.3 Fondants. In contrast to the previous case, fondants are characterised by a partially crystallised structure of sucrose in equilibrium with a liquid phase of sugars dissolved in a sugar syrup. The quantity

of dry matter in the product is around 11–13% and the proportion of crystalline phase is around 50% of the product. In order to obtain this partially crystallised structure, the confectioner destroys the crystalline structure of sucrose by dissolving it hot in a syrup which also contains glucose syrup, and then concentrates the product by heating under a vacuum, but to a lesser degree than for hard-boiled candies. The sugar solution is allowed to cool to around 45–50°C, and then beaten so as to bring about crystallisation of part of the sugar. Therefore, at equilibrium, when all the crystallisable sugar has been crystallised, the latter co-exists with a sugar-saturated syrup. Polyols have been used for a long time in fondants to lower their ERH. Sorbitol limits the tendency to thread on pouring. Fondants produce a fresh sensation in the mouth.

4.3.4.4 Chewing gum. Chewing gum is traditionally manufactured with a gum base which is mixed with sucrose and glucose syrup. If the gum base is increased, the part insoluble in water remaining in the mouth becomes greater; the chewing gum offers firmer chewing. Conversely, if the quantity of the gum base is reduced, the consistency becomes too low. If the quantity of glucose syrup is increased, softer, more flexible sticks are obtained which may become soft. Conversely, if the quantity of glucose syrup is lowered, the sticks are too rigid, lack elasticity and are brittle. If the glycerol content is modified, the stability of the sticks is influenced. An increase in the glycerol content produces a more malleable chewing gum which picks up moisture easily and adheres to the packaging.

Sugarless chewing gum can be manufactured using sorbitol and hydrogenated glucose syrup as well as other crystallisable polyols such as xylitol and mannitol. The sorbitol solution provides little elasticity and therefore the gum base content must be increased; the 25% mentioned here is a minimum—the content is often higher than 30%. In chewing gum, the liquid phase is very important since it determines the ‘machinability’ coefficient. If the solids are dissolved in the crystalline phase, the a_w or the ERH of the chewing gum will rise leading to an increase in the loss of water by the product. Sticking phenomena are observed during shaping and this requires powdering with mannitol (less hygroscopic than sorbitol) and a lower temperature than with sucrose (Figure 4.15).

4.3.5 Jams, jellies, fruit preserves

The technological problems are similar to those encountered in confectionery for the production of fondants or fudges (atmosphere with approximately 25% humidity). The main objective is to prevent the recrystallisation of sucrose during storage. For this purpose, invert sugar or glucose syrup is added to raise the solubility of the product or else

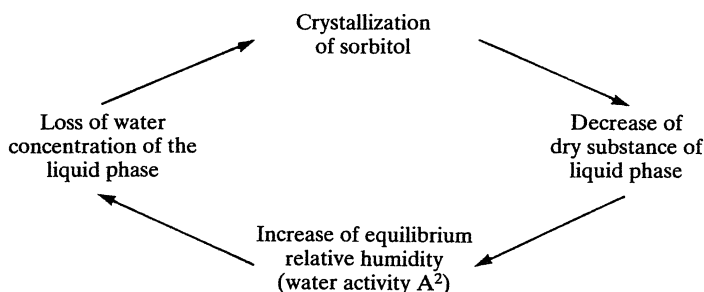


Figure 4.15. Cycle of evolution of a non-anti-crystallised chewing gum.

gelling agents or glucose syrups with a low DE are added in order to raise the viscosity of the mixture. Products must of course be well preserved. Polyols (sorbitol, xylitol, . . .) may be used. They have the same effect as the sugars used above and in addition they diminish browning (Emodi, 1982; Hyvonen and Koivistoinen, 1982; Voirol, 1985).

4.3.6 Dairy products

The dairy industry has developed 'low calorie' products with the same organoleptic properties as traditional products. Sucrose is replaced by sweeteners such as the polyols sorbitol, maltitol or xylitol (Hyvonen and Koivistoinen, 1982).

4.3.7 Pharmaceutical and cosmetic applications

Polyols can be used in many pharmaceutical preparations as excipients which are unreactive with other compounds, such as amino acids for example. Sorbitol as a humectant in creams and lotions helps to stabilise the water content providing better moisture control. The use of polyols is desirable since they are less cariogenic, suitable for diabetics and non-fermentable. Sorbitol, with the other polyols, provides a viscosity or bodying effect in many liquid preparations, elixirs, syrups, etc. It also helps to prevent caplock, a problem occurring with many liquid pharmaceutical preparations.

4.4 Legislative factors

It is difficult to compile an exhaustive report on the current state of legislation. The regulations on polyols as ingredients in various foodstuffs are evolving very rapidly throughout the EEC, United States and in other countries.

4.5 Cariogenic power

Dental caries is a localised intrabuccal disease resulting from the metabolic activity of bacteria colonising the dental surfaces (dental plaque). By glycolysis, these bacteria elaborate organic acids (lactic acid in particular) which can destroy the apatite crystals of dental enamel. The acidogenic flora at the origin of caries is customarily composed of the genera *Streptococcus*, *Actinomyces* and *Lactobacillus*. The cariogenic power of a foodstuff can be assessed by measurement of the pH variation recorded by telemetry on the dental surfaces for 30 min following the consumption of the tested foodstuff (Stephan, 1940; Armstrong and Wells, 1987). A foodstuff is considered non- cariogenic when the pH does not reach a value less than 5.2. This value is recognised as being the threshold at which dissolution of dental enamel commences.

The effect of polyols on the metabolic characteristics of cariogenic flora has been extensively studied *in vitro* and is discussed in detail in Chapter 8. From all this work it appears that buccal flora ferment polyols only slightly, if at all. A few strains of *Streptococcus* and *Actinomyces* can ferment polyols with low production of acid and extracellular polysaccharides. During regular and prolonged consumption of polyols, the number of these microorganisms is not modified. An adaptation phenomenon of strains can occur but its importance is not usually sufficient to be clinically significant.

In our diet, sugar alcohols cannot be completely substituted for fermentable sugars. As long as fermentable carbohydrates remain, there is a co-habitation which decreases the efficiency of the substitution.

On the basis of recent works, and in particular the results of epidemiological studies, it appears that sugar alcohols are to be considered as a factor contributing to lower carious frequency, to the same extent as bucco-dental hygiene and fluoride prophylaxis.

4.6 Health issues

The specific metabolism of sugar alcohols in man's digestive tract explains the interest for these products in human nutrition; it also explains the digestive discomfort which can be caused by the ingestion of these products and the need to understand and assess the factors which can influence their digestive tolerance.

4.6.1 Digestion of polyols in the human digestive tract

In the mouth, polyols are scarcely or not at all fermented by buccal flora organisms (see Section 4.5). It does not seem that there are any

grounds to envisage gastric absorption of polyols; on the contrary, their gastric emptying is a factor which can undergo wide variations depending on various factors which we will see. In the small intestine, some polyols can totally escape digestion; others can be hydrolysed there by pancreatic amylase and the disaccharidases of the brush border. Among the components of polyols, only D-glucose is actively transported from the intestinal lumen to the epithelial cells. Sorbitol, mannitol and xylitol are absorbed passively by facilitated diffusion. Compared with D-glucose, their absorption is relatively low. The intestinal hydrolysis of polyols and their absorption have been generally determined *in vitro* (biopsies of intestinal mucosa, everted intestinal loops, Ussing chambers). More recently, *in vivo* measurements in man of the intestinal absorption of polyols, by the hydrogen breath test, or by analysis of the intestinal content after intubation, or in ileostomates, have shown that the results obtained *in vitro* were in fact insufficient. It appears likely that *in vivo* the intestinal transit time will enable prolonged contact between the substrates, enzymes and absorbing surfaces. In the colon, the microbial flora ferments polyols with the production of gas (carbon dioxide, hydrogen and sometimes methane) and short-chain fatty acids (acetic, propionic, butyric and lactic acids).

4.6.2 Factors controlling the degree of digestion-absorption, clinical tolerance and energy value of polyols

4.6.2.1 Digestion-absorption. The efficiency and the exact site of the digestion-absorption of polyols (small intestine or colon) determine the digestive tolerance and the energy value of these products. It is therefore essential to know about the factors which are likely to modify the degree of digestion and absorption of polyols. The human small intestine has lost the properties to produce enzymes for the hydrolysis of lactitol; the digestion and/or the absorption of the other polyols in the small intestine is limited or slow. These polyols can be ingested straightaway in the form of hydrogenated mono- or disaccharides; with Lycasin, sorbitol and maltitol are released in the intestinal lumen during the hydrolysis of oligo- and polysaccharides. All polyols are therefore responsible in the intestinal lumen for an osmotic effect. In order to ensure isotonicity, the volume of intestinal liquid increases by reduction of the absorption of digestive secretions and also by secretion of water towards the lumen; the speed of transit in the small intestine accelerates and this aggravates the malabsorption even more by reducing, through a lack of time and also by dilution, the contact between the polyol and the digestion-absorption surface. The importance of the osmotic effect is determined by the polyol concentration leaving the stomach; the latter of course depends on the quantity of polyol ingested, but also on factors likely to slow down the distribution in the small intestine of this quantity, i.e. factors which, present in a mixed meal, slow down gastric emptying physiologically (caloric

content, solid content, viscosity). Therefore, the slower distribution in the small intestine of the ingested polyol reduces its osmotic load, favours its hydrolysis and/or its intestinal absorption, improves its digestive tolerance and raises its energy value.

In the opposite case, high quantities of polyol and water arrive in a short period of time in the colon and the colonic fermentation capacities can be exceeded with the consequential appearance of diarrhoeic stools containing a fraction of the polyol having escaped fermentation. However, when certain carbohydrates arrive regularly in the colon (such as lactose and lactulose), the capacities of the colonic flora to digest these substrates increase; it is therefore likely that these adaptation phenomena also occur with polyols and can therefore improve their digestive tolerance during prolonged ingestion. Finally, another way to reduce the osmotic load linked with the presence of a polyol in the intestinal lumen is to facilitate its absorption. It has recently been shown *in vitro* (similar to what we know about fructose) that the intestinal absorption of glucose stimulates that of sorbitol. By stimulating the absorption of sodium and water, glucose could favour the transport of sorbitol by a transmembranous flow effect (solvent drag), it could raise the intra-luminal concentration of sorbitol and it could therefore favour its diffusion or else act directly on its transport system. In addition to the effect of meals on gastric emptying, this fact could also explain why, at an equal level of ingested dose, the malabsorption of sorbitol is greater on an empty stomach than when this sugar is ingested in a foodstuff or with a meal providing glucose (in the form of starch).

4.6.2.2 Clinical tolerance. Polyol tolerance depends on the doses ingested, the presence of factors likely to reduce their osmotic load in the small intestine and the state of adaptation of the colic flora to digest them. The tolerance also depends on a last factor which is barely controllable as it is uncertain, i.e. the presence in the diet of other carbohydrates which are partially or totally indigestible in the human small intestine (lactose in the case of alactasia, stachyose, raffinose, dietary fibre). It is indeed likely that in some people, the concomitant intake of several indigestible carbohydrates will be responsible for digestive symptoms, whereas their ingestion in an equivalent quantity but separately will not lead to any symptoms. Therefore all these factors must be taken into account in the analysis of clinical tolerance studies and also when comparing tolerance thresholds for the various polyols. The first digestive symptoms appearing after the ingestion of polyols are excessive flatulence and borborygmi; abdominal pains and diarrhoea are later symptoms. Therefore, the determination of the diarrhoeogenic threshold is not sufficient.

4.6.2.3 Energy value. The great difficulty in determining the energy value of polyols stems from the fact that the metabolisable energy of these products is not the same after absorption in the small intestine and after fermentation in the colon. The colonic fermentation of polyols produces

energy and carbon necessary for bacterial maintenance and growth, and it also produces heat, gases and short-chain fatty acids. The latter are to a large extent absorbed by the colonic mucosa, used locally or metabolised in the liver; the energy recovered by the host during the fermentary processes is therefore in the form of short-chain fatty acids. The energy losses following colonic fermentation are linked with the production of heat and gas of course, but above all with the production of biomass. The respective value of these various losses is not known at present. Finally, in the organism, the production of ATP from the metabolism of short-chain fatty acids is 15–25% lower than from the metabolism of glucose. The previous reasoning has taken account of polyols alone. However, according to some authors, the digestibility in the small intestine of the other components of the dietary intake could be lower during the ingestion of polyols. Therefore, the energy provided by the usual diet would also be decreased.

Various methods can be used to determine the energy value of polyols. The energy balance studies require high ingested quantities, prolonged measurements, strict control of physical activity and environmental conditions, and finally knowledge of the corporal composition. This method is therefore almost inapplicable in man. All the other methods applicable to man always include an approximation of the colonic fermentation energy losses. Owing to the imprecision of the available methods, lack of knowledge of the site of digestion-absorption of most polyols, the existence of factors which are likely to modify this site, and interactions between polyols and the digestibility of the rest of the dietary intake, it presently appears illusory in human nutrition to ascribe a precise energy value to the various polyols. Finally, although the energy value of polyols is indeed lower than that of sucrose, this in fact has little influence insofar as the quantities ingested remain low. Similarly, the minor differences which exist between polyols in terms of energy value are without any nutritional consequence, which should limit the attempts made to classify them.

4.6.3 *The main polyols*

4.6.3.1 *Sorbitol.* The single intake on an empty stomach of 5 or 10 g sorbitol in solution leads to digestive symptoms (excessive flatulence, bloating, and also more rarely pains and diarrhoea) in 10–70% of the healthy volunteers depending on the studies (Hyams, 1983; Jain *et al.*, 1985; Rumessen and Gudman-Hoyer, 1987; Corozza *et al.*, 1988). On the contrary, when this product is consumed occasionally or regularly as a confectionery product, in other words repeatedly during the day and therefore most often in a post-prandial situation, minor digestive symptoms (excessive flatulence) appear for doses between 30 and 40 g per day (Steinke *et al.*, 1961; Pellier *et al.*, 1990). The quantities of

sorbitol absorbed in the small intestine can be determined by means of the hydrogen breath test using lactulose as the hydrogen production standard. This method is used on an empty stomach and therefore in the most unfavourable sorbitol absorption conditions. The degree of intestinal malabsorption of sorbitol assessed in two different groups of six volunteers ingesting on an empty stomach 20 g sorbitol in an isotonic solution is $66 \pm 11\%$ and $87 \pm 13\%$ (mean \pm SEM), which shows the relative imprecision of this method (Beaugerie *et al.*, 1989, 1990).

Interestingly, one study (Beaugerie *et al.*, 1990) demonstrates the influence of various factors on the degree of absorption of sorbitol; the same subjects ingested the same dose of sorbitol either in an isotonic solution or in a hypertonic solution (600 mmol/l), or in an isotonic solution with 20 g glucose (+80 kcal), or in an isotonic solution with 9 g oil (+81 kcal). The last two situations were able to slow down the gastric emptying and decrease the flow of sorbitol leaving the stomach. The percentages of malabsorbed sorbitol are as follows: $87 \pm 13\%$ (isotonic solution), $104 \pm 28\%$ (hypertonic solution), $62 \pm 13\%$ (sorbitol + glucose) and $70 \pm 10\%$ (sorbitol + oil). The intestinal absorption of sorbitol does not appear to have been studied in ileostomates. Beaugerie *et al.* (1990) have studied in six volunteers the intestinal absorption and colonic digestion of sorbitol; the subjects were previously adapted by ingestion of 30 g sorbitol per day during the three meals. The percentage of sorbitol absorbed in the small intestine, determined by intestinal intubation, was 79%. Its faecal excretion was negligible.

After absorption in the small intestine, sorbitol is transformed into fructose by hepatic polyol dehydrogenase (Wang and Van Eys, 1981). During intravenous perfusions of high doses of sorbitol (9–12 g/h), the urinary elimination can reach around 10%. The same does not apply after the ingestion of sorbitol; indeed, Felber and Renold (in Steinke, 1961) have measured the percentage of sorbitol excreted in urines in two subjects ingesting 14, 28, 42 and 56 g/day; this percentage is lower than 2%.

4.6.3.2 Xylitol. The Finnish studies on the prevention of dental caries have shown that, in adapted subjects, the ingestion of 50 g xylitol spread throughout the day leads to flatulence in half of them, but very few have diarrhoea. The tolerance can reach 100 g in certain subjects. The metabolism of xylitol in the human digestive tract is *a priori* close to that of sorbitol. After its absorption by the small intestine, xylitol is assimilated in the intermediary metabolism (Wang and Van Eys, 1981).

4.6.3.3 Mannitol. The determination of the mannitol clinical tolerance threshold has not been the subject of studies. According to some authors, mannitol is less well absorbed in the small intestine than sorbitol. However, the intestinal absorption determined in ileostomates ingesting on an empty

stomach 10 g mannitol (in 200 ml water) is 26% (Saunders and Wiggins, 1981); this value is close to that obtained in the small intestine of volunteers during the intragastric instillation of various doses of mannitol (Vidon *et al.*, 1983). Therefore, the degree of intestinal absorption of mannitol does not seem to be lower than that determined on an empty stomach for sorbitol using the hydrogen breath test.

Saunders and Wiggins (1981) have given two healthy subjects increasing doses of mannitol and they have determined its appearance in the stools. In one of the subjects, mannitol appeared after the ingestion of 18 g, whereas the other subject did not eliminate any, even after 50 g. Nasrallah and Iber (1969) have studied the metabolism of mannitol uniformly labelled with ^{14}C ; in ten subjects, after ingestion on an empty stomach of 28–100 g mannitol, 35% on average of the dose is excreted in the stools, but diarrhoea is frequently observed after ingestion of high doses.

After intestinal absorption, mannitol can be transformed like sorbitol into fructose, but dehydrogenase is far less efficient for mannitol (Wang and Van Eys, 1981). Therefore, a large part of mannitol is not metabolised in the organism and is excreted in urine. In the study with mannitol labelled with ^{14}C , the urinary excretion following the oral ingestion of 28–100 g mannitol is close to 20% (Nasrallah and Iber, 1969). The elimination of urinary mannitol after the ingestion of 2 g is a currently practised test to assess intestinal permeability; urinary elimination in these conditions is also close to 20% (Strobel *et al.*, 1984; Hamilton *et al.*, 1982). Therefore, urinary excretion expressed as a percentage of the dose gives comparable figures both after the ingestion of low and high doses. Examination of these values points to slightly higher figures for intestinal absorption than for urinary elimination. Given the different experiments and different doses, and bearing in mind the individual variations, it appears tempting to consider that the percentage of mannitol eliminated in urines is close to the percentage absorbed, and therefore that mannitol is not metabolised in the organism. This is corroborated by the study with mannitol labelled with ^{14}C in which the respiratory excretion of $^{14}\text{CO}_2$ is very low in subjects receiving 10 g mannitol intravenously (Nasrallah and Iber, 1969).

4.6.3.4 Maltitol. Beaugerie *et al.* (1989) have not observed digestive symptoms in six healthy subjects after the single intake on an empty stomach of 20 g maltitol; the ingestion of 57 g/day of maltitol after the three meals also did not lead to any symptoms in healthy subjects previously adapted for several days to receive this dose (Beaugerie, 1990).

The digestibility *in vitro* of maltitol by the disaccharidases of the intestinal mucosa is an established fact in animals and in man; however, at an equal concentration, it is observed that the digestibility of maltitol is equal to around one tenth that of maltose (Nilsson and Jagerstad, 1987). In the study performed in adapted volunteers ingesting 57 g maltitol during

the three meals (Beaugerie *et al.*, in press), the percentage of maltitol digested in the small intestine, determined by intestinal intubation, is $90 \pm 2\%$ (mean \pm SEM); nearly all of the glucose released is absorbed and $71 \pm 3\%$ of the sorbitol released is also absorbed. This means that the total quantity of hexoses absorbed in the small intestine represents 76% of the dose ingested and that 24% has penetrated the colon, either in the form of maltitol (10%), or in the form of sorbitol (14% of the weight of maltitol ingested). In this study, the faecal excretion of maltitol was almost nil. Rennhard and Bianchine (1976) gave 10 g maltitol per day for several days to four volunteers, then the subjects ingested a tracer dose of maltitol uniformly marked with ^{14}C with the unlabelled dose and breakfast; 3.6% of the radioactivity was found in the urines, mainly in the form of maltitol, and 4.9% of the radioactivity was found in the stools in a non-stated form.

4.6.3.5 Isomalt. The intake of 20 to 31.6 g isomalt on an empty stomach does not lead to any digestive symptoms (Thiebaud, 1984; Beaugerie *et al.*, 1989). In healthy subjects who ingest increasing doses of isomalt (12, 24, 48 g/day) evenly throughout the day for 12 weeks, there is a dose-dependent increase in the frequency of flatulence, whereas episodes of diarrhoea are no more frequent than in the control group (Spengler, 1987). Similarly, no volunteer suffered diarrhoea whereas the frequency of flatulence increased dose-dependently during the ingestion, for 7 days during two meals, of a daily dose of 20 to 50 g isomalt (Fritz, 1985).

In vitro, the brush border enzymes are capable of hydrolysing the two constitutive disaccharides of isomalt; however, the hydrolysis is lower than for maltitol (Nilsson, 1987). In ileostomates, 59% of the ingested isomalt (30 g on an empty stomach) escapes digestion in the small intestine; this means that 41% has been digested but not necessarily absorbed. Although the glucose released by hydrolysis can be rapidly absorbed in the small intestine, the same does not apply for sorbitol and mannitol. No measurement of digestibility in the human small intestine has been made during the ingestion of isomalt during meals.

4.6.3.6 Lactitol. In six adapted subjects, two had diarrhoea following the intake on an empty stomach of 20 g lactitol (Grimble *et al.*, 1988). In the study by Van Es *et al.* (1986), the fractionised ingestion during the meals of 50 g/day after an adaptation period was well tolerated in the eight subjects studied; however, two suffered excessive flatulence and four were previously excluded due to high digestive discomfort. The diarrhoeagenic dose is very high (74 ± 6 g/day) during the ingestion by volunteers of increasing doses of lactitol absorbed in two daily intakes after breakfast and dinner (Patil *et al.*, 1987).

Lactitol is not hydrolysed by the brush border enzymes and is not absorbed during studies made with jejunal segmental perfusion (Patil *et*

al., 1987). In ileostomates nearly 100% of the ingested dose is found in the ileal effluent.

Around 2% of the lactitol ingested is eliminated in urine; there is little available information on the quantities of lactitol which pass into the stools; they of course depend on the doses ingested and the way in which they have been ingested.

4.6.3.7 Lycasin. The single intake on an empty stomach, several times a day, of 50 g Lycasin can lead to diarrhoea in some non-adapted subjects (Kearsley and Birch, 1978; Kearsley *et al.*, 1982); on the contrary, in the study by Beaugerie *et al.* (1989), adapted volunteers did not have any symptoms following intake of 69 g per day of Lycasin during the meals (Beaugerie *et al.*, 1990). Studies with rat intestinal mucosa have shown that the various components of Lycasin are hydrolysed and release sorbitol and glucose (Rosiers *et al.*, 1985). In the study by Beaugerie *et al.* (1989), Lycasin has globally undergone digestion comparable to that of maltitol; the digestion of the oligo- and polysaccharides of Lycasin is close to 90%, maltitol present in Lycasin is digested as easily as maltitol ingested separately, and 72% of the sorbitol released is absorbed. In the stools, the faecal excretion of polyols is negligible.

4.6.4 Sugar alcohols and health

The specific metabolism of sugar alcohols in man's digestive tract explains to a large extent the advantages of these products for public health. Indeed, as they are not or only scarcely fermented in the mouth, polyols are barely cariogenic (see Chapter 8). Not being absorbed in the small intestine or only slowly, their metabolism is like that of slow sugars and therefore leads to a glycemic and insulinemic response lower than that of glucose (see Chapter 9). As they can be partly digested in the colon, their energy value is lower than that of the other sugars (see Chapter 10). In the colon, polyols therefore act like dietary fibres and could facilitate intestinal transit and may also have a beneficial role in the prevention of digestive, cancerous and metabolic diseases.

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5 Intense sweeteners

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5.1 Introduction

Intense sweeteners represent one of the most fascinating areas of food science. Chemically these products are extremely diverse, from amino acids (e.g. aspartame) to halogenated sugars (e.g. sucralose). Several of these products were discovered accidentally (e.g. saccharin), while others are the result of concerted efforts to develop a commercially viable high intensity sweetener (e.g. alitame).

They also vary significantly in their sweetness intensity (e.g. cyclamate is 30 times sweeter than sucrose, alitame is 2000 times sweeter). Table 5.1 provides the relative sweetness of the intense sweeteners discussed in this chapter. Sweetness is subjective and dependent upon a number of factors, including temperature, pH, the medium used, the concentration of the sweetener and the taster's sensitivity. Sucrose is the usual standard and is assigned a sweetness of 1. Sweetness is evaluated on a weight basis. The ideal sweetener does not exist. Even the premier sweetener sucrose does not fulfil all the needs for sweetness. The ideal sweetener should be at least as sweet as sucrose, colorless, odorless and non-cariogenic and have a pleasant untainted taste without a delay in onset or persistence in sweetness. It should be water soluble as well as chemically and thermally stable. Its safety profile must hold up to rigid regulatory scrutiny in order for it to be approved.

A new sweetener should be economically competitive with sucrose and other acceptable sweeteners. It should be easily manufactured and maintained in acceptable qualities. In certain uses (e.g. baked goods), bulk, browning properties, humectancy and texture also are important.

Having a variety of sweeteners available is essential, because no individual sweetener is perfect for all uses. With several intense sweeteners from which to choose, each can be used in the applications for which it is best suited. Manufacturers can overcome limitations of individual sweeteners by blending sweeteners together. Many sweeteners when used in combination have a synergistic effect. That is the sweetness of the combination is greater than the sum of the individual parts. A blend of cyclamate and saccharin

Table 5.1 Relative sweetness of the intense sweeteners.

Sweetener	Approximate sweetness
Acesulfame K	200
Alitame	2000
Aspartame	180
Cyclamate	30
1,1-Diaminoalkane compositions	300–1000
L-Sugars	1
L-Aspartyl-3-(bicycloalkyl)-L-alanine alkyl esters	1900
PS 99	1800
PS 100	2200
RTI-001	58
Saccharin	300
Sucralose	600

was the first practical application of the multiple sweetener approach. The primary advantage of this blend was that saccharin boosted the sweetening power of cyclamate while cyclamate masked the aftertaste that some people associate with saccharin.

The development and approval of a new sweetener is a long and tedious process. A number of compounds have been found to be sweet but only a few have been developed commercially. This chapter will discuss those sweeteners which are currently available or soon to be available and will briefly mention a few products which may someday appear in our food supply.

5.2 Aspartame

This peptide-based sweetener is discussed in Chapter 6 (Section 6.3.1)

5.3 Saccharin

Saccharin, discovered in the late 1870s, has been commercially available to sweeten foods and beverages since around the turn of the century. It was discovered by chemists Ira Remsen and Constantine Fahlberg who were working on the oxidation of *o*-toluenesulfonamide. These chemists discovered that the oxidation product was the condensed heterocycle *o*-sulfobenzimide rather than the expected *o*-sulfamoylbenzoic acid. The intense sweetness of the new compound was discovered when Fahlberg spilled the solution on his hands and later ate bread at dinner.

Saccharin is manufactured by a number of companies around the world. Most manufacturers use the synthetic route described by Remsen and Fahlberg. Toluene is treated with chlorosulfonic acid to produce *ortho*- and

para-toluenesulfonyl chloride. Treatment with ammonia then forms the corresponding toluenesulfonamides. The *ortho*-toluenesulfonamide is then separated from its *para* isomer which is oxidized to *ortho*-sulfamoylbenzoic acid, which when heated is cyclized to saccharin.

In the United States, saccharin is produced only by PMC Specialties Group which uses purified methyl anthranilate, a substance naturally occurring in grapes, to produce saccharin. PMC has refined an earlier procedure known as the Maumee process. The starting material, purified methyl anthranilate, is diazotized to form 2-carbomethoxybenzenediazonium chloride. Sulfonation followed by oxidation yields 2-carbomethoxybenzenesulfonyl chloride. Amidation of this sulfonylchloride followed by acidification, forms insoluble acid saccharin. Subsequent addition of the appropriate sodium or calcium compound produces the soluble sodium and calcium forms, respectively (Walter and Mitchell, 1986).

Saccharin is commercially available in three forms: acid saccharin, sodium saccharin and calcium saccharin. Several additional salts are possible, for instance silver, ammonium, magnesium and potassium, but none of these is commercially available today.

Saccharin occurs as a white, crystalline powder with a molecular formula of $C_7H_5NO_3S$ and a molecular weight of 183.18. Its melting point is approximately 229–230°C. It is generally odorless but may have a faint aromatic odor (Food Chemical Codex, 1981). Saccharin is extremely stable. In its bulk form, saccharin and its salts show no detectable decomposition over several years. Saccharin's versatility allows for its use in a wide variety of foods and beverages.

Saccharin is approximately 300 times sweeter than sucrose. When combined with other low-calorie sweeteners, synergism occurs so that the combinations are sweeter than the sum of the individual sweeteners. Saccharin has a bitter metallic aftertaste that becomes more noticeable in increasing concentrations. At normal levels of use, this aftertaste is detected by approximately 25% of the population. Combining saccharin with other sweeteners, however, helps eliminate the aftertaste.

Saccharin is used as a non-caloric sweetener in a broad range of foods and beverages, including: soft drinks; fruit juice drinks; other beverages and beverage mixes; tabletop sweeteners in powder, tablet or liquid form; processed fruits; chewing gum and confections; gelatin desserts, juices, jams; topping; sauces and dressings. Approximately 80% of the saccharin manufactured is used in beverages, foods and preparations such as mouthwashes and toothpaste. The remaining 20% is used in non-food applications such as nickel electroplating where sodium saccharin is added to the electrolyte to obtain a brighter finish.

Saccharin is not metabolized and is excreted from the body unchanged (Renwick, 1985). In its long history (over a century) of use, more than 20 human studies have been completed and no association between saccharin

intake and bladder cancer or any other ill effect has been found (Morgan and Wong, 1985). These data include a study of 9000 individuals conducted by the National Cancer Institute (NCI). NCI concluded that there was 'no evidence of increased risk with the long term use of AS [artificial sweeteners] in any form or with use that began decades ago.' (Hoover and Strasser, 1979).

Fourteen single generation animal feeding studies in which animals were fed saccharin for a lifetime have not shown saccharin to be a cancer causing substance. Several studies found bladder tumors in some male rats exposed to high doses of sodium saccharin for their entire life span (Oser, 1985). These doses of sodium saccharin were equivalent to a person drinking hundreds of cans of diet soda daily from birth.

Saccharin is currently available in more than 90 countries. In 1977, the US Food and Drug Administration proposed a ban on saccharin in the United States but a Congressional moratorium was placed on that ban to allow time for further research on the safety of saccharin. The moratorium has been extended five times based on the need for further scientific study, continued consumer demand and ongoing consideration of food safety policy in the United States. In testimony before the Senate Committee on Labor and Human Resources in 1985 then FDA Commissioner Frank Young stated that 'the actual risk, if any, of saccharin to humans still appears to be slight.' (Young, 1985).

Saccharin has been found to suppress the growth of oral microorganisms and therefore may inhibit the growth of plaque forming streptococci. Although the sweetener does not alter sugar metabolism directly, saccharin may raise the pH of the oral cavity and thereby aid in caries control (Linke, 1987). Saccharin has been reviewed and found safe by numerous regulatory and scientific bodies including the Joint Expert Committee on Food Additives of the World Health Organization, the UK Ministry of Agriculture, Fisheries and Food's Food Additives and Contaminants Committee, the American Medical Association and the American Diabetes Association. The extensive and growing body of scientific research on saccharin continues to support its safety for human use.

5.4 Acesulfame K

Acesulfame K also was discovered accidentally. While carrying out reactions with butyne and fluorosulfonyl isocyanate, Clauss and Jansen incidentally discovered the sweet tasting compound 5,6-dimethyl-1,2,3-oxathiazin-4 (3*H*)-one,2,2-dioxide. Through systematic research on the dihydrooxathiazinone dioxides, a number of sweet tasting compounds were discovered. Further research demonstrated that 6-methyl-1,2,3-oxathiazine-4 (3*H*)-one, 2,2-dioxide exhibited the most favorable taste proper-

ties and seemed the most easily synthesized of the dihydrooxathiazinone dioxides and was therefore chosen for development. To synthesize acesulfame K, ketones, β -diketones, derivatives of β -oxocarbonic acids and alkynes may be reacted with halogen sulfonyl isocyanates. The resulting compounds are transformed into *N*-halogen sulfonyl acetoacetic acid amide. This compound is cyclized to dihydrooxathiazinone dioxide ring system in the presence of potassium hydroxide by separating out the corresponding potassium salts. Because the dihydrooxathiazinone dioxides are extremely acidic compounds, salts of the ring system are formed. The production of the potassium salt requires potassium hydroxide.

Acesulfame K is a white crystalline powder. It has excellent stability. In aqueous solutions and when stored under cool dry conditions it has a virtually unlimited shelf-life. Low calorie soft drinks formulated with acesulfame K show no decrease in sweetness over a period of several months. Acesulfame K is also stable at high temperatures including those required for baked goods. Sterilization and pasteurization do not affect the taste of acesulfame K. At room temperature acesulfame K dissolves readily in water but is less soluble in alcohol.

Approximately 200 times sweeter than sucrose, acesulfame K has a clean quickly perceptible sweet taste. A bitter aftertaste may be detected when acesulfame K is used at high concentrations in aqueous solutions. This is not the case at lower concentrations. The sweetener mixes well with various other high intensity sweeteners including aspartame and cyclamate. It also mixes well with polyalcohols resulting in mixtures which are particularly suitable for sugarless confectionery (Lipinski, 1985)

Acesulfame K is sold under the brand name Sunette by the Hoechst Celanese Corporation in the United States and Sunett by Hoechst AG in Europe. It is currently approved for use in foods and oral hygiene products in more than 20 countries. This sweetener may be used in products such as tabletop sweeteners, puddings, soft drinks, cough syrups and toothpaste. In the United States, acesulfame K is approved for use in chewing gum, dry mixes for beverages, instant coffee, instant tea, gelatins, puddings, non-dairy creamers and as a tabletop sweetener. Internationally it is available in more than 100 products. Because of its excellent heat stability, acesulfame K is particularly suitable for low-calorie or sugar-free bakery products. When used for this purpose, bulking agents are required since high intensity sweeteners do not have the processing and bulking qualities of sugar. Suitable bulking materials would be polydextrose or polyalcohols.

Acesulfame K is not metabolized and is excreted unchanged from the body (Lipinski and Mayer, 1989). It does not promote tooth decay (Grenby and Saldanha, 1986).

More than 50 international studies have been completed supporting the safety of acesulfame K. These studies include four long-term animal feeding

studies (i.e. a two-year toxicity study in beagle dogs, a carcinogenicity study in mice and two long-term rat studies). The Joint Expert Committee of Food Additives has reviewed the research on acesulfame K and concluded that it is safe, allocating an acceptable daily intake of 0–9 mg/kg body weight (JECFA, 1983). It has been available for use in the United Kingdom since 1983. Acesulfame K was approved by the US Food and Drug Administration in 1988 and allocated an ADI of 15 mg/kg body weight (Federal Register, 1988). It is currently available in over 20 countries including Switzerland, France, Italy and Belgium and petitions are pending in a number of additional countries.

5.5 Cyclamate

The sweet taste of cyclamate was accidentally discovered in 1937 by a University of Illinois graduate student, Michael Sveda. The patent eventually became the property of Abbott Laboratories who performed the necessary studies and submitted an application to the US Food and Drug Administration for the use of its sodium salt in 1950. Cyclamic acid (cyclohexylsulfamic acid), sodium cyclamate and calcium cyclamate may be used as sweetening agents in food. Cyclamic acid is a white crystalline powder with a melting point of 169–170° C, good aqueous solubility and a lemon sour sweetness. Sodium and calcium cyclamate exist as white crystals or white crystalline powders. They are freely soluble in water at concentrations far in excess of those required for normal use. Cyclamate solutions are stable to heat, light and air throughout a wide pH range. Calcium cyclamate is somewhat less sweet than sodium cyclamate. Cyclamates are generally considered to have a sweetness 30 times that of sugar based on a weight for weight equivalence. Sweetener tablets containing a combination of cyclamate and saccharin had shown no diminution of sweetening ability or physical deterioration after more than 10 years. The stability data on cyclamate indicate that there is no need for an expiration date to identify the strength, quality and purity of the compound either in its bulk form or in foods and beverages containing cyclamate (Kasperson and Primack, 1986).

Although the sweetening power of cyclamates is generally listed at 30 times that of sugar, this value is only an estimate as the sweetening power of all sweeteners varies with the medium in which the compound is used and with the sweetener's concentration; as the concentration of cyclamate increases the relative sweetness decreases. Best results are usually obtained by using cyclamate and saccharin in combination where the saccharin boosts the sweetening power of cyclamate and the bitterness of saccharin is masked by the cyclamate. The sweeteners are also synergistic. They were very popular in low-calorie foods and beverages during the 1960s. The mixture

most commonly used contained 10 parts cyclamate to 1 part saccharin. Cyclamate is suitable for a number of applications including tabletop sweeteners, beverages, fruit juice drinks, beverage bases and mixes, processed fruits, chewing gums and confections, salad dressings, gelatin desserts, jellies, jams, toppings and may also be used in baked products. Cyclamate is generally used in combination with another sweetener, most frequently saccharin.

In late 1969, Abbott Laboratories, the principal US producer of cyclamate, was notified of bladder tumors in rats fed a cyclamate/saccharin mixture being studied by the Food and Drug Research Laboratories. All cyclamate was subsequently banned from the US market in 1970. Thirty additional studies did not confirm the result of the Food and Drug Research Laboratories study and in 1973 Abbott Laboratories petitioned the FDA for re-approval. This petition was denied in 1980. Both the American Statistical Association and the Society of Toxicology asked the FDA to reassess the statistical and scientific principles relied on in its 1980 decision. On the basis of these requests—plus new scientific evidence confirming the safety of cyclamate, the Joint Expert Committee on Food Additives' consistent approval of cyclamate, and its availability in over 50 countries—a new petition was submitted to FDA by Abbott Laboratories and the Calorie Control Council in 1982. Extensive additional testing has been completed since that date (Nabors and Miller, 1989). The FDA's Cancer Assessment Committee in 1984 found that cyclamate is not a carcinogen and stated 'After evaluation of all chronic bioassays performed with laboratory animals on cyclamate and its primary metabolite cyclohexylamine the CAC has reached the judgment that there is very little credible data to implicate cyclamate as a carcinogen at any organ/tissue site to either sex of any animal species tested.' And further, '[T]he collective weight of many experiments . . . indicates that cyclamate is not carcinogenic.' (CAC, 1984).

The multitude of short-term, acute, sub-chronic and chronic studies in animals, and the extensive data on pharmacokinetics, metabolism in man and animal, combined with epidemiologic evidence support the safety of cyclamate for human use. The Calorie Control Council and Abbott Laboratories have provided the FDA with a number of additional studies since 1982 and answered all the agency's questions. When reapproved in the United States, possibly within a year, cyclamate will most commonly be used in combination with other sweeteners due to its relatively low sweetening power.

5.6 Sucralose

In 1977, a Tate & Lyle chemist identified a halogenated sugar as a compound with commercial possibilities. By 1980, Tate & Lyle had

reached an agreement with Johnson & Johnson to undertake a joint development program for sucralose (trichlorogalactosucrose). Johnson & Johnson established McNeil Specialty Products Company to carry out the cooperative program with Tate & Lyle. Under the agreement, Tate & Lyle licensed McNeil to seek approval for and market sucralose in the United States, Australia, New Zealand, Central and South America and the Middle East. Tate & Lyle retained its rights to sucralose in Europe, Canada, Africa and the Far East (Broulik, 1987).

Sucralose is made by a multi-step process utilizing sucrose as the starting material. Three of the hydroxyl groups on the sucrose molecule are replaced with three atoms of chlorine, one at the 4-position on what is now the galactose moiety and two at the 1- and 6-positions on the fructose moiety.

Sucralose is a free-flowing, white crystalline solid which is freely soluble in water (20% at room temperature) and in ethanol and methanol. It is extremely stable. Dry sucralose has an estimated storage life of 4 years at 20°C, and dry products containing sucralose have an even longer shelf-life. Sucralose is more stable than sugar in aqueous solution; hydrolysis to its two constituents, 4-chlorogalactose and 1,6-dichlorofructose may occur at extreme conditions of acidity, temperature and time (Jenner, 1989).

These hydrolysis products are the only breakdown products and are not formed during the manufacturing process or in biological systems. Sucralose does not interact with other food ingredients in model food systems and is not affected by extreme conditions such as baking and pasteurization (Barndt and Jackson, 1990).

Sucralose has a clean, high-quality taste with an average sweetness intensity of about 600 times that of sugar. It is both non-caloric and non-cariogenic. Sucralose's remarkable stability, its high water solubility and high quality sweetness make it a very versatile sweetener for use in a wide range of products such as baked goods, beverages (Quinlan and Jenner, 1990), chewing gum, dairy product analogs, salad dressings, frozen dairy desserts, fruit and water ices, gelatins and puddings, jellies, jams, milk products and sugar substitutes.

Over 80 studies have been conducted to demonstrate that sucralose is without adverse effects at doses 400 times the projected maximal mean daily intake. Studies in man have shown no adverse health effects (McNeil, 1987).

In February 1987, Tate & Lyle submitted applications for sucralose approval in the United Kingdom and Canada and McNeil Specialties filed a food additive petition with the US Food and Drug Administration. Similar petitions have since been submitted in Denmark and Australia. In 1988, sucralose was reviewed by the Joint Expert Committee on Food Additives of the World Health Organization and found to be safe. A temporary ADI of 0–3.5 mg/kg body weight was granted (JECFA, 1989).

Although sucralose is not yet approved anywhere in the world, approval in the United States is expected by the end of 1991.

5.7 Alitame

Alitame is the result of a long-term project of Pfizer Central Research to discover and develop a new sweetener with unique qualities. Alitame, L- β -aspartyl-*N*-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide hydrate (2:5) is a member of the L- β -aspartyl-D-alanine amide series in which the alanine carboxyl group is terminated as an amide of a novel amine (2,2,4,4-tetramethylthietanyl amine). Alitame is a crystalline, odorless, non-hygroscopic powder. It is stable at elevated temperatures and in the neutral pH range (6–8). It is stable for practical purposes for more than a year at room temperature.

Alitame's sweetness potency is approximately 2000 times that of sucrose. It has a clean, sweet taste with no unpleasant aftertaste. Alitame's sweetness is synergistic in combination with acesulfame K and cyclamate and high quality blends may be obtained with these and other sweeteners, including saccharin.

Alitame is sufficiently stable for use in hard and soft candies, heat pasteurized foods, and in high-temperature-processed neutral pH foods such as sweet baked goods. It has exhibited excellent functionality and is compatible with freshly prepared foods that have been studied as well as refrigerated, frozen and dry food products. Prolonged storage of alitame and a few standard acidic liquid beverage recipes may result in an incompatibility as measured organoleptically. This is not reflected in storage stability as measured by chemical assay for alitame and its degradation products. Levels of off-flavorant(s) are below the limits of modern analytical detection. Adjustment of these formulations is expected to eliminate such problems. Substances which may produce off-flavors in storage with alitame in liquid products are hydrogen peroxide and sodium bisulfite. High levels of reducing sugars, such as glucose and lactose, may react with alitame in heated liquid or semi-liquid systems, such as baked goods, to form Maillard reaction products, depending upon the amount of heat, water and other factors. This latter reaction also has been reported for aspartame.

A food additive petition for broad clearance of alitame was filed with the US Food and Drug Administration, August 1986, requesting alitame's use in foods for which standards of identity do not preclude such use. Categories petitioned include baked goods and baking mixes, pre-sweetened, and ready-to-eat cereals, milk products, frozen desserts and mixes, fruit and water ices and mixes, fruit drinks, -ades, and mixes, jellies and jams, sweet sauces, beverages and tabletop sweeteners.

Alitame is well absorbed after oral administration. Most of the oral dose is excreted in the urine as a mixture of metabolites and the remainder is excreted in the feces. Alitame is partially caloric since the aspartic acid portion of the molecule is available for normal amino acid metabolism. The caloric contribution of 1.4 cal/g of alitame is clearly insignificant at use levels in the diet, contributing approximately 0.02% of the calories of the replaced sucrose.

Alitame has been evaluated in an extensive series of studies designed to establish its safety as a sweetener for human use. The compound has been studied in dogs, rats, mice and man with no adverse effects reported. Although not approved anywhere in the world, petitions are pending in a number of European countries as well as in the United States (Pfizer, 1986).

5.8 L-Sugars

In 1981 a US patent for the use of L-sugars, L-glucose, L-allose, L-fructose, L-galactose, L-altrose, L-idose, L-talose, L-tagalose and L-psicose, as low-calorie sweeteners in foods, beverages and drugs was granted to Biospherics Incorporated (Levin, 1981). These hexose monosaccharides are called left-handed sugars because they are the mirror images of their right-handed counterparts. As a result of this difference, the L-sugars are reportedly unavailable as food for microorganisms, animals and humans and are therefore not metabolized, are not subject to spoilage and are non-caloric. The L-sugars do occur naturally but rarely. For example, minor amounts of L-fructose and L-rhamnose occur in plantain seeds and L-galactose is found in flax seed gum, red algae and snail eggs. Because the L-sugars differ only in their mirror relationship from right-handed sugars, L and D forms of a particular sugar are said to have identical chemical and physical characteristics such as boiling point, melting point, solubility, viscosity, texture, hygroscopicity, density, color and appearance. Taste panels have found the L-sugars to be remarkably similar to their D-sugar isomers. The L-sugars should be non-cariogenic because microorganisms in the mouth should not be able to produce tooth decaying acids from L-sugars.

The L-sugars can be produced synthetically and work is currently under way to refine techniques to make the products commercially feasible for use in processed foods and beverages. They should be especially appropriate for baked products as they furnish bulk, texture, crystallinity and browning (Levin, 1986). The L-sugars are currently undergoing the extensive testing needed for regulatory approval and are not expected to be available until the late 1990s.

5.9 Sweeteners in their infancy

In 1985 the discovery of a new sweetener, D,L-aminomalonyl-D-alanine isopropyl ester (RTI-001) was announced. This peptide sweetener is reportedly 58 times sweeter than sucrose, stable in liquid and across food pH ranges, thermostable, non-toxic in preliminary testing and non-cariogenic. In addition, it is said to have a pure sweet taste comparable to sucrose. The compound is synthesized by coupling derivatives of the relatively uncommon amino acids, amino malonic acid and D-alanine, by standard peptide chemistry methodology (Seltzman *et al.*, 1985). Further development of RTI-001 is not currently being pursued (personal communication, 1990).

The 1,1-diaminoalkane derived sweeteners are a family of amino acid-based sweeteners with commercial potential. These sweeteners taste much like sucrose and vary in sweetness from 300 to 1000 times that of sucrose. They are reportedly extremely stable at high temperatures and across a wide pH range. The synthesis of the N-(L-aspartyl)-1,1-diaminoalkane-based sweeteners is based on 'retro-inverso' peptide modification (Fuller *et al.*, 1985).

General Foods has been assigned patents for two amino acid-based sweeteners currently known as PS 99 and PS 100. These L-aminodicarboxylic acid esters are 1800 and 2200 times sweeter than sucrose, respectively. They are both reported to be more stable than aspartame. Only preliminary toxicological studies have been carried out but early results are said to be promising (Anon., 1987). A new class of sweeteners was patented by The Coca-Cola Company in 1988. These L-aspartyl-3-(bicycloalkyl)-L-alanine alkyl esters are reportedly 1900 times as sweet as sugar. Their commercial significance remains to be determined (Iacobucci *et al.*, 1988).

Patents have been issued for numerous other sweet compositions. Many of these discoveries have not been commercially viable, while others are in early development.

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6 Natural high potency sweeteners

S.-H. KIM and G.E. DUBOIS

6.1 Introduction

It is almost certain that since early in time the sense of sweet taste has directed both man and animals to nutritive substances. Thus taste perception probably played an essential role for survival. From an evolutionary view point, it is also likely that plants took advantage of this aspect of sweet taste to propagate their species by producing sweet fruits and other edible parts. Thus, on a volume basis, most natural sweet substances are carbohydrates from plants. However, it is also apparent that some non-carbohydrate compounds have accidentally acquired a sweet taste with no nutritive intention. Most natural sweeteners belong to this category. In modern times, at least for most people of the Western world, the attainment of adequate nourishment has not been an issue, and sweet taste perception has been sought after for the alternative purpose of giving pleasure and enjoyment. In fact, twentieth-century man is more likely than not to consume excess calories. This overnourished population has increasingly succumbed to obesity and to illnesses which are favored by excess calorie consumption (e.g. cardiovascular disease, diabetes, cancer, etc.). Therefore non-nutritive sweeteners have assumed increasing importance in modern days. This chapter covers natural high-potency sweeteners, their synthetic modificants and high-potency sweeteners constituted of natural sub-units. Specifically excluded are carbohydrate sweeteners, which, though ubiquitous in nature, are of trivial sweetness potency. Among many reviews on sweeteners, a recent one (van der Wel *et al.*, 1987) gives extensive coverage to carbohydrate sweeteners as well as many non-natural sweeteners. This chapter covers protein sweeteners (by S.-H. Kim) and non-protein sweeteners such as peptide sweeteners, terpenoid sweeteners, and polyketide sweeteners (by G.E. DuBois). Since interest in sweeteners is proportional to their viability for use in food products, the sweeteners discussed in this review will generally be described relative to the properties requisite for commercial viability. A detailed dissertation on these properties is provided in Section 6.6 and the reader is referred to it for clarification of any points not apparent in earlier sections. In the

sweetener literature, various methods have been employed for reporting sweetness potencies. This complication is discussed in detail in Section 6.6. We have recalculated sweetness potencies in some cases, for the purpose of placing all data on the same scale. The recalculation methodology employed is described in Section 6.6.

6.2 Protein sweeteners

Among sweeteners, natural sweet proteins are unique in that they are natural, contain no modified or unusual amino acids, often have very high potency compared to sugar and decompose into a normal distribution of amino acids on hydrolysis. It can be argued, therefore, that protein sweeteners made up of all or most of the 20 natural amino acids have an advantage over other high-potency sweeteners, and even over peptide sweeteners such as aspartame, on a safety basis. Consumption of the protein sweetener would not result in change from normal exposures to any of the 20 common amino acids while, in principle, exposure to aspartic acid and phenylalanine could be elevated upon consumption of aspartame. For the specific case of aspartame, however, this concern is not important. It has been determined (Roak-Foltz and Leveille, 1984), that even if all sweeteners in the American diet were replaced by aspartame, aspartic acid and phenylalanine intakes would only increase by 5% and 12%, respectively. Nonetheless, protein sweeteners, like peptide sweeteners, are very attractive to consider as product candidates due to the high likelihood of safety. Thaumatin and monellin are the most studied of the protein sweeteners. Whether these proteins are produced by the plants for species propagation purposes or are sweet by accident is not known but they will be discussed in detail. Recently, several new natural sweet proteins have been discovered, for which brief descriptions are given.

Another advantage of high potency protein sweeteners is that they can serve as excellent probe molecules to study the basic science of taste perception. Although details of the molecular mechanism of the sweet signal induction are not known, the basic process is likely to be similar to many other molecular recognition processes. Like hormone-receptor interaction, sweet taste may be elicited by sweet-tasting compounds binding to receptors in taste buds. However, the similarity may end there. A 50% binding of hormone receptors usually occurs at a 10^{-8} – 10^{-11} M concentration of hormone, but the concentration to register half maximal sweet taste by sugar, for example, is about 10^{-1} M. Such high concentrations of sugar obscure specific interaction between receptors and sugar molecules from alternative sweet taste receptor activation by general non-specific mechanisms.

Two unusual proteins discovered in African berries possess the

interesting property of having a very high sweetness potency, registering sweet taste at a concentration of 10^{-8} M, thus showing activity similar to that of hormones. These proteins, monellin (Morris and Cagan, 1972; van der Wel, 1972) and thaumatin (van der Wel and Loeve, 1972) are approximately 100,000 times as potent as sugar on a molar basis and several thousand times more potent on a weight basis. Extensive reviews on both proteins and their practical applications have been published (van der Wel *et al.*, 1986; Higgenbotham *et al.*, 1981, 1983; Weickmann *et al.*, 1989).

6.2.1 Thaumatin and monellin

Thaumatococcus comes from a berry of a plant *Thaumatococcus danielli* Benth, locally known as the katemfe berry. This plant is common throughout tropical Western Africa. The fruit has been used for centuries by inhabitants of the region to sweeten foods such as bread and palm wine. The sweet substance is localized in a sweet aril of the fruit. There are two major sweet proteins in the fruit, thaumatin I and II,

Table 6.1a. Primary sequences of thaumatins.

Ala	Thr	Phe	Glu	Ile	Val	Asn	Arg	Cys	Ser	10
Tyr	Thr	Val	Trp	Ala	Ala	Ala	Ser	Lys	Gly	20
Asp	Ala	Ala	Leu	Asp	Ala	Gly	Gly	Arg	Gln	30
Leu	Asn	Ser	Gly	Glu	Ser	Trp	Thr	Ile	Asn	40
Val	Glu	Pro	Gly	Thr	Asn	Gly	Gly	Lys	Ile	50
Trp	Ala	Arg	Thr	Asp	Cys	Try	Phe	Asp	Asp	60
Ser	Gly	Ser	Gly	Ile	Cys	Lys	Thr	Gly	Asp	70
Cys	Gly	Gly	Leu	Leu	Arg	Cys	Lys	Arg	Phe	80
Gly	Arg	Pro	Pro	Thr	Thr	Leu	Ala	Glu	Phe	90
Ser	Leu	Asn	Gln	Tyr	Gly	Lys	Asp	Tyr	Ile	100
Asp	Ile	Ser	Asn	Ile	Lys	Gly	Phe	Asn	Val	110
Pro	Met	Asn	Phe	Ser	Pro	Thr	Thr	Arg	Gly	120
Cys	Arg	Gly	Val	Arg	Cys	Ala	Ala	Asp	Ile	130
Val	Gly	Gln	Cys	Pro	Ala	Lys	Leu	Lys	Ala	140
Pro	Gly	Gly	Gly	Cys	Asn	Asp	Ala	Cys	Thr	150
Val	Phe	Gln	Thr	Ser	Glu	Tyr	Cys	Cys	Thr	160
Thr	Gly	Lys	Cys	Gly	Pro	Thr	Glu	Tyr	Ser	170
Arg	Phe	Phe	Lys	Arg	Leu	Cys	Pro	Asp	Ala	180
Phe	Ser	Tyr	Val	Leu	Asp	Lys	Pro	Thr	Thr	190
Val	Thr	Cys	Pro	Gly	Ser	Ser	Asn	Tyr	Arg	200
Val	Thr	Phe	Cys	Pro	Thr	Ala				207

	46	63	67	76	113	
I	Asn	Ser	Lys	Arg	Asn	(Iyengar <i>et al.</i> , 1979)
II	Lys	Arg	Arg	Gln	Asp	(Edens <i>et al.</i> , 1982)
A	Asn	Ser	Lys	Arg	Asp	(Lee <i>et al.</i> , 1988)
B	Lys	Ser	Lys	Arg	Asp	(Lee <i>et al.</i> , 1988)

Table 6.1b. Primary sequences of monellin.

A chain										
Phe	Arg	Glu	Ile	Lys	Gly	Tyr	Glu	Tyr	Gln	10
Leu	Tur	Val	Tyr	Ala	Ser	Asp	Lys	Leu	Phe	20
Arg	Ala	Asp	Ile	Ser	Glu	Asp	Tyr	Lys	Thr	30
Arg	Gly	Arg	Lys	Leu	Leu	Arg	Phe	Asn	Gly	40
Pro	Val	Pro	Pro	Pro						45
B chain										
Gly	Glu	Trp	Glu	Ile	Ile	Asp	Ile	Gly	Pro	10
Phe	Thr	Gln	Asn	Leu	Gly	Lys	Phe	Ala	Val	20
Asp	Glu	Glu	Asn	Lys	Ile	Gly	Gln	Tyr	Gly	30
Arg	Leu	Thr	Phe	Asn	Lys	Val	Ile	Arg	Pro	40
Cys	Met	Lys	Lys	Thr	Ile	Tyr	Glu	Asn	Glu	50
	A22	A25	A26	A38	B49	B50				
I	Asp	Glu	Asp	Asn	Asn	Glu	(Frank and Zuber, 1972)			
II	Asn	Gln	Asn	Asn	Glu	Asn	(Bohak and Li, 1971)			
III	Asp	Glu	Asp	Asx ^a	Asn	Glu	(Hudson and Biemann, 1969)			
IV	Asp	Glu	Asp	Asn	Glx ^a	Asx ^a	(Hudson and Biemann, 1969)			

^a Asx, either Asn or Asp; Glx, either Gln or Glu.

with almost identical molecular weights of 22,000. They consist of 207 amino acids and have nearly identical amino acid sequences, differing only in five residues (Iyengar *et al.*, 1979; Edens *et al.*, 1982). Subsequent studies showed that there are more minor species (Higgenbotham *et al.*, 1977; Lee *et al.*, 1988). Amino acid sequences of these proteins are listed in Table 6.1. These sequence studies suggest that there is more than one copy of thaumatin genes; some of the gene products may have been modified post-translationally. Sequence analysis of one of the cDNAs suggests that the protein is synthesized as a precursor, which contains an additional 22 amino acids on its amino-terminal and 6 residues at its carboxy-terminal ends (Edens *et al.*, 1984). The purification procedures have been established (van der Wel and Bel, 1976; Lee *et al.*, 1988).

Monellin is also from a berry from Western Africa, *Dioscoreophyllum cumminsii*, also known as serendipity berry (Morris and Cagan, 1972; van der Wel, 1972). Its fruit comes in clusters with up to 100 red grape-like berries per bunch. The sweet tasting components were extracted from the white mucilaginous material surrounding the seed. The protein consists of two peptide chains, the A-chain of 45 and the B-chain of 50 amino acid residues with molecular weights of approximately 10,000 (Frank and Zuber, 1976). Whether the two peptides of mature monellin are derived from a precursor is not yet known. The amino acid sequence (Frank and Zuber, 1976; Hudson and Biemann, 1976; Bohak and Li, 1976) of the protein is shown in Table 6.1. A small fraction of the protein contains phenylalanine as the first amino acid of chain A.

6.2.1.1 Biochemical studies. The biological function of thaumatin is not known. When the fruit reaches maturity, there is an increase in thaumatin production relative to the total proteins present in the aril. In addition to its sweet taste, thaumatin has been known to have several other interesting properties: thaumatin inhibits the chemotaxis of *E. coli* (Barreau and van der Wel, 1983); the protein has the activity of protease, amidase and esterase when some of its disulfide bonds are reduced (van der Wel and Bel, 1980). Some of these activities may have come from contaminating materials rather than thaumatin itself (Beynon and Cusack, 1990). An interesting observation of functional implication was made recently; it has been found that a thaumatin-like protein (65% sequence similarity) is induced when tobacco plants are infected by the tobacco mosaic virus (Cornellissen *et al.*, 1986). Furthermore, a maize protease inhibitor has 52% sequence similarity to thaumatin and 57% to the thaumatin-like tobacco protein (Richardson *et al.*, 1987). It has been suggested that these proteins are probably produced in response to injuries and viral infections, and the sweet taste may be an accidental property of the protein.

Despite the absence of knowledge of its true biological function, there have been considerable biochemical studies to understand and develop thaumatin because of its potent sweet taste. The protein has no histidine or methionine, but contains twenty-three basic and eighteen acidic residues. There are sixteen cysteines forming eight disulphide bonds which are credited for the stability of the protein and its ability to elicit a sweet taste in a wide range of pH conditions and temperatures.

Like thaumatin, the amino acid composition of monellin lacks histidine, and has a large number of charged groups; sixteen basic amino acids and fourteen acidic residues. The protein, however, has one methionine and one cysteine. Monellin is not as stable as thaumatin on heating or in different pH ranges. The biological function of monellin in the plant is also not known. However, when monellin, with some of its ϵ -amino groups of lysine acetylated, is brought into a reducing environment, it also appears to have such enzymatic activities as protease, amidase and esterase (Hudson *et al.*, 1976). Whether these activities are also from some contaminating materials is not known.

Neither thaumatin nor monellin contains carbohydrates or modified amino acids. Several interesting observations have been made about the two proteins: (i) native conformations are essential for the sweet taste (van der Wel and Bel, 1972, 1976; Cagan and Morris, 1976; Morris *et al.*, 1978); (ii) although both proteins are potently sweet, there are no statistically significant sequence homologies (Edens *et al.*, 1982); (iii) despite the absence of sequence homology, antibodies raised against thaumatin compete for monellin as well as many other sweet compounds (Hough and Edwardson, 1978; van der Wel and Bel, 1978), but not for chemically modified non-sweet monellin (van der Wel *et al.*, 1978), and

similar immunological cross-reactivity was also found for antibodies raised against monellin (Kang, 1987); (iv) antibody-protein complexes lose their ability to elicit sweet taste (unpublished results); (v) the cross-adaptation of monellin and thaumatin in human taste experiments (van der Wel and Arvidson, 1978) and electrophysiological experiments (Brouwer *et al.*, 1973) suggest that they are recognized by the same receptor.

Since there is no *in vitro* assay for the sweet taste activity and no taste receptor molecule has yet been isolated, there has been interest in understanding the immunological cross-reactivity as a possible clue for the receptor binding site. This is based on the assumption that antibody and receptor binding sites are likely to be exposed and one may be a subset of the other. Despite the absence of sequence homology, it is possible that the two proteins may have similarity in their three-dimensional structure exposing a few key side chains in similar stereospecific arrangements. To explore these possibilities, to provide the structural basis for understanding the biochemical observations mentioned above and to search for the receptor binding sites of the two proteins, the backbone structures of thaumatin (Figure 6.1b, de Vos *et al.*, 1985) and monellin (Figure 6.1a, Ogata *et al.*, 1987) have been determined at 3 Å resolution, and refinement at a higher resolution is in progress. The topological and backbone structures of these two proteins are shown in Figure 6.1.

6.2.1.2 Crystallographic studies: thaumatin. Thaumatin crystallizes in space group $P2_12_12_1$ with cell parameters of $74.42 \times 53.38 \times 52.25$ Å. There is one molecule per asymmetric unit, and 50% of the crystal volume is protein. X-ray diffraction data were collected at 4°C, and the crystal structure determined by multiple isomorphous replacement (MIR) methods. An artist's drawing of the backbone structure is shown in Figure 6.1 (de Vos *et al.*, 1985). The structure has been refined at 3.0 Å resolution by a restrained-constrained least-squares method. The backbone structure of thaumatin shows that the molecule can be considered as made of three 'domains'; the central β -barrel and two 'finger' regions, similar to many membrane receptor binding proteins such as snake venom toxin, bungarotoxin, wheat germ agglutinin, cytotoxins and ragweed pollen allergens (Drenth *et al.*, 1980). All eight disulphide bonds have been identified and found to be mostly in the 'finger' regions. Topological and space-filling models of the structure are shown in Figure 6.2 b and d respectively.

We have recently collected 1.5 Å resolution diffraction data on thaumatin using a synchrotron generated X-ray source and are in the process of refining the thaumatin structure using this data set. It is expected that the final refined structure will provide reliable information about the conformation of most of the side chains, thus allowing us to search for the stereospecific arrangement of key functional groups on the surfaces of thaumatin similar to those on monellin surface.

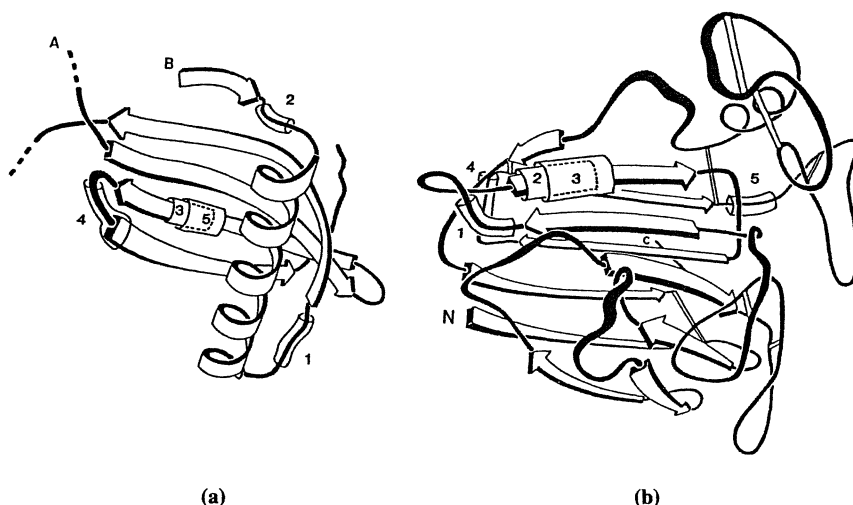


Figure 6.1. Schematic drawings of the backbone structure of thaumatin (right) and monellin (left). Tubed sections are the regions with tripeptide sequence similarities between the two proteins. The homologous tripeptide of each pair is identified by the same number in both structures. Broken lines represent disordered residues that could not be seen in the electron density maps.

6.2.1.3 Crystallographic studies: monellin. Monellin crystallizes in space group $P2_1$ with cell parameters $a = 39.6 \text{ \AA}$, $b = 71.8 \text{ \AA}$, $c = 86.9 \text{ \AA}$ and $\beta = 107.1^\circ$ containing four independent monellin molecules consisting of eight peptide chains (Figure 6.2). A 3.0 \AA data set for the native crystal and 3.5 \AA data sets for five heavy atom derivatives have been obtained using an ω -step scan on a Nicolet diffractometer at 4°C . Crystal structure was determined by a combination of the multiple isomorphous replacement (MIR) method and electron density averaging methods (Ogata *et al.*, 1987). Two amino terminal residues of the A chain and three carboxyl terminal residues of the B chain are disordered and could not be seen in the map. Topological and space-filling models of the structure are shown in Figures 6.2 a and c respectively.

The backbone structure (Figures 6.1b and 6.2b, Ogata *et al.*, 1987; Ogata, 1986) reveals that there is one five-stranded anti-parallel β -sheet made of two strands from the B chain and the remaining three from the A chain. The β -sheet is twisted in the same handed sense as observed in β -sheets of many other proteins. The only α -helix nestles in the gently twisted concave side of the β -sheet, and runs almost perpendicular to the β -strands.

6.2.1.4 Structural comparison between monellin and thaumatin. The crystal structures of monellin and thaumatin show clearly that there is no similarity of significance at the level of backbone structures except the β -sheet regions. Thaumatin (Figures 6.1b and 6.2b) is made of a core of two β -sheets, one on top of the other, and two large complex looped 'domains' are located on the

same side of the double β -sheets (de Vos *et al.*, 1985). Each β -strand is only six amino acids long on average. Monellin, on the other hand, has a single β -sheet of five strands, each strand containing an average of ten residues (Figures 6.1a and 6.2a). It has a few, small tight loop regions as compared to several large loops with disulfide bonds in the thaumatin molecule (Ogata *et al.*, 1987; Ogata, 1986). Thus in overall structure there appears to be little similarity between the two molecules.

At the level of small local conformation, we examined five pairs of homologous tripeptide sequences (Edens *et al.*, 1982): residues B28–30, B6–8, A22–24, A29–31 and A21–23 in monellin (labeled 1–5 in Figure 6.1a) and have the same tripeptide sequences at residues 94–96, 100–102, 101–103, 118–120 and 128–130 (also labeled 1–5 in Figure 6.1b) in thaumatin, respectively. Although the tripeptide homologies are not statistically significant, the immunological cross-reactivity (Hough and Edwardson, 1978; van der Wel and Bel, 1978; Kang, 1987) and cross-adaptability (van der Wel and Arvidson, 1978; Brouwer *et al.*, 1973) imply the existence of common chemical and structural features, and the possibility exists that a joint site made of two or more of these short homologous regions may act as an antigenic determinant and sweet determinant. Gross conformations of three of the five pairs, regions 1, 4, and 3 of monellin, are found to be similar to their counterparts in thaumatin at the current resolution of 3 Å, i.e. the former two are located in looped regions and the third in a β -strand. Of these, region 3 was not considered to be an antigenic site because it is located within the β -sheet, and only the side chain of one out of three points toward the exterior of the protein.

About regions 1 and 4, since the separation of these two regions are different in the two proteins (26 Å in monellin and 18 Å in thaumatin), we can rule out the possibility that they jointly form the common single antigenic site. The only remaining possibility is that several smaller regions (shorter than three residues) and/or functional groups of side chains, distributed in the same way in both proteins, jointly form the common antigenic site.

6.2.1.5 Immunochemical identification of cross-reacting antibody binding sites. In addition to the structural approach of searching for the cross-reacting antibody binding sites on both proteins, we have also taken an immunochemical approach (Kang, 1987). So far, two tryptic peptides have been identified from monellin that bind to thaumatin antibodies. Western dot blots of monellin peptides with cross-reacting thaumatin antibodies gave two strong positives. The amino acid composition and N-terminal four residue sequencing revealed that the two peptides correspond to the amino acid sequences of 5–17 of A chain and 1–17 of B chain, respectively (Kang, 1988). Similar experiments on thaumatin peptides using cross-reacting monellin antibodies showed that thaumatin peptides corresponding to residues 1–8 and 54–67 cross-reacted immunologically (Kang, 1988).

The locations of these peptides in the a acid sequence and three-dimensional structures are shown in Figure 6.3.

Visual examination of these regions does not suggest any obvious conformation similarities except two loops; a loop within the peptide 54–67 of thaumatin and a loop within the peptide 5–17 of the A chain of monellin. It is likely that one has to examine the three-dimensional arrangement of side chains or functional groups of these regions, especially the loops, on the molecular surfaces in high resolution structures of the two proteins. High resolution studies of both proteins are in progress.

6.2.1.6 Re-designing monellin In addition to the structural and biochemical

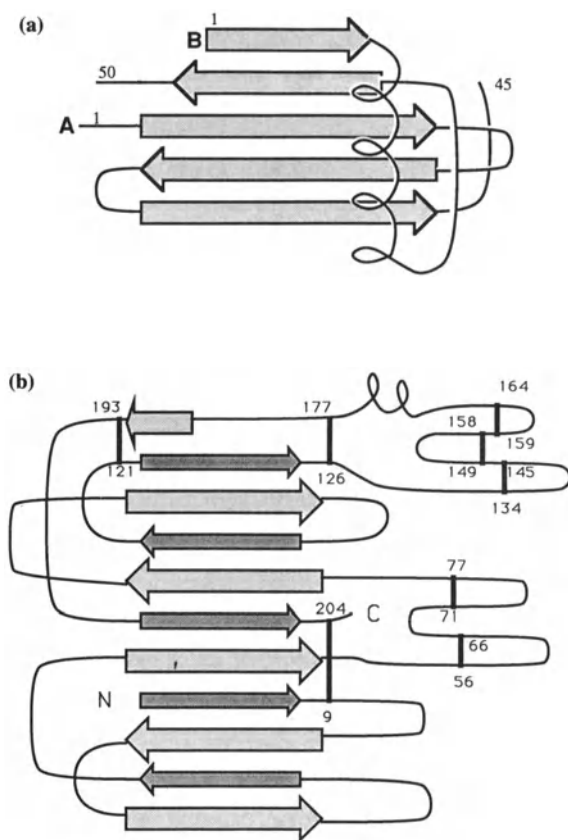
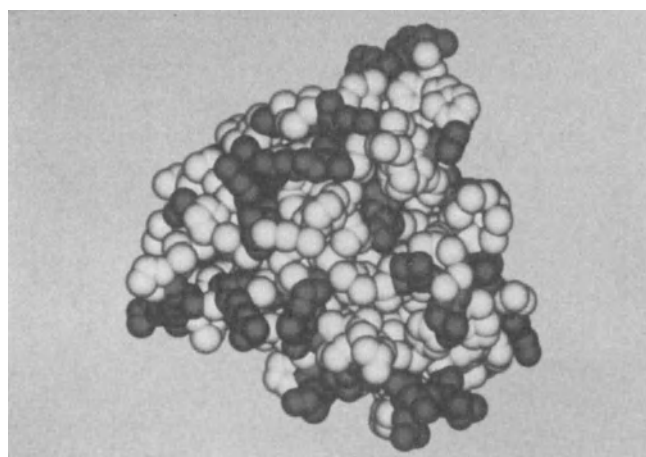
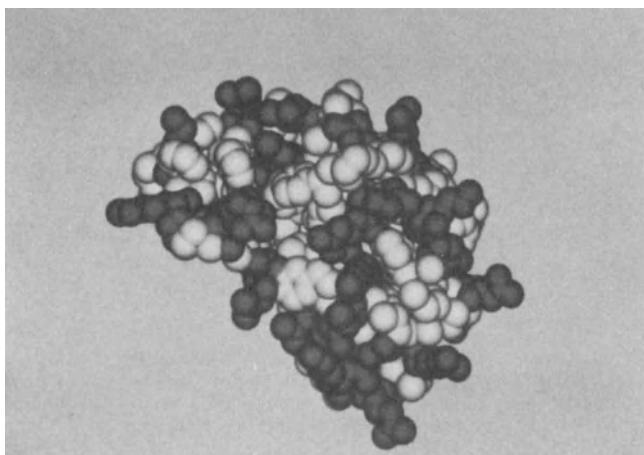


Figure 6.2. (a,b) Topological structure of monellin (a) and thaumatin (b) based on the crystal structures of both proteins at 3 Å resolution. β -strands are represented by flat arrows, α -helix by a coil and loops by curved lines. The beginning and ending residues of A and B chains of monellin are indicated. The beginning and ending residues and residues which are involved in disulfide bonding in thaumatin are indicated.

studies described above, we have taken site specific mutagenesis methods to identify the receptor binding sites on monellin. For this purpose we have chosen to clone the gene for the single chain monellin (Kim *et al.*, 1989) for the following reasons. Thaumatin contains eight disulfide bonds. It is quite thermally stable. However, once the molecule is denatured, it loses most or all of the sweet taste, presumably because it is difficult to remake all eight disulphide bonds correctly and fold them into the sweet, native conformation. Monellin on the other hand has no disulfide bond, is only half the size of

(c)



(d)

Figure 6.2. (c,d) Space-filling models of monellin (c) and thaumatin (d).

thaumatin, but contains two peptides. To facilitate the mutagenesis, we first designed a new monellin molecule in which the B chain is fused to the A chain. We assumed that if we clone and express A and B chains separately, there may be the problem of renaturation when the two chains are mixed, since it has been shown that heat denatured natural monellin loses its sweet taste.

(a) *Design.* The crystal structure of monellin suggested a very natural and obvious way of fusing the two peptides of monellin together. Figure 6.4 shows the topological structure based on the backbone structure of the monellin molecule as determined by X-ray diffraction studies at 3 Å resolution (Ogata *et al.*, 1987). In the structure the carboxy-terminal β -strand of the B chain forms an anti-parallel β -sheet with the amino-terminal β -strand of the A chain. At the current resolution, the last three residues of the B chain and the first four residues of the A chain are partially disordered. However, we can clearly see that isoleucine-46 of the B chain is in register and hydrogen-bonded to glycine-6 of the A chain. We have tested several sequences of different lengths as a linker joining the two chains. Of these, the most conservative design is a junction of eight residues long. It contains the same amino acid sequence as natural monellin except for the first residue, phenylalanine, of the A chain. Thus the new peptide bond formed is between Glu-50 of B chain and Arg-2 of A chain (see Figure 6.4), resulting in a single chain 94 residues long.

(b) *Expression and purification.* The genes coding for the various fused monellin molecules were chemically synthesized. A total of 14 oligonucleotides were synthesized, coding for each fused monellin gene. The dideoxy DNA sequencing method was used to check the correctness of the base sequence. The oligonucleotides were mixed, annealed and ligated to produce a synthetic gene, which was then introduced into an expression vector carrying the *trp* promoter and operator (Russell *et al.*, 1982). The plasmid vector was then introduced into *E. coli* W3110 (ATCC27325). The cells were induced with indoleacrylic acid and the protein was purified using CM-25 Sephadex. Since the fused monellin has a very high content of basic residues, a single ion-exchange chromatography gave highly pure protein. We subsequently also cloned the synthetic fused monellin gene into a yeast expression vector, which also gave a high yield of pure fused monellin (unpublished results).

(c) *Characterization of the new protein.* The *E. coli*-expressed single-chain monellin has an apparent molecular weight corresponding to the sum of the two chains (A and B) of natural monellin on SDS gel, and binds specifically to the antibody raised against natural monellin (Figure 6.5).

N-Terminal sequence indicated that the first residue, methionine, was cleaved off in the final protein. Circular dichroism spectra of a fused monellin

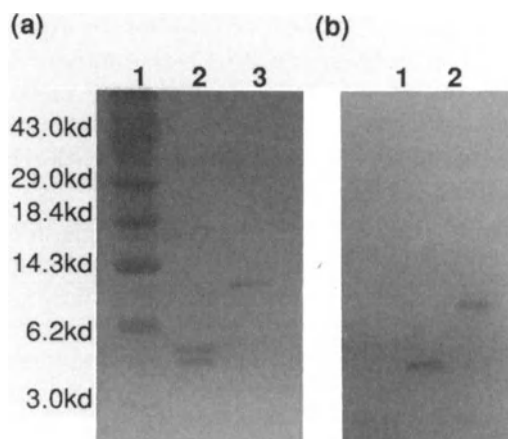


Figure 6.5. (a) Polyacrylamide slab gel electrophoresis of monellin and fused monellin. Lane 1, marker proteins: insulin (3 kDa), bovine trypsin inhibitor (6.2 kDa), lysozyme (14.3 kDa), β -lactoglobulin (18.4 kDa), carbonic anhydrase (29 kDa), ovalbumin (43 kDa); Lane 2, natural monellin which is composed of A-(45 residues) and B-chains (50 residues); Lane 3, fused monellin, which is composed of 94 residues. (b) Western blot using antibody raised against natural monellin: Lane 1, natural monellin band corresponding to the B-chain; Lane 2, fused monellin.

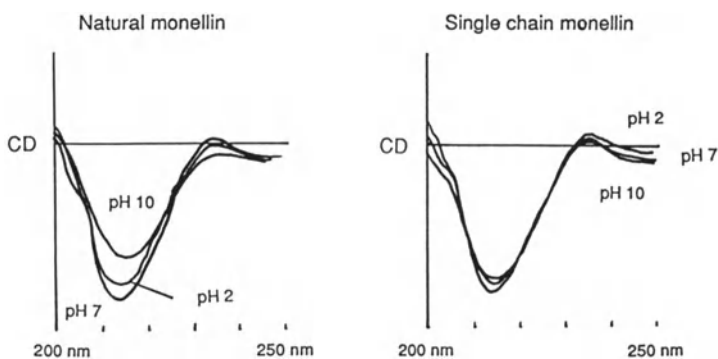


Figure 6.6. Circular dichroism spectra of (a) natural monellin at pH = 2, 7 and 10; and (b) of single chain monellin at pH = 2, 7 and 10.

are practically identical to those of natural monellin in the pH range 2–10 (Figure 6.6) although natural monellin shows slight partial denaturation at pH 2. Furthermore, compared to sucrose solutions at various concentrations, the potencies of sweetness of fused monellin are just as high as the natural one. The thermal stability of fused monellin is substantially improved over that of natural monellin. For example, when natural monellin is heated to

50°C at pH 2 and allowed to cool down to room temperature, it loses its sweet taste. However, when fused monellin is heated to 100°C at the same pH for several minutes and allowed to cool down to room temperature, it recovers its sweet taste completely (Table 6.2). With this fused monellin molecule, we are in the process of doing the site directed mutagenesis. The results of this study should complement our biochemical results.

Taste and smell are two of the least understood sensory perceptions at the molecular level. Monellin and thaumatin are the only known proteins that elicit potent chemical sensory perception, and thus are two of the best systems to study the chemical sense of taste at the molecular level. Furthermore they also are the only two proteins with known crystal structures that have immunological cross-reactivity without sequence or structural homology. The structural information at high resolution may provide the most solid foundation on which future molecular experiments can be designed and existing biochemical and mutational results interpreted to answer questions regarding the chemical sense of taste and immunological cross-reactivities.

6.2.1.7 Practical aspects The concentration versus intensity (C/I) function and flavor profile analysis data for thaumatin (Pecore *et al.*, 1989) are illustrated in Figure 6.7. From the equation describing the thaumatin C/I function, it may be calculated that $P_w(2) = 8600$, $P_w(5) = 5600$ and $P_w(8) = 2600$. Inspection of the flavor profile histogram leads to the prediction that thaumatin will be judged to be of low acceptability as a sugar substitute in food products. However, it may be of use in other applications as a food additive and flavoring agent as well as a sweetener in food products in which the flavor profile of thaumatin has advantages over sugar.

6.2.2 Mabinlin, pentadin, curculin and others

The occurrence of sweet albumins in the seeds of *Capparis masaikai* Levl. (Capparidaceae) has been reported (Zhang, 1986). This plant is used in Chinese herbal medicine for the treatment of sore throats. From the defatted seeds of this plant, two sweet proteins mabinlin I and mabinlin II have been isolated in 13% yield. These proteins are estimated to have molecular weights of 11,600 and 10,400, respectively with approximately 80% sequence homology. Although detailed sensory evaluation study results have not been reported, the mabinlins are said to be similar in quality to thaumatin and monellin but to be somewhat less potent.

The identification of a sweet protein in the fruits of *Pentadiplandra brazzeana* Baillon (Pentadiplandraceae), a plant indigenous to tropical Africa, has been reported (van der Wel *et al.*, 1989). This protein has been named pentadin and is estimated to have a molecular weight of 12,000. As with the mabinlins, careful sensory panel studies have not yet

Table 6.2. Heat stability of monellin (M) and fused monellin (PS)

Temperature (°C)	pH 2		pH 4		pH 6	
	M	PS	M	PS	M	PS
Taste at given temperature						
40	+	+	+	+	+	+
50	—	+	+	+	+	+
60	—	—	+	+	+	+
70	—	—	—	+	+	+
80	nd ^a	nd	—	+	+	+
90	nd	nd	nd	+	(+)	(+)
100	nd	nd	nd	(+)	—	(+)
Taste at room temperature						
40	+	+	+	+	+	+
50	—	+	+	+	+	+
60	—	+	+	+	+	+
70	—	+	+	+	+	+
80	nd	+	+	+	+	+
90	nd	+	+	+	+	+
100	nd	+	+	+	+	+

Key: +, heat stable; — not heat stable
^and, not detectable.

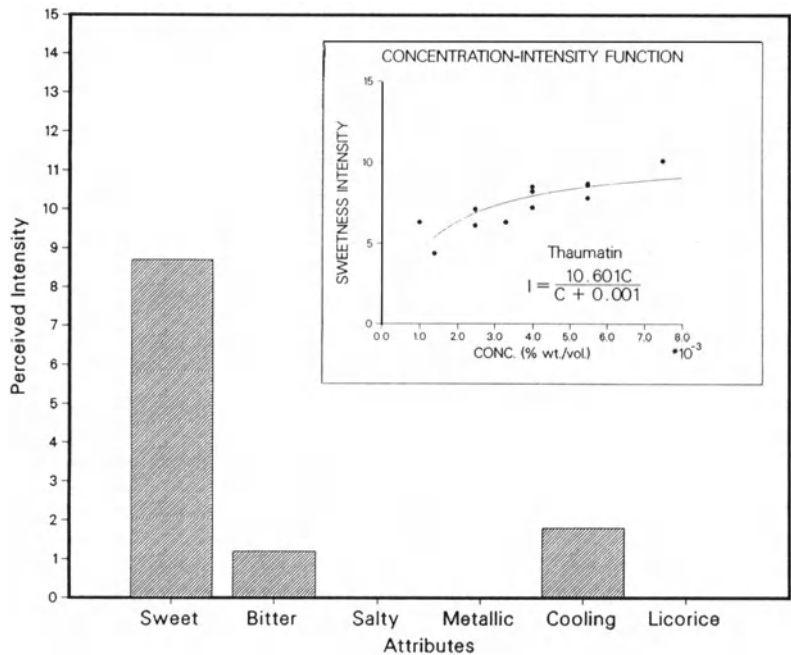


Figure 6.7. Flavor profile and relative concentration-intensity function for thaumatin.

been conducted. It is said, however, to be similar to monellin in taste characteristics with a potency of approximately 500 times that of sucrose.

Another sweet protein which has recently been described comes from the plant *Curculigo latifolia* which grows wild in Western Malaysia (Yamashita *et al.*, 1989). The sweet protein occurs in the pulp of the fruit and has been named curculin. This protein has been shown to have a molecular weight of approximately 24,000 and to be constituted of two sub-units which are of equivalent size. No systematic sensory panel studies have been reported on this sweetener. Curculin differs from the other known sweet proteins in that, in addition to being sweet itself, it causes sour stimuli to be sweet and also causes water to taste sweet.

Finally, a sweet protein has been demonstrated to be present in the white portion of chicken eggs (Eguchi, 1988). Very little information is available on the specific properties of this sweet protein. It is clear, however, sweet proteins are not uncommon in nature, and the process of discovery then would speed up tremendously once the sweet taste receptor molecule(s) is identified.

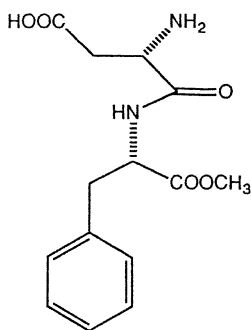
6.3 Peptide sweeteners

6.3.1 Aspartame

The sweetener discovery of greatest commercial significance is that of aspartame (1), a peptide-based sweetener. Aspartame exhibits a taste quality nearly indistinguishable from sucrose, and allowed, for the first time, formulation of many low-calorie food products without hedonic sacrifice. Due to this near perfect sucrose mimicry, it is not a surprise that, following regulatory approval, aspartame has revolutionized the low-calorie section of the food industry. Strictly speaking, it must be pointed out that aspartame, due to its natural amino acid constituency, is a nutritive rather than a non-nutritive sweetener. Aspartame is metabolized to provide energy proportional to the amount ingested. However, since more than 100 g of sucrose may be replaced by 1 g of aspartame, it functions, in effect, as a non-nutritive or non-caloric substance.

Peptide type sweeteners were unknown until the first, the dipeptide ester L-aspartyl-L-phenylalanine methyl ester, was serendipitously discovered in 1965 (Mazur *et al.*, 1969). This sweetener is not found in any natural source but is constituted of the natural protein building blocks aspartic acid and phenylalanine and also methanol, a component present in substantial amounts in fruit and vegetable juices. It is known generically as aspartame, often abbreviated as APM, and is very well known to consumers under the brand name NutraSweet*. The sweet taste of APM was discovered by

*NutraSweet is a registered trademark.



1

James Schlatter while working under the direction of Robert Mazur of G.D. Searle & Company. APM was prepared by Schlatter as an intermediate in a drug discovery program aimed at finding new treatments for ulcers. Interestingly, this compound had been prepared some years earlier by chemists at ICI in the United Kingdom. Its sweet taste had not been noted, however. It can only be speculated as to the number of known organic compounds which possess sweet taste or other useful properties which are yet to be discovered. It is ironic that even though substantially more than 1000 sweet analogues of aspartame have been prepared since 1965, none is better than aspartame when all the properties requisite for commercial viability are considered.

6.3.1.1 Taste properties. The sweetness potency of aspartame is significantly dependent on the sucrose reference concentration. Thus, from the equation of the APM concentration (C)/intensity (I) function given in Figure 6.8, it is calculated that $P_w(2)=280$, $P_w(8)=150$ and $P_w(10)=110$ (Pecore *et al.*, 1989), where $P_w(X)$ refers to the sweetness potency by weight as compared to an $X\%$ solution of sucrose.

The phenomenal commercial success of aspartame is easily explained by its exceptional sucrose mimicry. As suggested by its flavor profile in Figure 6.8, it is essentially indistinguishable from sucrose; no significant non-sweet flavor attributes are observed (Pecore, 1989). Interestingly, however, the temporal profile of aspartame is different from that of sucrose. Both a slightly delayed appearance time, AT, [5 s (APM) vs. 4 s (sucrose)] and a protracted extinction time, ET, [19 s (APM) vs. 14 s (sucrose)] have been observed (DuBois and Lee, 1983). These differences are not sufficient, however, to adversely affect acceptability in food products.

An interesting and useful manner in which aspartame has an advantage over other high potency sweeteners is that it has the ability to enhance

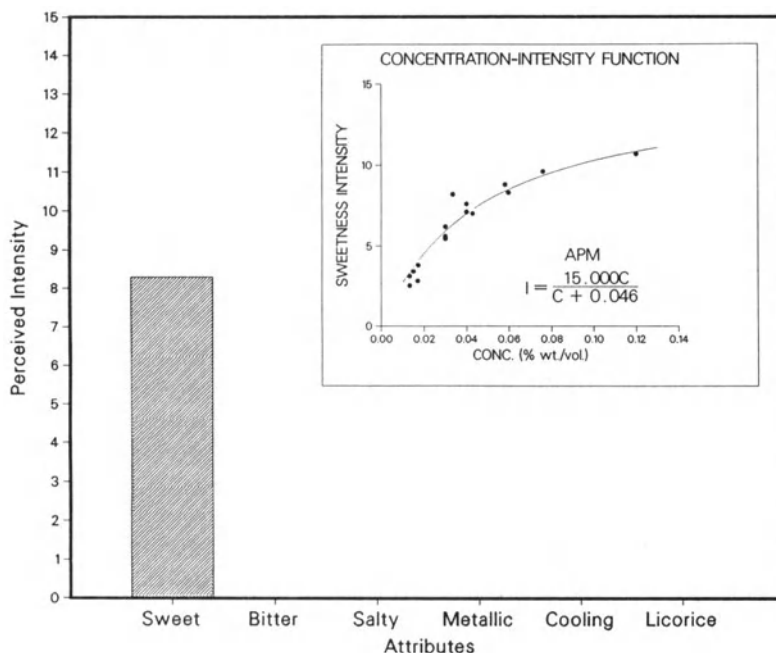


Figure 6.8. Flavor profile and relative concentration-intensity function for aspartame (APM).

other desirable flavor attributes. This effect was noted early on and has been systematically studied (Baldwin and Korschgen, 1979; Wiseman and McDaniel, 1989). It has been hypothesized that this unique advantageous property of aspartame is due to its atypical temporal profile (DuBois, 1989). More specifically, aspartame's flavor enhancement properties are suggested to be consequences of its slightly delayed AT relative to that of sucrose. Thus, in food products, greater temporal resolution of sweetness (taste) and flavor (olfaction) neural signals occurs for APM than is the case for sucrose. This increased resolution of neural olfactory and taste signals for APM over sucrose may result in the flavor intensity to be judged higher in the better resolved aspartame-sweetened food system. This effect is then interpreted as a flavor enhancement effect of aspartame.

6.3.1.2 Safety. The safety of aspartame has been more extensively studied than that of any other food additive. Most of the principal metabolism, pre-clinical and clinical studies have been reviewed (Stegink *et al.*, 1984; Butchko and Kotsonis, 1989). On the basis of evaluation of all of the safety assessment studies, aspartame was approved by the FDA in 1981 with an acceptable daily intake (ADI), level of 20 mg/kg body weight. This ADI is based on a no observed effect level (NOEL) in preclinical studies of

2000–4000 mg/kg body weight. In 1984, based on clinical studies, the ADI for aspartame was raised by the FDA to 50 mg/kg body weight. The ADI established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for aspartame is 40 mg/kg body weight. No effects were noted in the clinical studies at doses many times greater than potential human consumption. Consideration of aspartame metabolism explains why this is the case. Upon ingestion, aspartame is completely broken down to the two natural amino acid building blocks and methanol. The safety of the amino acids L-aspartic acid and L-phenylalanine is, of course, no surprise since dietary protein provides substantially greater quantities of these nutrients. Methanol also is not a safety concern when exposure occurs as a metabolite of aspartame. Beverages formulated with aspartame to a sweetness level matching 10% sucrose contain the equivalent of 60 mg/l of methanol; this is substantially less than the average of 140 mg/l content for fruit juices. Thus, the demonstrated safety of aspartame should be no surprise to anyone. Consumption of aspartame does not result in exposure of the internal body tissues to any novel substances. Natural sub-unit assembly and metabolic sub-unit disassembly is a unique high potency sweetener concept presently exemplified only by aspartame and the protein sweeteners.

In spite of the safety of aspartame, however, products containing it are required to carry an informational statement for people with a rare genetic disease known as phenylketonuria. Approximately one in 15,000 people have the disorder which involves an inability to metabolize phenylalanine. Unchecked, phenylketonuria results in mental retardation. Today, in the United States, this disorder is detected at birth, if present. The normal treatment is a phenylalanine restricted diet from infancy through childhood and sometimes into adulthood. It should be emphasized, however, that phenylalanine consumption is only restricted, not eliminated, since it is an essential amino acid and, as such, is necessary for life. Such people must carefully monitor their consumption of phenylalanine from all dietary sources including aspartame.

6.3.1.3 Physical properties. Aspartame exhibits sufficient solubility for all food applications. At ambient temperature in water, a solubility of $\geq 1.0\%$ can be attained at any pH of 4 or less and also at neutral pH; solubility reaches a minimum at pH 5.5, the isoelectric point (NutraSweet Technical Applications Manual, 1987). Thus, using $P_w(8)=150$, it can be calculated that aspartame is greater than 18 times more soluble than necessary to provide an 8% sucrose level of sweetness intensity.

If aspartame has a drawback, it is hydrolytic stability. At 25°C it exhibits hydrolytic half-lives in aqueous buffer of 116, 260, 242, 82 and 2 days for pHs of 3, 4, 5, 6, and 7, respectively (Homler, 1984). Clearly maximum utility would be expected in the pH range of 3–5. Fortuitously,

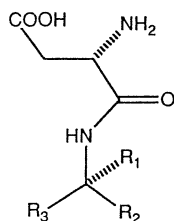
this is a representative range for most food and beverage systems. It is particularly fortuitous that none of the three principal degradation products, L-aspartyl-L-phenylalanine (AP), 3-benzyl-6-carboxymethyl-2,5-diketopiperazine (DKP) and β -L-aspartyl-L-phenylalanine methylester (β -APM), exhibit either 'off' tastes in aged food or beverage products or safety problems. All of the degradation products have been demonstrated to be safe. Interesting, the β -amino acid (β -AP) has recently been demonstrated to be naturally produced on metabolism of normal dietary protein (Burton *et al.*, 1989). In summary, it can be stated that aspartame exhibits sufficient stability for use in acidic food and beverage products. On the other hand, the neutral pH range encountered in baked goods is problematic. An encapsulated form of aspartame has been developed, however, to address this limitation (Tsau *et al.*, 1987). FDA approval of the use of aspartame in baked goods based on encapsulated aspartame is pending.

6.3.2 Other peptide sweeteners

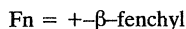
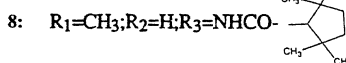
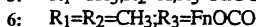
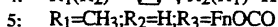
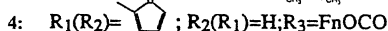
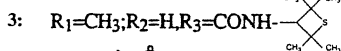
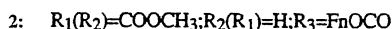
The discovery of the sucrose-like sweet taste of aspartame in 1965 provided the impetus for a substantial amount of research in numerous industrial and academic laboratories on *peptide* type sweeteners. This effort was particularly enhanced by the report by chemists of Takeda Chemical Industries that aspartame analogue **2** exhibited a clean sweet taste with $P_w(8.3)=33,200$ times sucrose (Fujino *et al.*, 1976). This represents more than a 200-fold potency increase over aspartame. Dipeptide ester **2** is constituted of the natural building blocks, L-aspartic acid, D-alanine and (+)- β -fenchol. Problematical, however, was that **2** was substantially less hydrolytically stable than aspartame. Nonetheless **2** provided an 'exciting lead compound for a number of research programs. Efforts at Pfizer led to the selection of **3** as a preferred compound (Brennan *et al.*, 1983). This compound was assigned the generic name of alitame and reportedly exhibits $P_w(10)=2000$ times sucrose. Alitame is said to exhibit a clean flavor profile much like aspartame but to be advantaged over aspartame by greater hydrolytic stability. A food additive petition was filed on alitame by Pfizer in 1986 and, at present, approval is pending.

Another program based on **2** as a lead was initiated at Procter & Gamble Company. Dipeptide ester **4** was synthesized as a part of this program (Blum *et al.*, 1987). It is reported to exhibit $P_w(8-10)=16,450$ and to be substantially more stable than aspartame. Problematical for **4**, however, is a substantially delayed AT and a prolonged ET. A third program based on the Takeda discovery was conducted at General Foods Corporation. Preferred compounds identified in this program are **5** and **6** which have been reported to be hydrolytically stable and to exhibit $P_w(8.6)=2300$ and $P_w(8.5)=1133$ times sucrose, respectively (Zanno *et al.*, 1988). A fourth

dipeptide sweetener program influenced by **2** was initiated at The Coca Cola Company (Iacobucci *et al.*, 1988). The most attractive sweetener synthesized in this program is **7** with a sweetness potency of 1930 times that of sucrose.

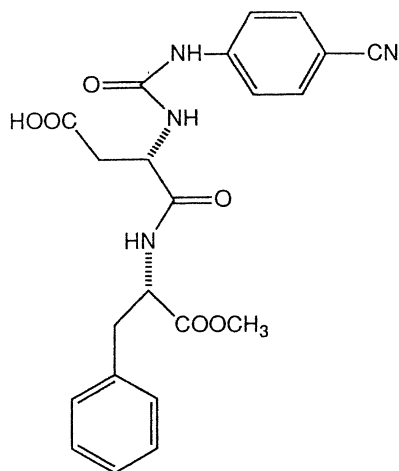


2-8



Finally, an effort by Cumberland Packing Corporation was also, at least in part, influenced by the discovery of **2**. This program resulted in the identification of **8** as the preferred product candidate (Verlander *et al.*, 1986; Fuller *et al.*, 1985). This substance was reported to be 800–1000 times as potent as sucrose and also to be much more stable than aspartame.

In summary, a very substantial amount of effort has been expended in the *peptide* sweetener class in an effort to identify a sweetener advantaged over aspartame. To date, however, only Pfizer's alitame has been developed to the point of the filing of a Food Additive Petition (FAP).

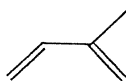


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Another sweetener discovery program which was influenced by *peptide* sweetener structure-activity-relationships was initiated in the laboratory of Professor Claude Nofre at the Université Claude Bernard in Lyon, France. This program resulted in the discovery of a number of extremely potent sweeteners, a representative example of which is **9** (Nofre *et al.*, 1987). This sweetener is reported to exhibit $P_w(2)=7,800$ times sucrose. To date, no FAP has been filed on a sweetener of this structural type. Clearly, however, the very high potency of this peptide derivative suggests that costs of high potency sweeteners may ultimately approach 0 cents/lb on a cost per sucrose equivalent (CSE) basis.

6.4 Terpenoid sweeteners

An extensive variety of naturally occurring sweet compounds has been discovered over the last half of the twentieth century. The preponderance of these sweet natural products are related by their common ontological ties to the biosynthetic precursor isoprene (**10**). Isoprene-derived natural products are sometimes called isoprenoids. More commonly, however, they are referred to as terpenoids. Sweeteners of the terpenoid type may be subdivided into *monoterpenoid*, *sesquiterpenoid*, *diterpenoid* and *triterpenoid* structural classes. Members of these classes are biosynthetically derived from 2, 3, 4 and 6 isoprene subunits and therefore are C_{10} , C_{15} , C_{20} and C_{30} compounds, respectively. In all cases, the sweet terpenoids which have been found in nature have been further elaborated in structure by secondary metabolic processes such as oxidation and glycosylation.

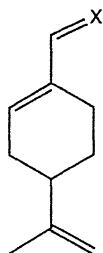


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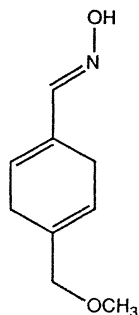
6.4.1 Monoterpenoids

A well-known monoterpenoid class sweetener is perrillartine (**11**), the syn oxime of perillaldehyde (**12**) which occurs in the volatile oil of *Perilla frutescens* (L.) Britton (Labiatae). While perillaldehyde is said to be slightly sweet, its oxime derivative has been reported to exhibit a P_w (8.6) 770 times that of sucrose (Acton *et al.*, 1976) and to be used as a sweetening agent for tobacco in Japan (The Merck Index, 1983). At this potency, it can be calculated that a concentration of 110 ppm is required to provide an 8.6% sucrose equivalent level of sweetness. Problematic, however, is the fact that the solubility limit of **11** is only 82 ppm. In

addition, it has been reported that the flavor profile of **11** while sweet, is appreciably bitter and accompanied by licorice, menthol and gingery aftertastes. By the Quantitative Descriptive Analysis (QDA) technique (Stone *et al.*, 1974), in which the taste quality of a solution having a total taste intensity equivalent to 8.6% sucrose is quantitatively sub-divided into percentages of the various taste quality components, **11** was reported to have a sweet/bitter/other (S/B/O) taste intensity ratio of 60/25/15. In an attempt to address these shortcomings of **11**, Acton and Stone at Stanford Research Institute (Acton and Stone, 1976) carried out an extensive programme aimed at the identification of a viable analogue. The preferred compound identified in this effort was oxime **13**. This substance exhibited a rather clean flavour profile with S/B intensity ratio of 98/2 and $P_w(8.6) = 450$. In addition, the solubility of **13** was determined to be 3400 ppm which may be calculated to be some 18 times as soluble as needed to match 8.6% sucrose in sweetness. The results of preliminary studies on the metabolism and safety assessment of **13** have been reported (Mitoma *et al.*, 1985). As a consequence of concerns raised by these studies, it is apparent that further development of this sweetener is not in progress.



11: X=NOH **12:** X=O

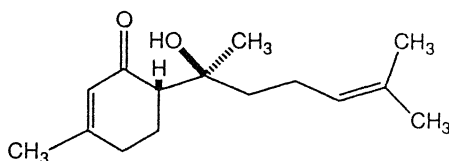


13

6.4.2 Sesquiterpenoids

In the laboratory of Professor A. Douglas Kinghorn at the University of Illinois at Chicago, an intensive program has been specifically aimed at the search for, and structure elucidation of, natural sweeteners. As an outcome of this program, the only known example of a sesquiterpenoid sweetener was discovered (Compadre *et al.*, 1985). One of the methods employed by Kinghorn and co-workers was the search of the botanical literature for references to sweet tasting plants. While examining a monograph entitled *Natural History of New Spain* authored in 1651 by the Spanish physician

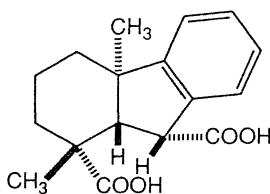
Francisco Hernandez, reference was made to a sweet plant known to the Aztecs as *Tzonpelic xihuitl* which is translated as sweet herb (Compadre *et al.*, 1986). This plant has subsequently been determined to be *Lippia dulcis* Trev. (Verbenaceae) and the sweet component identified as **14**. In recognition of Hernandez's first written notation of this sweet plant, **14** has been named hernandulcin. This sesquiterpenoid is a member of the bisabolane class. No untoward effects were noted in preliminary safety studies. Hernandulcin has been reported to exhibit $P_w(8.6) = 1800$ times that of sucrose. Problematical, however, is the presence of a significant bitter taste component and of a lingering aftertaste. The solubility of **14** has been reported to be 500 ppm (Kingham *et al.*, 1989). Thus, it can be calculated that it is some 10 times as soluble as need be to provide an 8.6% sucrose level of sweetness. While quantitative data on the hydrolytic stability of hernandulcin are lacking, qualitatively it is reported to be quite stable. Under extreme conditions (140°C), it does, however, undergo degradation via a retroaldol reaction (Compadre *et al.*, 1987). In an effort to understand the minimum requisite functionality for sweet taste in **14**, numerous analogues have been prepared (Compadre *et al.*, 1988). None is advantaged over hernandulcin as a sweetener, however. It is not likely that hernandulcin will be commercially developed as a sugar substitute as a consequence of its rather poor flavor profile.

**14**

6.4.3 Diterpenoids

Sweet taste is ubiquitous among diterpenoids. All but one of the known sweet diterpenoids are glycosides. The one non-glycosidic Diterpenoid sweetener which has been reported is the aromatic dicarboxylic acid **15** (Tahare *et al.*, 1971). This substance occurs naturally in pine tree rosin. From the original data, it may be calculated that **15** exhibits a $P_w(1)$ of

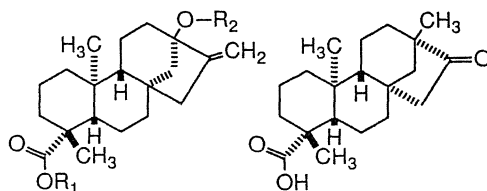
approximately 1600 times that of sucrose. At a concentration sufficiently high to provide the level of sweetness necessary for use in foods, **15** is reported to exhibit strong bitterness. Thus, as a consequence of its poor flavour profile, **15** has little utility as a food additive.

**15**

Of diterpenoid sweeteners, the most exhaustively studied are the diterpenoid glycosides occurring in the leaves of the plant *Stevia rebaudiana* Bertoni (Compositae) which is indigenous to the northeastern part of Paraguay. A number of comprehensive reviews of the sweeteners of this plant have appeared in recent literature (Phillips, 1987; Crammer and Ikan, 1987). Some of the background information on these sweeteners is reviewed here without further reference. The *Stevia rebaudiana* (Bertoni) plant grows to approximately 0.4–0.8 m in height. In early work attempting to propagate *Stevia rebaudiana*, it was found to be difficult from cuttings and that the seeds were generally infertile. Subsequently, as an outcome of substantial efforts by the Japanese which began in 1954, *Stevia rebaudiana* was commercially developed as a source of a natural high potency sweetener. The Japanese government provided the impetus for the development of this industry. The country of Japan was a net importer of sucrose and the government hoped to assist in the development of an indigenous solution to this problem of dependency on foreign supply. Today, the *Stevia rebaudiana* plant is very successfully propagated from root stock and is commercially cultivated in Japan, Singapore, Taiwan, Malaysia, South Korea, China, Israel and Brazil as well as in Paraguay. In addition, while still only on a laboratory scale, progress has been made on the preparation of *Stevia rebaudiana* sweeteners by tissue culture technology.

Significant progress on elucidation of the structures of the sweeteners of *Stevia rebaudiana* was not described until 1931 when Bridel and Lavieille described the isolation of a crystalline glycoside which they called stevioside. They estimated stevioside to be 300 times as potent as sucrose. In addition, they determined that acid hydrolysis of stevioside yielded an aglycone and glucose as the only carbohydrate component. Particularly interesting was their finding that enzymatic hydrolysis with the digestive juice of the snail *Helix pomatia* yielded an aglycone which

was isomeric with the acid hydrolysis product. The enzymatic and acid hydrolysis products were named steviol and isosteviol, respectively. Finally, Bridel and Lavieille also concluded from their analyses that stevioside was constituted of one steviol aglycone conjugated by three glucose units.

**16, 18, 19****17**

16: R₁=R₂=H

18: R₁=β-D-Glu; R₂=β-D-Glu 2-β-D-Glu

19: R₁=H; R₂=β-D-Glu 2-β-D-Glu

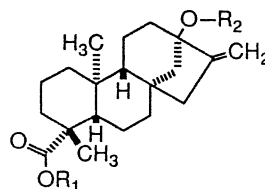
Further progress on the structure elucidation of stevioside awaited the contributions in 1955 of Mosettig and Wood and their co-workers at the US National Institutes of Health. They had interest in the potential medicinal uses of stevioside. These workers confirmed the structural formula of C₃₈H₆₀O₁₈ suggested earlier by Bridel and Lavieille and demonstrated that alkali treatment of stevioside removed one glucose unit to provide a new substance constituted of steviol and two glucose sub-units which they named steviolbioside. Finally, they also assigned the structures of steviol as **16** and isosteviol as **17** and the structures of the glucose conjugates stevioside as **18** and steviolbioside as **19**. As a part of this work, the interconversion of steviol and (–)-kaurene was demonstrated. The sweeteners of *Stevia rebaudiana* have therefore been assigned to the ent-kaurene class of diterpenoids.

Advances in the development of chromatographic and spectroscopic methods facilitated the isolation and structure elucidation of several additional sweet steviol glycosides from crude extracts of *Stevia rebaudiana*. Major contributions were made by Tanaka and his co-workers at Hiroshima University in Japan who identified rebaudiosides A (**20**), B (**21**), C (**22**), D (**23**) and E (**24**). At about the same time, Kobayashi and co-workers at Hokkaido University identified two steviol glycosides which they named dulcosides A (**25**) and B. Dulcoside B was determined subsequently to be identical with rebaudioside C (**22**). Steviolbioside and rebaudioside B, although isolated from some plant extracts have been demonstrated to be artifacts of the isolation procedures rather than naturally occurring themselves.

In summary, *Stevia rebaudiana* has been demonstrated to contain a

complex mixture of six steviol glycosides. Dependent on the sources, the individual steviol glycoside contents may vary considerably. Stevioside is invariably the major component, however, making up 5–22% of the weight of the dry leaves with rebaudioside A second in abundance at 1.5–10% and the other four glycosides present only in low to trace amounts. Companies involved in the commercial development of *Stevia rebaudiana* have made significant progress in the identification of strains which produce proportionately higher levels of rebaudioside A which exhibits an improved flavor profile over stevioside. Companies which have been particularly active in this venture are Morita Kagaku Kogyo of Japan and the Stevia Company in the United States.

- 2-1 β -D-Glu
- 20: $R_1=\beta$ -D-Glu; $R_2=\beta$ -D-Glu 3-1 β -D-Glu
- 2-1 β -D-Glu
- 21: $R_1=H$; $R_2=\beta$ -D-Glu 3-1 β -D-Glu
- 2-1 α -L-Rham
- 22: $R_1=\beta$ -D-Glu; $R_2=\beta$ -D-Glu 3-1 β -D-Glu
- 2-1 β -D-Glu
- 23: $R_1=\beta$ -D-Glu 2-1 β -D-Glu; $R_2=\beta$ -D-Glu 3-1 β -D-Glu
- 24: $R_1=\beta$ -D-Glu 2-1 β -D-Glu; $R_2=\beta$ -D-Glu 2-1 β -D-Glu
- 25: $R_1=\beta$ -D-Glu; $R_2=\beta$ -D-Glu 2-1 α -L-Rham
- 26: $R_1=R_2=\beta$ -D-Glu
- 27: $R_1=H$; $R_2=\beta$ -D-Glu



20–27

Substantial effort has been expended on a search for steviol glycoside sweeteners from other botanical sources. Particularly active in this regard have been Kinghorn and co-workers who in 1984 reported the results of screening 110 species of *Stevia* (Soejarto *et al.*, 1982). Only one of these species (*Stevia phlebophylla*) was found to contain low levels of stevioside, however, and it is now believed to be extinct. A novel steviol glycoside **26** was reported in 1981 as having been isolated by Tanaka and co-workers from the leaves of *Rubus chingii* Hu which are used in the southern part of China in making a sweet tea (Tanaka *et al.*, 1981). This sweet steviol glycoside has been named rubusoside and under alkaline conditions was shown to yield steviolmonoside (**27**).

Many reports have appeared in the literature concerning the sweetness potencies of the aforementioned steviol glycosides. Only a few reports are available in which attempts to quantitatively describe the flavor profile of

these compounds have been made. The available data are those obtained by the QDA method relative to a 10% sucrose level of taste intensity. Potency and QDA assessments which have been reported are summarized for steviol (16) and compounds 18–27 in Table 6.3. From these data, a number of observations may be made and conclusions drawn. First, since all of 19, 21 and 27 are sweet, the glucosyl ester functionality of the steviol glycosides is not necessary for sweet taste. Second, since steviol (16) is not sweet at all, it appears that the glucosyl ether functionality of steviol glycosides is essential for sweet taste. Third, since stevioside and rebaudioside A appear to be of equivalent potency while the latter exhibits a more sucrose-like QDA profile, it appears that taste quality may be optimized by modulation of molecular hydrophilic/lipophilic balance. Finally, the relatively clean QDA profile of rebaudioside A (20) makes clear why commercialization activities have focused on *Stevia rebaudiana* varieties which are high in 20 production. Sensory evaluation data obtained by the flavour profile analysis method for rebaudioside A (Pecore *et al.*, 1989) are given in Figure 6.9. From the equation of the *C/I* function, it can be calculated that $P_w(2)=770$, $P_w(5)=440$ and $P_w(8)=100$. In addition, the flavour profile of rebaudioside A is illustrated in Figure 6.9 in histogram form. The significant bitter taste

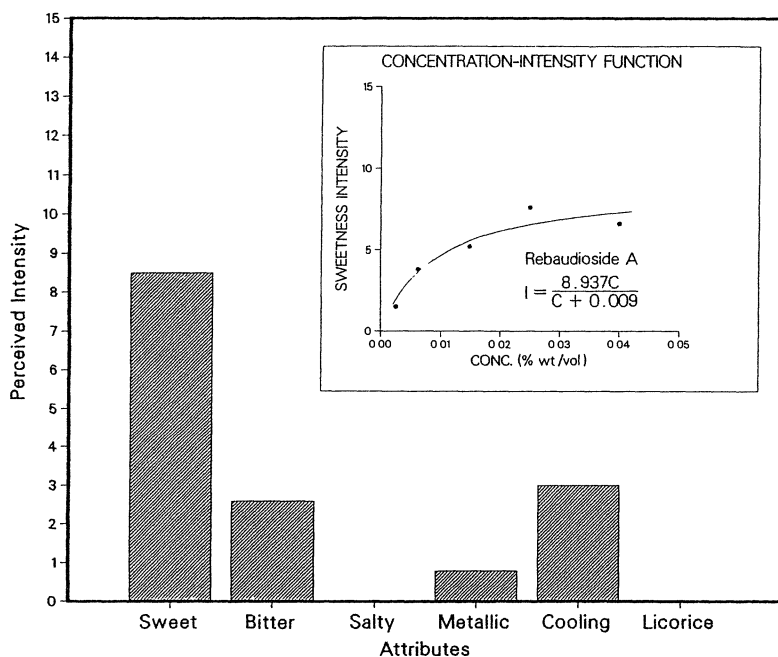


Figure 6.9 Flavor profile and relative concentration-intensity function for rebaudioside A.

component suggests that rebaudioside A may have minimal utility in food products except for those with significant bitter taste intensity.

Of the naturally occurring sweet steviol glycosides described above, temporal profile studies have been reported only for stevioside. In the case of stevioside, a sucroselike AT of 6 s (sucrose 4 s) was determined. The ET (22 s) was somewhat delayed, however, relative to sucrose (14 s). Although the other steviol glycosides have not been rigorously studied, it appears that these sweeteners generally exhibit reasonably rapid sweetness appearance times but may be disadvantaged by somewhat protracted extinction times.

Studies which have been conducted to evaluate the safety of the sweet components of *Stevia rebaudiana* have been reviewed (Phillips, 1987). As a result of the review of these studies and consideration of the long history of human exposure in Paraguay, *Stevia rebaudiana* sweeteners have been commercialized in Japan since 1973. In addition, they have been commercially developed in Brazil, Paraguay, South Korea and China. Nonetheless some concerns have been expressed due to the results of some *in vitro* studies on steviol. Specifically, Vignais and co-workers demonstrated steviol to be an inhibitor of oxidative phosphorylation (Vignais *et al.*, 1966). Also, Kinghorn and co-workers found steviol to be mutagenic following activation with liver microsomes (Pezzuto *et al.*, 1985). However, no evidence exists that steviol is produced *in vivo* in man and, as such, data on the safety of steviol may have little relevance.

The solubility of stevioside in water has been determined to be 9300 ppm (Mikulec *et al.*, 1990). Employing the potency $P_w(10)=190$, it can

Table 6.3. Potencies and QDA profiles of steviol and natural steviol glycosides

Compound	$P_w(R)$	Taste quality (S/B/O)
16 (Steviol-K salt)	25(10) ^a	0/100/0 ^a
18 (Stevioside)	190(10) ^a 143(3.5);149(7.5);89(9.0) ^e	62/30/8 ^a
19 (Steviolbioside-Na salt)	100(10) ^a	65/35/0 ^a
20 (Rebaudioside A)	170(10) ^a 242(6);149(7.5);85(8.5) ^e	85/12/3 ^a
21 (Rebaudioside B-Na salt)	150(10) ^c	88/4/8 ^c
22 (Rebaudioside C/dulcoside B)	36(2.0) ^d	
23 (Rebaudioside D)	221(5.5);163(8.0);89(9.0) ^e	
24 (Rebaudioside E)	174(4.5);125(6.0);85(8.5) ^e	
25 Dulcoside A	54(0.7) ^d	
26 Rubusoside	150(10) ^b	69/26/5 ^b
27 Steviolmonoside-Na salt	160(10) ^b	33/61/6 ^b

^aDuBois *et al.*, 1981d. ^bDuBois *et al.*, 1984. ^cDuBois *et al.*, 1985a. ^dYoshikawa *et al.*, 1979.

^eKasai *et al.*, 1981.

be calculated that stevioside is some 18 times as soluble as need be to provide a 10% sucrose level of sweetness intensity. The other more highly glycosylated steviol glycosides are more water soluble than stevioside.

The hydrolytic stability of rebaudioside A has been evaluated by Chang and Cook (1983). Under acidic conditions as would occur in phosphoric or citric acid based carbonated soft drinks, they found no degradation after 3 months at ambient temperature. Stability at pH 7 is also very good. Thus *Stevia rebaudiana* sweeteners are viable as commercial products in so far as they exhibit adequate hydrolytic stability.

In Japan, there are many companies involved in the commercial production of *Stevia rebaudiana* sweeteners. In 1985, sales reached 300 metric tons. Many of these companies have banded together to form the Stevia Association which promotes the use of these sweeteners. As of 1985, this association had eleven members. Most of the producers of *Stevia rebaudiana* sweeteners are members of the association. Examples of commercial *Stevia rebaudiana* derived products are Sato Stevia* (Sato Science Laboratories), Stevion* (Morita Kagaku Kogyo Co. Ltd.) and Murumilon 50* (Maruzen Kasei Co. Ltd.). Marumilon 50 is a mixture of 50% stevioside and a 50% mixture of the rebaudiosides. Of particular interest with respect to Marumilon 50 is the report that its sweetness potency is amplified by a factor of two or three by use in a 5% salt solution (Maruzen Kasei Co. Ltd., 1980). Another Maruzen Kasei Co. Ltd. product is a blend of Marumilon 50 and glycyrrhizin called Marumilon A*. This sweetener is said to provide a closer reproduction of sucrose taste than Marumilon 50. In the case of Marumilon A, sweetness potencies are reported to be increased by a factor ranging from four to six upon evaluation in 5% salt solution (Maruzen Kasei Co. Ltd., 1980).

Substantial efforts have been expended since the late 1970s on evaluation of the structure-activity-relationships of steviol glycoside sweeteners through the synthesis of novel compounds. One group at Shizuoka Women's University in Japan has investigated the effects of replacement of the β -D-glucosyl ester moiety of stevioside with alternative mono- and di-saccharide groups (Kamiya *et al.*, 1979; Esaki *et al.*, 1984). Specifically evaluated have been the 2-O- α -L-rhamnopyranosyl- β -D-glucopyranosyl (28), the 2-O- α -L-rhamnopyranosyl- β -D-galactopyranosyl (29), the 2-O- α -L-quinovopyranosyl- β -D-glucopyranosyl (30), the β -D-xylopyranosyl (31), the α -L-arabinopyranosyl (32), the α -D-mannopyranosyl (33), the β -L-glucopyranosyl (34), the α -L-rhamnopyranosyl (35) and the β -L-quinovopyranosyl (36) ester analogues of stevioside. These compounds were reported to be sweet exhibiting sweetness potencies $P_w(0.6)$ of 300, 300,

*Sato Stevia, Stevion, Murumilon 50 and Marumilon A are registered trademarks.

substituents attached to steviolbioside esters has little, if any, effect on taste quality or potency (cf. **18** vs. **31**, **32**, **33** and **34**) so long as substituent hydrophilicity remains comparable. Decrease in substituent hydrophilicity results in the introduction of a significant level of bitter taste quality (cf. **35** and **36**), and (b) the structure of disaccharide substituents attached to steviolbioside esters has little effect (cf. **18** vs **28**, **29** and **30**), either positive or negative, on taste quality or potency.

Conclusions which may be drawn from Shizuoka Women's University work are as follows.

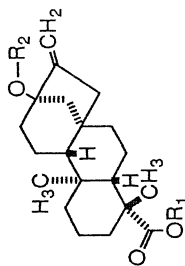
- (a) The olefinic functionality of steviolglycoside sweeteners is essential for significant activity and is likely involved in a critical interaction with the taste receptor.
- (b) The structure of the ester substituent of steviol glycoside sweeteners is of little significance for activity and is not involved in specific receptor interactions.

Over the last ten years, substantial efforts have been expended on the study of hexose conjugates of the natural steviol glycoside sweeteners. Particularly active in this effort have been Professor Osamu Tanaka and his co-workers at Hiroshima University in Japan. In early work (Kasai *et al.*, 1981), it was reported that transglucosylation of steviolbioside and stevioside from starch could be efficiently carried out using the enzyme cyclodextrin glucosyltransferase (cGT) isolated from *Bacillus megaterium*. Thus **19** yielded the mono-, di-, and tri-glucosyl derivatives **42–44**, the first two of which were both estimated to exhibit $P_w(5)=99$. Similarly, **18** yielded a mixture of 30% mono- (**45** and **46**), 32% di- (**47–49**) and 20% tri- (**50–53**) glucosylation products. Of these products, only **45** was evaluated as a pure compound. It was reported to exhibit $P_w(7.1)=142$. The **45/46**, **47/48/49** and **50/51/52/53** mixtures were evaluated in sensory panel studies with $P_w(6.4)=128$, $P_w(6.9)=138$, $P_w(6.6)=131$, respectively, being reported. In later work (Fukunaga *et al.*, 1989), products **45–53** were separated and reported to exhibit $P_w(3.3)=133$, $P_w(3.6)=180$, $P_w(3.4)=136$, $P_w(4.1)=205$, $P_w(3.0)=121$, $P_w(3.8)=150$, $P_w(3.6)=146$ and $P_w(2.9)=117$, respectively. Of these compounds, both the mono- (**46**) and the di- (**49**) glucosylation products were reported to be substantially improved in taste quality over both stevioside (**18**) and rebaudioside A (**20**). At the same time, triglucosylation products **50** and **53** were judged to be comparable in taste quality to **20** while all of the remaining compounds exhibited significantly more intense bitter, or other objectionable, taste qualities. It was concluded in this work that optimum taste properties are obtained by conjugation of one or two glucose units to the 4-hydroxyl of

the terminal glucose of the steviol glycosyl ether moiety. Due to the concomitant glucosylation of the glycosyl ester moiety, however, such compounds cannot be efficiently prepared. For this reason, the cGT-mediated glucosylation of **54**, the galactosyl ester analogue of stevioside, was evaluated (Mizutani *et al.*, 1989) since it was expected that cGT would glucosylate only the sophorosyl ether moiety. Stevioside analogue **54** was reported to exhibit $P_w(3.2)=129$ and a taste quality significantly inferior to stevioside. This reaction was successful yielding a mixture of mono- (**55**), di- (**56**), tri- (**57**) and tetra- (**58**) glucosylation products which were reported to exhibit $P_w(5.9)=236$, $P_w(7.2)=289$, $P_w(3.6)=143$ and $P_w(3.8)=152$, respectively. The taste qualities of **55** and **56** were both indicated to be substantially improved over stevioside while those of **57** and **58** were somewhat inferior to stevioside.

The cGT-mediated glucosylation of rubusoside (**26**) has also been described. Thus a complex mixture of mono- (**59** and **60**), di- (**61–63**), tri-, tetra-, penta- and hexa-glucosyl products, which individually were obtained in 9.0, 11.0, 13.5, 4.5, 9.0 and 4.4% yield, respectively, was obtained. The individual mono- and di-glucosyl products exhibited $P_w(3.6)=142$, $P_w(2.8)=113$, $P_w(7.4)=298$, $P_w(2.6)=104$ and $P_w(2.2)=88$, respectively, while the tri- to hexa-glucosyl products, evaluated as mixtures, exhibited $P_w(4.0)=158$, $P_w(1.7)=68$, $P_w(2.7)=107$ and $P_w(1.3)=53$, respectively. Of these products, only monoglucosyl conjugate **59** and diglucosyl conjugate **61** exhibited a taste quality equivalent to or better than that of rebaudioside A. It was concluded from this effort that only products of glucosylation of the steviol glycosyl ether moiety would yield products with improved sweetness potency and taste quality. For this reason, as was described above with the galactose ester analogs of stevioside analog **54**, Tanaka and co-workers evaluated the glucosylation of the galactose ester analogue **64** of rubusoside (Mizutani *et al.*, 1989). This substance exhibited $P_w(2.6)=104$ and was inferior in taste quality to rubusoside and very substantially inferior to stevioside. As expected, glucosylation proceeded smoothly to provide a mixture of the mono- (**65**), di- (**66**), tri- (**67**) and tetra- (**68**) glucosylation products which were reported to exhibit $P_w(4.2)=167$, $P_w(7.8)=312$, $P_w(5.1)=203$ and $P_w(2.8)=111$, respectively. Of these derivatives, only diglucosyl conjugate **66** exhibited an improved taste quality over rebaudioside A. Thus, again, the recurring conclusion is demonstrated that an intermediate level of glucosylation of the steviol glycosyl ether substituent results in improvement of both taste quality and sweetness potency.

In the above discussion, it is noted that the products of mono- and di-glucosylation of the steviolglycoside ether moieties of both rubusoside and stevioside are of increased sweetness potency and improved taste quality. Problematical with respect to commercial viability is that such



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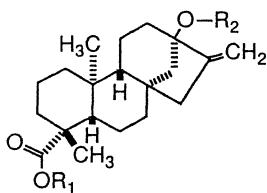
compounds would be of high production cost due to the lack of selectivity in the glucosylation. In order to address this problem, Tanaka and co-workers have investigated the potential for selective protection of the stevioside glucosyl ester moiety by galactosylation. While both α -galactosidases (Kitahata *et al.*, 1989a) and β -galactosidases (Kitahata *et al.*, 1989b) have been investigated, only the β -galactosidase from *Bacillus circulans* has, to date, allowed the selective protection of the 4-hydroxyl of the glucosyl ester moiety of rubusoside to give the protected intermediate **69**. Although it has not yet been reported, it is expected that these results will lead to efficient three-step enzymatic conversions of the natural sweeteners rubusoside and stevioside to the much improved glucose conjugates **59/61** and **44/49**, respectively. Though cost is a concern, it is apparent that on the basis of all other properties, these two product mixtures are viable candidates for commercial production. Strictly speaking, however, these sweeteners cannot be referred to as natural sweeteners. This may be a modest detractant from a marketing perspective.

While the substantial efforts recounted above have illustrated the effects on taste properties of modification with alternate glycoside functionality in steviol glycosides, significant efforts have also been reported on the substitution of the carbohydrate functionality by other polar groups. Efforts aimed at this objective were conducted in the laboratory at Dynapol Company in California. Preceding this program, it had been demonstrated at Dynapol Company (Wingard *et al.*, 1980) that stevioside was rapidly converted to steviol on incubation with rat cecal contents and that steviol was efficiently absorbed in rats. Due to concerns that a similar absorption and metabolism would be observed in man and since, as was discussed above, steviol has been found to inhibit oxidative phosphorylation and to be mutagenic in bacteria, efforts were mounted to identify sweet steviol glycoside analogues which could not be metabolized to steviol (DuBois *et al.*, 1981d; DuBois and Stephenson, 1985a). This objective was realized in the form of steviolbioside and rebaudioside B esters **73–81** which were determined to be sweet while exhibiting $P_w(10)/(S/B/O)$ properties of 160/(92/4/4), 140/(80/19/1), 120/(79/17/4), 160/(84/2/14), 200/(60/35/5), 170/(79/18/3), 120/(87/8/5), 120/(96/0/4) and 170/(96/1/3), respectively. Clearly, as one conclusion of these results, the glycosyl ester moieties of steviol glycoside sweeteners are not at all important for interaction with the sweet taste receptor. In fact, given the structural diversity of the glycosyl ester replacements, it is clear that practically any polar ester substituent will satisfy the structural requirements for sweet taste activity. In comparison of the QDA S/B/O taste quality ratios for stevioside, steviolbioside, rebaudioside A, rebaudioside B and **73–81**, a high correspondence between molecular hydrophobicity, as quantitated by reverse

phase chromatographic k' values and degree of bitterness was noted. It was concluded that the optimum level of hydrophobicity was attained in the steviolbioside sulfopropyl ester **73** since minimal non-sweet taste quality was observed and the further decrease of hydrophobicity exemplified in **80**, although not adversely affecting taste quality, did result in diminished potency.

As a part of the above investigation, it was learned that the sulfopropyl ester of steviol exhibits a purely bitter taste. Interestingly, it was noted that aqueous solutions of sulfopropyl steviolbioside (**73**) became increasingly bitter if prepared and stored under non-sterile conditions (DuBois, 1981b). This effect was completely inhibited in the presence of sodium benzoate. Thus, it is likely that products containing steviol glycoside sweeteners will require storage under sterile or bacteriostatic conditions.

Clearly from the results discussed above, the ester carbohydrate moiety present in steviol glycoside sweeteners is not a requisite for sweet taste. Studies have also been conducted to determine the level of importance of the ether carbohydrate functionality. Thus the sulfopropyl ether **82** and the carbohydrate-modified analogues **83** and **84** were prepared (DuBois *et al.*, 1984). These compounds exhibited $P_w(10)/(S/B/O)$ properties of 110/(18/82/0), 16/(8/67/25) and 50/(23/27/50), respectively. Compounds **83** and **84** are of particular interest since their high molecular weights (**83**, $M=846$; **84**, $M=1130$) and high anionic charges were expected to render them non-absorbable through the gastrointestinal tract wall. Problematical, however, were the very poor taste qualities of these steviol derivatives where, in each case, sweet taste was only a minor component of the

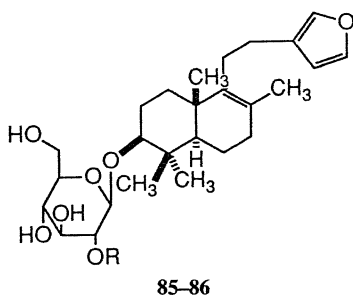


73-84

73: $R_1=(CH_2)_3SO_3Na; R_2=\beta\text{-D-Soph}$ 74: $R_1=CH_2COOH; R_2=\beta\text{-D-Soph}$ 75: $R_1=(CH_2)_4SO_3Na; R_2=\beta\text{-D-Soph}$ 76: $R_1=CH(COOH)CH_2CH_2COOH; R_2=\beta\text{-D-Soph}$ 77: $R_1=(CH_2)_2CH(NH_2)COOH; R_2=\beta\text{-D-Soph}$ 78: $R_1=CH(COOH)CH_2CH_2COOH; R_2=\beta\text{-D-Soph}$ 79: $R_1=(CH_2)_3PO(OH)ONa; R_2=\beta\text{-D-Soph}$ 80: $R_1=(CH_2)_3CH(SO_3Na)_2; R_2=\beta\text{-D-Soph}$ 81: $R_1=(CH_2)_3SO_3Na; R_2=\beta\text{-D-Soph}$ 2 $\beta\text{-D-Glu}$ 82: $R_1=R_2=(CH_2)_3SO_3Na$ 83: $R_1=(CH_2)_3SO_3Na; R_2=\beta\text{-D-Glu}$ 2 $(CH_2)_3SO_3Na$ 2 $(CH_2)_3SO_3Na$ 84: $R_1=(CH_2)_3SO_3Na; R_2=\beta\text{-D-Glu}$ 2 $\beta\text{-D-Glu}$ 3 $(CH_2)_3SO_3Na$

taste profile. No firm conclusion regarding the importance of the ether carbohydrate functionality of steviol glycosides is possible from this work. The great diversity of structure in the many sweet variants of carbohydrate ether functionality discussed above and the fact that **82** exhibits some sweet taste suggests, however, that the carbohydrate ether functionality of steviol glycoside sweeteners is not involved in any specific receptor interactions. Further studies will be required to unambiguously determine the significance of the ether carbohydrate functionality.

The identification of a novel subclass of the diterpenoid glycosides was reported by Tanaka *et al.* (1983). As part of a general program aimed at the identification of sweet principles in Chinese plants, they isolated and determined the structure of a labdane type glycoside from the plant *Phlomis betonicoides* Diels (Labiatae). This sweet diterpenoid was named baiyunoside after the Chinese name 'Bai-Yun-Shen' for the drug isolated from this plant. Structural studies indicated baiyunoside to be **85**. This substance is reported to be 500 times more potent than sucrose (no reference concentration given) but to be disadvantaged by having a very much prolonged sweet aftertaste which lasts for more than 1h. More recently (Tanaka *et al.*, 1985), a second sweet labdane glycoside called phlomisoside I (**86**) has been isolated from this same source. Phlomisoside I is reported to be similar in taste to baiyunoside with the persistence of the sweet taste. Although rigorous quantitative data are not available, it appears from the minimal sensory commentary given that neither baiyunoside nor phlomisoside I is a commercially viable sweetener.



85: R=β-D-Xyl

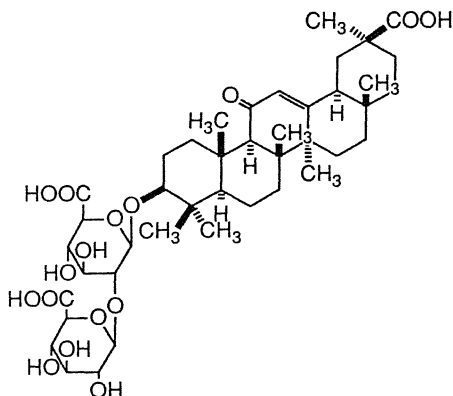
86: R=α-L-Rham

6.4.4 Triterpenoids

All of the known triterpenoid sweeteners are glycosides and the most well known is glycyrrhizin. Glycyrrhizin, a mixture of calcium, magnesium and potassium salts of glycyrrhizic acid occurs at a level of 6–14% in the roots of the European and Central Asian shrub *Glycyrrhiza glabra* L.

(Fabaceae). The crude extract of the plant is well known as licorice. Glycyrrhizin is obtained from licorice extract by sulfuric acid precipitation. Ammoniated glycyrrhizin (AG) is obtained by dissolution of glycyrrhizin in aqueous ammonia, concentration and recrystallization from alcohol. AG, which contains more than one molar equivalent of ammonia, may be converted to the monoammonium salt known as monoammonium glycyrrhizinate (MAG) by careful recrystallization. Glycyrrhizic acid is a triterpenoid glycoside of the oleanane type of structure **87**. The original structural work leading to this structural assignment was completed in 1950 (Lythgoe and Trippett, 1950).

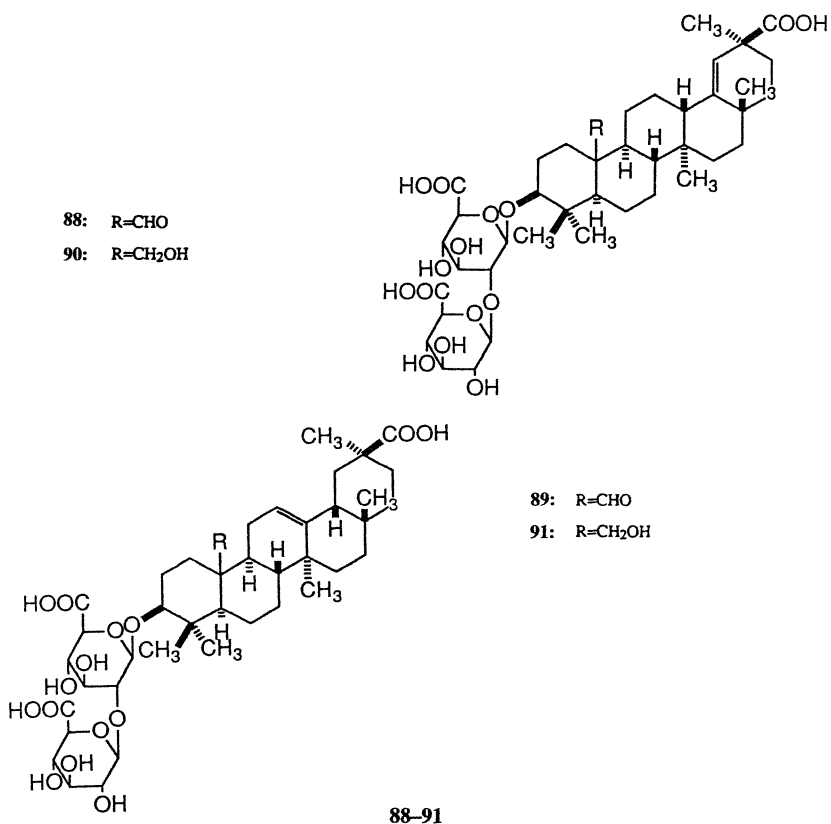
MAG has been reported to have $P_w(10)=33$ times that of sucrose and to exhibit significant bitter, licorice and cooling flavor attributes (DuBois, 1982a). The temporal profile of MAG is not at all sucrose-like. A very slow AT of 16 s (sucrose AT=4 s) and a very prolonged ET of 69 s (sucrose ET=14 s) have been reported (DuBois and Lee., 1983). As a consequence of these strong deviations from sucrose-like sweetness, MAG and the related licorice-derived products have only very limited utility in food applications. These limited applications have recently been reviewed (O'Brien-Nabors and Inglett, 1982).

**87**

In 1985, the glycyrrhizic acid derived sweeteners were approved in the United States as GRAS (Federal Register, 1985). However, the approval is specific with regard to their use 'as flavoring agents, flavor enhancers, and surfactants.' Specifically excluded from this approval is 'as a component of sugar substitutes.' As a consequence of this limited approval, the applications of glycyrrhizin type sweeteners are extremely limited. Limiting utility also is the lack of sucroselike taste quality.

Although *Periandra dulcis* Mart. (Leguminosae), also known as Brazilian

licorice, was originally reported (Machado, 1941) to contain glycyrrhizin, more recent work has demonstrated (Hashimoto *et al.*, 1980; Hashimoto *et al.*, 1982a, b, 1983) that the sweet principles of this plant are also, like glycyrrhizin, oleanane-type triterpenoid glycosides. From the roots of this plant were isolated the four triterpenoid glycosides which were named periandrins I (88), II (89), III (90) and IV (91) in yields of 2.3×10^{-3} , 5.7×10^{-4} , 7.7×10^{-5} and $3.0 \times 10^{-5}\%$, respectively. These four compounds were reported to exhibit $P_w(0.9)=90$, $P_w(0.95)=95$, $P_w(0.92)=92$ and $P_w(0.85)=85$. By the same methodology, glycyrrhizin was reported to exhibit $P_w(0.93)=93$. It is claimed (Hashimoto *et al.*, 1982)

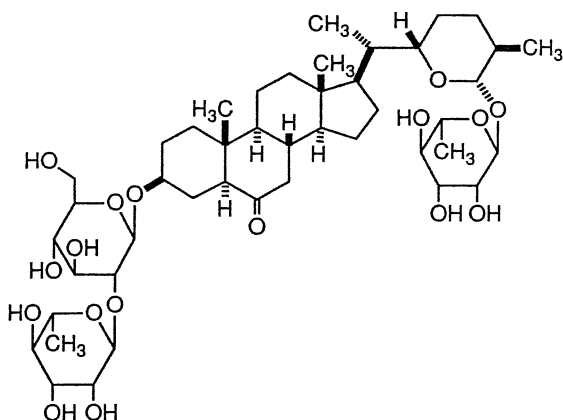


that the periandrins have a more rapid onset of sweetness than glycyrrhizin. Thus, the periandrins may have utility as preferred ingredients in food applications where glycyrrhizin is used. Problematical for the commercial exploitation of the periandrins is that they are quite difficult to separate from the bitter components of *Periandra dulcis* root. In addition, their

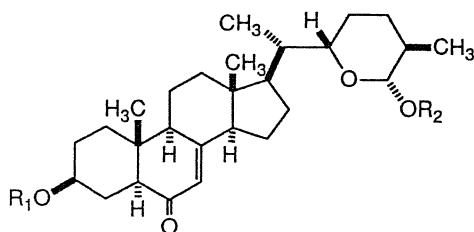
very low natural abundance is suggestive of high production cost rendering commercial exploitation unlikely.

Following the naturally abundant glycyrrhizin, the next sweet triterpenoid glycoside to be identified in nature was osladin, the sweet component of the rhizomes of the fern *Polypodium vulgare* L. (Polypodiaceae). Osladin and the other sweet compounds described in this section are based on a steroid-type triterpenoid nucleus. Although in very early work (Guignet, 1885), it was reported that glycyrrhizin was the sweet component of this plant, it was subsequently demonstrated (Jizba and Herout, 1967, Jizba *et al.*, 1971) that the sweetness was due to **92**, which was given the name osladin, after 'osladic', the Czech name for polypody. Although the structure of osladin is given as **92**, the configuration of the monosaccharide L-rhamnosidic bond has never been established. Osladin was first isolated in a low yield of 0.03% from the roots of *Polypodium vulgare*. More recently (Reisch and Dawidar, 1978), it has also been isolated from other parts of this plant. In this later work, osladin is said to be 300 times (no reference concentration given) more potent than sucrose. Sweet compounds which are structurally related to osladin have recently been identified by Kinghorn and co-workers in the dried rhizomes of the related plant *Polypodium glycyrrhiza* DC. Eaton (Kim *et al.*, 1988; Kim and Kinghorn, 1989). This plant, which is also known as licorice fern, has a long history of human use in the Pacific northwest region of the United States. The first sweet compound isolated from *Polypodium glycyrrhiza* was obtained in about 10 times the yield that osladin occurs in *Polypodium vulgare* and was named polypodoside A. Studies on polypodoside A indicated it to have structure **93** and to exhibit $P_w(6)=600$. The taste of **93** was reported to be disadvantaged, however, by the presence of 'licorice-like off-taste and some lingering aftertaste'. The somewhat less potently sweet polypodoside B (**94**) and the tasteless polypodoside C (**95**) were also isolated from *Polypodium glycyrrhiza*. It was also determined that the monoglycoside analogue **96**, which occurs naturally in *Polypodium vulgare*, is not sweet. Thus, it was concluded that the structures of the glycoside moieties of **92-96** are important for sweet taste with any alteration profoundly affecting activity. Although specific data have not been reported, it has been stated that polypodoside A, and by inference osladin, are sparingly water soluble. In summary, consideration of the known properties of osladin and the sweet polypodosides relative to the criteria necessary for sweetener commercial viability, leads to the conclusion that these interesting steroidal glycosides are not at all commercially viable as sweeteners.

A number of triterpenoid glycosides have been described in which the aglycone is of the cucurbitane class of triterpenoids. The first of these compounds to receive significant attention in the literature was that derived from the fruits of the vine known to the Chinese as 'Lo Han Kuo'. This plant is now known by the systematic name *Thladiantha grosvenorii* (Swingle)



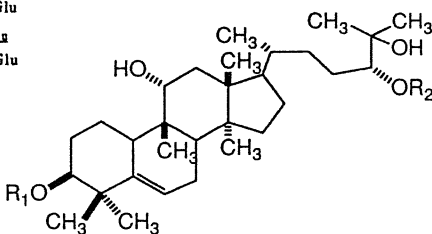
92

93: R₁=β-D-Glu 2-1 α-L-Rham; R₂=α-L-Rham94: R₁=β-D-Glu; R₂=α-L-Rham95: R₁=β-D-Glu; R₂=α-L-Rham 1- CH₃96: R₁=β-D-Glu 2-1 α-L-Rham; R₂=H

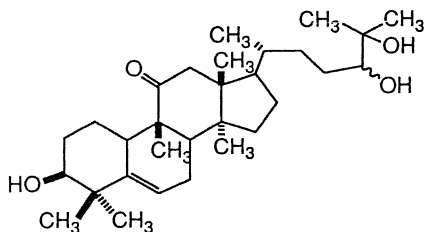
93-96

C. Jeffrey (Cucurbitaceae) while it was formerly named *Mamordica grosvenorii* (Swingle) and its fruits have a long history of use in Chinese herbal medicine. In early work (Lee, 1975), a sweet glycoside was isolated from this source and said to be some 150 times (no reference given) as potent as sucrose. Later the isolation and structure elucidation of the two triterpenoid glycosides **97** and **98** were described (Takemoto *et al.*, 1983a,b,c). These sweet glycosides were named mogrosides IV and V, respectively. The most abundant component, occurring at a level of 1% in the dried fruits, is mogroside V and was estimated (Kingham and Soejarto, 1986) to exhibit $P_w(5.1)=256$. No information is available on the flavor profile, temporal profile, water solubility or hydrolytic stability of the mogrosides. Lee indicated, however, that the sweetness of the *Thladiantha grosvenorii* sweetener is accompanied by a lingering licorice-like aftertaste. The long history of use in China is suggestive, however, of a reasonable level of taste acceptability and also of safety for human consumption.

- 97: $R_1 = \beta\text{-D-Glu}$ $\alpha\text{-D-Glu}$; $R_2 = \beta\text{-D-Glu}$ $\alpha\text{-D-Glu}$
 98: $R_1 = \beta\text{-D-Glu}$ $\alpha\text{-D-Glu}$; $R_2 = \beta\text{-D-Glu}$ $\alpha\text{-D-Glu}$
 100: $R_1 = \beta\text{-D-Glu}$; $R_2 = \beta\text{-D-Glu}$ $\alpha\text{-D-Glu}$
 101: $R_1 = \beta\text{-D-Glu}$; $R_2 = \beta\text{-D-Glu}$ $\alpha\text{-D-Glu}$



97, 98, 100, 101



99

A second sweet triterpenoid glycoside from the cucurbitane class has been isolated from the roots of the plant *Bryonia dioica* Jacq. (cucurbitaceae) and partially characterized as a trisaccharide derivative bryodulcoside of the aglycone bryodulcosigenin (**99**) (Tunmann *et al.*, 1959, 1966a,b). Clearly, bryodulcoside and the mogrosides are related in structure. No further information on the properties of bryodulcoside is available. From the rhizomes of another Chinese medicinal plant, *Hemsleya carnosiflora* C.Y. Wu and Z.L. Chen (Cucurbitaceae), have been isolated (Kasai *et al.*, 1987), by Professor Tanaka and co-workers at Hiroshima University, the two new sweet cucurbitane glycosides V (**100**) and VI (**101**). These glycosides were isolated in 0.02 and 0.04% yields, respectively. No information is available at this time with respect to the sweetness potency, flavor profile, temporal profile or other properties of these sweet cucurbitane triterpenoid glycosides.

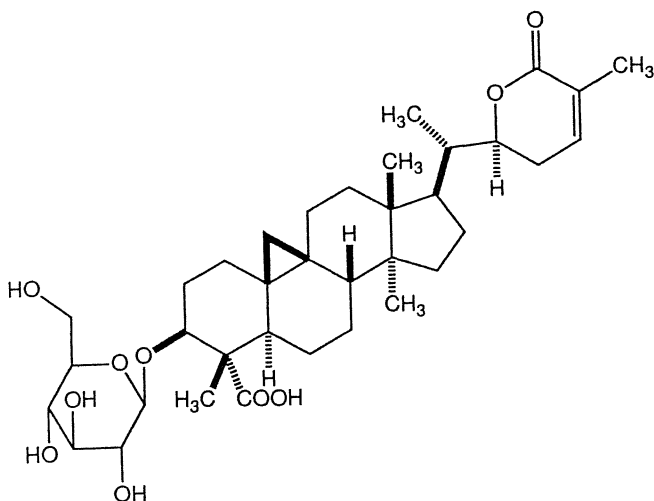
In 1989, the first triterpenoid glycoside sweetener in which the aglycone is based on the cycloartenol carbon skeleton was described by Professor Kinghorn and co-workers at the University of Illinois at Chicago (Choi *et al.*, 1989). These workers isolated the sweet component, which they named abrusoside A, from the leaves of *Abrus precatorius* L. (Leguminosae) in 0.033% yield. By a combination of spectroscopic and X-ray crystallographic studies, abrusoside A was determined to have structure **102**. Further information on this sweetener has not yet been reported.

6.5 Polyketide sweeteners

In recent years, a number of sweet natural products have been identified in nature which are biosynthetically derived from acetate and other carboxylic acids. Through the intermediacy of acetyl- and other acyl-coenzyme A derivatives, poly- β -ketomethylene chains are assembled and cyclized in enzyme-facilitated processes to provide many natural products or intermediates which through secondary metabolic processes are further processed to the polyketide-derived compounds found in nature. Classes of sweeteners which are ontogenetically derived in this way are the closely related dihydroisocoumarins, flavanones and dihydrochalcones.

The first of the polyketide-type sweeteners to be identified in nature was phyllodulcin, a 3,4-dihydroisocoumarin. This sweet natural product occurs most prevalently in the leaves of the 'Amacha' plant [*Hydrangea macrophylla* Seringe var. *thunbergii* (Siebold) Makino (Saxifragaceae)]. In Japanese, 'Amacha' means 'sweet tea'. At 'Hamatsuri', a Japanese festival celebrating the birth of Buddha, the fermented leaves of this plant are used to prepare a sweet tea. Phyllodulcin was first isolated from this plant in 1916 (Asahina and Ueno, 1916), its structure determined in 1930 (Asahina and Asano, 1931) and the absolute configuration of the one chiral center established in 1959 as *R* (Arakawa and Nakazaki, 1959). Thus natural phyllodulcin is D-(+)-phyllodulcin **103**. A biosynthetic-type total synthesis of racemic phyllodulcin has been reported (Takeuchi *et al.*, 1980) and, in addition, production in cultured plant cells has been described (Suzuki *et al.*, 1977). Phyllodulcin has also been identified in other species of *Hydrangea* (Tachibana *et al.*, 1974). Flavor and temporal profile or QDA data on **102** have not been reported. However, phyllodulcin has been indicated to exhibit $P_w(3)=400$ and to be disadvantaged by a slow onset of sweetness and by a lingering sweet aftertaste (Yamato *et al.*, 1977). Phyllodulcin is additionally disadvantaged by low water solubility thus rendering difficult the preparation of intensely sweet solutions. In summary, although phyllodulcin is commercially available and is consumed in Japan, as a consequence of its non-sucrose-like temporal profile and poor solubility characteristics, it has little viability as a general utility sweetener.

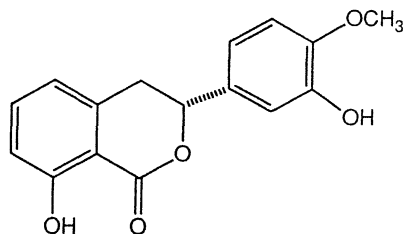
Although 3,4-dihydroisocoumarins which are structurally related to phyllodulcin have been found in nature, phyllodulcin is the only sweet natural 3,4-dihydroisocoumarin. Yamato and his co-workers at Okayama University were intrigued by this rather specific structure-activity-relationship and, as a consequence, in the 1970s began an intensive study of synthetic analogues in an attempt to identify the essential pharmacophore of **103**. Over 100 compounds were synthesized as part of this program, the results of which have been reviewed (Yamato and Hashigaki, 1979). Early on in this effort, it was learned that dihydrostilbene **104** [$P_w(3)=300$] was nearly as active as phyllodulcin and thus clearly contained the essential



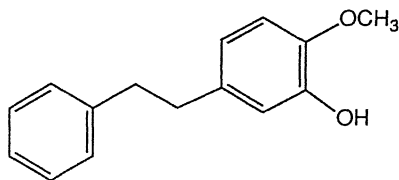
102

pharmacophore. Subsequent efforts focused on the preparation of variants in which one of the three groups Ph, CH_2CH_2 , and 3-OH-4-OCH₃-Ph were monadically altered. Essentially none of the analogues prepared exhibited significant water solubility and thus commercial viability was not attained in any case. Taste testing of these substances was accomplished with the aid of ethanol as a co-solvent.

A sub-class of polyketide sweeteners which bears a striking similarity to the dihydroisocoumarins is the dihydrochalcone class. The first substance of this type to be isolated was obtained from the Australian plant *Smilax glycyphylla* Sm. (Lilliaceae) and is called glycyphyllin **105** (Rennie, 1886). The sweet taste of **105** is said to be accompanied by a strong bitterness (Horowitz *et al.*, 1974). Tanaka and co-workers have demonstrated the presence of sweet dihydrochalcones in certain species of *Symplocos*. In specific, phlorizin (**106**) was isolated from the leaves of *Symplocos*



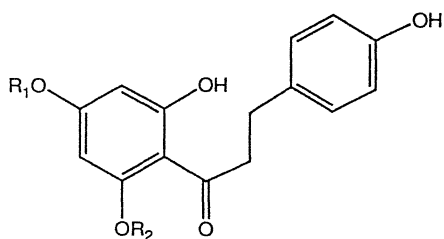
103



104

lancitolia Sieb. and Zucc. in 0.6% yield and from the leaves of *Symplocos spicata* Roxb. in 0.7% yield (Tanaka *et al.*, 1982). In addition, trilobatin (107) was isolated in 0.6% yield from the leaves of *Symplocos microcalyx* Hyata (Tanaka *et al.*, 1980). Phlorizin and trilobatin were also isolated from the leaves of *Lithocarpus litseifolius* (Hance) Chun (Fagaceae) in 1.5 and 1.2% yield, respectively. *Lithocarpus litseifolius* leaves are used to make a sweet tea in Yunnan, China. Sweetness potency and flavor profile or QDA data on these natural dihydrochalcone sweeteners are not available.

The more interesting dihydrochalcone sweeteners, from a perspective of commercial viability, were discovered accidentally in 1963 by Robert Horowitz and Bruno Gentili at the USDA laboratory in Pasadena, California (Horowitz and Gentili, 1963). Efforts in the USDA laboratory have been reviewed (Horowitz, 1986). In the early 1960s, Horowitz and Gentili were studying the bitter taste structure-activity relationships of



105-107

105: $R_1=H; R_2=\alpha\text{-L-Rham}$

106: $R_1=H; R_2=\beta\text{-D-Glu}$

107: $R_1=\beta\text{-D-Glu}; R_2=H$

the citrus flavonoid glycosides. Based on a model they were developing, they hypothesized that naringin dihydrochalcone (**108**), the product of hydrogenolysis of naringin (**109**) the bitter component of grapefruit (*Citrus paradisi*) rinds, would be of increased bitterness. They were surprised to find, however, that it was not at all bitter, but rather potently sweet. Subsequently (Horowitz, 1964; Horowitz and Gentili, 1969), it was determined that optimal properties in this sub-class of sweeteners were reached in the form of neohesperidin dihydrochalcone (**110**). This sweetener may be obtained by hydrogenolysis of the flavanone neohesperidin (**111**) which occurs naturally in the bitter (Seville) orange (*Citrus aurantium*). However, since Seville oranges are not grown in abundance, **110** may be more economically obtained from naringin and isovanillin in a three-step process.

The flavor profile and C/I function for **110** are given in Figure 6.10 (Pecore *et al.*, 1989). From the C/I function, it can be calculated that $P_w(2)=2410$, $P_w(5)=1430$, and $P_w(8)=410$. The flavor profile of **110** suggests that it may, as a consequence of many non-sweet taste components, have minimal utility in food systems. The major drawback of **110**, however, is its atypical temporal profile (DuBois and Lee, 1983). It exhibits AT=9 s (sucrose=4 s) and an ET=40 s (sucrose=14 s). In

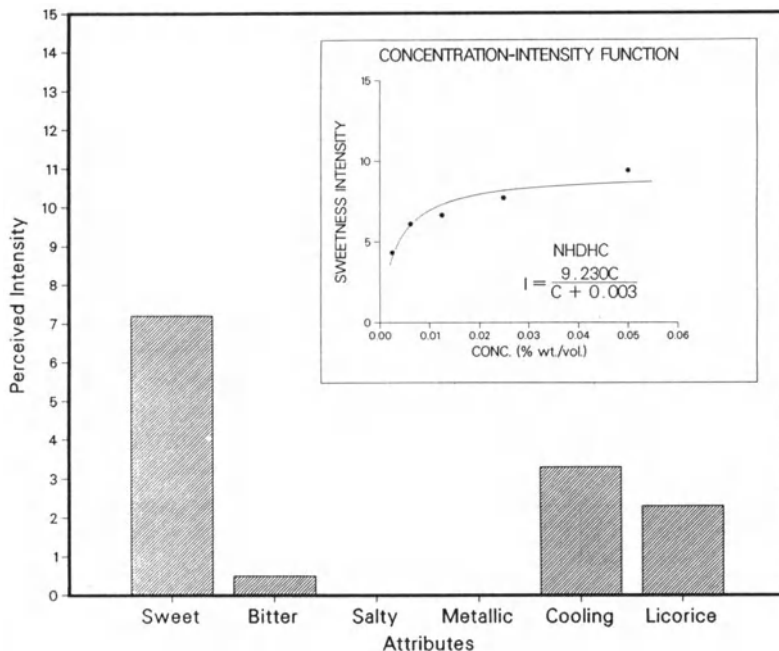
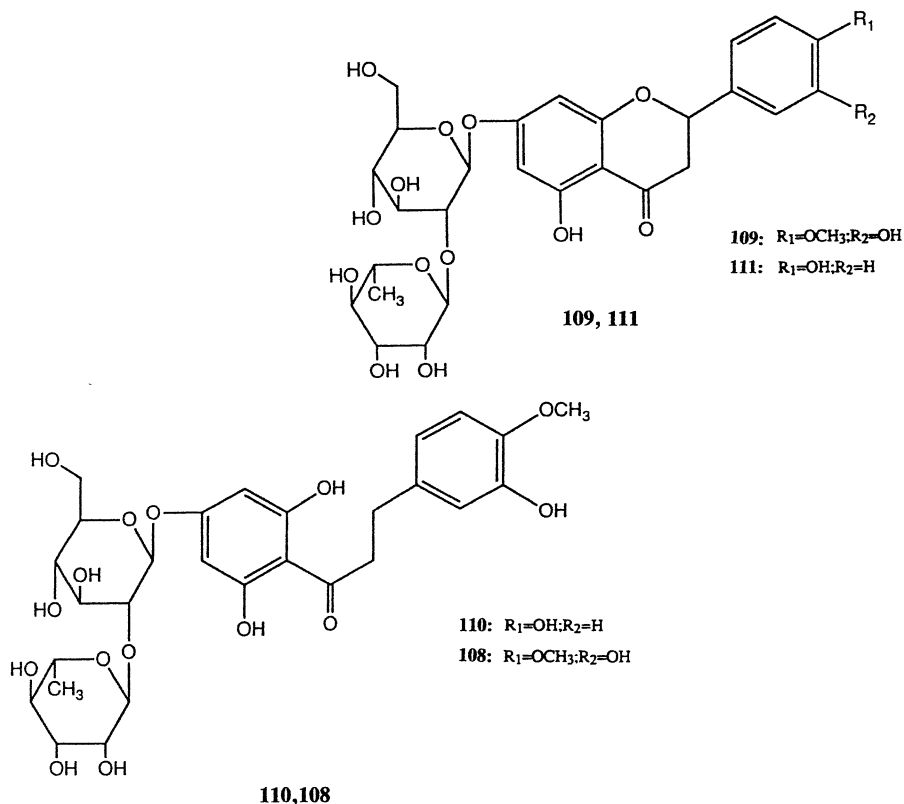


Figure 6.10 Flavor profile and relative concentration-intensity function for neohesperidin dihydrochalcone (NHDHC).

food systems, this temporal profile combines with that of other flavor components to result in a most unnatural taste.

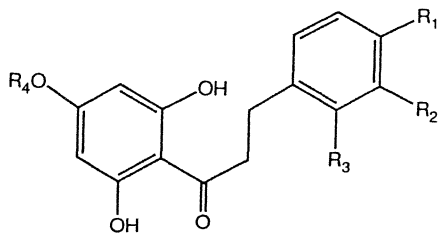
The solubility of **110** is reported to be 500 ppm at ambient temperature (Horowitz, 1986). Since $P_w(8)=410$, it can be calculated that **109** is 2.6 times as soluble as necessary to provide an 8% sucrose level of sweetness intensity. Neohesperidin dihydrochalcone is quite stable to the conditions which would be encountered in food systems with no sweetness of solutions being lost over a period of years (Horowitz, 1986). This qualitative statement is supported by quantitative studies which have been reported (Inglett *et al.*, 1969).

Neohesperidin has been approved for use as a sweetener in Belgium and a few other countries. The limited regulatory approval is likely due mainly to the minimal interest in the use of this sweetener in foods. It is strongly disadvantaged over other sweeteners by its marginal taste quality, in particular its atypical temporal profile, and also its limited water solubility and low rate of dissolution. Thus, neohesperidin dihydrochalcone is not likely to be used anywhere unless as a component in combination with more sucrose-like sweeteners.



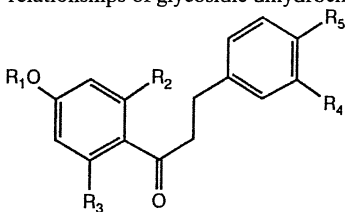
In early studies on the structure-activity-relationships in dihydrochalcone sweeteners, it was established that the neohesperidosyl carbohydrate portion of **110**, which in this work was estimated to exhibit $P_w(2.8)=4600$, was important for activity (Horowitz and Gentili, 1969). Substitution of the β -rutinosyl moiety to give **112** resulted in loss of all sweetness. In addition, it was determined that the rhamnosyl moiety of **110** was not required for activity since the β -D-glucosyl analogue **113** was found to be active [$P_w(2.8)=300$]. Although a comparison of the activities of **110** and **112** suggests that the 6-OH of the glucosyl moiety of **110** may be essential for activity, it was found that the 6-OCH₃ derivative **114** was equivalently potent as **110**. Thus, since **110**, **113** and **114** are all active, it was hypothesized that the 3- and 4-hydroxyl substituents of the glucose moiety of **110** are required for sweetness. Consistent with this hypothesis, it was found that xylosyl analogue **115** was also active [$P_w(2.8)=620$]. The effects of aromatic ring substitution were also evaluated (Krbecek *et al.*, 1968). Thus naringin dihydrochalcone (**108**), and analogue **116** were found to exhibit $P_w(5)$ values of 100 while **117** was noted to be not sweet. These workers reported neohesperidin dihydrochalcone to exhibit $P_w(5)=1000$ and interestingly that analogues **118** and **119** exhibited $P_w(5)$ values of 1000 and 2000 respectively.

Intrigued by the early correlation of sweet taste activity and dihydrochalcone carbohydrate moiety structure, Esaki and co-workers at Shizuoka Women's University in Japan began an intensive study of this matter. The results of their efforts are summarized in Table 6.4. Observations made,



112-119

- 112:** R₁=OCH₃;R₂=OH;R₃=H;R₄= β -D-Glu α -L-Rham
113: R₁=OCH₃;R₂=OH;R₃=H;R₄= β -D-Glu
114: R₁=OCH₃;R₂=OH;R₃=H;R₄= β -D-Glu CH₃
115: R₁=OCH₃;R₂=OH;R₃=H;R₄= β -D-Xyl
116: R₁=R₃=H;R₂=OH;R₄= β -D-Glu α -L-Rham
117: R₁=R₂=H;R₃=OH;R₄= β -D-Glu α -L-Rham
118: R₁=OCH₂CH₃;R₂=OH;R₃=H;R₄= β -D-Glu α -L-Rham
119: R₁=OCH₂CH₂CH₃;R₂=OH;R₃=H;R₄= β -D-Glu α -L-Rham

Table 6.4. Structure-activity relationships of glycosidic dihydrochalcones.


Com pound	R ₁	R ₂	R ₃	R ₄	R ₅	Sweetness potency (R) ^a
110	β-Neohesperidosyl	OH	OH	OH	OCH ₃	5200 (1.3)
108	β-Neohesperidosyl	OH	OH	H	OH	270 (1.3)
113	β-D-Glucosyl	OH	OH	OH	OCH ₃	340 (1.3)
120	β-Sophorosyl	OH	OH	H	OH	0
121	2-O-β-D-glucosyl-β-D-galactosyl	OH	OH	H	OH	0
122	β-Kojibiosyl	OH	OH	OH	OCH ₃	Slightly sweet
123	β-Maltosyl	OH	OH	OH	OCH ₃	54 (1.3)
124	β-Cellobiosyl	OH	OH	OH	OCH ₃	130 (1.3)
125	β-Lactosyl	OH	OH	OH	OCH ₃	103 (1.3)
126	β-L-Quinovopyranosyl	OH	OH	OH	OCH ₃	720 (1.3)
127	2-O-α-L-Quinovopyranosyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	870 (1.3)
128	2-O-β-L-Quinovopyranosyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	85 (1.3)
129	2-O-α-L-Quinovopyranosyl-β-D-galactopyranosyl	OH	OH	OH	OCH ₃	870 (1.3)
130	2-O-β-L-Quinovopyranosyl-β-D-galactopyranosyl	OH	OH	OH	OCH ₃	85 (1.3)
131	2-O-α-L-Rhamnopyranosyl-β-D-galactopyranosyl	OH	OH	OH	OCH ₃	2500 (1.3)
132	2-O-α-L-Mannopyranosyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	49 (1.3)
133	2-O-α-D-Mannopyranosyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	0
134	2-O-β-D-Arabinopyranosyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	0
135	β-L-Glucopyranosyl	OH	OH	OH	OCH ₃	270 (1.3)
136	2-O-Methyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	650 (1.3)
137	3-O-Methyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	260 (1.3)
138	6-O-Methyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	1300 (1.3)
139	β-D-Glucopyranosuronyl	OH	OH	OH	OCH ₃	0
140	β-Maltotriosyl	OH	OH	OH	OCH ₃	280 (1.3)
141	2-O-β-L-Glucopyranosyl-β-D-glucopyranosyl	OH	OH	OH	OCH ₃	5 (1.3)
142	2-O-α-L-Rhamnopyranosyl-β-D-galactopyranosyl	H	OH	OH	OCH ₃	1200 (1.3)
143	2-O-α-L-Rhamnopyranosyl-β-D-galactopyranosyl	H	H	OH	OCH ₃	1.1 (1.3)
144	2-O-α-L-Rhamnopyranosyl-β-D-galactopyranosyl	H	OH	H	OH	0
145	2-O-α-D-Fucopyranosyl-β-D-galactopyranosyl	OH	OH	H	OH	0
146	2-O-β-D-Fucopyranosyl-β-D-galactopyranosyl	OH	OH	H	OH	0
147	2-O-α-L-Fucopyranosyl-β-D-galactopyranosyl	OH	OH	H	OH	400 (0.8)
148	2-O-α-L-Fucopyranosyl-β-D-galactopyranosyl	OH	OH	OH	OCH ₃	1300 (0.8)
149	2-O-β-L-Fucopyranosyl-β-D-galactopyranosyl	OH	OH	H	OH	280 (0.8)
150	2-O-β-D-Galactopyranosyl	OH	OH	H	OH	320 (0.8)

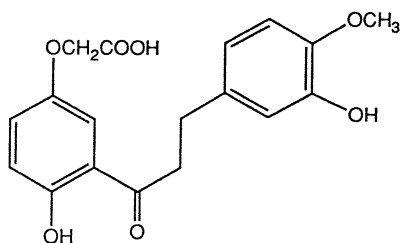
^aSweetness potencies in the original literature (Esaki *et al.*, 1975; Kamiya *et al.*, 1976; Kamiya *et al.*, 1978; Esaki *et al.*, 1983) were reported relative to various saccharin references; these values have been recalculated with the aid of the equation $I = (11.416C)/(C + 0.013)$, given in Figure 6.1, in order to place them relative to sucrose references.

and conclusions which may be drawn from them, upon review of these data are as follows:

- (a) Since substitutions of the 2- (e.g. **110** and **136**), 3- (e.g. **137**), 4- (e.g. **123–125** and **140**) and 6- (e.g. **138**) hydroxyls of the glucopyranose moiety of **113** have no significant effects on sweetness, it is concluded that none of these substituents is involved in essential receptor binding interactions. This conclusion is supported by the equivalency of the β -D- and β -L-glucosyl dihydrochalcones **113** and **135**.
- (b) Since only 2-*O*-L-substitution of the glucosyl moiety of **113** results in potent sweet taste, it is concluded that only L-sugars which exist in a ¹C conformation can potentiate the sweetness of **113**. The α -L-quinovopyranosyl and α -L-fucopyranosyl substituents are particularly effective α -L-rhamnopyranosyl equivalents.
- (c) Since 2-*O*-L-substitution of the glucosyl moiety of **113** with α -L-mannopyranosyl is ineffective, it is suggested that the rhamnose CH₃ substituent in **110** may be involved in an important receptor binding interaction.

In the work discussed above by Horowitz and Gentili and also by Esaki and co-workers, it was implicitly assumed that the functionality important for receptor binding interactions was embodied in the carbohydrate moieties. In 1973, however, chemists from the Hungarian Academy of Sciences reported (Farkas *et al.*, 1973) that dihydrochalcone **151** is 'intensely sweet'. This was the first suggestion that the complex carbohydrate functionality of **110** and of all of its analogues in Table 6.3 was not involved in essential receptor binding interactions. This lead was then intensively investigated by a co-author of this review (GED) at Dynapol Company. It was specifically an objective of the Dynapol program, to identify analogues of **110** which would exhibit sucrose-like temporal characteristics while still retaining a high quality flavor profile. Results from this effort which further developed dihydrochalcone structure-activity-relationships are summarized in Table 6.5. In this work, four hypotheses were investigated in attempt to understand the rationale for the common atypical temporal profile of dihydrochalcone sweeteners. First, it was suggested that dihydrochalcones may require some metabolic activation before they may activate the sensory system. Second, it was proposed that the dihydrochalcone active conformation may be a minimally populated conformation and that time may be required for build-up of a sufficient concentration of the active conformer. Third, it was proposed that dihydrochalcones are tenaciously bound to the receptor by a chelate-type binding; formation of the chelate may be slow after which dissociation of the receptor-sweetener complex could be very slow. Fourth, it was suggested that dihydrochalcones diffuse slowly to the receptor due to non-specific interactions with non-receptor tissue

and then bind tightly to the receptor due to hydrophobic interactions which are only slowly dissipated. In the final analysis, none of these hypotheses was supported. Nonetheless, a dihydrochalcone with a very significantly improved temporal profile, relative to **110**, was identified. The improved compound which was identified is the homoserine conjugate **205**. This sweetener was found to exhibit AT=8 s (sucrose, 4 s; **110**, 9 s) and ET=29 s (sucrose, 14 s; **110**, 40 s). In addition, as can be seen from Table 6.5, the taste quality of **205** is quite acceptable. Interestingly, it was demonstrated that the two enantiomers of **205** were indistinguishable in sweet taste activity (DuBois and Stephenson, 1982b). Problematical, however, is the fact that **205** is only sparingly soluble in water.



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Observations which may be made from the results in Table 6.5 and conclusions which may be drawn from them are as follows:

- (1) The A-ring 2,6-dihydroxyketone moiety, though not essential for weak to moderate potency sweet taste, is required in entirety for high sweetness potency.
- (2) The unit $-\text{CO}(\text{CH}_2)_n-$ bridging the gap between the two aromatic rings results in optimum sweetness potency with $n=2$.
- (3) The B-ring methoxy-hydroxy functionality is essential for clean sweet taste.
- (4) The A-ring C-4 hydroxy moiety may be substituted with an apparently limitless number of hydrophilic side-chains without loss of sweet taste so long as the net molecular hydrophobe-hydrophile balance remains within a narrow range.

Interest in the dihydrochalcone sweeteners was piqued at Dynapol by the relatively high molecular weight of these sweeteners and the likelihood that they would not be substantially degraded metabolically. It was hoped that sweeteners of this type may have an enhanced probability of safety

since minimal absorption across the gastrointestinal wall may result. This objective was in fact realized in the form of dihydrochalcone dimer **209** (Wingard *et al.*, 1978). This substance of $M=984$ (H-form) was prepared in ^{14}C -labeled form and demonstrated in rats to be less than 1.2% absorbed. Dihydrochalcone **209**, though effectively a non-absorbable sweetener, is not a viable product candidate, however. The sweet taste of this substance was exceptionally slow to develop

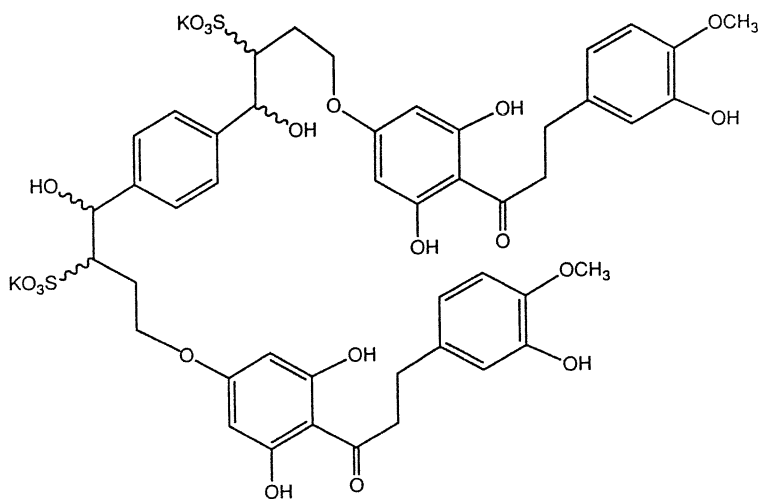
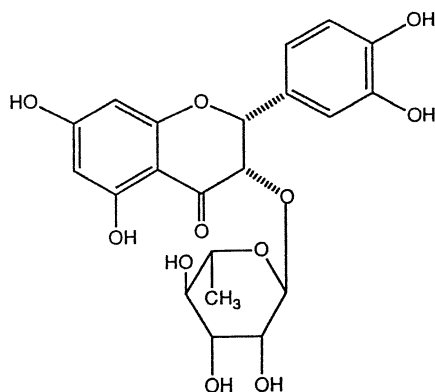
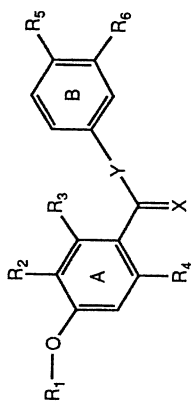
**209****210**

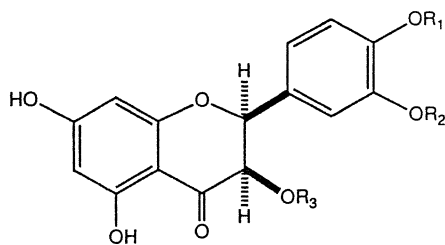
Table 6.5. Structure-activity relationships of nonglycosidic dihydrochalcones

Com pound	R ₁	R ₂	R ₃	R ₄	X	Y	R ₅	R ₆	P _w (10)	S/B/O
110	b-Neohesperidosyl	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	660	88/5/7
152	CH ₂ COONa	H	H	OH	O	(CH ₂) ₂	OCH ₃	OH	63	42/32/26
153	H	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	660	84/11/5
154	CH ₂ COONa	H	H	H	O	(CH ₂) ₂	OCH ₃	OH	0	-
155	CH ₂ COOH	H	H	OCH ₂ COOH	O	(CH ₂) ₂	OCH ₃	OH	0	-
156	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	500	82/8/10
157	CH(CH ₂)COONa	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	120	22/57/21
158	CH(COONa) ₂	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
159	(CH ₂) ₃ COOK	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	310	74/11/15
160	CH(COOH)CH ₂ CH ₂ COOH	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
161	CH ₂ SO ₃ Na	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	430	84/10/6
162	(CH ₂) ₂ SO ₃ K	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	700	83/7/10
163	(CH ₂) ₃ SO ₃ Na	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	500	80/9/11
164	(CH ₂) ₄ SO ₃ K	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	230	50/19/31
165	(CH ₂) ₂ CH(COOH)SO ₃ K	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	150	72/13/15
166	(CH ₂) ₂ CH((CH ₂) ₃ SO ₃ K)SO ₃ K	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
167	(CH ₂) ₃ SO ₃ K	H	H	OH	O	(CH ₂) ₂	OCH ₃	OH	180	81/15/4
168	(CH ₂) ₃ SO ₃ K	H	OCH ₃	OH	O	(CH ₂) ₂	OCH ₃	OH	240	57/25/18
169	(CH ₂) ₃ SO ₃ K	H	OCH ₂ Ph	OH	O	(CH ₂) ₂	OCH ₃	OH	240	28/38/34
170	(CH ₂) ₃ SO ₃ K	H	O(CH ₂) ₃ SO ₃ K	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
171	H	H	O(CH ₂) ₃ SO ₃ K	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
172	H	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
173	H	SO ₃ Na	OH	OH	O	(CH ₂) ₂	OCH ₃	OSO ₃ Na	0	-
174	H	CH ₂ SO ₃ K	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	130	7/70/23
									0	-



175	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	OH	OH	52	3/33/64
176	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	H	OH	76	38/49/13
177	H	H	OH	OH	O	CH ₂	OCH ₃	OH	170	11/70/19
178	CH ₂ COONa	H	H	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
179	CH ₂ COONa	H	H	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
180	(CH ₂) ₃ SO ₃ K	H	H	OH	O	(CH ₂) ₂	OCH ₃	OH	38	23/59/28
181	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	H	H	0	-
182	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	-OCH ₂ O-	OH	0	-
183	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	O-n-Pr	OH	240	39/38/23
184	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	OCH ₃	NH ₂	200	5/79/16
185	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	Cl	OH	27	11/70/19
186	CH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	NHCH ₃	OH	0	-
187	CH ₂ COONa	H	OH	OH	OH	(CH ₂) ₂	OCH ₃	OH	0	-
188	CH ₂ COONa	H	OH	OH	OH	(CH ₂) ₂	OCH ₃	OH	0	-
189	H	H	OH	OH	OH	(CH ₂) ₂	OCH ₃	OH	89	7/88/5
190	CH ₂ COONa	H	OH	OH	H ₂	(CH ₂) ₂	OCH ₃	OH	0	-
191	CH ₂ COONa	H	OH	OH	H ₂	(CH ₂) ₂	OCH ₃	OH	0	-
192	(CH ₂) ₃ SO ₃ K	H	OH	OH	-O-	(CH ₂) ₂	OCH ₃	OH	32	66/22/12
193	H	H	OH	OH	-O-	(CH ₂) ₂	OCH ₃	OH	41	41/38/21
194	CH ₂ COONa	H	OH	OH	OH	NH	OCH ₃	OH	0	-
195	CH ₂ COONa(CH ₂) ₂ COONa	H	OH	OH	O	NH	OCH ₃	OH	0	-
196	CH(COONa)CH(OH)CH ₂ OH	H	OH	OH	O	(CH ₂) ₂	O-N-Pr	OH	0	-
197	CH(COONa)CH(OH)CH ₂ OH	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
198	CH(COONa)(CH ₂) ₃ OH	H	OH	OH	O	(CH ₂) ₂	O-n-Pr	OH	20	20/80/0
199	CH ₂ COCH ₂ COONa	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	34	69/31/0
200	(CH ₂) ₂ NH ₂	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	310	85/3/12
201	(CH ₂) ₃ NH ₂	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	86	75/25/0
202	(CH ₂) ₃ PO(OH)OK	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	180	50/50/0
203	(CH ₂) ₃ NHSO ₃ K	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	280	80/7/13
204	(CH ₂) ₂ CH(OH)COONa	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	350	89/6/5
205	(CH ₂) ₂ CH(NH ₂)COOH·HCl	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	440	92/8/0
206	CH ₂ CH(NH ₂)CH ₂ COOH	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	400	85/5/10
207	CH ₂ (COOH)(CH ₂) ₂ NH ₂ ·HCl	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	210	82/14/4
208	4-Propyl·HCl	H	OH	OH	O	(CH ₂) ₂	OCH ₃	OH	0	-
									70	60/13/27

^aDuBois *et al.*, 1977a,b; 1981a,b.



211–214

- 211: $R_1=R_2=H; R_3=COCH_3$
 212: $R_1=CH_3; R_2=H; R_3=COCH_3$
 213: $R_1=CH_3; R_2=H; R_3=H$
 214: $R_1=R_2=R_3=H$

and then was observed to linger for up to 1 h. In fact, the sweet taste of **209** is so slow to develop that at first it was thought not to be sweet.

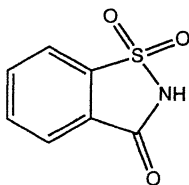
The last of the polyketide type sweeteners found in nature are of the flavanone subclass. In 1988, it was reported by Tanaka and his co-workers (Kasai *et al.*, 1988) that the leaves of the sub-tropical tree *Engelhardtia chrysolepis* Hance, which grows in the wild of Guangdong, Guangxi and Fujian, China, contain the sweet dihydroflavonol rhamnoside **210**. Further information on the sweetness potency, flavor profile and temporal profile has not yet been reported.

Subsequent to this report, Kinghorn and co-workers reported that the sweet constituent of *Tessaria dodoneifolia* (Hook and Arn.) Cabrera (Compositae), which is indigenous to Paraguay, is the dihydroflavonol acetate **211** (Nanayakkara *et al.*, 1988). They observed, however, that **211** underwent slow spontaneous autoxidation at neutral pH thus rendering it of limited utility for commercial applications. In recognition of this limitation, synthetic dihydroflavonol derivatives **212–214** were prepared and evaluated. It is reported that **211–214** exhibit $P_w(2)$ values of 80, 400, 40 and 0 times that of sucrose, respectively. Flavor and temporal profile information on these sweet compounds have not been reported.

6.6 Requirements for commercial viability of non-nutritive sweeteners

The discovery of the potent sweet taste of saccharin **215** (Fahlberg and Remsen, 1879) in 1878 made possible, for the first time, the formulation of no- or low-calorie equivalents to formerly calorie-laden food products. Since the discovery of saccharin, chemists have synthesized, or identified from natural sources, thousands of novel potentially sweet

organic compounds. Many of these represent substantial improvements over saccharin which is limited in acceptance due to the presence of pronounced bitter and metallic flavor attributes.



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Aspartame, saccharin and other substances which are substantially more potent than sucrose are generally termed 'high potency' sweeteners. Such sweeteners are frequently, in the current literature, errantly referred to

Table 6.6. Comparative viability of natural, natural-derived and natural sub-unit sweeteners presently used in food.

Sweetener name/ mol. wt.	Sweetness potency [$P_w(X)$]	Taste quality (S/B/O)	Temporal profile AT	Solubility [X MRS(X)] ^a ET	Stability	Regulatory status/U.S. (ADI)
Aspartame/ 294	280 (2) 150 (8) 110 (10)	100/0/0	5s 19s NA	18 (8)	Fair	50 mg/kg body weight
Thaumatococin/ 5600 (5) 2600 (8)	8600 (2) 5600 (5) 2600 (8)					
Monellin/ Perillartine/165	770 (8.6)					
Stevioside/804	190 (10)	60/25/15	NA	0.74 (8.6)	Good	GRAS flavor for chewing gum only
Rebaudioside A/966	770 (2) 440 (5) 100 (8)	85/12/3	NA	18 (10)	Good	No approval
Glycyrrhizic acid ammonium salt/839	33 (10)	84/3/13	16s 69s	NA	Good	No approval
Phyllodulcin/ Neohesperidin dihydro- chalcone/612	400 (3) 2410 (2) 1430 (5) 410 (8)	NA	NA	Poor	Good	GRAS as a flavoring agent
		81/3/16	9s 40s	2.6 (8)	Good	No approval

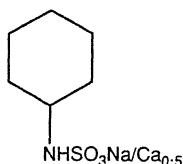
^aMRS(X) is the multiple of the required solubility to match the sweetness intensity of $X\%$ sucrose.

NA, not applicable

as 'intense' or 'high intensity' sweeteners. This terminology is confusing and therefore not recommended, since no sweet substances are known which exhibit a higher intensity of sweetness than may be exhibited by sucrose. Occasionally, sweeteners are referred to as 'artificial' sweeteners and 'natural' sweeteners. This terminology is also disfavored since sweet taste as an observation from any source is real and bears no naturalness or artificiality. It is recommended, however, that sweeteners be referred to as nutritive, non-nutritive or high potency as accurately describes the particular substance. Since interest in sweeteners is proportional to their viability in food products, a discussion of the properties requisite for commercial viability will be given. Some of the properties of representative sweeteners are given in Table 6.6.

6.6.1 Taste quality

Since the 1960s, we have seen strong growth of food products sweetened with non-nutritive sweeteners. The most successful of these products have been beverages, especially carbonated soft drinks (CSDs). As an example, non-nutritive sweeteners allowed the creation of non-caloric alternatives to the common sucrose-sweetened CSDs which carry approximately 150 cal per 12 oz serving. This commercial success was only made possible, however, by the identification of a sweetener composition which enabled the formulation of beverages which consumers judged to approach the sucrose-sweetened counterparts in acceptability. Consumers have never shown any willingness to sacrifice taste quality for other advantages (e.g. reduced caloric content). A key discovery which significantly facilitated the burst of growth in the low-calorie food product sector in the 1960s was made at Abbott Laboratories (Helgren, 1957). It was found that the combination of sodium saccharin with sodium or calcium cyclamate (216) in a ratio such that each sweetener contributed equal sweetness to the mixture resulted in a product which exhibited improved taste quality over either sweetener taken separately. This combination was one of approximately ten parts of the cyclamate salt to one part of the saccharin salt since the latter is roughly ten times more potent as a sweetener than the former. Cyclamate and especially saccharin salts exhibit 'off' flavors which are often described as bitter and metallic. As a result of these 'off' tastes, neither saccharin- nor cyclamate-sweetened products approached the sugar-sweetened counterparts in acceptability. It was found, however, that these 'off' flavors were substantially ameliorated in the 10:1 cyclamate saccharin mixture. In fact, the attenuation was so significant that for the first time, it became possible to formulate non-caloric or low-calorie alternatives to many food and beverage products without a major hedonic compromise.



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The taste quality of a sweetener is really only meaningful in the context of the food product in which it will ultimately be used. The taste quality of a food product is best measured by consumer studies of product acceptability. A widely used technique in food product development is flavor profile analysis (Meilgaard *et al.*, 1987). By this technique, which was pioneered at the Arthur D. Little Company in the 1940s, expert sensory panels are trained to break down complex multiple flavor attribute systems and to rate the intensities of the components. This technique has also been employed to describe the flavor attributes exhibited by sweeteners. Examples of data obtained by this technique are given in this article for the sweeteners discussed.

A number of sweeteners have been identified that exhibit flavor profiles not substantially different from that of sucrose. A finding of a sucrose-like flavor profile might lead one to conclude that the sweetener would allow formulation of food products with acceptability equivalent to the sucrose-sweetened products. In many cases, however, this is not found to be true. One identified factor which may be the cause for such a disparity is difference in temporal profiles (DuBois and Lee, 1983). This effect has been comprehensively studied in the flavonoid glycoside structural class of sweeteners (DuBois and Lee, 1983). The most well known member of this class is neohesperidin dihydrochalcone (**110**). In this case, 9 s are required for the maximum of sweetness intensity to be realized for a solution isosweet with 10% sucrose. This time has been defined as the sweet taste appearance time or AT. For comparison, 10% sucrose exhibits an AT of 4 s. In addition to a delayed AT, neohesperidin dihydrochalcone also exhibits a prolonged sweet aftertaste. This effect has been quantitated as the time required for the perceived sweetness intensity to decline from a 10% sucrose to the low, but greater than threshold, 2% sucrose equivalent level of sweetness. This time has been termed the extinction time or ET. Neohesperidin dihydrochalcone exhibits an ET of 40 s in comparison to 14 s for isosweet 10% sucrose. These temporal effects combine to cause neohesperidin dihydrochalcone to be substantially less acceptable as a sweetener than sucrose in most food systems. Thus, it is not sufficient to

know only a sweetener's flavor profile to predict viability. The temporal profile must also be known. Ultimately, of course, the taste quality of a sweetener may only be appreciated following food product consumer acceptability studies in the food category of interest.

6.6.2 Safety

A major impetus behind the interest in natural sweeteners is the perception of safety. First order logic would suggest that sweeteners with a long history of human exposure without the observation of toxicity are safe. Indeed it is this kind of rationale which is employed in the regulation of food additives in Japan. In Japan, unless safety studies have been conducted which demonstrate adverse effects of a natural sweetener, it may be used in food. In other countries, however, due largely to concerns following chronic exposure, natural sweeteners are regulated in the same way as any other food additives.

In the United States, the use of sweeteners is regulated by the 1958 Food Additives Amendment to the Food, Drug and Cosmetic Act of 1938. This legislation and its effects on the regulation of sweeteners and other food additives has been reviewed (Ronk, 1978; Schultz, 1981). By this Act, saccharin and cyclamates were exempted as Generally Recognized As Safe (GRAS) food ingredients. Not all sweeteners are included on the GRAS list, however, which in its original form, named 675 food ingredients. Surprisingly, even sucrose is not included. However, the omission of sucrose and many other obviously safe food ingredients prompted the following official FDA comment: 'It is impractical to list all substances that are generally recognized as safe for their intended use. However, by way of illustration, the Commissioner regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder and monosodium glutamates as safe for their intended use' (Code of Federal Regulations, 1988).

In order to achieve GRAS status, a sweetener, or any food ingredient, may be Generally Recognized As Safe, among 'experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (a) scientific procedures or (b) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food' (Code of Federal Regulations, 1989).

For the cases of substances not included on the GRAS list, two tracks toward approval for use in foods are defined. First, and, in principle, somewhat simpler, is the 'GRAS Affirmation' process. To qualify for this track, it must be either (a) demonstrated that the substance was in common use in food in the United States prior to 1958 or (b) be based on expert judgment of safety demonstrated by published safety studies. If the available safety data are not sufficient to support the requested uses or

increase in projected exposure levels, the FDA may affirm GRAS status but limit levels of use until further safety data are established.

The second track toward approval of a sweetener for use in foods is the 'Food Additive Petition' (FAP) process. The FAP process requires extensive safety studies in test animals. One objective of these studies is the determination of the highest dose which may be given without the appearance of adverse effects. This dose is termed the 'no observed effect level' or NOEL. The NOEL in the most sensitive animal species evaluated is then used by the FDA to regulate the level at which the food additive may be used in foods. This level is termed the 'acceptable daily intake' or ADI, and is defined as 1/100 of the NOEL in the most sensitive animal species. These NOEL and ADI exposure levels are given in milligrams per kilogram of body weight. Thus, as an example, if the NOEL of a proposed new sweetener was determined to be 500 mg/kg in the most sensitive animal species evaluated, then an ADI of 5 mg/kg would be allowed. The question then naturally arises as to the meaning of a 5 mg/kg ADI allowance of a new sweetener for use in foods. The ADI allowance level is then employed by the FDA to determine the food categories and levels in those categories in which the new sweetener may be used. The objective of this exercise is to ensure that the 5 mg/kg exposure level is not exceeded on a chronic basis. In order to do this, 90th percentile, 14-day average food category consumption data are employed. Approval of the new sweetener may then be granted for use in food categories where, in the aggregate, 90th percentile/14-day average consumption data on these categories does not exceed the ADI.

Internationally, although individual countries assume responsibility for the regulation of food additives within their boundaries, there has been an attempt at harmonization of food additive regulation. This process has been reviewed (Vettorazzi, 1989). In 1956, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) established the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The fundamental objective of JECFA is the establishment of ADIs for food additives following the assembly and interpretation of all relevant biological and toxicological data. Over its lifetime, approximately 700 intentional food additives have been evaluated by JECFA.

6.6.3 Solubility

Many sweeteners are known which are insufficiently water soluble to be of general utility. Commonly, sweetness intensity levels equivalent to at least

10% sucrose are required in food systems. In some systems (e.g. frozen desserts), soluble sweetener levels are required which allow a sweetness intensity match of 15–20% sucrose. In addition, for many food systems, manufacturers may require that the sweetener dissolve sufficiently rapidly so as to not interfere with current manufacturing processes. A particularly relevant illustration of requirements imposed by manufacturing processes is that for CSD products. In this case, it is necessary that a concentrate solution of the sweetener/flavour system complex be rapidly attainable. Thus high solubility and rapid dissolution rates are very desirable properties for non-nutritive sweeteners.

6.6.4 Stability

In order for a sweetener to be commercially viable, it must be stable to the intended conditions of use. Degradation may be from hydrolytic, pyrolytic or photochemical processes depending on the food application. Stability is required for two reasons. First, the sweetener must not degrade such that the level of sweetness of the food product would be substantially reduced during the product lifetime. As a corollary to this functionality mandate, the sweetener must not break down to produce any 'off' flavor substance. The second reason for the stability requirement principally relates to sweeteners which are defined as food additives. By the FAP process, degradation products which form in the requested applications require safety demonstration. Generally, for the cases of most types of organic compounds, if exposure to the degradation product would be expected to reach or exceed 0.0063 mg/kg body weight, then the requisite safety assessment studies would be equal to those mandated for the sweetener itself (US Food and Drug Administration, 1982). As such, stability is an extremely important property for a sweetener being considered for development as a new food additive.

6.6.5 Cost

Sweeteners are almost invariably considered as potential sucrose replacements. With sucrose in plentiful supply, presently at 36 cents/lb (Chemical Marketing Reporter, 1989), potential replacements must either be cost competitive or present sufficient advantages such that a cost premium is warranted. The effective cost to the food product manufacturer of an alternative sweetener is equivalent to the wholesale price divided by the alternate's potency in delivering the desired property. In most cases,

the desired property is sweet taste intensity. Thus the effective cost or cost per sucrose equivalent (CSE) is the quotient of wholesale price and sweetness potency (P). Sweetness potency is generally expressed as a multiple of the potency of sucrose where the latter is defined as unity. Although for some purposes, sweetness potencies are expressed on a molar basis, most commonly P is expressed on a weight basis (P_w). It is important to recognize, however, that P values for sweeteners are not constants (Moskowitz, 1983). P , for a sweetener, varies according to the concentration of sucrose which is used as reference. As an example, P_w for aspartame has been reported to be 400 times that of sucrose relative to a 0.34% sucrose reference [i.e. $P_w(0.34)=400$] but to be only 133 times as potent as sucrose relative to a 10% sucrose reference [i.e. $P_w(10)=133$] (Homler, 1984).

In addition to the reference concentration effect on P , substantial effects can also be found due to food systems and temperature. As an example of the food system effect, it has been reported that in water, aspartame is 133 times more potent than sucrose (10% reference) whereas in CSDs, it is 180 times sucrose in potency at the same reference concentration level (Homler, 1984). As an example of the temperature effect, it has been reported and is generally accepted that fructose is more potent than sucrose (Hyvonen and Koivistoinen, 1982). At elevated temperatures, however, sucrose is more potent than fructose. Thus a computation of sweetener CSE requires specification of the application so as to allow determination of the relevant value of P_w .

Though not a natural sweetener, for the purpose of illustrating the calculation of effective sweetener cost, consider sodium saccharin. This sweetener is currently available in the United States for a wholesale price of \$2.75/lb (Chemical Marketing Reporter, 1989) and exhibits a $P_w(8)$ in water of 260 times sucrose (Pecore *et al.*, 1989). The CSE of sodium saccharin, used in water at an 8% sucrose sweetness intensity level, would therefore be $\$2.75/260=1.0$ cents/lb. This contrasts quite sharply with sucrose which at year-end 1989 was available for a wholesale price of 36 cents/lb. Clearly such cost factors attainable with high potency sweeteners offer food manufacturers the potential of substantial increased profitability in the low calorie sector of their product categories. Although sweetener cost is an important consideration in assessing a sweetener's viability, it will not be further discussed in this review. Only a very few of the sweeteners discussed have been commercially developed and of those, none are commodities such as saccharin with stable prices. Nonetheless, cost must be seriously considered when entertaining the thought of commercially developing any sweetener.

6.7 Conclusion

Most of the 'natural' non-nutritive sweeteners discussed in this chapter were discovered by accident and are, therefore, rather limited in number. This situation will change dramatically once the sweet taste receptor or receptors are isolated since at that time systematic searching which does not involve human tasting will be possible. The key to the discovery of the receptor(s) is the receptor ligand(s). Several sweeteners with very high sweetness potency and which may be useful in receptor isolation have been described in this chapter. Thus, it is reasonable to expect that within a few years, the identity of the receptor gene(s) will be known. This will then allow the gene(s) to be cloned and expressed in systems which will allow the molecular mechanism of sweet taste to be fully elucidated. In the interim, the characterization of taste properties of various 'natural' non-nutritive sweeteners continues. A comparison of the properties of nine 'natural' sweeteners is given in Table 6.6.

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7 Sweetener markets, marketing and product development

M.G. LINDLEY

7.1 Introduction

Sweetener markets are dynamic and have changed dramatically over the last two decades. The development of new products and the increased level of awareness of health and nutrition have both contributed significantly to these changes, although in the latter case not always with sound underlying logic.

Consumer understanding of nutritional issues is not always helped by food marketing strategies which frequently incorporate messages concerning the benefits or virtues of a particular product from the standpoint of what can broadly be termed the health of the consumer. Thus, in recent years we have seen the development of very many food products whose formulations rely, at least in part, on some positive nutritional feature or another. For example, consumer foods have been developed and marketed on the basis of being low in sodium, low in gluten, high in fibre, high in soluble fibre, low in certain amino acids, high in calories, low in fat, low in saturated fat, low in calories and containing 'modified' carbohydrates. In other words, there have been a multitude of often conflicting messages consumers have had to comprehend in making food selection decisions. So much so that there has been concern expressed recently in the United States of the danger stemming from a failure to separate 'health' from 'hype' (Freundlich *et al.*, 1989).

Some of the more consistent marketing messages of recent years have centred on the energy content of the diet and the contribution that sugars make to total energy, as well as the role played by fermentable carbohydrate in the etiology of dental disease. These features have underpinned much new product development and research into new speciality ingredients capable of supplying what are seen as the desirable properties of sugars, but which do not contribute calories or contribute to dental caries. It has been deemed necessary to develop these speciality ingredients because throughout the last 30 years consumers have remained remarkably consistent in the amount and composition of sweet products they eat. The amount of specific individual sweeteners consumed has changed significantly, but the total per capita,

as illustrated in Table 7.1 (Anon., 1986) has altered little during this decade.

Table 7.1. Per capita sweetener consumption in the United States (kg/head).

Year	Consumption (kg/head)
1980	50.0
1981	49.0
1982	49.4
1983	51.8
1984	54.6
1985	56.6
1986	55.4

Source: Anon. (1986).

As has been noted, the desire to consume sweet foods appears strong. Therefore, almost irrespective of the 'nutritional' message, consumers will demand that the sweet products that they eat or drink meet their criteria of quality. To ensure that these criteria are met, it is vital that product developers understand fully the functionality of the sweetener(s) used in what can be termed the normal or standard product. Only then will they be able to successfully utilise whichever alternative sweetener they are being required to use and to develop products which are as similar as possible to the standard.

Advances in technology, notably in the production of high fructose corn syrups, ensure that economic factors have had an important impact on the formulation and marketing of sweet products. But even in these instances, manufacturers must strive to ensure that the quality of the finished food is not compromised. Almost irrespective of the economic factors, if foods and beverages do not meet consumer quality expectations, they will not succeed in the market.

The trends in sweetener consumption, and the reasons for them as they relate to consumer product marketing are reviewed in this chapter. The functionality of sweeteners, and their role in product development are also discussed.

7.2 Trends in sweetener markets

7.2.1 Introduction

Two new sweetener product developments have had a dramatic impact on the composition of world sweetener markets. Firstly, in 1970 the world

production of high fructose syrups from corn could be measured in tens of thousands of tonnes. World production in 1988 was more than 7 million tonnes on a dry basis (Anon., 1988a). Secondly, the regulatory approval of aspartame in the early 1980s has influenced not only the consumption of saccharin, but also has almost certainly slowed the rate of growth of the high fructose syrup market, particularly in North America.

These changes merely serve to illustrate that although the general perception of the sweetener industry might be that it is basically static, the reality is quite different. There have been recent dynamic and far reaching changes in its make-up that have had equally important consequences for food product manufacturers and hence for consumers.

7.2.2 Nutritive sweetener markets

The two major market volume nutritive sweeteners are sucrose and high fructose corn syrups (HFCS). Overall, there has been a steady increase in sucrose consumption during the decade of the 1980s and a much faster, though currently slowing, growth in the consumption of fructose syrups. These trends are illustrated in Table 7.2 (Anon., 1989a) where it can be seen that in 1980 sucrose accounted for 97.1% of the total, but this had fallen to 93.8% in 1988.

Table 7.2. World sucrose and high fructose syrup consumption (millions of tonnes, dry basis).

Year	Sucrose consumption		HFCS consumption	
	Amount (M tonnes)	% of total	Amount (M tonnes)	% of total
1980	88.6	97.1	2.6	2.9
1982	93.7	96.0	3.9	4.0
1984	96.5	94.8	5.3	5.2
1986	100.3	93.9	6.5	6.1
1988	107.3	93.8	7.1	6.2

Source: Anon. (1989a).

The United States has consistently been the largest volume producer of HFCS, currently producing approximately three quarters of the world's output. Japan is the second largest producer accounting for almost 10% of the total in 1988. The EEC, with its quota system controlling production, comes a distant third in the production league table with less than 4% of the total.

Simply analysing the total picture in this way can be misleading, however, for there are significant regional variations in consumption patterns. For example, world consumption of sucrose has increased by more than 20% during the 1980s, but consumption has fallen in the United States from 8.6 million tonnes in 1980 to 6.9 million tonnes in 1988 (Anon., 1989b), a drop of 20%. The reason for the drop is very clearly due to a switch from sucrose use in beverages to cheaper high fructose syrup (Table 7.3).

Table 7.3. Nutritive sweetener use in beverages (United States: millions of tonnes, dry basis).

Year	Sweetener consumption (M tonnes)		
	Sucrose	HFCS	Total
1980	1.9	1.0	2.9
1982	1.5	1.8	3.3
1984	0.85	2.4	3.25
1986	0.3	3.25	3.55

Source: Anon. (1989b).

It was the development in the United States during the late 1970s of techniques to further enrich enzymically produced 42% HFCS with fructose, so producing 55% HFCS, that led to the almost wholesale switch from sucrose in beverages. This switch received further impetus because of the existence of duties and quotas designed to protect indigenous sucrose producers. One consequence of the protective measures was that it gave a financial incentive to the HFCS manufacturers through the existence of artificially raised sucrose prices. As a result they were able to undercut sucrose prices, while generating substantial profits.

While HFCS has all but eliminated the use of sucrose in the U.S. beverage industry, small but steady market growth in the use of sucrose in other sectors of the food industry has occurred. Market penetration by HFCS has been much less in sectors such as baked products and confectionery due to the inherent functional difficulties of its use (see Section 7.4).

While dramatic shifts in the U.S. sucrose and HFCS markets have occurred, markets for the other principal nutritive sweeteners have changed little. Dextrose markets and those of glucose syrups have grown slowly throughout the 1980s at rates roughly equivalent to the growth in population (Anon., 1989b).

Crystalline fructose is a recent entrant into the market at prices which, in certain circumstances, make it competitive with sucrose. Markets remain small, estimated to be around 30,000 tonnes worldwide (Anon., 1989c), but it does have its niches, some of which may turn out to be substantial.

7.2.3 High intensity sweetener markets

In contrast to the markets for nutritive sweeteners where the imposition of quite complex (and sometimes apparently arbitrary) quota, support pricing and duty policies can greatly influence market development, those of intense sweeteners are governed by regulatory approval considerations and technical performance criteria only. The steady, but generally unspectacular growth in nutritive sweetener markets contrasts with the more rapid increase in intense sweetener markets since the mid-1970s (Table 7.4) (Anon., 1988b). Over this period, intense sweetener markets have almost doubled from 3.7 million tonnes sugar equivalent in 1974 to approximately 6.8 million tonnes sugar equivalent in 1987.

Table 7.4. World consumption of high intensity sweeteners (millions of tonnes, sugar equivalent)

Year	total consumption (M tonnes sugar equivalent)
1974	3.7
1976	4.15
1978	4.6
1980	5.2
1982	5.6
1984	6.6
1986	6.8

Source: Anon. (1988b).

Within this total there have been some interesting fluctuations, however. Saccharin exhibited a steady increase in consumption until 1982. The first regulatory approval of aspartame led to dramatic increases in saccharin consumption as soft drink manufacturers began by using a blend of the two sweeteners. Soon, however, the switch to all-aspartame sweetened soft drinks took place, leading to a fall in saccharin consumption. The levels now appear to have stabilised (Table 7.5) (Anon., 1988c).

The most dramatic change has obviously been in the consumption of aspartame (Table 7.6) (Anon., 1988d). Clearly, for any product to have exhibited the rate of growth that aspartame has is truly remarkable. Again, the switch from saccharin-aspartame blends to all-aspartame in beverages is reflected in the jump in consumption in 1985. Growth has since slowed, but remains steady.

Table 7.5. World saccharin consumption (millions of tonnes, sugar equivalent)

Year	Saccharin consumption (M tonnes sugar equivalent)
1974	3.4
1976	3.8
1978	4.3
1980	4.9
1982	5.2
1984	5.7
1986	5.15

Source: Anon. (1988c).

Table 7.6. World aspartame consumption (millions of tonnes, sugar equivalent)

Year	Aspartame consumption (M tonnes sugar equivalent)
1981	0.03
1982	0.05
1983	0.18
1984	0.42
1985	0.98
1986	1.10
1987	1.25

Source: Anon. (1988d).

7.2.4 Conclusions

The world markets for both nutritive and high intensity sweeteners have grown steadily in recent years and still continue to grow. Within this pattern of steady growth there have been some quite dramatic fluctuations, both in terms of regional consumption patterns and in the composition of the sweeteners within particular territories. These fluctuations illustrate the dynamic nature of the sweetener industry generally, and occur in response to a variety of market and marketing forces that exist.

7.3 Sweetener marketing

7.3.1 Introduction

If the criteria of sweetener selection for almost any product were based simply on performance characteristics, sucrose would almost invariably be the sweetener of choice. This is hardly surprising when we consider that

the development of all traditional food products occurred in an era when sucrose was almost the only sweetener available for use. Thus, the functional characteristics of sucrose have been built into very many food products, and alternatives will almost invariably compare unfavourably (Section 7.4).

However, performance is not the only selection criterion used by the food industry when choosing a sweetener for any particular product. Obviously, economic factors are an important consideration, and increasingly we have seen sweeteners, and the products containing them, marketed on a nutritional basis. Image, both from the positive and negative standpoints, is a major part of the nutrition message that marketeers have structured around sweeteners in general.

7.3.2 Sweetener economics

It is only when sucrose or high fructose syrups are being used in liquid food systems that sweetener selection is based simply on economic factors. In some market territories, notably in the United Kingdom, combinations of sucrose and saccharin may also be used on occasion for cost-saving reasons. Apart from these examples, other factors such as sweetness quality, sweetener functionality and image or nutritional aspects will be part of the equation governing the selection of a particular sweetener for any food or beverage product.

As has already been discussed, it is in the United States where the most dramatic shifts in sweetener consumption have occurred, with the substitution of sucrose by high fructose being the best example (see Table 7.3). The economic sense of that industry switching in the way that it has is illustrated in Table 7.7 (Anon., 1989d). Here, the effect of supporting the sugar price artificially has been to ensure that HFCS prices consistently undercut sucrose prices in that market.

The very low prices of these bulk carbohydrates have had one important consequence. That is, the difficult and very slow market development for speciality bulk sweeteners such as crystalline fructose, palatinit and lactitol. Generally speaking, any functional or image benefit these alternatives have will rarely outweigh the economic disadvantages of their use in place of sucrose or high fructose syrups.

Because of their high sweetening power, intense sweeteners will almost inevitably be cheaper on a sweetness equivalence basis than the bulk sweeteners. For example, in the United States saccharin is currently around 5% of the price of refined sugar. The incentive to use intense sweeteners can be further strengthened through the use of mixed sweeteners in certain circumstances, hence capitalising on the sweetness synergies that can result (Frijters, 1987).

Table 7.7. Price trends for sucrose and high fructose syrup (cents/lb, dry basis)

Year	Sweetener prices		
	Sucrose (cents/lb)	HFCS (cents/lb)	HFCS/sucrose(%)
1980	30.8	23.6	77
1981	28.3	21.4	76
1982	28.1	14.3	51
1983	26.4	17.7	67
1984	25.8	19.6	76
1985	23.4	18.2	78
1986	23.6	18.1	77
1987	23.8	16.5	69

Source: Anon. (1989d).

7.3.3 Nutritional aspects of sweetener marketing

Collectively, as much (if not more) will have been written about the nutritional consequences of consuming sweeteners than about any other class of food ingredient. These range from the concept of sucrose being 'pure, white and deadly' which was particularly prevalent in the 1970s (Yudkin, 1979), through the almost magical qualities attributed to honey as a sweetener (Stein, 1973), to the many articles written on the consequences of consuming high intensity sweeteners (for a review, see Renwick (1983)). Much has also been made of the role of fermentable carbohydrate in general and sucrose in particular in the development of dental caries, and the potential benefits of sugar alcohols as alternatives (Grenby, 1983).

From the standpoint of sweetener marketing, the strategies that have been developed and followed are relatively straightforward. The commodity nature and distribution characteristics of sucrose render advertising the base product pointless, particularly so since customers are rarely offered a choice of brands on the supermarket shelves. Generic advertising to try to offset some of the negative perceptions of sucrose has had some success in parts of the world, notably Australia, but in general it has had little impact. As a result, if it is possible to manufacture products without using sucrose or using lower quantities, and without compromising on quality, it will almost certainly be considered a positive marketing feature to describe the resulting product as low in sugar or sugar free. This strategy has been followed for sugar-free confectionery, but with only limited success, probably because dental caries is not clearly as much of an issue now as it used to be. Fluoridation of water supplies and of toothpastes has reduced the problem significantly.

The strategy has also been abused to some extent by the marketing of products such as apple or grape juices as suitable for the manufacture of

'sugar-free' or 'no added sugar' products. There have even been instances of fruit juice syrups containing 70% solids being promoted as having 30% fewer calories than sucrose!

Marketing food and beverage products sweetened with intense sweeteners presents the marketer with something of a dilemma. On the one hand it is clearly going to be advantageous to focus on the reduced or low-calorie features of the finished product; on the other hand it must be acknowledged by label declaration that the product is sweetened with an artificial ingredient. In addition, in the United States, products sweetened with saccharin must also carry a warning that saccharin has been found to cause cancer in laboratory animals. Obviously, this is hardly the sort of marketing message companies wish to project about their products. Wherever feasible, therefore, saccharin has been removed from ingredient lists.

Aspartame marketing by the NutraSweet Company has focused on the quality of sweetness delivered and that the sweetener is composed of two common amino acids, thus developing a perception of 'naturalness'. Aggressive branding with the objective of tying product manufacturers to the sweetener has also been part of the strategy. The success of this has yet to be really tested and it will be interesting to observe just how effective the branding strategy remains into the 1990s.

7.3.4 Conclusions

Creative marketing strategies can almost always be developed around any product or ingredient, and those developed for sweeteners have been no exception. There is little question that the continuing high degree of consumer interest and awareness in sweeteners will necessitate the maintenance of secure marketing positions for both new and established sweeteners and the foods and beverages they sweeten.

7.4 Sweeteners in product development

7.4.1 Introduction

It is frequently stated and has already been repeated in this chapter that to maximise the acceptability of any food product sweetened with an 'alternative' sweetener, it is critical that the resulting food differ as little as possible from the standard, probably sucrose sweetened, product. This remains a critical requirement because of the innate conservatism of consumers and because sucrose remains the gold standard against which all alternatives are compared. It would be interesting to speculate why sucrose

is the preferred sweetener in this way; is it an innate or learned preference; but that discussion is probably outside the scope of this chapter. However, it is important to identify and discuss the crucial features that must be considered by food product development personnel when they are seeking to create 'alternatively' sweetened products.

This is not a straightforward task. Very many foods and beverages have been optimised over decades in an empirical and iterative manner, and part of this optimisation has centred around the sensory and physical characteristics of, usually, sucrose. There is certainly nothing magical about the functionality of sucrose. It is simply that sucrose was the first sweetener available in bulk quantities to a developing processed food industry. Naturally, the resulting foods and beverages reflect sucrose's versatility. Unfortunately, from the point of view of the product developers of today, it is a hard act to follow.

In considering the use of sweeteners in products, both as single ingredients and as mixed sweeteners, the sweetness intensity desired in the finished food must be clearly understood, account must be taken of the functional properties delivered by the quantity of sweetener for that desired sweetness, as well as sweetness quality, stability, temporal characteristics and flavour interactions. In other words, it is probably true to say that the easiest part of new product development using alternative sweeteners is the matching of sweetness intensity. That is the single feature over which control may be simply exerted.

However, in order to have some possibility of controlling the other important functional parameters mentioned, it is vital that the properties of alternative sweeteners are thoroughly understood.

7.4.2 Sweetness intensity

Details of the relative sweetness intensity of both bulk and intense sweeteners are given elsewhere and will not be repeated here. It is a crucial parameter for product developers to understand, however, not least because the perceived intensity of all sweeteners relative to sucrose varies dramatically depending on their concentration, temperature of tasting, pH, and on the flavour and texture of the product in which they are incorporated.

For example, the sweetness of aspartame is normally quoted as ranging from 180 to 200 times as sweet as sucrose (Beck, 1974). In reality, however, the intensity can range from around 400 times sucrose at threshold concentration down to less than 100 times sucrose in confections at around 90% sucrose equivalent (Beck, 1978). Coupled with that simple effect of concentration are the reported complicating effects of other formulation changes such as flavour or viscosity. Small amounts of gelatin or methyl cellulose reportedly raise the perceived sweetness of aspartame in

Table 7.8. Fructose-sucrose sweetness synergy (%)

Sucrose (parts)	Fructose (parts)	Relative sweetness (x sucrose)
100	0	100
75	25	118
50	50	128
25	75	121
0	100	117

Source: Batterman *et al.* (1987).

buffered aqueous solution (Pangborn *et al.*, 1973; Cloninger and Baldwin, 1974).

Perhaps the most important practical consideration on sweetness intensity from the product development standpoint, is whether or not there are any significant advantages to be gained from exploiting the intensity synergy that can be a consequence of mixing sweeteners. Sweetener mixtures have been used in foods for many years; perhaps the most familiar example is the saccharin/cyclamate (1:10) combination that has been in use for around 30 years. Although it is not used in the United Kingdom or North America because cyclamate is banned in these territories, it remains a very widely used sweetener mixture. The sweetness synergy that results from this combination makes it a very economic mixture with the additional benefit that the cyclamate greatly enhances sweetness quality through reducing the bitterness which stems from saccharin.

More and more, for reasons of sweetness synergy, manufacturers routinely examine the opportunities that might stem from using mixtures of sweeteners in their products. For example, aspartame is synergistic with saccharin (Cloninger and Baldwin, 1970; Scott, 1973; Beck, 1974), cyclamate (Yamaguchi *et al.*, 1972), sucrose and glucose (Moskowitz, 1974). Acesulfame K is also reported to be synergistic with cyclamate and aspartame (von Ryman Lipinski, 1988), the latter combination being particularly attractive because of the assurance of stability of at least a portion of the sweetness in the mixture.

Synergy is not a phenomenon that is only exhibited by mixtures of sweeteners that contain at least one intense sweetener. Pure fructose, which is now commercially available at competitive prices, is synergistic with sucrose to a quite significant degree (Table 7.8). Maximum synergy is achieved with equal weight mixtures of the two sweeteners, and such combinations are therefore able to ensure reductions in sweetener costs that can be commercially significant (Batterman *et al.*, 1987).

Fructose is also synergistic with aspartame and there can be economic

advantages of using such mixtures. For example, sucrose at 10% concentration is equi-sweet with 667 ppm aspartame. With the addition of 3% fructose, the amount of aspartame needed to be equi-sweet with 10% sucrose is 160 ppm, a 76% reduction (Hyvonen, 1978). Obviously, it would be possible to make 'no calorie' claims with such mixtures, but useful savings can be made in the formulation of 'reduced calorie' or 'diet' products, which can be positioned accordingly.

The potential economic advantages of using mixed sweeteners in food products can be significant, but this is not necessarily the only advantage to be had. Reference has already been made to sweetness stability benefits, and there are other functional benefits to be had, particularly with the use of bulk sweeteners.

Table 7.9. Influence of pH and temperature on the decomposition of saccharin.

pH	Saccharin decomposition after 1 h (%)		
	100°C	125°C	150°C
2.0	2.9	8.5	18.6
3.3	0	1.0	1.9
7.0	0.3	0.3	1.6
8.0	0	0	0

Source: DeGarmo *et al.* (1952).

It is a relatively straightforward matter of correct dosing to ensure that formulated foods deliver the desired degree of sweetness. However, it is far from straightforward to determine what is the optimum degree of sweetness, from the consumer viewpoint, that finished products should have. To do this, food manufacturers establish the optimum sweetness level of particular products within their ranges and adjust the level of sweetener to ensure that pre-determined sweetness intensity is achieved. Hence, the absolute level of sweetness in any product is more important to consumers than the intensity of the sweetener used in that product.

The influence of the sweetness intensity of products on their acceptability has been studied quite extensively. For example, it has been shown (Elias, 1980) that carbonated cola beverages are actually more preferred at higher sweetness levels than are normally provided commercially. Similar findings in other types of food product have been reported. In ice-cream, a broad optimum of acceptability at a sweetness intensity higher than that prevailing commercially has been reported (Pangborn *et al.*, 1957). A very wide variation in preference distribution, ranging from 6% to 24% sucrose, has also been demonstrated in a sucrose sweetened lemonade product with the most preferred level being 10% (Pangborn, 1980).

The probable reason for these observations is that during the kind of

methodology employed in generating these data, satiety will not influence acceptability. In the normal eating situation, a lower sweetness intensity of the product might prove to be more acceptable. The benefits of such a course are obvious. Firstly, using less sweetener generally means lower cost of ingredients, and secondly, lower sweetness levels probably mean that satiety is reached after higher levels of consumption (and hence product sales).

7.4.3 Sweetness stability

If consumers use sugar or an intense sweetener to sweeten their tea or coffee, they can be quite confident of the resultant beverage being essentially constant in sweetness from one drink to the next. In other words, as their drinks are prepared daily and drunk immediately, it does not matter whether or not the sweetener they are using has long-term stability. Providing they dose the beverage with reasonable precision, it will taste as expected.

Obviously, consumers also expect a degree of constancy in the prepared foods they purchase. Unfortunately, however, with prepared foods and beverages, that will not always be possible. They may be required to have shelf stability in excess of 12 months, and the ingredients used must all be sufficiently stable for any changes which do occur on storage to be difficult to detect.

Although high fructose syrups are the most widely used sweeteners in beverages manufactured in the United States, more sucrose is used to sweeten beverages generally than any other sweetener. This is in spite of the fact that its sweetness is not constant because of inversion to glucose and fructose during storage. For example, at pH 2.5, about 50% of the sucrose inverts within 4 weeks of storage at 20°C (Jacobs, 1959). During inversion, the perceived sweetness will change, although in this particular case, the change will normally be small because the sum of the sweetness of glucose and fructose is almost the same as that of sucrose. The actual perceived sweetness will depend more on factors such as the temperature at which the product is consumed. This is particularly true with fructose since the state of the equilibrium of anomeric forms depends very much on temperature.

At refrigerator temperatures, the fructose equilibrium favours the β -pyranose form, which is almost twice as sweet as sucrose. As the temperature rises, the equilibrium shifts to the furanose form and this form is believed to have little or no sweetness (Shallenberger, 1971). Therefore, any food or beverage sweetened directly with fructose, or with a sweetener such as sucrose which releases fructose on storage, will be subject to some variation in sweetness intensity. Such variations are outside the control of the manufacturer.

The sugar alcohols, in contrast, are essentially inert in food systems. This is due to the absence of a reducing group, which is a centre of lability, and in the

case of the 'disaccharide' sugar alcohols (palatinit, lactitol and maltitol) to the relative stability of the linkage between the moieties of those molecules. Therefore, manufacturers of products sweetened with sugar alcohols can be assured that their products will have constant sweetness.

In contrast, however, the principal intense sweeteners all show some degree of instability in food products, although saccharin, cyclamate and acesulfame K do require exposure to some quite extreme process and storage conditions before breakdown becomes measurable.

The effects of subjecting saccharin to extremes of temperature and pH have been studied quite extensively (DeGarmo *et al.*, 1952; Kroyer and Washuttl, 1979a, b). It is only at low pH and very high temperatures that significant decomposition occurs (Table 7.9) (DeGarmo *et al.*, 1952). The decomposition product is ammonium-*o*-sulphobenzoic acid. The influence of water-soluble vitamins, amino acids and olive oil on saccharin's stability have also been examined (Kroyer and Washuttl, 1979a,b), but it was again only at extremes of temperature that decomposition occurred. In summary, saccharin is stable under the normal range of conditions employed in food processing and storage.

Cyclamate is also a stable sweetener although it is less stable than saccharin (Talmage *et al.*, 1968; Hrdlicka and Janicek, 1971; Maruyama *et al.*, 1971; Kroyer and Washuttl, 1979a,b). Hydrolysis to sulphuric acid and cyclohexylamine occurs at very slow rates proportional to the hydrogen ion concentration, but the rate is so slow that for all practical product development purposes, it can be regarded as stable.

Similarly, acesulfame K is also stable enough in foods to present manufacturers with no particular problems (Arpe, 1978). On the other hand, aspartame is well known for its instability in solution and this instability has presented manufacturers with some critical formulation challenges.

The effect of pH on the stability of aspartame is well understood. In carbonated beverages, for example, it has been necessary to develop products of slightly higher pH than was normal for saccharin sweetened drinks, and it has been difficult if not impossible to incorporate aspartame successfully in beverages of a neutral pH. This has meant that chocolate milks, as an example, do not have sufficient sweetness stability if aspartame is the sole sweetener, and to date it has not been possible to incorporate aspartame in reduced calorie baked products. The development of an encapsulated version of aspartame which is claimed (Tsau and Bell, 1989) to be suitable for baking may offer new challenges for the developers of reduced calorie cakes and biscuits.

It is clear that sweetness stability is a crucial issue for food product formulators. To tackle the development of sweet products with an appropriate degree of assurance, it is critical that the effects of the formulation composition and the processing and likely storage conditions are completely

understood. Only then will the consumer receive products of the required quality that will meet his expectations.

7.4.4 Sweetener functionality

Both bulk and intense sweeteners contribute a range of functional properties to foods and beverages in addition to the sweet functionality. In much the same way that the sweetness quality of sucrose makes it the standard against which other sweeteners are compared, so its functional characteristics are also frequently regarded as optimum. Consequently, if there were no external pressures or internal marketing advantages resulting from the development of foods and beverages designed for 'special dietary' purposes, the sweetener of choice from a technical viewpoint would usually be sucrose.

Alternative bulk sweeteners to sucrose have been used in a very wide range of specialty food products including jams and preserves, chocolate, yoghurt, cakes, biscuits, puddings and beverages. In almost all cases, sucrose out-performs the alternatives in virtually every functional measurement (Lindley, 1983). In reality, however, this is hardly surprising when one considers the range of functional properties and the vital roles played by sucrose in the development and maintenance of food product structures. These are summarised in Table 7.10.

Table 7.10. Functional characteristics of sucrose in food products.

Market sector	Technical reasons for using sucrose				
	Sweetness	Bulk	Texture	Humectancy	Preservation
Beverages	X				
Baked goods	X	X	X	X	
Hard candy	X	X	X		
Soft candy	X	X	X		
Dairy frozen	X	X	X		
Desserts	X	X			
Preserves	X	X			X
Spreads	X	X			X
Canned products	X				

Source: Lindley (1983).

That being said, there are many product examples where alternatives to sucrose do function very well, particularly in liquid food systems such as beverages and canned fruit where the use of invert sugar and/or high fructose syrups results in perfectly acceptable products. This is, as has been noted, particularly true in the case of high fructose syrups which are functionally

equivalent to sucrose in liquid systems, but whose cost is frequently lower than that of sucrose. Generally speaking, none of the functional attributes of these particular products is compromised by the use of these alternative sweeteners. The manufacture of equivalent products sweetened with intense sweeteners is also relatively straightforward since the bulk in such products is provided by water. The sweetener only provides a single functionality; namely sweetness. In fact, canned fruits may actually benefit texturally from the use of intense sweeteners. In these cases, osmotic pressure will force fluid into the fruit cells, thus ensuring a more juicy texture than would be the case if high (dehydrating) concentrations of sucrose were used in the syrup (Lindley, 1983).

The use of intense sweeteners in the major markets of confectionery and baked products has been almost completely precluded on functionality grounds. Sucrose has many functional roles in the development of baked product structure, most of which centre around its control of the temperatures at which starch gelatinises and proteins denature, and around its affinity with water (Back *et al.*, 1979; Wooton and Bamunuarachchi, 1980). These features have a major influence on the development of cake and biscuit structure, as well as having an impact on the staling and drying out of baked goods (Lindley, 1987).

Alternative carbohydrates and sugar alcohols such as sorbitol, palatinit, lactitol and xylitol are generally able to influence product structure in a similar manner to sucrose, but rarely with the same versatility and without exhibiting some negative features. For example, glucose and fructose virtually mimic sucrose in their ability to control the temperature of starch gelatinisation, and hence development of structure, in cake batters. However, the resulting products will be darker in colour since glucose and fructose participate in browning reactions to a greater extent than does sucrose. Product volume may also be less (Bean *et al.*, 1978; Hardy *et al.*, 1979). Therefore, when the use of bulk alternatives to sucrose results in less acceptable foods than would be produced by using the sucrose standard, it is hard to imagine the intense sweeteners finding more than specialist applications in baked goods.

Similarly, it is difficult to imagine that intense sweeteners will ever find widespread use in confectionery products, principally because of the bulking and textural properties normally provided by sucrose, or in the case of 'sugar-free' or 'diabetic' confectionery, sorbitol and other sugar alcohols.

Sugar alcohols are quite widely used. They are non-cariogenic (Sicard, 1982) and their functional acceptability in most forms of confectionery is well documented (Sicard and Leroy, 1983).

Despite the many inherent difficulties in the technical development of 'alternatively' sweetened foods and beverages, the rewards for overcoming the technical problems are substantial. Thus, food manufacturers will continue to strive to formulate high quality special dietary foods, despite

the many inherent problems in being limited to the ingredients currently at their disposal.

7.5 Conclusions

For all food ingredient and consumer product companies to survive and grow commercially, they must continue to provide consumers with the products that meet their requirements. This truism is behind many of the dynamic changes in the growth and constitution of sweetener markets in recent decades. Despite many of the negative criticisms of what can be regarded as traditional sweeteners, such as sucrose and saccharin, there has been steady growth in most territories. New sweetener developments continue, and are rationalised on the basis that they provide the consumer with one benefit or another, usually the production of foods for special dietary purposes. Thus we have seen a substantial increase in such foods on the market.

It is critical, however, that products manufactured with 'alternative' sweeteners are of equal quality to traditionally sweetened products. Special dietary foods that are viewed as being of inferior quality will be strongly resisted by consumers who will probably view them as being unacceptable. Therefore, it is generally insufficient for manufacturers to develop new products in which the 'traditional' sweetener has simply been replaced by an 'alternative'. In general, a completely new formulation will be required in which the goal is more likely to be equal acceptability rather than an exact duplication.

For these reasons and despite the pressures that have been exerted on sucrose in particular, the markets for sucrose, high fructose syrups, saccharin and aspartame will continue to predominate. Niche markets for alternatives will develop, but the extent of their penetration of the major markets will always depend on the development of new food formulations around their unique functionalities.

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8 Sweeteners and dental health

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During the last decade the prevalence of dental caries in children, teenagers and young adults has declined substantially in most industrialised countries. This has led to an increasing proportion of caries-free individuals and to improved dental health. However, recent epidemiological data show that caries continues to be a problem in adult populations, where about 10–15% may still be considered at high risk of caries.

8.1 Dental caries: a multi-factorial disease

Dental caries is a complex sugar-dependent bacterial disease in which three main factors can be identified: the host with susceptible teeth, the substrate and the cariogenic microorganisms. The presence and interaction of all three factors are necessary if dental caries is to develop. Within each of these factors there is, however, a large number of both local and general predisposing factors which to different degrees can influence the occurrence of dental caries. Evaluation of all these so-called risk factors is therefore essential for identification of those persons who are particularly susceptible to caries.

In the development of dental caries, susceptible surfaces of the teeth are colonised by microorganisms in a mat of dental plaque. The bacteria ferment dietary carbohydrates and convert them to acidic end products, particularly lactic acid, which can cause a rapid drop in pH and lead with time to the initiation of caries by de-mineralisation of the tooth surface.

There is substantial support for the involvement of specific microorganisms in the caries process (Emilson and Krasse, 1985; Loesche, 1986). Studies have shown that mutans streptococci and lactobacilli play a key role. The cariogenic potential of the mutans streptococci group is probably due to their unique combination of properties. They colonise the teeth in a localised pattern (Lindquist and Emilson, 1990) and produce extracellular polysaccharides from sucrose, so-called glucans, which support the formation of adherent colonies on the tooth surface (Hamada and Slade,

1980). The mutans streptococci tolerate very high sucrose concentrations, are aciduric and produce acid from sucrose in higher quantity and more rapidly than other microorganisms in the plaque (Ikeda and Sandham, 1972; Hoeven and Franken, 1982). These properties give the mutans streptococci an ecological advantage over other plaque bacteria on a sucrose-containing diet and may be the explanation for the close correlation between the mutans group of streptococci and dental caries.

8.2 Evaluation of cariogenic potential

Assessment of the cariogenic potential of foods and sweeteners has involved, besides clinical studies, three main methods: (1) the measurement of acidic production in dental plaque; (2) experimental caries formation in rodents and monkeys; and (3) enamel de-mineralisation/remineralisation tests. Nowadays, these methods are preferred since it is not feasible to carry out long-term experimental clinical studies in man for ethical and practical reasons. Plaque pH and animal caries models have gained wide acceptance and are considered valid approaches. When used in combination they are good enough for predicting the cariogenic potential (DePaola, 1986).

The importance of repeated acid production in plaque for formation of caries was emphasised more than 50 years ago (Stephan, 1940). In a series of *in vivo* studies, it was demonstrated that after carbohydrate mouth rinsing, the pH of dental plaque fell to levels at which enamel de-mineralisation could take place. After reaching a minimum, the pH slowly returned to the original level. The plaque pH responses, which reflect the acidic challenge to the teeth, are influenced by a large number of host, microbial and dietary factors. Saliva and its buffering capacity and the bacterial composition may be important determinants, but a more important influence may be provided by the substrate to which the plaque is exposed.

There are basically three main types of plaque pH techniques for measuring the acidogenic potential of various foodstuffs. With the 'sampling technique', plaque is removed from a large number of accessible sites at regular time intervals after the ingestion of a specific food item and the pH is measured *in vitro*. The 'touch technique' implies that direct *in vivo* readings can be made by placing the microelectrode on or between the plaque-covered tooth surfaces and then monitoring pH changes after ingestion of the substrate. In 1966, a 'telemetry technique' was introduced which continuously records plaque pH changes *in vivo* at the enamel-plaque interface (Graf and Mühlemann, 1966). Whereas the first two methods can be used only on accessible tooth surfaces, the telemetry method can be used on caries-prone interproximal sites with undisturbed plaque and where pH readings can be made from the inner side of plaque.

Plaque pH measurements are of value for assessing the acidogenic

potential of food and the results obtained are essentially in agreement with those seen in animal and clinical studies. Although there is no single perfect test to predict the cariogenic potential of foods or beverages, substances which have been found to be non- or hypo-acidogenic in plaque pH measurements, have not been shown to be cariogenic in experimental animal studies or clinical trials. Moreover, food items cariogenic in animals have also been shown to have high acidogenic potential by plaque pH data. Utilisation of plaque pH measurements have helped to develop products with decreased acidogenic potential and in Switzerland carbohydrate-containing products which do not cause plaque pH values in vivo below 5.7 within 30 min after consumption may be advertised as 'safe for teeth' (zahnschonend).

8.3 Sucrose

Of all sugars, sucrose has been considered the 'arch-criminal' of dental caries (Newbrun, 1967). A large number of epidemiologic, animal and clinical studies have discriminated sucrose as a crucial microbial substrate, essential for the development of dental caries (Newbrun, 1982; Sheiham, 1983; Rugg-Gunn and Edgar, 1984; Glinsmann *et al.*, 1986). The consumption of sucrose and other sugars in many industrial countries during the last two decades has remained almost unchanged at a high level (Burt and Ismail, 1986; Honkala and Tala, 1987; Birkhed *et al.*, 1989). At the same time, a marked decline in caries prevalence has been noted indicating that sugars no longer should be as important for dental caries as they have been. However, the extensive use of fluoride, especially in toothpaste, reduces the susceptibility of the tooth surface against acid attacks and has to a large degree counteracted the effect of sucrose consumption on dental caries.

8.3.1 Bacteriological studies

The main property which makes sucrose more cariogenic than other common sugars is its ability to act as a substrate for acid production at low pH and for the synthesis of extracellular polysaccharides such as glucan polymers rich in α -(1-3)-linkages. These insoluble polymers are of special importance since strains of mutans streptococci which have lost the ability to produce glucans from sucrose also lose their cariogenic virulence. Sucrose is a more energy-giving substrate than other simple carbohydrates that the microorganisms can utilise. The energy is present in the link between the glucose and fructose component of sucrose. The mutans streptococci have at least two transport systems for sucrose into the cells and several enzymes for the metabolism of sugars. High sucrose concentrations give these

bacteria an ecologic advantage compared with other oral microorganisms. The presence of sucrose can confer adhesiveness to dental plaque and mediate the binding of mutans streptococci to the tooth surface through the glucans (Rölla *et al.*, 1983). The sucrose-dependent colonisation of tooth enamel by mutans streptococci is an important aspect of the cariogenicity of sucrose. Effective mechanisms for the prevention of dental caries would be to interrupt the process of the adherence of mutans streptococci to the tooth surface or to use sweetening agents that are not used by oral bacteria for the synthesis of insoluble glucans or for the production of acids.

8.3.2 *Animal studies*

Strains of mutans streptococci initiate rampant decay when implanted into the mouths of experimental animals fed sucrose-containing diets (Hamada and Slade, 1980). Results have shown that the development of caries is closely correlated with the frequency and amount of sucrose ingested and that sucrose produces more caries than other carbohydrates (Tanzer, 1981).

8.3.3 *Clinical studies*

The classical Swedish Vipeholm study (Gustafsson *et al.*, 1954) provided convincing evidence that consumption of sugar can increase caries activity in man; the result influenced the prevention of dental caries for a long time. The main finding in this study was that sugary foods consumed in a sticky form at high frequency between meals were highly cariogenic, whereas the quantity of sucrose consumed during meals had little influence on the caries rate. The second longitudinal study which deserves special mention was conducted at Hopewood House in Australia (Harris, 1963). The study showed that institutionalised children given a diet in which sucrose and other refined carbohydrates were virtually absent had an extremely low caries incidence compared with age-matched neighbouring children on less restrictive diets. Further evidence for the major role of sucrose in dental caries was provided in experimental caries studies in students (von der Fehr *et al.*, 1970). Rinsing with a 50% sucrose solution nine times a day over a 23-day period resulted in initial caries lesions on buccal tooth surfaces.

Information on sugar supplies in 47 countries has demonstrated a significant positive correlation for 12-year-olds between the quantity of available sugar per person and the caries prevalence in a country (Sreebny, 1982). The data also indicated that 50 g of sugar per person per day may represent the upper limit of acceptable sugar consumption. Thus to achieve improved dental health, where most of the population will not get caries, it has been suggested that the level of sugar consumption in a population

should be reduced to about 15 kg/person per year, assuming the widescale use of fluoride (Sheiham, 1983).

8.4 Low- or non-cariogenic sugar substitutes

Most people in modern societies enjoy eating sweet-tasting foods and therefore it is not realistic to eliminate sucrose from the diet. Attention has instead turned to finding alternative low or non-cariogenic sugar substitutes. Today there are both non-caloric and caloric sweeteners available, which are interesting from a cariological point of view. Table 8.1 shows different monosaccharides, sugar alcohols and polysaccharides, which belong to the group of caloric sweeteners.

Table 8.1 Caloric sweeteners used as sucrose substitutes.

Sugars	Glucose, fructose, invert sugar lactose, Palatinose, sorbose
Sugar alcohols	Acyclic polyols
	Xylitol (pentitol)
	Sorbitol (hexitol)
	Mannitol (hexitol)
	Disaccharide alcohols
	Lactitol (12-carbon polyol)
	Maltitol (12-carbon polyol)
	Palatinit (mixture of two 12-carbon polyols)
	Lycasin (mixture of sorbitol, maltitol and high molecular weight sugar alcohols)
Starch hydrolysate	

8.5 Glucose, fructose and invert sugar

8.5.1 Bacteriological studies

The acid production from glucose, fructose and invert sugar is of the same order of magnitude as from sucrose (Neff, 1967; Birkhed, 1978). Glucose is used, however, by the oral microorganisms in a similar manner as sucrose and there is similarity in acidogenicity of sucrose and glucose in terms both of plaque pH in situ (Frostell, 1973; Edgar, 1976) and of acid production in vitro (Birkhed and Edwardsson, 1978).

8.5.2 Animal studies

Studies in rats and hamsters inoculated with mutans streptococci have

demonstrated that glucose, fructose and invert sugar give less caries than sucrose, especially on smooth buccal and lingual tooth surfaces (Krasse, 1966; Birkhed *et al.*, 1981). In monkeys, however, animals fed a fructose diet had a somewhat higher caries incidence than those fed sucrose or glucose (Colman *et al.*, 1977).

8.5.3 Clinical studies

A mouth rinse with fructose and glucose induces a fall in plaque pH similar to that for sucrose (Frostell, 1973). Persons with frequent consumption of the monosaccharides fructose and glucose develop less voluminous plaque compared to those exposed to sucrose (Carlsson and Egelberg, 1965). In the Turku Sugar Studies (Scheinin *et al.*, 1975a), the caries incidence was about 30% lower in the volunteers who consumed a fructose diet than in those who were given a sucrose diet. The cariogenic potential of invert sugar has been examined in two studies. In the Gustavbergs study (Frostell *et al.*, 1981) children who, during 2 years, consumed food products containing invert sugar in combination with sucrose restriction developed significantly less caries (35%) than children who were allowed a sucrose diet. The mechanisms behind the invert sugar effect were considered to be reduced polysaccharide production and decreased formation of plaque. In the Malmö study (Frostell *et al.*, 1991) sucrose was partially replaced in between-meal products with invert sugar. This study is interesting since it was a double blind trial, where pre-school children were given either sucrose- or invert sugar-sweetened food items for 2–3 years. The caries reduction was approximately 20–25%.

8.6 Lactose

Lactose gives a smaller drop in plaque pH and has a lower cariogenic potential than glucose or sucrose (Frostell, 1973). Milk, which contains lactose, gives even smaller falls in pH than pure lactose, probably owing to the buffering action of its protein content. Human breast milk, with higher lactose content (6–9%) than cow's milk (4–5%), is somewhat more acidogenic than cow's milk but less acidogenic than lactose or sucrose solutions (Roberts *et al.*, 1984). Human milk is unlikely to be cariogenic in humans except perhaps in rare instances when frequent on-demand suckling is permitted. An adaptation of the oral microflora to both lactose and milk in man after frequent use has recently been observed (Birkhed, Edwardsson and Imfeld, unpublished observations).

8.7 Palatinose (isomaltulose)

The metabolism of Palatinose by oral microorganisms has recently been reviewed (Takazoe, 1989). The mutans streptococci cannot use Palatinose for insoluble glucan synthesis as they can with sucrose. Palatinose is rarely fermented by the mutans streptococci (Ooshima *et al.*, 1983), but it is decomposed into glucose and fructose by sucrase-isomaltase complex in the intestinal mucosa (Goda *et al.*, 1983). The acid production from isomaltulose in dental plaque suspensions is markedly lower than that from sucrose (Maki *et al.*, 1983). Addition of Palatinose to sucrose mixtures inhibits the production of insoluble glucans by glucosyltransferase (Ooshima *et al.*, 1983).

8.7.1 Animal studies

Palatinose has been found to be non-cariogenic in rats infected with *Streptococcus mutans* (Ooshima *et al.*, 1983). Replacement of 50% of the sucrose content in the diet with Palatinose resulted in significantly decreased caries development compared with a control diet containing sucrose alone, indicating that even partial substitution of dietary sucrose with Palatinose is interesting in caries prevention.

8.7.2 Clinical studies

There is a risk for adaptation of the oral microflora to metabolise Palatinose in a more cariogenic way after frequent use. Thus, daily mouth rinses over a 6-week period with a 15% solution have resulted in a more pronounced pH decrease in dental plaque than before rinsing, but the pH did not fall below 6.2 (Topitsoglou *et al.*, 1984).

8.8 Sorbose

8.8.1 Bacteriological studies

Few oral bacteria are able to ferment sorbose to acid. Only strains within the species *Lactobacillus casei* have been identified as sorbose fermenters (Havenaar *et al.*, 1979; Lohmann *et al.*, 1981). An adaptation, resulting in a rapid acid production rate, has been observed with this microorganism. Mutans streptococci, however, do not ferment sorbose either before or after adaptation periods (Havenaar *et al.*, 1979).

8.8.2 *Animal studies*

Experimental caries studies in rats indicate that sorbose has little or no cariogenic potential (Firestone *et al.*, 1980; Hefti, 1980). It has been observed that sorbose suppresses the cariogenicity of sucrose in contrast to Lycasin 80/55 and sorbitol, suggesting an anticariogenic effect of this hexose (Havenaar *et al.*, 1984).

8.8.3 *Clinical studies*

Sorbose seems to have a reducing effect on both the plaque formation and on the glucose-fermenting capacity of dental plaque (Mühlemann *et al.*, 1977a).

8.9 Xylitol

8.9.1 *Bacteriological studies*

The majority of the oral bacteria, including mutans streptococci and lactobacilli, lack the ability to metabolise xylitol to acids (Gehring *et al.*, 1975; Edwardsson *et al.*, 1977; Platt and Werrin, 1979). Unlike sorbitol, xylitol seems to have a growth inhibitory effect on pure cultures of some oral bacteria. Thus, the addition of xylitol to a glucose-containing medium reduces the growth of mutans streptococci (Knuuttila and Mäkinen, 1975; Assev *et al.*, 1980; Vandeboncoeur *et al.*, 1983). This inhibition by xylitol appears to be related to the accumulation of xylitol-5-phosphate within the cells (Waalder *et al.*, 1985). Xylitol causes no drop in plaque pH (Birkhed *et al.*, 1979). Mostly, a pH increase is found probably depending on the salivary stimulating effect of the sweet taste itself.

8.9.2 *Animal studies*

Many experimental studies in rats have demonstrated no or an extremely low caries rate in the presence of a xylitol-containing diet. There are even indications that xylitol can exert an anti-cariogenic effect (Mühlemann *et al.*, 1977b). Even partial substitution of dietary sucrose by xylitol seems to reduce the cariogenicity compared to sucrose (Leach and Green, 1980).

8.9.3 *Clinical studies*

The Turku Sugar Study involved an almost complete substitution of sucrose by xylitol for 2 years (Scheinin *et al.*, 1975a). A highly significant reduction

in caries (85%) was found in the xylitol group compared with the sucrose group. A supplementary study, also conducted in Turku, on the effects of xylitol- versus sucrose-containing chewing gum over a 1-year period, showed that 4–5 pieces of gum per day gave a dramatic reduction of dental caries (82%) compared to the sucrose group (Scheinin *et al.*, 1975b). Subsequent studies have supported these data and have shown that consumption of 2–3 xylitol-sweetened chewing gums per day for 2 years in addition to basic preventive measures resulted in close to 50% lower incidence of caries compared to a group receiving only basic prevention (Isokangas *et al.*, 1988, 1989). Collaborative WHO field studies conducted in Thailand, Hungary and French Polynesia showed, in comparison to systemic or topical fluoride treatment, that partial substitution of sucrose with xylitol and sorbitol was associated with a cariostatic effect (Scheinin, 1985).

Short- and long-term use of xylitol decreases the levels and proportions of mutans streptococci in plaque or saliva (Gehring *et al.*, 1975; Loesche *et al.*, 1984; Bánóczy *et al.*, 1985; Wennerholm and Emilson, 1989). A mixture of xylitol and sorbitol seems to have the same or almost the same positive effect as pure xylitol (Birkhed *et al.*, 1983; Topitsoglou *et al.*, 1983; Söderling *et al.*, 1989). A xylitol-sorbitol-sweetened gum showed a significantly better ability to resist pH drops in plaque induced by sucrose rinse than a pure sorbitol gum (Söderling *et al.*, 1989). No general adaptation of the oral flora to xylitol has been observed so far (Birkhed *et al.*, 1979), but strains of mutans streptococci with reduced sensitivity to xylitol have been isolated in xylitol consumers (Mouton *et al.*, 1984).

8.10 Sorbitol

8.10.1 Bacteriological studies

The cariogenic potential of sorbitol has recently been reviewed (Birkhed and Bär, 1991). The usual explanation offered for the lower cariogenicity of sorbitol compared with easily fermentable sugars such as sucrose, glucose and fructose is that it cannot be metabolised at all or not as easily by oral microorganisms (Edwardsson *et al.*, 1977; Havenaar *et al.*, 1979). Only 10–20% of the plaque flora, belonging almost exclusively to species of mutans streptococci, *Lactobacillus* and *Actinomyces* are able to ferment sorbitol (Edwardsson *et al.*, 1977; Platt and Werrin, 1979; Havenaar *et al.*, 1979; Kalfas and Edwardsson, 1990). Sorbitol is fermented by oral bacteria at a slower rate than glucose and its catabolism is affected to a greater extent by the extracellular pH (Kalfas *et al.*, 1990a).

Frequent sorbitol consumption gives rise to a certain microbial adaptation, which may be due to an increased production of sorbitol-degrading enzymes, to an increased acid production by dental plaque and to elevated

numbers of mutans streptococci and lactobacilli (Birkhed *et al.*, 1990b). In persons with dry mouth and frequent consumption of sorbitol-sweetened products, the risk of adaptation may lead to a small acid production (Kalfas *et al.*, 1990b). For persons with normal salivary flow and for people with moderate consumption of sorbitol-containing food items, the consequences of such an adaptation are probably of little clinical importance.

8.10.2 Animal studies

Sorbitol has shown a low cariogenic potential in animal studies. When tested in monkeys for example, sorbitol was found to be far less cariogenic than sucrose (Cornick and Bowen, 1972). Furthermore, it failed to support the growth of mutans streptococci in dental plaque and during a 2-year period the capacity of the plaque flora to produce acid from sorbitol remained low or unaltered.

8.10.3 Clinical studies

Clinical trials with sorbitol-sweetened chewing gums or sweets indicate that sorbitol is more or less non-cariogenic (Møller and Poulsen, 1973; Bánóczy *et al.*, 1981; Glass, 1983). For example, when children were chewing three pieces of the gum per day for 2 years, the caries increment was significantly less than in a control group who did not chew any gums (Møller and Poulsen, 1973).

8.11 Mannitol

8.11.1 Bacteriological studies

Mannitol gives a small fall in pH compared to sucrose (Ahldén and Frostell, 1975). Mutans streptococci and *Lactobacilli casei* are unique among the plaque flora in their ability to ferment mannitol and sorbitol. The enzymes, mannitol 6-phosphate and sorbitol 6-phosphate dehydrogenase, involved in hexitol catabolism are, however, inducible and their synthesis is inhibited by the presence of glucose (Brown and Wittenberg, 1973).

8.11.2 Animal studies

The few animal studies, which have been carried out on mannitol, have shown a low cariogenic potential of mannitol in comparison with sucrose (Shaw, 1976).

8.12 Lactitol

8.12.1 Bacteriological studies

Interdental plaque pH measurements have shown that lactitol is hypo-acidogenic (Imfeld, 1983). A number of plaque bacteria can metabolise lactitol, for example mutans streptococci and certain strains of *S. sanguis*, *Lactobacillus* and *Actinomyces* (Havenaar *et al.*, 1979). The acid production is much slower from lactitol than from lactose, fructose and sucrose (Gehring, 1978). Frequent subculturing of *S. mutans* in lactitol medium can temporarily increase its fermentation (Havenaar *et al.*, 1979). Lactitol proved to have superior dental properties compared to sorbitol and mannitol, with lower acid and polysaccharide formation by oral micro-organisms and less attack on enamel mineral (Grenby *et al.*, 1989; Grenby and Phillips, 1989).

8.12.2 Animal studies

There are only a few studies on the cariogenicity of lactitol in animals. In rats, lactitol has shown a cariogenic potential similar to that of sorbitol, but significantly less compared with sucrose (Hoeven, 1986). Replacement of sucrose in biscuits with lactitol has resulted in a very low level of caries (Grenby, 1989).

8.12.3 Clinical studies

A 3-day trial in man, where two groups were given either conventional sucrose sweets or sweets made with lactitol as the only sweetener, showed a significant reduction in the amount of plaque in the lactitol group, indicating a dentally beneficial effect (Grenby and Desai, 1988).

8.13 Maltitol

8.13.1 Bacteriological studies

Maltitol is slowly fermented by *S. mutans* and *Actinomyces viscosus* and by some species of the genus *Lactobacillus* (Edwardsson *et al.*, 1977; Havenaar *et al.*, 1979).

8.13.2 Animal studies

The cariogenic potential of maltitol has been studied in rats, inoculated with *A. viscosus* and *S. mutans*, by applying it to the molar teeth of the animals five times daily (Firestone *et al.*, 1980). At the same time, the rats were fed a low cariogenic diet *ad libitum*. The results showed that maltitol has non-cariogenic properties in this animal test model.

8.13.3 Clinical studies

Little experimental work has been reported on maltitol alone, although it is the major constituent (50–55%) of Lycasin. Maltitol lozenges eaten four times a day for 3 months did not affect the plaque and acid formation or the number of mutans streptococci and lactobacilli in dental plaque (Birkhed *et al.*, 1979).

8.14 Palatinit

8.14.1 Bacteriological studies

Acid production from Palatinit by pure cultures of *S. mutans* is low (Imfeld, 1983). The available microbiological data indicate that Palatinit has characteristics similar to sorbitol. Some strains of mutans streptococci, actinomyces and *L. casei* are able to ferment Palatinit in vitro and lower the pH to levels less than 5.5 (Hoeven, 1979). Like other sugar alcohols, the rate of degradation is presumably slower than that of sucrose (Havenaar *et al.*, 1984).

8.14.2 Animal studies

Several rat experiments have shown that a Palatinit-containing diet has low or no cariogenicity (Firestone *et al.*, 1980; Havenaar *et al.*, 1984). Following the administration of Palatinit to rats, no increase in the numbers of the Palatinit-fermenting flora was found (Hoeven, 1980).

8.15 Lycasin

The cariogenic potential of Lycasin has recently been reviewed (Rugg-Gunn, 1989). Different types of Lycasin have been tested. The most common type today is Lycasin 80/55 (manufactured in France), which contains 6–8% sorbitol, 50–55% maltitol, 20–25% maltotriitol and

10–20% polysaccharide alcohols. Lycasin, product 6563, which was earlier produced in Sweden, contained much more high molecular weight components than Lycasin 80/55 and seemed to be more cariogenic than French Lycasin (Frostell and Birkhed, 1978).

8.15.1 Bacteriological studies

The acid production rate from Swedish Lycasin is about 65% and from French Lycasin 40% of that obtained from glucose and sucrose (Frostell and Birkhed, 1978). Bacteriological studies show that only some plaque bacteria, such as streptococci and lactobacilli, slowly ferment Swedish Lycasin (Edwardsson *et al.*, 1977). Mutans streptococci and lactobacilli produce acid from Lycasin 80/55 (Havenaar *et al.*, 1979). However, the fermentation rate is slow, even after an adaptation period. The Lycasin can be hydrolysed by salivary amylase with the formation of di- and oligosaccharides, but at a lower rate than starch (Birkhed and Skude, 1978).

8.15.2 Animal studies

From a series of rat experiments (Birkhed and Frostell, 1978; Havenaar *et al.*, 1984), in which the animals had been inoculated with strains of mutans streptococci or a combination of mutans streptococci and *A. viscosus*, one may conclude that: (1) Lycasin 80/55 could be classified as being non-cariogenic; (2) no adaptation of practical importance develops in dental plaque; and (3) the combination of xylitol and Lycasin 80/55 has anti-cariogenic properties.

8.15.3 Clinical studies

The substitution of sucrose by Swedish Lycasin in candies has been studied in a 2-year clinical study of 3–6-year-old children (Frostell *et al.*, 1974). A control group received conventional sucrose-sweetened products. The reduction in caries was about 25%. No evidence of adaptation of the oral microflora to Lycasin was found after 3 months frequent consumption of lozenges containing Swedish Lycasin (Birkhed *et al.*, 1979).

8.16 Starch hydrolysate

Raw starch gives a low decrease of plaque pH when compared with both glucose and sucrose (Neff, 1967). Starch modified by different

processes shows an increasing susceptibility to hydrolysis by α -amylase, and correspondingly produces an increased pH drop in dental plaque compared with raw starch; the more severe the processing conditions the more the pH falls (Lingström *et al.*, 1989; 1991). Extensive animal studies have shown that starchy foods have a very low caries inducing potential in comparison with sucrose (Stephan, 1966). In rats, which were fed the nutrients through gastric intubation and given the test substance through a programmed feeder, starch was found to have less than half the cariogenic potential of sucrose (Bowen *et al.*, 1980). Mixtures of starch and sucrose such as sweetened cereals or chocolate cookies with soft filling gave higher caries score than sucrose alone, indicating that starch can increase the cariogenic potential of sugar by increasing its retentiveness in the mouth. In humans, the cariogenic potential of starch can only be indirectly assessed. Persons with hereditary fructose intolerance who avoid fructose and sucrose but tolerate starch are almost free from caries (Newbrun *et al.*, 1980). The Vipeholm study showed that when the sweets were withdrawn and the subjects received only starchy foods at meals, the caries activity was markedly reduced (Gustafsson *et al.*, 1954). However, there is no study in man, where starch products have been given frequently.

8.17 Non-caloric sweeteners

This group of sugar substitutes includes a wide variety of compounds, e.g. saccharin, cyclamate, acesulfame-K and aspartame. Since they are non-nutritive sweetening agents and thus have no caloric value, these products are not expected to be fermented by microorganisms of the oral cavity. Accordingly, saccharin and aspartame do not cause acid production in plaque or bacteria at the concentration at which they are employed (Bibby and Fu, 1985). Inhibitory actions of these two intense sweeteners on bacterial growth and metabolism have been suggested (Linke and Chang, 1976; Best and Brown, 1987; Grenby and Saldanha 1986). When two or three sweeteners were combined (acesulfame K, cyclamate and saccharin) a strong synergism on inhibitors on acid production by oral microorganisms was found (Ziesenitz and Siebert, 1986; Brown and Best, 1988). Several rat experiments have also shown that these non-caloric sweeteners are completely non- or even anti-cariogenic (Tanzer and Slee, 1983; Siebert *et al.*, 1987; Lout *et al.*, 1988). The use of a non-caloric sweetener containing glucose and saccharin, reduced the plaque formation over a 3-day period (Grenby, 1975). Pure aspartame has also been found to reduce adherent plaque formation in vitro (Olsson, 1977).

8.18 Comparison of various groups of sweeteners

When evaluating the cariogenic potential of sugar substitutes, different aspects should be considered. Of course, it is desirable that the sweeteners are tested in clinical studies. Since this is not possible for practical and other reasons, one may judge the sugar substitutes from microbiological and animal experiments and from short-term studies in man. Valuable information about the cariogenicity can be received from plaque pH measurements. Results from such studies make it possible to classify the sugar substitutes into different groups.

- (1) The first most suitable group seems to consist of non-caloric sweeteners and xylitol. These substitutes are fermented by no or only a few oral bacteria and no negative adaptation of the oral flora has been observed.
- (2) A second intermediate group consists of sorbitol, lactitol, lactose, Lycasin®, mannitol, maltitol, Palatinit and Palatinose. These substitutes are fermented by certain groups of organisms within the oral genera *Actinomyces*, *Lactobacillus* and *Streptococcus*. Adaptation of oral bacteria to lactose, Palatinose and sorbitol, resulting in a more pronounced acid production, has been observed.
- (3) A third, least suitable, group consists of fructose, glucose, invert sugar and starch hydrolysate. Most oral bacteria ferment these sugars, in the case of starch after cleavage of amylase, and the acid production rates approximate that of sucrose.

8.19 Sweeteners and caries prevention

There is much evidence showing that frequent consumption of sugar-containing products often results in high caries risk. It is therefore important in the caries prevention to reduce consumption of snacks and try to teach people to use sugar in a more rational way, i.e. to eat sweet products infrequently. In some products there may also be justification in replacing sucrose with low or no cariogenic sweeteners. Relatively few clinical cariological studies have been carried out on the caries reducing effect of sugar substitutes. There are only some such data available on the use of fructose, invert sugar, xylitol and sorbitol. Even if the results are promising, most experience and conclusions come from short-term studies and animal experiments. From this standpoint, the results are promising. Thus, there are many sugar substitutes on the market today, both caloric and non-caloric, which show low or even no cariogenic potential.

The availability of sugar-free products in most countries today is relatively low, but will hopefully increase in the future, for example in beverages, as sweeteners in coffee and tea, in chewing gums, lozenges and medical drugs. Without doubt, the use of such sugar-free food items is a helpful tool in caries prevention.

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9 Sweeteners and metabolic disorders

N. FINER

9.1 Introduction

It is only recently that man has been exposed to a significant intake of sweetness in the diet. The pattern of sweetener use is rapidly changing; use of sucrose from cane and beet is giving way to corn-derived syrups (which contain fructose as well as sucrose), new nutritive sweeteners such as sorbitol and xylitol, and a rapidly growing use of intense, non-nutritive (or artificial) sweeteners such as saccharin and aspartame. This chapter considers the metabolic fate of this variety of sweet-tasting dietary components, how they may affect health, and the problems they pose for individuals already suffering from certain metabolic diseases such as diabetes mellitus and phenylketonuria.

9.2 Nutritive sweeteners

This term is used to describe those substances that when present or added to foods to provide a sweet taste also provide a source of energy (Table 9.1). In the main these are mono- or disaccharides (sugars) such as sucrose or fructose, or polyols (sugar alcohols) such as sorbitol or xylitol. A recent suggestion (Panel on Dietary Sugars Committee, 1989) that it would be helpful for dietary research to distinguish sugars intrinsic to the cellular structure of the food from those which are free or added would seem to add confusion. Some intense sweeteners such as aspartame do have a caloric value, but are used in such small quantities that they are effectively non-nutritive.

9.2.1 Normal metabolism of sugars and polyols

Much early work on the metabolism of foods necessarily relied on studying the fate of single doses, often unphysiological, of single substances, and provided the basis of most of our knowledge about food metabolism. However the physical state of food profoundly affects its digestion,

Table 9.1. Metabolic classification of sugars, polyols, and sweeteners. Brackets signify compounds insufficiently sweet for use as sweetener.

Nutritive		Non-nutritive	
Insulin-requiring		Non-insulin requiring	
Sugar component	Monosaccharides		
Glucose	Glucose		Aspartame
Starch	Glucose		Saccharin
[Maltose]	Glucose		Cyclamate
Sucrose	Glucose + fructose	Fructose	Acesulfame K
Corn syrup	Glucose + fructose	Sorbitol	Thaumatococin
[Lactose]	Glucose + galactose	?Lactitol	Alitame
		Maltitol	
		Xylitol	
		[Mannitol]	

absorption and subsequent metabolism, areas which have only recently have been addressed (Jenkins *et al.*, 1988). Even fewer data on how nutrients in a mixed meal interact are available (Macdonald, 1972). Consumption of sugars in the United States averages 91 g daily (21% of total calorie intake and nearly 50% of total carbohydrate intake); 41 g is from sucrose, 19 g from high-fructose corn syrup, 16 g from fructose and 15 g from lactose (Glinsmann *et al.*, 1986). Adults in the United Kingdom consume more sugars: 100–120 g daily, representing 20% of total energy intake, and half of which comes from non-milk added sugars (Panel on Dietary Sugars Committee, 1989).

9.2.1.1 Digestion and absorption. Starches and disaccharides are digested in the gut by enzymes secreted by the pancreas and gut. The completeness of digestion within the small bowel depends upon the food source: starch from rice is virtually completely digested, while 20% of starch from beans remains unabsorbed from the small bowel (Wursch, 1989). The monosaccharides released are absorbed across the intestinal wall by diffusion, active transport, or metabolism within the gut wall to lactate. The extent to which any individual food is absorbed depends upon its rate of gastric emptying and digestion, time of transit through the gut and the efficacy with which it crosses the gut wall into the portal circulation. As much as 50 g of sucrose in a single test dose can be completely absorbed; the same quantity of fructose as a 20% solution, but not as a 10% solution, is frequently malabsorbed (Ravich *et al.*, 1983).

Polyols (sugar alcohols) are less well utilised. Only about 75% of the total calorific value of sorbitol and xylitol (Beaugerie, 1987) and 60% of maltitol (Rennhard and Bianchine, 1976) are available, the precise

figures depending upon the methods used to estimate absorption, and the conditions of administration (Wursch and Anantharaman, 1989). Undigested polyols (or carbohydrates) are fermented in the large bowel by bacteria to metabolites, including carbon dioxide, hydrogen and methane. This results in abdominal pain, diarrhoea and flatulence if more than 30–50 g daily are consumed. As little as 10 g of sorbitol may cause such symptoms (Crapo, 1988).

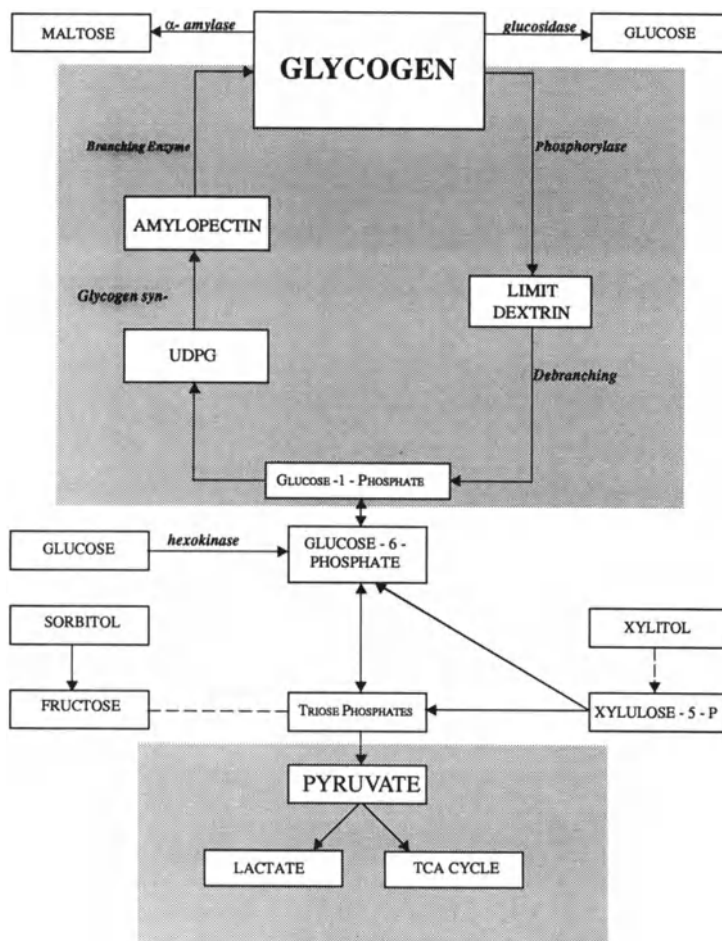


Figure 9.1 Glycogen and caloric sweetener metabolism. The shaded areas represent pathways that are regulated by insulin. Enzymes are in *italics*.

9.2.1.2 Metabolism. **Glucose** plays a central role in providing a readily available substrate for energy requiring processes within most tissues

(Figure 9.1). It is oxidised by the enzymes of the tricarboxylic acid cycle to carbon dioxide and water, thus releasing ATP. Glucose levels in blood are closely controlled. Glucose is taken up by the liver to form glycogen and fatty acids by insulin-stimulated glucokinase activity. Insulin also inhibits hepatic glucose output from the breakdown of glycogen and gluconeogenesis. Glucose stimulates insulin release from the pancreatic β -cell by direct interaction with insulin receptors. It has long been known, however, that when glucose is given by mouth it elicits a much greater insulin response than when given intravenously in amounts that produce an equivalent blood glucose level (McIntyre *et al.*, 1964). The term entero-insular axis describes these gut factors that enhance insulin release after a meal (Geekie *et al.*, 1981).

Of the various intestinal peptide hormones that influence the entero-insular axis, gastric inhibitory polypeptide (glucose-dependent insulinotropic polypeptide, GIP) is the most important and has recently been reviewed (Morgan *et al.*, 1988). GIP is only stimulated by carbohydrates after they have been converted to actively absorbed sugars, and not by disaccharides, fructose or polyols (Sykes *et al.*, 1980; Salminen *et al.*, 1982). GIP itself only stimulates insulin secretion from pancreatic β -cells when blood glucose levels are raised above fasting. This, for example, occurs following a mixed meal, but not after fat alone. The importance of the physical form of food on insulin secretion, first described by Jenkins *et al.* (1978), is in part explained by GIP secretion.

The re-discovery of Moskovitz's findings that blood glucose responses are not proportional to the carbohydrate content of food eaten (Moskovitz, 1937), has been refined into the concept of a glycaemic index (Jenkins *et al.*, 1951). The glycaemic index relates the increment in blood glucose after a standard quantity of a food, to a fixed quantity of standard comparator, either glucose, wholemeal bread or most recently white bread (Jenkins *et al.*, 1988). Clearly the post-absorptive metabolism of the carbohydrate ingested will also determine the food's glycaemic index, as will its rate of digestion and absorption.

Fructose is rapidly phosphorylated in the liver by the enzyme fructokinase, independently of insulin. Peripheral tissues lack the enzymes necessary for fructose metabolism. Fructose 1-phosphate is then split by aldolase to triose phosphates (glyceraldehyde, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate); these intermediates of the glycolytic-gluconeogenic pathway are converted in the liver to glucose and glycogen. Fructose can, in this way, significantly contribute to hepatic glucose output and so raise blood glucose levels and increase insulin secretion (Felber *et al.*, 1959).

About 30% of the triose phosphates formed are converted to pyruvate and lactate. Lactate can accumulate to cause acidosis, especially in liver disease, or under anaerobic conditions (Kreisberg and Wood, 1983).

Phosphorylation of fructose is an energy dependent step so that high substrate levels of fructose (in practice only usually seen with intravenous infusion) can deplete ATP levels, so interfering with adenine nucleotide catabolism (Maenpaa *et al.*, 1968) to raise plasma uric acid levels. This effect, however, is not usually clinically significant because the carrier system responsible for uptake from the hepatic circulation is saturable (Sestoft and Fleron, 1974). Studies of dietary fructose on uric acid have given variable results (Fox and Kelly, 1972; Macdonald *et al.*, 1978); over 2 weeks, a diet containing 20% of dietary carbohydrate from fructose failed to alter serum uric acid levels (Turner *et al.*, 1979). However, after 5 weeks, a diet which provided 7.5% of calories from fructose increased uric acid levels after a sucrose, but not a starch meal. This effect was more marked in hyperinsulinaemic men, although uric acid levels remained in the normal range throughout (Hallfrisch *et al.*, 1986).

Diets high in fructose may also increase serum triglycerides, more than when the carbohydrate source is starch, by increasing hepatic synthesis and release of triglyceride, and decreasing removal from the circulation by peripheral adipocytes (Nikkila, 1974).

Sucrose is digested to provide equal parts of glucose and fructose, and can therefore be considered in terms of the metabolism of both compounds. There is little evidence to show that sucrose aggravates post-prandial hyperglycaemia to any greater degree than comparable amounts of other carbohydrates (Bantle, 1989); it has a glycaemic index of 85–92%, unexpectedly lower than wholemeal bread (93–106%) or Weetabix (109%) (Jenkins *et al.*, 1988).

Sorbitol and **mannitol** are poorly absorbed from the gut; 70–80% can be recovered from the ileum. Little mannitol is metabolised (Nasrallah and Iber, 1969). In contrast, sorbitol is rapidly metabolised by the liver to fructose. All enter the liver cell independent of insulin. Dietary sorbitol does not appear in plasma, and even when infused directly into the circulation does not diffuse across membranes and so does not contribute to cataract formation (Gabbay, 1973), a common complication of diabetes mellitus.

Xylitol is rapidly converted to D-xylulose by polyol dehydrogenase, and then enters the pentose-phosphate shunt pathway to form glyceraldehyde 3-phosphate. These initial steps are not regulated by insulin; its further metabolism to glucose, glycogen or lactate is similar to fructose (Froesch, 1976). Because xylitol is slowly absorbed from the gut, it does not affect lactate homeostasis or have other unwanted metabolic effects at doses that can be consumed without side effects.

Maltitol is poorly digested and absorbed in the gastrointestinal tract (Wursch, 1989), and undergoes bacterial fermentation causing diarrhoea and flatulence at doses below those affecting plasma sorbitol or glucose levels to any significant extent (Secchi *et al.*, 1986).

Effects of sugars on blood glucose levels are of most importance to persons with diabetes mellitus, and will be discussed later. There have been a few studies of their chronic effects in healthy subjects. Fasting mean serum glucose and insulin in 19 healthy volunteers were higher after 6 weeks during which they were fed 30% of calories as sucrose compared to starch (Reiser *et al.*, 1979) but this was solely due to a fall in glucose levels on the starch diet. Two other studies found no significant differences in fasting glucose or levels when 24% (Crapo and Kolterman, 1984) or 33% (Bossetti *et al.*, 1984) of calories came from fructose compared to sucrose.

Non-enzymatic glycosylation of proteins, particularly haemoglobin (measured as glycated haemoglobin) is used as an index of overall blood glucose control in diabetes, and may directly reflect the adverse effects of elevated blood glucose levels on body tissues (Gonen and Rubenstein, 1978). Fructose more rapidly glycosylates haemoglobin than glucose (Bunn and Higgins, 1981), although plasma levels of fructose in man are probably too low for this to be clinically significant (Bantle *et al.*, 1986).

9.2.2 Nutritive sweetener metabolism in disease states

The metabolic basis for the various diseases affecting carbohydrate, lipid, protein and purine metabolism are well characterised, and allow a rational dietary approach to their management. It is to be emphasised that these diseases do not just include the esoteric disorders of glycogen metabolism, for example, but also include the commonest diseases to affect the populations of the 'developed' countries such as hyperlipidaemia and diabetes mellitus.

9.2.2.1 Diabetes mellitus. Diabetes mellitus is a syndrome characterised by hyperglycaemia (elevated blood glucose level) and the propensity of patients with the disease to develop specific clinical complications of microvascular disease damaging the kidney, retina and peripheral nerves. Macrovascular disease, affecting the circulation to the heart, brain and limbs, is also much commoner amongst diabetics than the normal population, and a major cause of mortality and morbidity (Garcia *et al.*, 1974).

Insulin-dependent diabetes mellitus (IDDM) is characterised by complete failure of the pancreatic β cell, with an absence of endogenous insulin production. The disease usually develops in childhood, or early adulthood and has a prevalence in the United Kingdom of about 0.2%. Patients with IDDM must take insulin, by injection, to survive; without it they die from the metabolic consequences of their inability to metabolise glucose, i.e. ketoacidosis.

Non-insulin-dependent diabetes mellitus (NIDDM) is a more common

disease affecting 1–2% of the population. It is strongly associated with obesity, which may act permissively on an underlying genetically determined metabolic disorder (Bennett *et al.*, 1976; Zimmet and Whitehouse, 1978), and a variable resistance to endogenous insulin. Thus, in the face of hyperglycaemia, insulin production may be high, normal or reduced. The mainstay of the treatment of NIDDM is dietary: the obese should reduce their weight to restore insulin sensitivity, and all should avoid foods (or meals) that rapidly supply glucose into the systemic and portal circulation. Dietary measures suffice to control the disease in many, so obviating the need for drugs that either increase pancreatic insulin output (sulphonylureas), or increase peripheral disposal of insulin (biguanides).

There is now general agreement that sugar is not a cause of diabetes mellitus (Glinsmann *et al.*, 1986; Panel on Dietary Sugars Committee, 1989), or impaired glucose tolerance (a more minor defect of glucose metabolism, not in itself associated with any disease, but which may progress to diabetes mellitus) (Anderson *et al.*, 1973; Thompson *et al.*, 1978), except in as much as it may contribute to obesity.

Control of blood glucose lessens the chances of an individual with diabetes mellitus developing complications of the disease (Tchoubroutsky, 1978; Skyler, 1979). Until the 1970s it was traditional to limit carbohydrate and eliminate refined sugar, in the belief that this would minimise hyperglycaemia (Joslin, 1928). Compliance with these diets was poor (West, 1973), and they were necessarily high in fat, if they provided sufficient energy. Concern about the effect of diets high in fat on the development of macrovascular disease (Panel on Diet, 1984) prompted a re-appraisal of dietary therapy, and for the past decade it has been advocated that carbohydrate, mainly in the form of polysaccharides, should be the main source of food energy for diabetics (American Diabetes Association, 1979; Nutrition Subcommittee, 1982). It is also now recognised that small amounts of sucrose (up to 50 g daily) taken with meals have no adverse effect on blood glucose levels. There is evidence that high complex carbohydrate diets may even improve glycaemic control (Simpson *et al.*, 1979a, b).

Patients with IDDM control their blood glucose by balancing the amount and type of insulin they inject to 'cover' their dietary intake. This is usually achieved by calculating the carbohydrate intake from carbohydrate exchanges (based on a food's carbohydrate content by weight rather than its glycaemic index), by using multiple injections of insulin, and increasingly now, by measuring capillary blood glucose at home with test strips and reflectance meters. This allows an artificial 'feedback loop' controlling blood glucose to be externally 'closed'. Well-motivated and well-instructed patients can achieve near-physiological control of their blood glucose levels.

Several short-term studies (Bantle *et al.*, 1983; Erkelens *et al.*, 1985; Vorster *et al.*, 1987) have shown that replacing a portion of carbohydrate in a single meal, or covering larger amounts of sucrose from either ice-cream (Nathan *et al.*, 1984) or cornflakes and banana (Weyman-Daum *et al.*, 1987), with small doses of short-acting insulin, is possible without any loss of glycaemic control. In one study, either 42 g of sucrose, fructose, wheat or potato starch, were incorporated into breakfast, together with 30 g rice starch. There was little difference in subsequent blood glucose levels (Figure 9.2) (Bantle *et al.*, 1983). Similar results have been shown for patients with NIDDM (Slama *et al.*, 1984; Erkelens *et al.*, 1985; Vorster *et al.*, 1987; Abraira and Derler, 1988).

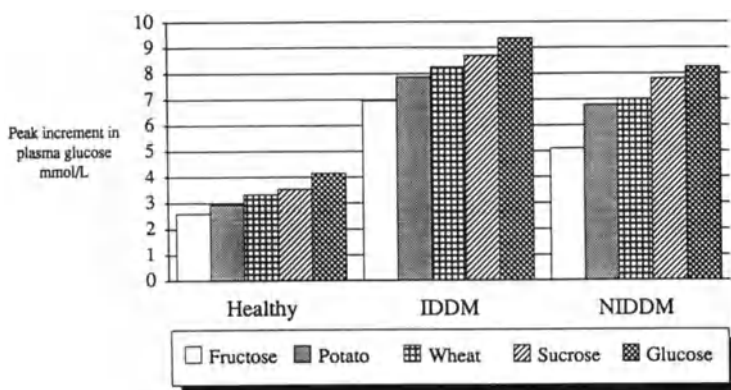


Figure 9.2. The glycaemic response to breakfasts containing 25% of calories from carbohydrates from various sources, in healthy ($n=10$), insulin-dependent diabetics (IDDM, $n=12$), and non-insulin-dependent diabetics (NIDDM, $n=10$). There was no significant difference in response between F,P,W,S and P,W,S,G for healthy controls, F,W,P,S,G for IDDM, F,S and S,P,W,G for NIDDM. Data from Bantle *et al.* (1983).

Longer-term studies of adding sucrose to the diet of diabetics for 8 days to 6 weeks, mainly have failed to show deleterious changes in blood glucose levels (Jellish *et al.*, 1984; Hollenbeck *et al.*, 1989). A diet of 60% carbohydrate+10% sucrose given to patients with NIDDM increased blood glucose, triglyceride and insulin concentrations compared to a diet in which 40% of total calories came from carbohydrate and 3% from sucrose (Coulston *et al.*, 1987). However the differences were small (1–2 mmol/l) and were, surprisingly, only apparent for a few hours after the midday meal; fasting blood glucose levels remained unchanged. Triglyceride and VLDL-cholesterol increased, however, while HDL-cholesterol decreased (see Section 9.2.2.2).

If sucrose in small amounts is no longer proscribed to the diabetic, what is the place of the nutritive sweeteners such as fructose and sorbitol? It would

seem attractive to use these sweeteners since they do not require insulin for their metabolism and therefore should not adversely affect blood glucose levels. However, as has been stated above, they do contribute to the body's 'glucose pool', and offer only a slight calorific advantage to the overweight patient with NIDDM. Furthermore their use is practically limited by their effects on the gut.

Single meal studies have mainly failed to show benefit for sugar substitutes in IDDM (Akerblom *et al.*, 1972; Arvidsson-Lenner, 1976; Bantle *et al.*, 1983) or NIDDM (Nikkila, 1974; Arvidsson-Lenner, 1976; Steel *et al.*, 1983). Short-term studies have given conflicting results. Substituting small amounts of fructose for starch into the diet of diabetic children was studied under home conditions; it did not alter glucosuria (Akerblom *et al.*, 1972). In another study, Pelkonen *et al.* (1972) found no change in fasting blood glucose or glucosuria in patients with IDDM, well or poorly controlled, given 75 g fructose in place of starch daily for 3 weeks. Providing 21% (an unrealistically high amount) of energy from fructose gave significantly lower blood glucose levels over 8 days compared to 23% sucrose or 100% starch diets (Bantle *et al.*, 1986), but patients were not allowed to change their insulin or sulphonylurea dosage—an unrealistic restriction in clinical terms. A 1-year study compared a 55% carbohydrate diet to diets in which 30 g starch daily was substituted for either fructose or glucose (Blayo *et al.*, 1988). No differences in body weight, mean, fasting, or post-prandial blood glucose or glycated haemoglobin levels were found. The authors of this unique long-term study concluded that substituting a moderate intake of refined sugars for starch had no adverse effect.

There are few studies which give useful information about the place of sorbitol or xylitol for diabetics (Brunzell, 1978).

It seems therefore that, at best, nutritive sweeteners may allow the patient with diabetes to add a modest amount of sweetener to the diet without influencing glycaemic control. The use of alternatives to sucrose are, however, limited by their tendency to cause diarrhoea if more than 30–50 g is consumed in a day. Furthermore they offer no caloric dilution advantage to the overweight diabetic. Indeed, to achieve the same texture of biscuits and chocolate, manufacturers in the past increased the fat content of foods sweetened with sorbitol and mannitol (Crapo, 1988), although this practice is no longer permitted in the United Kingdom.

9.2.2.2 Hyperlipidaemia. Suggestions from epidemiological data that sucrose may be causally related to the development of cardiovascular disease (Yudkin, 1964) have not been supported by expert committees that have reviewed the available data (Glinsmann *et al.*, 1986; Panel on Dietary Sugars Committee, 1989). Experimental data on the effects of sugars on lipids are controversial (Bantle, 1989); studies show either no

change or an increase in serum cholesterol or triglyceride levels in healthy subjects in whom sucrose replaced other dietary carbohydrates. There is similar disagreement in the literature for their effect on diabetics (in whom the increased risk of vascular disease remains an important clinical problem). Thus even when patients with IDDM or NIDDM ate 21% of their energy as fructose, or 23% as sucrose, or 100% as starch for 1 month (Bantle, 1989), there were no significant changes in fasting cholesterol or triglycerides or post-prandial triglycerides. Abaira and Derler had similar results when feeding diets with a 75-fold variation in dietary sucrose (from 3 to 220 g, but keeping total carbohydrate and fat constant) over 1 month to patients with NIDDM (Abaira and Derler, 1988).

9.2.2.3 Glycogen storage diseases. Glycogen storage diseases have an incidence of about 1:40,000 (Huijing, 1968) and are classified by their enzyme defect and clinical picture (Forfar and Arneil, 1984) (Table 9.2). Glycogen is a glucose polymer which is an important source of energy for most tissues. It differs from plant starches by its more branched structure which confers greater solubility. Figure 9.1 outlines glycogen metabolism and the enzyme defects associated with the clinical disorders known as glycogen storage diseases. In the liver, the first step of glycogen synthesis in the liver involves the phosphorylation of glucose by hepatic glucokinase; in muscle this conversion is catalysed by a non-specific hexokinase. Glucose 6-phosphate is readily interconverted with glucose 1-phosphate; the latter combines with the nucleotide uridine triphosphate to act as a substrate for the glycogen synthetase enzyme complex. Straight chains of glycogen are built up by α -1,4 linkages between glucose molecules. Branching develops by α -1,6 linkages between glucose moieties and is catalysed by a specific 'branching enzyme'. Glycogen breakdown requires a phosphorylase to hydrolyse 1,4-linked glucose molecules, and a 'debranching enzyme' for the 1,6 links.

Type I disease, due to glucose 6-phosphatase deficiency, presents at birth with hepatomegaly, and hypoglycaemia that fails to respond to glucagon or infusion of fructose or galactose. The brain, however, adapts to ketone body utilisation so that symptoms of hypoglycaemia are often absent. The inability to produce glucose rapidly leads to severe acidosis, hyperuricaemia and hyperlipidaemia. To maintain life, 2-hourly glucose feeds, glucose polymers and starch must be given, usually to provide 50–70% of energy needs (Francis, 1987). Galactose, fructose and sorbitol, and large quantities of sucrose and lactose must be avoided because they are metabolised to lactate (Fernandes, 1975) and so increase the risk of lactic acidosis developing.

Types III and VI 'debranching' enzyme deficiency give a milder clinical picture than Type I disease. Hypoglycaemia, hyperlipidaemia and hepatomegaly all occur because of the failure to break down

glycogen from the liver, but plasma glucose levels can be maintained by utilising other pathways. Amino acids, galactose and fructose can be all metabolised normally to glucose. A high protein diet, with frequent meals or drinks, with starch, especially at night, is usually successful at normalising growth.

Table 9.2. Classification of the glycogen storage disorders.

Type	Name	Clinical features	Enzyme defect	Diet
I	Von Gierke	Hypoglycaemia, hepatomegaly, lactic acidosis, hyperlipidaemia	Glucose 6-phosphatase	Frequent glucose + starch, avoid fructose, galactose
II	Pompe	Cardiomegaly, muscle weakness, death in infancy	Lysosomal α -glucosidase	Nil
III	Forbes	Variable hypoglycaemia, muscle weakness and hepatomegaly	Debranching enzyme	High protein + starch, to enhance gluconeogenesis
IV	Andersen	Portal cirrhosis, death in infancy	Branching enzyme	Nil
V	McArdle	Pain and stiffness on exertion, myoglobinuria	Muscle phosphorylase	Nil
VI	Hers	Hepatomegaly	Phosphorylase	High protein + starch
VII	Tauri	Pain and stiffness on exertion	Phosphofructo kinase	Nil
VIII	Huijing	Spasticity, decerebration, death in infancy	Phosphorylase	Nil
IX		Hepatomegaly kinase	Phosphorylase	Nil
X		Rare; hepatomegaly	Phosphorylase kinase	
XI		Hepatomegaly, renal tubular disease	?	
O		Mental handicap, hypoglycaemia	Glycogen synthetase	Frequent protein feeds

9.2.2.4 Fructose metabolism disorders. These have been reviewed by Froesch (1976). The most common disorder, hereditary fructose intolerance (HFI), is an autosomal recessive which affects about 1:20,000 live births. It results from an almost total lack of fructose 1-phosphate splitting activity by liver aldolase (Figure 9.3). Gluconeogenesis is still possible under fasting conditions by residual, although reduced, activity of fructose 1,6,-diphosphatase which converts triose phosphates to glucose. Fructose ingestion leads to an accumulation of fructose 1-phosphate which secondarily inhibits further phosphorylation of fructose by fructokinase, so

increasing blood fructose levels. Fructose also inhibits phosphorylase activity (decreasing glycogenolysis) and fructose 1,6-diphosphatase (decreasing gluconeogenesis) resulting in hypoglycaemia.

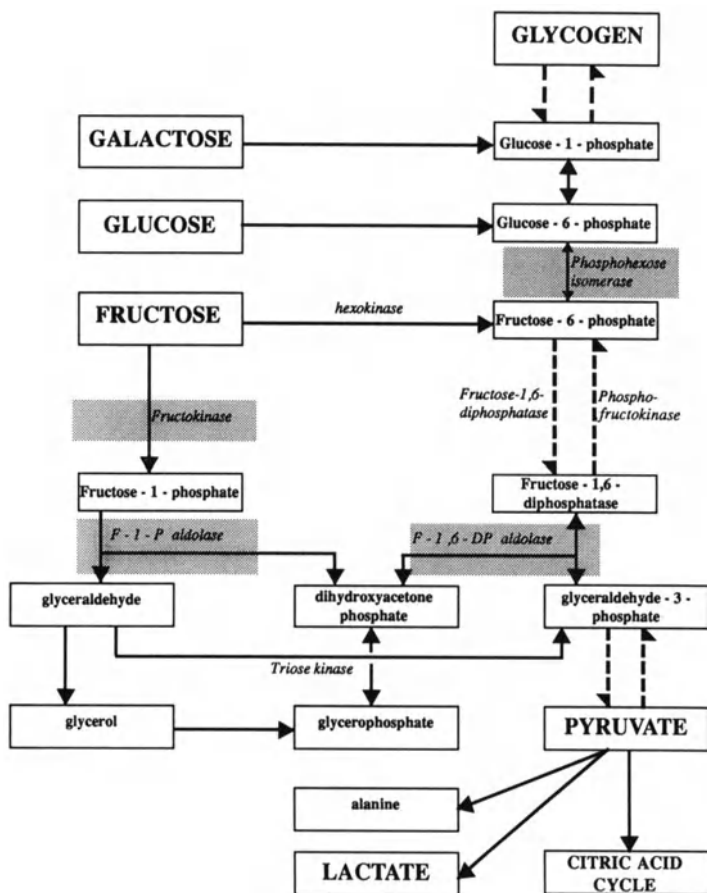


Figure 9.3. Fructose metabolism and its inherited disorders. The shaded areas represent the key enzymes which are defective in hereditary fructose intolerance.

The use of formula milk feeds that contain fructose in the form of sucrose means that the condition often now presents in babies during their first days of life. Breast-fed children present with the disease when they are introduced to sweetened drinks such as orange juice. Babies with HFI develop hypoglycaemia and vomiting within 30 min of a fructose-containing feed. They subsequently fail to thrive. Hepatomegaly, hyperbilirubinaemia

and later renal tubular dysfunction develop, probably secondarily to tissue damage resulting from ATP depletion.

Diagnosis may be difficult and delayed in neonates. It should be suspected in all newborns who vomit for no obvious reason and the urine tested for fructose, and blood for hypoglycaemia and hypophosphataemia. The diagnosis can be confirmed by challenging with intravenous fructose and demonstrating hypoglycaemia. Interestingly intravenous fructose does not cause vomiting, which is thought to be secondary to disordered fructose (and thus ATP) metabolism within the small bowel mucosa.

Treatment aims to eliminate fructose, and fructose precursors, from the diet. Affected children rapidly develop an aversion to sweet foods, and dietary adherence is not usually a problem.

Fructose 1,6-diphosphatase deficiency is a rarer autosomal recessive disorder, and presents with a similar clinical picture to HFI, but less acutely. Presentation is often precipitated by intercurrent infection, as a sick child with hypoglycaemia, lactic acidosis and hepatomegaly. If the diagnosis is suspected it is best not to challenge with fructose which may precipitate a deterioration; fasting, spontaneous hypoglycaemia, which does not respond to glucagon, followed by assay of fructose 1,6-diphosphatase activity in a liver biopsy will confirm the diagnosis. The condition is treated similarly to HFI by replacing dietary fructose, sucrose and sorbitol by glucose.

9.2.2.5 Other inborn errors of carbohydrate metabolism. A large variety exist and are described in the standard textbooks (Forfar and Arneil, 1984).

Lactase deficiency is an important and frequently occurring condition that may be congenital (Holzel *et al.*, 1959) or acquired. The inability to digest lactose (the main source of which is milk) results in diarrhoea, pain and weight loss. It may present in babies with all the above, and/or failure to thrive. In adults it may be more insidious. Treatment is to exclude lactose from the diet.

Sucrase-isomaltase deficiency presents with similar diarrhoeal symptoms to lactase deficiency, particularly after sucrose intake (Weyers *et al.*, 1961). Sucrose must be eliminated in the diet of children, but can be liberalised in older children and adults. Starch does not usually need to be restricted.

Glucose-galactose malabsorption is a rare disorder first described in Sweden (Lindquist and Meeuwisse, 1962). The intestinal brush border is deficient for the glucose and galactose transport system blocking absorption of these sugars. Fructose, however, is normally absorbed. Treatment aims to exclude glucose, galactose and all complex carbohydrates containing them from the diet, requiring specially formulated diets containing protein and fat.

9.3 Non-nutritive sweeteners

9.3.1 Aspartame

The accidental discovery in 1969 of aspartame's sweet taste (Mazur *et al.*, 1969) led to an investigation, on an unprecedented scale, of its mode of action, metabolism and safety. The first dipeptide ester, aspartame has generated whole families of related compounds, none of which yet has come to the market. It was approved for use in the United States in 1981, only after extensive investigation of its metabolism and possible toxicity, and considerable controversy. It has now been approved by the regulatory authorities of more than 70 countries.

Any substance taken in massive excess is likely to be harmful, and so the safety of food additives is based on the concept of an acceptable daily intake (ADI). This usually is at least 100 times less than the quantity that can be shown to have any ill-effect. For aspartame the ADI has been set at 50 mg/kg body weight daily (Food and Drug Administration, 1984), somewhat less than this 100-fold safety factor. The remarkable similarity of its taste to sucrose, together with very successful marketing has made it ubiquitous in modern manufactured foods and drinks. Despite its popularity, intake by adults and children remains well below the ADI (Heybach and Allen, 1988). A persistent rumble of largely anecdotal reports of adverse effects or possible toxicity from aspartame's three main metabolites has persisted ever since (Farber, 1990; Roberts, 1990).

9.3.1.1 Normal metabolism. Aspartame (L-aspartyl-L-phenylalanine *O*-methyl ester) is a dipeptide composed of two naturally occurring amino acids, aspartic acid and phenylalanine. Its metabolism and toxicology have been subject to detailed and extensive study in non-human primates and man (Opperman *et al.*, 1973; Ranney *et al.*, 1976; Stegink *et al.*, 1981, 1987, 1988; Matthews, 1984; Stegink, 1984, 1987). as well as in a variety of pathological conditions in man (Stegink *et al.*, 1979; Filer and Stegink, 1989). Like proteins, it is metabolised to amino acids, and thus has an energy value of 4 kcal/g. However, its intense sweet taste means that only milligrams are needed to sweeten foods and so it is effectively 'calorie-free'.

Aspartame is absorbed and metabolised in two ways. It may be hydrolysed in the gut by hydrolytic and proteolytic enzymes to release the aspartate, phenylalanine and methanol which are then absorbed to enter the portal circulation in the same way as amino acids and methanol derived from protein and polysaccharides (Opperman *et al.*, 1973; Ranney *et al.*, 1976; Matthews, 1984). Aspartame is also transported into gut mucosal cells to be metabolised to aspartate, phenylalanine and methanol within the cell.

About 10% by weight of aspartame is released as methanol, which is subsequently slowly oxidised to formaldehyde and formate by a variety of pathways. Consumed in quantity, methanol is a potent toxin causing metabolic acidosis and blindness (Tephly and McMartin, 1984). However, 200–500 mg/kg body weight of methanol is estimated to be necessary to produce any significant accumulation of formate, the metabolite which is responsible for toxicity (Food and Drug Administration, 1984); 600–1700 cans of diet drink would have to be consumed all at once to produce these levels. At aspartame intakes of 34 mg/kg body weight, blood methanol does not reach detectable levels (<0.04 mg/dl) (Filer and Stegink, 1989). Although blood methanol levels do rise at abuse levels of 200 mg aspartame/kg body weight blood methanol, they reach no higher levels than seen after other naturally methanol-containing drinks such as tomato juice, wine and brandy (Stegink, 1987). Formate production from aspartame is balanced by excretion so that levels in blood do not alter (Filer and Stegink, 1989) even in long-term studies (Leon *et al.*, 1989).

Aspartate levels do not rise in adults until after doses of more than 100 mg/kg body weight aspartame (Stegink *et al.*, 1988), and even after doses twice this, blood aspartate levels do not rise to potentially toxic levels (Stegink *et al.*, 1981). Aspartate has been shown to interact with monosodium glutamate to cause hypothalamic neuronal necrosis in rats (Reynolds *et al.*, 1976), but in man the combination does not raise levels above that seen after a protein meal.

Phenylalanine increases in concentration in blood after aspartame ingestion (Filer and Stegink, 1988) and is utilised in the liver for protein synthesis or metabolised to tyrosine by phenylalanine hydroxylase (phenylalanine-4-monooxygenase) (Figure 9.4). It, as well as other amino acids (including its metabolite tyrosine) are taken up across the blood-brain barrier and act as substrates for neurotransmitter synthesis (for reviews see Harper, 1984; Pardridge, 1988). Disorders of phenylalanine metabolism are well recognised, and much research has been directed to the effects of phenylalanine from aspartame use in these groups of individuals (Anon., 1988).

9.3.1.2 Phenylketonuria. Phenylketonuria was one of the classical 'inborn errors of metabolism' first recognised by Folling (1934). He discovered the presence of large quantities of phenylpyruvic acid in the urine of mentally defective (as they were then termed) patients. It is now known that phenylpyruvic acid is excreted because of an inherited defect or absence of the gene coding for the synthesis of phenylalanine hydroxylase. Several variants of PKU are now also well described, which involve alterations in the enzymes that synthesise tetrahydrobiopterin needed as a co-factor for phenylalanine hydroxylase. The main clinical features of phenylketonuria

(PKU) probably arise from both the toxic effects of phenylalanine and its metabolites, as well as tyrosine deficiency. Low levels of tyrosine deplete the brain of dopamine and noradrenaline. Tyrosine is also a precursor for melanin, and adrenaline and noradrenaline synthesis in the adrenal medulla.

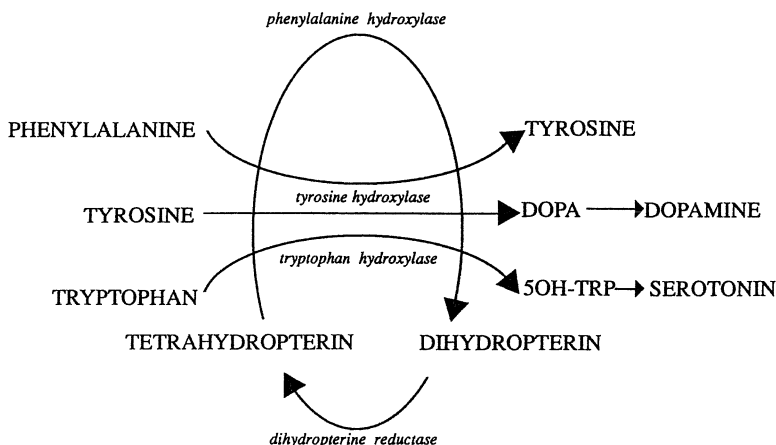


Figure 9.4. Metabolism of phenylalanine and other aromatic amino acids.

PKU sufferers appear normal at birth, but, if untreated, become developmentally retarded in early childhood. They have a diluted skin and hair colour (from melanin deficiency), microcephaly and commonly have seizures. Because the gene is common, about 1 in 60, all children are screened for hyperphenylalaninaemia 6 days after birth, by the Guthrie test. Early detection of homozygotes allows a protein-restricted diet to be instituted, with the aim of keeping the blood phenylalanine levels between 0.1 and 0.24 mmol/l (2–4 mg/dl), until the age of 10, below 0.7 mmol/l (11.6 mg/dl) until age 18 and below 1.2 mmol/l (20 mg/dl) thereafter (Watts, 1987). Heterozygotes for PKU have no symptoms, despite slightly elevated blood phenylalanine levels. They do show exaggerated plasma phenylalanine responses to oral phenylalanine loading.

It can be appreciated that aspartame represents a significant dietary source of phenylalanine. PKU homozygotes will know their diagnosis and their need to avoid protein foods or drinks containing phenylalanine, as well as those sweetened with aspartame. Much research has been concerned with the possible adverse effects of aspartame in heterozygotes for PKU, especially in pregnancy, since they metabolise the phenylalanine portion of aspartame less well than normal.

Phenylalanine was given to 8 subjects heterozygous for PKU and 12 normal subjects at a dose of 34 mg/kg. This dose was chosen as representing the 99th centile of projected 24 h ingestion of aspartame if used to fully replace dietary sucrose on an equivalent sweetness basis (Stegink *et al.*, 1979). Since aspartame cannot replace sugar in baked or cooked foods (because it is unstable at baking temperatures), this is probably a considerable overestimate of the likely 99th centile of consumption. Mean phenylalanine levels reached 16.0 ± 2.25 $\mu\text{mol/dl}$, significantly higher than 11.1 ± 2.49 $\mu\text{mol/dl}$ in normal subjects. At abuse doses of 100 mg/kg, heterozygotes had plasma levels that were double that of normal subjects (41.7 ± 2.33 versus 20.2 ± 6.77 $\mu\text{mol/dl}$) (Figure 9.5), but still well below the range associated with neurotoxicity in PKU. Others have confirmed that aspartame does not result in excessively high blood phenylalanine levels in PKU heterozygotes (Caballero *et al.*, 1986), nor result in accumulation after 21 weeks of 1800 mg/day (Koch *et al.*, 1976).

Despite the evidence that blood phenylalanine levels do not reach toxic levels, there has been speculation that increasing plasma phenylalanine could lead to alterations with aspartame that could have neurochemical consequences (Wurtman, 1988). Phenylalanine competes with other large neutral amino acids (LNAA) for uptake into the brain (Pardridge, 1988). Thus the rate of tryptophan and tyrosine entry to the brain, and their subsequent availability as precursors for synthesis of the neurotransmitters

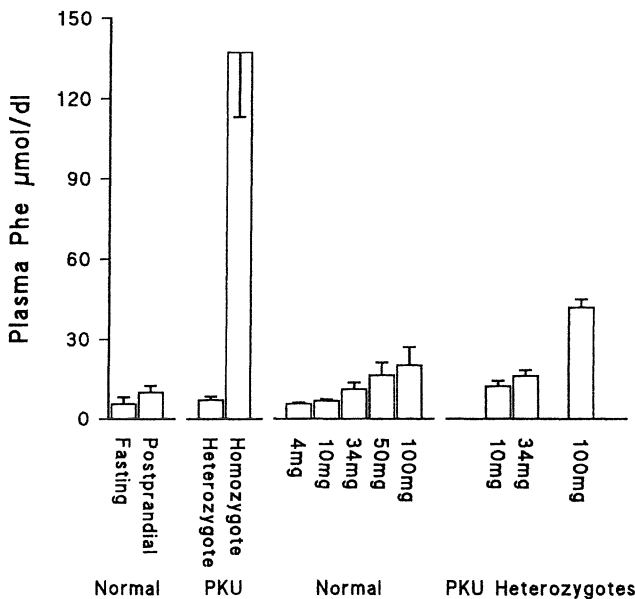


Figure 9.5 Plasma levels of phenylalanine (mean \pm SD) in normal subjects and homozygous phenylketonurics, and in response to varying oral doses of aspartame in heterozygous and normal subjects. Data from Caballero *et al.* (1976), Stegink (1984 and Stegink *et al.* (1987).

serotonin, dopamine and noradrenaline, depend not on each amino acid's absolute blood level, but on its concentration relative to the competing amino acids. It has been suggested that although the absolute level of blood phenylalanine after aspartame may be no higher than after a meal, the absence of competing amino acids liberated from protein in the meal may cause a much higher phenylalanine/LNAA ratio. A similar, or enhanced effect could occur after meals containing carbohydrate meals, from the fall in plasma LNAA due to their uptake into liver and muscle mediated by insulin release (Wurtman, 1983).

Animal studies have failed to confirm that this effect significantly alters neurotransmitter levels (Fernstrom *et al.*, 1986) or more importantly neuronal function (Garattini *et al.*, 1988). In man, direct measurement of brain biochemistry is clearly not possible; however most studies have failed to find behavioral or cognitive changes following aspartame ingestion (Leiter *et al.*, 1988; Lieberman *et al.*, 1988).

9.3.1.3. Diabetes mellitus. As discussed under the heading of nutritive sweeteners, there is no overwhelming necessity to replace moderate amounts of sucrose, provided that the sucrose is taken as part of a meal. However, there is a widespread belief by mothers of diabetic children, that artificially sweetened foods will help their children maintain a 'diabetic diet' (Court, 1976). A survey in 1977 found that 91% of all diabetic patients were using saccharin (Committee for a Study on Saccharin, 1978). Few diabetics are prepared to go without any sweet foods (Mehnert, 1978).

In the main, the issues over the use of aspartame by diabetics do not differ from those concerning individuals without diabetes (Crapo, 1988). Not surprisingly, perhaps, a study of 43 adults with NIDDM found no change in fasting blood glucose levels when 1.8 g of aspartame was given daily, divided into capsules given with each meal, compared to placebo (Stern *et al.*, 1976). There were no unwanted effects. Other studies, reviewed by Horwitz (1984), have shown that aspartame taken in this way has no significant metabolic effects in diabetics. There is little data, however, on the effects of aspartame when used as a sweetener. There are no data to answer the question of whether the use of aspartame (or other non-nutritive sweeteners) improves dietary compliance or blood glucose control.

9.3.2 Other non-nutritive sweeteners

Saccharin, cyclamate and acesulfame are less widely used now. While concern about possible toxic effects of the first two compounds remains, there are no known metabolic conditions that preclude their use.

9.4 Conclusions

A variety of metabolic disorders determine the acceptability and safety of both nutritive and non-nutritive sweeteners. Sugars have become a major macro nutrient in the human diet, and sweetness something that most can freely enjoy. Like all enjoyable things there is a danger for puritanism to limit their use. There is however little scientific evidence to support such a stance. However the converse is also true, that is that there is little evidence of benefit from replacing dietary sucrose, in most cases. The words of the old music hall song are perhaps relevant: 'A little of what you fancy does you good'.

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10 Sweeteners and body weight

D.A. BOOTH

10.1 A behavioural issue

Sweeteners and body weight are both matters of the (bio)chemical sciences—the compositions of foods and of human tissues. Yet the chemistry and biology on their own can make no sense of the relationships between sweeteners and body weight. Even when we take into account food and drink sales statistics or dietary surveys, we lack the information needed to ascertain how or even whether uses of intense or bulk sweeteners affect body weight. We also have to find out the scientific facts about how people actually behave towards sweeteners and weight. These depend not only on effects of sweetener consumption on the body, but also on how people feel and think about those effects, about these and other food constituents, and about their own bodies.

Body weight is little if at all affected by the cumulative results of diverse behaviour in total amounts of sweeteners consumed, whether calorific bulk sweeteners or the intense sweeteners carrying few or no calories. Rather, the evidence is that the role of sweeteners in weight depends on the individual consumer's timing of ingestion of sugars and hence of their low-calorie substitutes.

10.1.1 The chemistry of sweeteners

The chemistry and food technology of bulk and intense sweeteners are dealt with elsewhere in this book. However, we should start by challenging some vague but pervasive assumptions about the chemistry of sweetness, because these influence attitudes in the industry (and among health professionals and the general public) to the roles of sugars and artificial sweeteners in obesity and slimming.

There is an extraordinary contrast of structure between glucose or other sweet sugars and the intensely sweet aromatic sulphamine derivatives, saccharin and cyclamate. Remarkably sweet proteins and peptides have also been discovered, including the substituted dipeptide aspartame.

Some amino acids and peptides are very bitter; saccharin also can have

a bitter side-taste. We have hypothesised that these chemical associations between sweetness and bitterness are no coincidence but they have biological significance that depends crucially on behaviour (Booth, 1990a; Booth *et al.*, 1987). This biological function of sweetness would not fit at all with some commonly assumed roles for low-calorie sweeteners in weight control.

The structure/activity relationships illustrated above for sweetness receptors make no sense as a biological system to detect sugars. The usual notion is that the reason why we have sweetness receptors and a liking for what stimulates them is in order to get us to eat calories; this is also biologically most implausible. Nobody has yet specified a substantial reproductive advantage that our ancestors could have gained from eating fruit only when it was ripe, let alone from challenging their immune systems with bees' stings during honey hunts (Booth, 1990b)! This consideration also undermines the view that the liking for sweetness is liable to contribute to obesity nowadays wherever sugary foods are commonly available, on the grounds that the only conceivable use in the past for a sweet tooth was somehow to enable deposition of fat that nourishes a foetus through the winter or improves the chance of surviving to breed more later.

Some realistic biological and behavioural science may make a lot more sense out of the chemistry of sweeteners. Human beings and other omnivorous mammals are born with a reflex mechanism in the brainstem by which sweeteners facilitate ingestive movements (Chapter 1). This congenital preference is unique to the sweetness receptor(s). Yet, as Conner shows in Chapter 1, the mature liking for sweetness in foods and drinks is not at all unique to that sensory characteristic and, furthermore, the learned food-sweetness preferences completely suppress the biologically endowed sweetness preference reflex. This entirely acquired character of the preferences for sweetness and all other salient characteristics of familiar foods also applies to other omnivores (Booth, 1985). Why then are we born with these strange sweet receptors, attached to a distinctive reflex? The suggestion is that they never have conveyed advantage on the genes of adults or even of children but that the newborn suckling needs them, for the following reasons (Booth, 1990a).

Weaned omnivores, when foraging on their own, must be able to avoid toxic doses of plant alkaloids. This presumably is the function of the bitter receptors, which are strongly stimulated by nitrogenous compounds and are linked to reflex expulsive movements of the mouth.

Yet mother's milk is rich in essential nitrogen, in the form of protein, peptides and free amino acids and amino sugars. The suckling may therefore be at risk of spitting out its sole source of nutrition, unless some receptor(s) for amino acids counter the alkaloid receptor(s) (and indeed also the aversive reaction that occurs to dissociated carboxylic acid groups and regurgitate hydrochloric acid).

This amino acid receptor thus has to be specific for aliphatic C—OH groups in close proximity to moderately polar groups such as C=O and N—H. Then this sweet receptor must compete in the suckling infant with the bitter receptor needed in later life. It could do this by peripheral and/or central interaction of its stimulation with stimulation of bitterness receptors (what the psychophysicists call 'mixture suppression'). It may also compete behaviourally; the bitterness rejection reflex could be countered by means of an acceptance reflex attached to the amino acid receptor. The cat family do not have a sugar preference; consistently with our hypothesis, these species are obligate carnivores and so do not need to avoid poisonous plants.

10.1.2 The biochemistry of body weight

Highly mobilisable lipid deposits in abdominal adipose tissue, an aspect of the chemical composition and metabolism of the body, are more important for this chapter than simply body weight. The widespread interests that connect sweeteners and body weight are slimming and avoiding getting fat, i.e. reducing or keeping down the content of triacylglycerols in adipose tissue.

Yet this biochemistry also plunges us immediately into psychological science. For we cannot adequately relate sweeteners to the control of adiposity without allowing for what people believe and wish for about their body weight, shape and composition, and what they do to try to obtain it. These are objective facts determinable only by measurement of the mechanisms through which consumers perform in the mental and behavioural tasks they face in dealing with foods and beverages.

Often what concerns people is not so much their weight as their appearance, the size and shape of parts of the body or of the clothes that fit it. Changes in the amounts of fat subcutaneously and in some deeper depots can affect body shape substantially, although muscular changes may also contribute. Nevertheless, except in those who are seriously underweight or overweight, most aspects of shape and size come from structures of bone, muscle and fat that are virtually unalterable. In particular, mature women have fat deposits in the upper leg that seem to mobilise only during lactation (Rebuffe-Scrive *et al.*, 1985). Thus, one must question the good sense of efforts to change from the normal pear-shaped tendency of the female figure or from a chunky shape at a normal weight in either sex, whether by the use of intense sweeteners or by any other means.

A rotund, apple-shaped physique is another matter, however. Its social significance for physical attractiveness and self-esteem varies with time

and place (probably largely according to the general level of affluence). However, the medical significance appears to be relatively constant, at least in north European stock: larger deposits of fat in the abdominal region are associated with premature death from degenerative disorders such as diabetes and heart disease, especially in younger men. The waistline readily spreads out, especially if physical activity decreases and sociable eating increases, as it tends to in our society from the 30s or even in the 20s age range. This fat is turning over metabolically as much as any, so it may well encourage the circulation of fats, which are readily converted to deposits on the walls of arteries such as those that supply blood to heart muscle. By the same token, this fat can be lost more readily than some, and indeed its deposition prevented in the first place, to reduce the risk to the heart.

Weight relative to height, in particular Quetelet's Body Mass Index (BMI, weight in kilogrammes divided by the square of height in metres), has been the most common and convenient way to monitor obesity, i.e. adiposity in sufficient excess to be a risk to health ($\text{BMI} > 27$ or thereabouts). Of course, if there was unusually great muscle development, as from body building for example, a high BMI would not indicate obesity (at that time at least). Even excluding such cases, however, neither BMI nor desirable weight for height is a very good measure of body fat content, although even results from specialised laboratories using densitometry or fat-insoluble isotopes are hardly precise. There is some prospect of satisfactory and more widely usable measures based on electrical impedance, but at present the general public (and the epidemiologist) has only the bathroom scales and the tape measure.

Since abdominal fat deposits seem to be the component of body weight that is the main risk to health (Björntorp, 1987), inches read off a tape should probably be taken more seriously in health promotion: men beware who have a longer waistline than hipline! Weighing may be favoured because it is thought to be a more sensitive measure than measuring the waist or a visible roundness. All the talk about weight control encourages such an attitude. It may also be reinforced by the ups and downs read off the scales from day to day or week to week. That impression can be delusory, though, because of short-term changes in water balance or differences in clothing or timing relative to eating or excretion. Waistline self-measurement can be made unreliable, however, by the perhaps almost unintentional behaviour of tightening the tape or even pulling in the muscles. Also, the desirable ratios of waist and hip circumferences are so far based on fewer statistics than the weight-for-height criteria. Nevertheless, long-term changes in waistline are the nearest we are likely to get to a domestic measure of the health-relevant fat content of body tissue.

10.1.3 The psychology of sweeteners and body weight

The primary issue for this chapter is therefore whether any human uses of sweeteners can under any specifiable conditions affect fat storage in the body, in practice or in principle. This of course goes far beyond the reach of food science and technology and indeed of market data. Moreover medical research does not provide the answers either. Unfortunately, the struggle to identify the relevant behaviour, thoughts and feelings is still in its early stages, because few psychologists have addressed the combination of chemical, physiological and cognitive issues and, in Europe at least, neither the food nor the health establishments recognise the central role of behavioural science.

10.1.3.1 Population associations. Epidemiological analyses, whether between populations or among individuals, are ill-equipped to address questions about sweeteners and weight, even when augmented by adequate dietary assessments. Choices between bulk and intense sweeteners are certain to be affected by the user's perception of his or her own fatness. This will undermine an interpretation of any observed associations of intake with body weight as the effects of sweeteners on weight.

As it turns out, sugar intake when separated from intake of fat (and of intense sweeteners) has no association at any level with obesity, while higher intakes of intense sweeteners have if anything been associated with greater body weights (Garn *et al.*, 1980; Richardson, 1972; Walker *et al.*, 1982). The strong fattening image of sugar (Booth and Blair, 1989) should make higher body weight strengthen the tendency to avoid sugar. The efforts of the heavier people to slim by drinking and eating 'diet' products should also increase their intake of intense sweeteners. So these associations are no more evidence for an appetite-stimulating effect of saccharin than they are for a slimming effect of sucrose! To the contrary, they are rather good evidence that fatter people's current ways of avoiding sugar and using low-calorie sweeteners do little if anything to help them lose weight (Booth, 1987). This interpretation is supported by the lack of clear association between weight changes and intense sweetener intake (then mainly saccharin) in careful analyses of prospective data from a large number of middle-aged women in the United States (Stellman and Garfinkel, 1988).

10.1.3.2 Laboratory effects. There have been suggestions that the sensory effects of sweetness may have physiological effects that influence energy intake or expenditure and hence body weight. Yet even if such effects of sweetener consumption on appetite or thermogenesis are one day clearly demonstrated in the laboratory, the consequences for weight in everyday life would still need to be ascertained by analysis of the outworkings of such

mechanisms during spontaneous behaviour over the long term. Moreover, most of the methods used to seek evidence for physiological effects of sweeteners on intake have been inadequate to exclude all the psychological mechanisms that may be involved. Obviously it will always be difficult to find adequate laboratory models of normal human behaviour or to collect data from real life that can be used to test hypotheses. The most serious limitation at present on either of these approaches, nevertheless, is poor conceptualisation of candidate mechanisms and hence of the measurements needed to test them, whether in the laboratory or in the field.

The scientific question is whether the sensing and assimilation of any bulk or intense sweeteners act via identifiable processes to affect human eating behaviour, or on thermogenesis or physical activity, in a way and to an extent that reliably yields substantial changes in a person's balance between energy intake and energy expenditure in everyday life.

Energy expenditure. Metabolic effects of sweeteners can affect body fatness only if they affect the balance of the body's energy input and output. Effects on fatty acid synthesis or circulating triglycerides are not relevant to obesity or slimming unless a change in energy output results.

From this thermodynamic point of view, sugars would be as advantageous as starches to replace fat in the diet, because both sorts of carbohydrate are converted into storage triglyceride less efficiently than fat.

There has been one report claiming that the palatability of a meal had a thermogenic effect (LeBlanc and Brondel, 1985); however, size of meal was confounded with palatability and such effects have proved hard to replicate (Weststrate *et al.*, 1990).

Energy intake. Increases in appetite ratings or food intake for some hours after consuming a low-calorie sweetener have been found in some (Rogers *et al.*, 1989) but not all (Birch *et al.*, 1989) comparisons of sweetened and unsweetened foods eaten in the laboratory. However, to date such studies have not included measurements of mechanisms in the mind or in the body that might mediate effects lasting hours. Thus there are no data to explain the differing reports of experimenter-induced effects of sweetness, let alone to provide the requisite theoretical basis for extrapolating experimental results to specified conditions in everyday life (Booth, 1988).

The mere timing of an effect of sweetness provides no basis for attributing it to cognitive or physiological mechanisms, nor to inhibitory (satiating) or excitatory (appetising) reactions rather than to effects on intentions to restrain intake or even to gain extra nutriment. The only way to determine a mechanism of action is actually to measure valid and sensitive indexes of potential causes and to observe a pattern that implicates some mediating processes and excludes other candidates (Booth, 1990c). A so-called balanced experimental design, crossing high and low sweetness with

high and low calories in an acceptable food, does not isolate effects of sweetness from those of carbohydrate energy. This is because neither the interaction between high sweetness and perceived lack of calories nor that between low sweetness and the satiating effect of high calories need be summative, at either physiological or cognitive levels. Cognitively, calories without sweetness is likely to be a familiar phenomenon (as with starches for example) while, for some at least, sweetness without calories may be quite unexpected in a food.

Behaviour is strongly influenced by what people believe and expect. The name of a food and its taste (in both strict and loose senses of that word) trigger expectations that may or may not be matched by the effects of its assimilation over minutes or even hours after consumption. Furthermore, the effects of what the subject supposes to be the caloric intake (Polivy, 1976) on subsequent food intake are highly contingent on the eater's current or chronic participation in the practices, attitudes and emotionality of dieting (Herman and Polivy, 1980).

Cognitive mechanisms capable of stimulating long-term food intake clearly exist. Indeed, Hill *et al.* (1989) have observed a well-known example of their short-term manifestation—the laboratory phenomenon known as 'breakdown of dietary restraint' (Herman and Mack, 1975). The conditions for this effect are currently under intensive re-evaluation and are proving more complex even than initially envisaged (Herman, 1978). The 'breakdown' is a stimulatory effect on shortly subsequent intake by the consumption of a snack thought by the eater to be high in calories; this contrasts with the intake suppression, or 'cognitive satiety' (Wooley, 1972; Booth, *et al.*, 1982), that can be obtained in many people. The increase in intake relative to no snack or to a supposed low-calorie snack may be most likely to occur in someone who is on a diet and perhaps has the attitudes of a chronic dieter. Such dieters, having been inveigled by the experimenter (as may also happen in real life) into eating a strongly sweet food, for example, may well thenceforth give up on their diet and begin consistently to eat considerably larger amounts of food. This would be a long-term effect of cognitive interpretation of the intensity of sweetness in the food, not some highly protracted physiological after-effect of the sweet taste. The identification of such a mechanism for a stimulating effect of sweetness on appetite requires assessment of not only the subject's dieting attitudes and status but also the changes in expectations and evaluations of intake later in the day that may be occurring as a result of the experiences in the laboratory.

There is clear evidence that, when no sweetness-calorie expectations are operative, low-calorie sweeteners do not stimulate spontaneous intake of familiar foods in the subsequent hours. In several studies of both normal-weight and obese people in good health, supplied with a high-sugar diet under conditions in which all food and drink intakes could be exactly

measured, Porikos and Van Itallie (1984) found that the groups' average quantities of foods consumed remained at the baseline level for the first day that sugar was covertly replaced by aspartame. Intake did increase in subsequent days but no more than would be expected from the loss of calories in main meals as a result of sweetener substitution (Booth, 1987, 1988, 1989a).

10.2 Mechanics of action of sweeteners on intake

10.2.1 Intake of plain saccharin or sugars

Stimulation of intake by sweetness is not a very robust phenomenon. Booth and Quartermain (1965) tested a brain mechanism of eating for a susceptibility to the taste of saccharin similar to that thought to be shown in the obesity classically produced by hypothalamic lesions. It is remarkably difficult to get a rat to eat more of its ordinary food by sweetening it with saccharin at any concentration, as Sclafani and Toris (1981) emphasised when re-investigating this phenomenon. This is despite the fact that rats (like us) have an innate ingestive reflex to the taste of saccharin and sucrose (Grill and Norgren, 1978) and will drink, choose and work for the taste of pure saccharin solution with great enthusiasm and persistence.

Furthermore, rats drink enormous volumes of a 'cocktail' of saccharin and dilute glucose (Valenstein *et al.*, 1967). The glucose may mask a bitter note in saccharin and the innate attractiveness of sweetness is reinforced by metabolism of the glucose (Booth *et al.*, 1972). Nevertheless, these rats do not get fat. Energy expenditure required of the tongue and the kidney may not be negligible. However, much or all the regulation of null energy balance is attributable to caloric compensation by cutting back on intake of maintenance food. The prompt metabolic satiety from rapidly absorbed sugar rather precisely suppresses the rat's caloric intake, both by delaying meals and by reducing their size (Booth, 1972a). (Note that this classic effect illustrates that there is a single behavioural concept of a state of satiety and a process of satiation and that it must refer to any inhibition of eating by any effect of food, not to a particular mechanism in consciousness, brain or body nor to effects either only on intervals between meals or only on sizes of meals, contrary to many recent definitions and models.)

It is also much more difficult to fatten a rat by giving it a sugar solution to drink than by giving it access to a metabolisable oil or fat or indeed bread (Rogers and Blundell, 1984) or low-glucose maltodextrin (Sclafani, 1987). The attractiveness of the sweet taste to a naive rat is countered during the drinking of hypertonic sugar solutions by an appetite-suppressing distention of the duodenum (McCleary, 1953; Booth, 1972a). In addition, a sort of

boredom limits the amount of drinking of the same taste (Mook and Wagner, 1988). The basic satiety mechanism of hepatic oxidation (Booth, 1972a, b; Booth and Jarman, 1976; Tordoff and Friedman, 1986) is also operative.

Human subjects show the same satiety or even nausea generated by osmosis into the gut (Cabanac and Fantino, 1979), the same taste-specific loss of interest in repeated tastes of sweetness (Wooley, *et al.*, 1972), and probably the same metabolic satiety based on energy supply to the liver (Booth, 1981; Booth *et al.*, 1970, 1976, 1982). There are thus several immediate feedback mechanisms preventing large intakes of high-sugar foods, even if their sweetness is highly attractive, at least initially.

10.2.2 Acquired dislikes and likings for sweetness

Nevertheless, the main influences on food intake are acquired from personal experience. If we drank concentrated sugar solutions, we presumably would learn within a few drinks to dislike whatever strength of sweetness is associated with osmotic bloat, just as the rat does (Booth *et al.*, 1972). Furthermore, dissolved or fast-dissolving sugars rasp the throat as well as cloying rapidly.

The more usual sugary foods, however, are consumed in physiologically and socially rewarding contexts (Booth, 1985, 1990a; see Chapter 1). Even the rat is rapidly conditioned to prefer the particular intensity of sweetness (or other taste or aroma) which is associated with the rapid assimilation of metabolisable energy, such as from ethanol (Sherman *et al.*, 1983) or from carbohydrates that do not produce osmotic effects in the gut (Booth *et al.*, 1972; Tordoff and Friedman, 1986; Sclafani, 1987). In exactly the same way, when people choose between two flavours associated with the effects of disguised higher and lower concentrations of maltodextrin ingested on different occasions before lunch, they prefer the flavour of the more concentrated carbohydrate (Booth *et al.*, 1982; Booth and Toase, 1983).

Thus a liking for strong sweetness, even in combination with other sensory features, can justly be regarded as unsophisticated. It is in fact literally infantile, because the contribution of sometimes quite moderate levels of sweetness to the palatability of many ordinary foods and drinks, like the flavour principles of any familiar cuisine (Rozin, 1981), is motivation that is acquired by experience. Plain sweetness indeed is rather tedious: pressed-sugar confectionery had to have another tastant and perhaps colour or aroma added, and table sugar is always added to a more flavoursome beverage or to an interestingly textured food.

10.2.3 Context-dependence of acquired sensory preferences

After the consumption of at least some types of foods, the rat will show a carbohydrate-conditioned flavour preference only on re-instatement of the degree of gastrointestinal distension during which it had learned the preference (Booth, 1972c; Gibson and Booth, 1989). Similarly, people whose preference for the flavour of a food had been conditioned by maltodextrin before lunch showed that preference only before lunch, not when they were tested after lunch, and *vice versa* for another group of people who had learned after lunch (Booth and Toase, 1983).

Thus, we are built not only to learn automatically from earliest life (Harris and Booth, 1988; Harris *et al.*, 1990) to like the particular combinations of sensory characteristics in the cuisine presented to us, but we are also built to acquire the desire to eat or drink those materials in the usual bodily or social context (Booth, 1990d). Thus we elaborate situational appetites or dietary habits throughout adulthood (Booth *et al.*, 1982).

Rats even learn to start eating on presentation of a signal that has been paired repeatedly with food (Weingarten, 1984; Weingarten and Martin, 1989). Quite young children begin to recognise that what adults regard as breakfast foods are more appropriate at breakfast-time (Birch *et al.*, 1984). Many of us have learned to like better at the appropriate time certain drinks and foods (sweets, both desserts and confectionery) that are commonly served at the ends of meals and between meals, and not necessarily to like in those contexts the sweet vegetables and fruit that fit other habits (Conner and Booth, 1988; Conner *et al.*, 1988; and Chapter 1). Hence, where sweetened drinks and foods are provided during breaks between meals, then people will tend to crave such items at such times.

This is not a carbohydrate craving, specific to sugar (and starch). It is a liking for commonly experienced snackfoods and drinks, whatever they may be in our culture (Booth *et al.*, 1987; Conner and Booth, 1988). If people are deprived or deprive themselves of what they are used to, they are liable to crave the missed experience when they remember it. This is even more likely if someone is emotionally wrought. There is therefore no good reason to assume that a wish for familiar (or strange) foods reflects any verbally uninstructed, innate or learned ability to select foods containing a nutrient that we need (Booth and Gibson, 1988).

10.3 Theoretical implications for body weight control

These well-established and mostly long-known behavioural mechanisms can be used to predict everyday uses of sweeteners that could have an impact on energy balance.

10.3.1 How might sugar consumption cause obesity?

The calories in sugar are as good as those in starch (and possibly better than those in fat) at suppressing appetite. The sweetness of sugar creates no greater subsequent appetite than a similar amount of unsweetened starch. The sweetness of a snackfood or drink is no more of a temptation than its crispness, saltiness or creaminess. So how can sugar consumption contribute to weight problems?

The only possibility that has thus far been identified is that the calories in sugar, no differently from the calories in starch or protein, fat or alcohol, may be consumed in amounts and at times when they are very inefficient at suppressing subsequent energy intake—namely in drink, confectionery or snack item sizes at an hour or more before a meal (Booth, 1988). These few dozen calories (or even as much as 200 kcal) will be absorbed before the next meal and so will not suppress appetite during it. Hence they will not be compensated for but will add to the intake side of energy balance.

The results of Porikos and Van Itallie (1984) provide experimental evidence that drink-break energy is not compensated by the subsequent spontaneous intake over many days (Booth, 1987). More than half the sugar intake during the 'baseline' phase of these studies was in the soft drinks that the subjects were required to consume and in snacks taken in the evening. The limited and apparently asymptotic increase in caloric intake for up to 10 days after the sugar was replaced by aspartame corresponds to the lower energy content of the meals. Hence the lack of further compensation of caloric intake can be attributed to the failure of sugar energy between meals to suppress intake in the baseline period. (The same would be expected of between-meal energy from starch, protein, alcohol or fat.) This interpretation is also consistent with the tendency for the subjects to gain weight while they were inveigled initially into consuming as much sugar as possible between meals (for the experimental purpose of obtaining as large an effect of sweetener substitution as realistic).

If only, say, 20 kcal in a drink is taken between or after meals three or four times a day for most days of the week (and the extra energy is not spent by adding a long walk to the day), the sugar and milk in those cups of tea or coffee each day could fatten at a rate sufficient to account for middle-aged spread. Often eating a biscuit with the cup of tea or coffee could do the job much more quickly and effectively by adding 50–100 kcal of fat, starch and sugar. Thus, very common habits at drink breaks are likely to be making a major contribution to the very prevalent insidious accumulation of *avoiirdupois* at the waistline, which is one of our greatest public health problems at present.

This prediction from laboratory studies of satiety and of eating habit development is strongly supported by recent preliminary field evidence (Blair *et al.*, 1989). Similar correlational analyses of poor automatic control

of caloric intake in our data and in American studies (de Castro and Orozco, 1990) implicate moderate alcohol consumption, quite likely at the ends of and after meals. Indeed, a recent field experiment in the United States (Tordoff and Alleva, 1990) found greater weight gain and reported caloric intake over 3 weeks when fructose-sweetened soda was provided than when apparently indistinguishable aspartame-sweetened soda was; unfortunately neither the theory nor data relevant to timing of calorie consumption are presented but presumably much of the distributed soda was drunk between meals, replacing the usual drink that may have been calorific but less so. In our English samples, only those who avoid calories between meals and at the ends of meals are able to keep off lost weight.

10.3.2 Could low-calorie sweeteners help to reduce weight?

There is an obvious implication that consumption of sugar and other calorific sweeteners between and towards the end of meals creates substantial difficulties in weight control. On the face of it, substitution by low-calorie sweeteners would reduce those difficulties. This theoretical prediction has to allow for a number of technological and cultural complexities, however.

The calories are sugar only in soft drinks, sweetened black hot drinks, sugar confectionery and fruit and fruit juices. Even in 'sweets' such as biscuits and chocolate, fat is by far the greater energy source. Starch also contributes to energy intake between meals, in biscuits as well as in crisps and corn snacks. Bulking and other textural effects of sugar are essential to these solid food items, and may in some instances be more important than sometimes allowed for in flavour enhancement and the body in beverages.

Therefore, what would most help those with weight problems is to switch all sweet and non-sweet food items and calorific beverages out of drink breaks. That is, low-calorie sweetening could in principle be of help in weight control but only for those who consume nothing but a sweetened hot drink or a soft drink between meals. It is essential, of course, that these consumers use the low-calorie drink as a substitute for the traditional drink, and not as a medicine that somehow cuts into the fat more than the lowering of energy intake relative to energy expenditure.

10.3.3 Can we prevent obesity and still use sweeteners?

If the above theory is correct, it is quite baseless to fear that sugar, the sweet tooth or artificial sweeteners must in principle be causing obesity, regardless of what roles they play in an individual consumer's pattern of eating behaviour. No such overriding mechanism has yet been theoretically specified, let alone demonstrated to exist. This concern may therefore be

no more than a middle-class rationalisation of the Manichean ideology that any pleasure of the flesh is evil, which in this materialist age can only mean bad for your health (Fischler, 1987).

Current efforts merely to provide alternative drinks and foods with metabolisable bulk sweeteners substituted by low-calorie sweeteners could be self-stultifying healthwise, however. The practicable substitutions in drinks and desserts would maintain the contribution of sweetness to the palatability of alternative items that are still available and whose energy content is largely caloric sweeteners. In such circumstances, many people are liable to find it impracticable to make a clean break with the fattening use of the traditional version.

Commercial implications are obvious. For example, soft-drink manufacturers may be loath to promote low-calorie versions, for fear of undermining the positive perceptions of the sugar drinks which currently have a much larger market. Yet if consumers susceptible to obesity consume sugar sodas habitually when only a drink is needed, commercial support for such a habit contributes to ill-health.

10.3.4 Implications for marketing of sweetened foods and beverages

These roles of sweeteners in weight control mean that manufacturers and retailers will increasingly face two distinctions bearing on the provision of sweeteners and other putatively weight-relevant constituents and labels.

10.3.4.1 Marketing effective weight control The first important distinction is between satisfying any and all demands for bulk or intense sweeteners within the bounds of established safety and health regulations and focusing on formulating and marketing products that support those uses by consumers which actually do help the individual to avoid or reduce unwanted body weight and to maintain the leaner shape indefinitely. Such a distinction could become commercially viable and even highly profitable. First, though, it is a big challenge to technological ingenuity and to demand development (Azlyn *et al.*, 1989; Booth, 1989b).

10.3.4.2 Marketing healthy weight control. The second distinction extends beyond the uses of sweeteners and opens up even greater long-term challenges.

Current policy is to market to weight concerns without regard to objective need. Another approach would be to market not only to those weight-reducing uses that can be effective but also only to weight reduction of a sort that would improve health (Booth, 1989b). This requires positioning products that support effective weight-reducing habits so that they are taken up primarily by unhealthy overweight users (or by still-lean

users who may have a genetic vulnerability to obesity) and directing those products away from those who want to lose weight but do not need to in order to reduce risk to health.

In these two sorts of consumer motivation, probably the same habits can help weight reduction (Blair *et al.*, 1989). The difference in product positions would depend on avoiding an undifferentiated appeal to the motivation to improve appearance regardless of health implications.

The difficulties of such a commercial policy have to be faced because of the seriousness of the social and medical problems. At the present point in their history, the affluent Western cultures are plagued by an oppressive and even dangerous cult of scrawniness without regard to physical individuality (Blair *et al.*, 1989; Booth, 1988, 1990c). This pressure to be slim for appearance's sake bears particularly hard on the female population (Orbach, 1978), even from a very young age (Hill *et al.*, 1989; Salmons *et al.*, 1987). Arguably, therefore, seeking without restraint to satisfy any sort of demand for purported or effective aids to slimming is unacceptably exploitative.

10.4 Conclusion

In summary then, many consumers are concerned about their bodily shape, the figure or perhaps fitness. This is commonly expressed and monitored in terms of weight but this is recognised as surrogate for shape, commonly the waistline and in women thighs and buttocks. This concern has been reinforced by medical advice to avoid obesity, based on BMI relative to the range associated in past insurance statistics with longevity and reduced risk of some major degenerative diseases (Garrow, 1988).

There is only one clear mechanism by which sugars could contribute to unhealthy overweight and fatness. This operates when they are a substantial component of the energy consumed habitually in or with beverages after and between meals by those who are susceptible to weight gain. Furthermore, the only fully established risk to health from consumption of sugars and fermentable forms of starch arises from a not dissimilar dietary pattern, i.e. leaving them on the teeth frequently at intervals of 1–2 h or less.

There is therefore an objective benefit to health available by substitution of intense sweeteners for bulk sweeteners in food or beverage products that will be used either by those individuals susceptible to obesity to replace most of their between-meal caloric intake (and not expect anything more from the products) or by those susceptible to dental caries to reduce greatly the frequency of challenges to the teeth. These legitimate targets are, however, probably only a modest segment of the existing market,

because the starch and fat and indeed the sugar in many commonly used snackfoods and desserts cannot be replaced by non-caloric and non-fermentable substitutes. Also, maintaining the sensory qualities of existing calorific products may do little to help prevent obesity, or at least not help as much as developing attractive new types of zero-calorie drinks and 'nibbles'. To resolve this issue, we need mechanistically relevant behavioural data.

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11 Sweeteners: statutory aspects

D.J. SNODIN

11.1 Introduction

For any urbanised society to be adequately nourished there must be a way of ensuring a good standard of food supply. In recognition of this, dating back at least 2000 years, codes of practice were established to control commercial malpractice such as giving short weights and adulteration of staple foodstuffs. The Romans used food inspectors to take samples of imported grain which were kept in sealed bags as a check against later adulteration. In the Netherlands, as early as 1196, inspectors supervised the Utrecht fish markets. Today, when we have adequate food to meet our basic requirements, the emphasis of regulatory activity has switched to the control of health risks. In respect of additives this trend has led to the development of an increasingly complex web of legislation controlling the introduction of new ingredients and the surveillance and periodic re-evaluation of substances already present in the food supply.

Although sugars, mainly in the form of honey, have been used for sweetening food since antiquity, until the second half of the 19th century they were too expensive to become significant constituents of the diet. In most developed countries, the average *per capita* consumption of refined sugars peaked in the 1960s and early 1970s and since that time has been static or, as in the United Kingdom, has been gradually declining. The factors contributing to this situation are complex, though one significant influence has been the increasing popularity of low- and reduced-energy foods, especially soft drinks, containing non-sugar sweeteners. Although sugars and sweeteners can perform similar functions in foodstuffs, in legislative terms the two groups are sharply distinct from each other. Whereas the common sugars are considered as foodstuffs, sweeteners are regulated as additives. Thus, in principle, sweeteners should be evaluated and legally controlled no differently from any other group of food additives. However, bearing in mind such things as the potentially high level of consumer exposure, the intense public interest and the checkered safety and regulatory track record of several sweeteners, it is not surprising that the regulatory authorities have tended to be particularly exacting in their evaluation requirements for sweeteners.

For new sweeteners, although their precise legal positioning and the procedures for approval may differ from one country to another, there is a remarkable similarity in the data requirements particularly on safety testing. It now appears commonplace for government officials from different countries to discuss with each other their data evaluations on new additives during the review process. In fact government delegates from the United States, Canada and the United Kingdom attend regular formal tripartite meetings for discussion of such common issues, and attendance may be extended to include Japanese, EEC and other representatives. These developments go hand-in-hand with the globalisation of the sweetener business. The financial investment required to develop a new sweetener is now so large that marketing the product on a world-wide basis is more or less essential in order to guarantee a satisfactory return on the investment. In commercial terms, it is necessary to obtain approvals for a new sweetener in a way that maximises access to the largest markets. This means that approvals in the United States and EEC are the primary goals. Another important objective is an evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the key advisory committee to Codex Alimentarius on additive safety and purity criteria. Although the opinion of JECFA is only advisory, and does not give direct access to any markets, it is usually of considerable influence on the evaluations and/or approvals in individual countries.

11.2 Sweeteners in commercial use and in development

Sweeteners that have been approved in the United States or positively evaluated by the EEC or by JECFA and are in commercial use are shown in Table 11.1. For convenience, the sweeteners are divided into two groups (FACC, 1982):

- (a) *Intense sweeteners*: those with a sweetness many times that of sucrose
- (b) *Bulk sweeteners*: those with a sweetness similar to that of sucrose

Additionally, three sweeteners (all intense) still under development are shown in the table.

11.3 Regulation and evaluation of sweeteners

11.3.1 General

11.3.1.1 Regulation. Laws setting out the general principles applying to the safety, quality, sale, labelling, hygiene, etc. of foodstuffs exist in most

Table 11.1. Sweeteners in commercial use and in development.

Sweetener	Sweetness factor (sucrose = 1)
Intense sweeteners	
Acesulfame K	150
Aspartame	200
Cyclamate	30
Neo-DHC	250
Saccharin	350
Thaumatin	2000
Bulk sweeteners	
Hydrogenated glucose syrup	0.3–0.8
(maltitol)	0.9
Lactitol	0.3
Mannitol	0.4–0.5
Palatinit (isomalt)	0.5
Sorbitol	0.4–0.5
Xylitol	1.0
Sweeteners in development	
Alitame	2000
Stevioside	300
Sucralose	600

developed countries. Regulations drawn up under the auspices of such laws indicate the detailed legal requirements applying to individual aspects such as permitted additives and quality criteria for individual foodstuffs. Sweeteners are normally considered as additives and sweetener regulations would specify:

- a 'positive list' of approved sweeteners
- foods in which the sweeteners can be used and/or foods in which they cannot be used
- maximum use levels of sweeteners in groups of products, e.g. soft drinks, table top-preparations, desserts
- specifications of identity and purity for each permitted sweetener
- labelling of sweeteners sold as such and labelling of products containing sweeteners; this aspect may also be covered by food labelling regulations

The addition of a new sweetener to the positive list is subject to scrutiny by government scientists and in many cases also by committees of independent experts. Any company wishing to market a new sweetener, or wishing to extend the use of an approved sweetener to additional product areas, must submit a food additive petition to the appropriate national regulatory authorities. A petition for a new sweetener would contain:

- (i) Information from the manufacturer and potential industrial users demonstrating the need for the sweetener, i.e. better technical performance than existing approved products and benefits to the consumer
- (ii) physicochemical, analytical and manufacturing information on the sweetener itself, analysis in foodstuffs, extensive data on the proposed uses including data on stability and breakdown products and estimates of consumer intakes
- (iii) toxicological and other data on the sweetener itself and on any significant impurities and/or breakdown products, necessary to evaluate the safety-in-use of the sweetener
- (iv) data relevant to the likely environmental impact of the sweetener

Most countries require a case for the need of the sweetener to be demonstrated before any other data are evaluated in order to avoid the application of often scarce government resources to evaluating substances with poor commercial prospects and little consumer benefit. A few countries, however, such as the United States, do not operate this principle. In some countries only a summary of the toxicological data may be required if the sweetener has already been evaluated favourably by an international committee such as JECFA. At the time of writing, only the United States has a formal requirement for environmental studies.

11.3.1.2 Toxicological evaluation. Tests of different types are performed, mainly in laboratory animals, but also with *in vitro* systems and in human volunteers, in order to establish the toxicity profile of the sweetener (DHSS, 1982a; WHO, 1978). The principal animal species employed are rat, mouse and dog. The rabbit and monkey are used to a lesser extent. Selection of dose levels is of critical importance. Especially in shorter term tests, it is important to employ at least one dose which is high enough to elicit some signs of toxicity, for example a decrease in growth rate compared with that in control animals. For dietary administration of non-nutritive materials, into which category a number of sweeteners fall, it is usually accepted that in long-term studies the highest dose should not exceed 5% of the diet in order to avoid nutritional imbalance. This criterion has not been applied to bulk sweeteners which are partially or totally caloric, nor to the nutritive intense sweetener aspartame. The mid- and low-dose levels are normally one-third and one-tenth of the highest dose respectively, though other combinations such as one-half and one-quarter have been used.

In order to meet national and international quality standards, the principles of Good Laboratory Practice (GLP) need to be applied to the conduct of toxicology tests (Code of Federal Regulations, chapter 21, part 58). In essence, this means it is necessary to lay a paper trail documenting

the identity and competence of the investigating scientists, the study plan (protocol), detailed descriptions by standard operating procedures (SOPs) of the methods used, data recording, test material identity, stability and homogeneity in the animal diet, etc. Serious deviations from GLP can lead to the rejection of a study by regulatory authorities.

Test material purity and batch-to-batch variation require careful consideration. The ideal situation would be to have sufficient material of reasonable purity in one large homogeneous batch with which to conduct all of the toxicological programme. Thus, this level of purity would be the minimum acceptable for the commercial material, assuming that the sweetener is eventually approved. In practice, several batches of slightly varying purity and impurity profile could well be used in an extensive toxicological programme, and in these circumstances weighted mean values for purity and impurity levels would need to be assessed.

Over the last three decades a number of regulatory authorities have issued toxicity testing guidelines with varying degrees of rigidity. In some cases the detailed procedures specified for certain tests in a number of countries were sufficiently dissimilar to force companies to enter into protracted negotiations with several regulatory authorities in order to establish a mutually acceptable test procedure. Fortunately these problems have been virtually eliminated by the programme of harmonisation of testing methods carried out by the Organization for Economic Co-operation and Development (OECD). Founded in 1960, the OECD has 25 member countries from North America, Western Europe and Australia. As the *OECD Guidelines for the Testing of Chemicals* (OECD, 1981) have been endorsed by all member countries, their use provides a common basis for the international acceptance of test data. The guidelines, which include the principles of Good Laboratory Practice, are designed to allow some flexibility for expert judgement and are subject to updating when and if appropriate.

Acute toxicity tests involve the assessment of adverse effects occurring within a short time, e.g. after 7 or 14 days, of administration of single, or occasionally multiple doses of a test material given over 24 h. Such studies normally are of two main types. The median lethal dose, LD₅₀, 'a statistically derived expression of a single dose of material that can be expected to kill 50% of the animals' (Gehring, 1973), is determined in groups of 5–10 animals/sex per group at a range of mathematically related doses and the data are evaluated by one or more statistical techniques, e.g. probit analysis (Miller and Tainter, 1944). The unnecessary suffering of the large number of animals required to determine a precise LD₅₀ value has led to the adoption of 'limit tests' where a series of doses is given up to a pre-determined value, e.g. 5 g/kg.

Sub-acute and chronic toxicity tests involve repeated exposure of groups of test animals, usually rats, mice or dogs, to the test material for periods

normally ranging from a few weeks to 2 years or more. These tests provide information on the major toxic effects of a compound and on the target organs affected. They form the backbone of toxicological testing.

Sub-acute tests are traditionally carried out over 28 or 90 days in rats (using not less than 20 animals/sex per group) or in dogs (using not less than 4 animals/sex per group). One control and three test groups are the norm. Doses are selected on the basis of results from acute tests, from short, high-dose 'range finding' tests and from metabolic and pharmacokinetic studies. For a sweetener, the preferred route of administration would be via the diet though with an unpalatable material, the use of oral gavage in the case of the rat or capsules in the case of the dog may be necessary. Throughout the tests, food consumption and body weight are measured and clinical condition monitored. Haematological, clinical chemistry and urine tests are carried out on test and control animals during the study, pre-test examinations being added in dog studies. Organ function tests and/or metabolic studies may also be included, the latter often involving the use of satellite groups. At the end of the test period, all surviving animals are killed and subjected to a detailed necropsy. The major organs are weighed and samples of all organs are taken and processed for histopathological examination by cutting thin sections and applying a stain such as haematoxylin and eosin. In the first instance microscopic examination of tissue sections is normally restricted to the highest dose group and the control group, tissues from the other two dose groups being examined only if any significant differences, possibly treatment-related, are noted in the first review. Where appropriate, e.g. in the case of body weight, food and water consumption, organ weights, haematological and clinical chemistry parameters, the data are evaluated statistically.

Chronic toxicity tests are carried out in the rat over 18 months or in the dog over 12, 18 or 24 months. Minimum numbers of animals are 20 and 4/sex per group in the rat and dog, respectively. Doses are set on the basis of results from sub-acute tests. The techniques and procedures applied to chronic toxicity tests are similar to those described above for sub-acute tests. Highly sensitive advanced techniques such as electron microscopy and immunocytochemistry can be applied in either sub-acute or chronic tests in order to follow up on initial macroscopic or microscopic observations.

Carcinogenicity is, in essence, just one manifestation of chronic toxicity. Animal tests to evaluate carcinogenicity are similar to those for chronic toxicity except that no interim examinations are carried out in order to avoid subjecting the animals to any unnecessary stress. Tests would normally be carried out using at least 50 animals/sex per group in rats over 24 months or longer (depending on the strain used) and in mice over 18 or 24 months. More animals should be used in the control group e.g. $\sqrt{n} \times 50$ where n = no. of dose groups, or two control groups may

be used in some cases as a safeguard against finding an unusual tumour pattern in a single control group. Conventionally, the highest dose used is the maximum tolerated dose (MTD), i.e. a dose that does not shorten life expectancy of the test animals nor produces signs of toxicity other than those due to cancer. Operationally the MTD has been set as the maximum dose level at which a substance induced a decrement in weight gain of no greater than 10% in a sub-acute test. The rat test is often modified in two ways. Firstly, the chronic toxicity and carcinogenicity components are amalgamated in a combined study, and secondly test animals are additionally exposed to the sweetener during the neonatal period. The use of *in utero* exposure is thought by some to increase the sensitivity of test animals to tumour induction. The number of animals surviving to terminal sacrifice should be sufficient to enable adequate statistical analyses to be conducted on tumour types and rates. Histopathological examinations should be conducted on the basis of predetermined criteria for tumour classification and description. Non-neoplastic lesions are also normally determined in carcinogenicity studies (DHSS, 1982b).

Tests for reproductive toxicity are designed to identify adverse effects on the embryo, foetus or neonate. In practical terms the tests employed fall into two main types. Multigeneration studies, normally conducted in the rat, involve the administration of test material to groups of male and female animals over at least two generations, with at least two litters being produced in each generation. The results are evaluated to check for any effects on growth, mating performance and fertility, gestation length, gestation index and litter responses such as litter size, live birth index, offspring viability, male/female ratio and offspring bodyweight. Teratogenicity studies, conducted in a rodent species (normally the rat) and a non-rodent species (normally the rabbit), are designed to examine in detail the occurrence of toxic effects on the foetus during organogenesis. A rat teratogenicity study may form part of a multigeneration study. The test material is administered to pregnant animals during the most susceptible period of foetal development (days 6–15 of pregnancy in the rat and days 7–18 in the rabbit). Females are killed just before term and their uteri examined for evidence of resorption sites (i.e. indication of embryo lethality). The young are then examined for skeletal and soft tissue abnormalities and anomalies.

Studies on absorption, distribution, metabolism and elimination (excretion) (sometimes called ADME studies, metabolic and pharmacokinetic studies or just metabolic studies) provide information on the behaviour of the test material as it passes through the body. Several tests would be conducted using radiolabelled test substance (commonly involving the ^{14}C isotope) during the development of a sweetener. Single-dose balance studies (i.e. determination of radioactivity excreted via the urine, faeces and expired air, in comparison with the dosed radioactivity), metabolic

studies, whole-body autoradiography and determination of plasma levels would be conducted in rodents and some of these in dogs also. Exploratory balance and metabolic studies in humans would be essential in the early part of the toxicology programme. These results would enable the major routes and rates of absorption, distribution, metabolism and elimination to be determined and for comparisons to be made between humans and the principal animal species employed in the toxicity tests. This would ensure that the animal species used in these tests would be appropriate (or reasonably appropriate) surrogates for humans, that appropriate doses would be used and that the investigators were alerted to any unusual features, such as possible bioaccumulation, requiring further studies. More sophisticated ADME studies would, most probably, be carried out later in the sweetener testing programme in order to obtain more precise data on plasma concentration profiles and metabolite characterisation. It may be necessary to perform certain biochemical studies especially if the test substance is metabolised to any significant extent leading to the possibility of metabolic adaptation through enzyme induction.

Mutations in human genetic material present a potential health hazard due both to the possibility of inherited disorders and to the induction of malignancies. Genetic toxicity tests are designed to assess the mutagenic properties of a chemical. Information on three levels of mutation, namely gene, chromosome and aneuploidy (missing or extra chromosomes), is necessary to provide comprehensive coverage for the assessment of mutagenic and carcinogenic potential. As no single validated test can provide information on all three end points, each substance is normally subjected to a battery of tests arranged in tiers. Firstly, screening tests for gene mutation would be carried out in bacteria (e.g. *Salmonella typhimurium*) and in cultured mammalian cells. Tests for clastogenicity (chromosome damage) in mammalian cells (e.g. peripheral human lymphocytes) would also be conducted. Where appropriate, these *in vitro* tests are carried out in the presence and absence of an exogenous metabolic activation system such as the microsomal fraction of rat liver homogenate. Any compound showing one or more positive results in this first tier, or a compound such as a sweetener to which prolonged human exposure is expected, would be subjected to *in vivo* testing. Normally a bone-marrow test—metaphase analysis for chromosome damage in rodents or a (mouse) micronucleus assay—and another test such as the sex-linked recessive lethal assay in the fruit fly (*Drosophila melanogaster*), unscheduled DNA synthesis (UDS) in rat liver or a DNA binding assay would be undertaken. In special cases, where indicated by pharmacokinetic and other data, *in vivo* tests for germ cell effects may also be required for which the most useful and convenient test is the dominant lethal assay, though it does suffer from low sensitivity (DOH, 1989).

Human studies would be carried out at different points in the safety testing programme of a sweetener. As well as various human ADME studies already described, multiple-dose tolerance and toxicity studies up to 3 or 6 months in duration, and possibly studies in special sub-groups such as diabetics, would be conducted. All such studies must take account of ethical and legal as well as scientific aspects of the investigations. The World Medical Association's 1964 'Declaration of Helsinki', updated in 1975, established most of the key principles involved, such as the need for informed consent, the right of a subject to withdraw from the investigation at any stage and the review of the experimental protocol by an independent committee (DHSS, 1982a).

Although the extent of toxicological testing required to enable an adequate evaluation of the safety of an additive varies to some extent from one regulatory authority to another, in the case of most sweeteners however, a comprehensive set of tests would be required owing to the high potential intake and widespread use over all sectors of the population including children. Nevertheless, the natural intense protein sweetener thaumatin has been approved by several authorities including those in the EEC and Canada, on the basis of studies lasting up to 90 days, no chronic studies or multi-generation studies being deemed necessary.

11.3.1.3 Safety assessment. The principal objective of the evaluation of toxicological data on food additives by regulatory authorities is, presuming that no significant toxic effects preclude such an evaluation, the establishment of an acceptable daily intake (ADI) for humans. The ADI is 'the daily intake of a chemical which during an entire lifetime appears to be without appreciable risk on the basis of all known facts at the time.' It is expressed in milligrams of the chemical per kilogram of body weight (WHO/FAO, 1962). Generally, compounds that exhibit significant adverse effects in the pivotal safety studies, e.g. carcinogenicity, teratogenicity, or show any tendency to bioaccumulation, would not be allocated an ADI, though there may be special cases such as saccharin where epidemiological and other data may reduce the relevance of such findings to humans and enable a (temporary) ADI to be allocated (IPCS, 1987).

The first stage in the establishment of the ADI is the determination of the no-observed-adverse-effect level (NOAEL), also called the no-effect level, the no-observed-effect level or no-adverse-effect level. A decrement in weight gain has often been used for setting an effect level, though a reduction in growth rate coupled with decreased food consumption is difficult to interpret as an adverse effect since high levels of sweetener may be affecting the palatability of the animal chow. In these circumstances studies could be conducted in order to ascertain whether food conversion efficiency had been impaired, although if the results of such studies were in any way inconclusive, the effect would probably be attributed to toxic

anorexia rather than unpalatability. Other adverse effects could be related to organ-weight changes especially when associated with histopathological evidence, though some effects such as caecal enlargement that almost always occur when rodents are fed high levels of poorly absorbed materials, are not considered to be relevant to humans. In many cases it is often very difficult, if not impossible, to judge the toxicological relevance of an observed effect (Berry, 1988), though most toxicologists tend to feel that they cannot ignore any significant effect unless it can be shown to be toxicologically irrelevant (Lu, 1988). The dose level below that producing an 'adverse' effect is then designed as the NOAEL. It is possible for the highest level tested to be the NOAEL, which is the case for several sweeteners. It is normal to calculate NOAELs for each important animal study. Conventionally, the NOAEL used for calculating the ADI is that from the animal study displaying a toxic effect at the lowest dose, the species involved then being considered as the 'most sensitive species'. In practice, extra weight is given to long-term (in relation to the expected life-span of the species), better conducted studies and to studies conducted in species that are metabolically and pharmacokinetically equivalent or closely related to humans (IPCS, 1987). The allocation of the overall NOAEL is a matter of professional judgment, but as it lies right at the heart of food additive regulatory toxicology, it can cause considerable controversy. For example, in the case of the intense sweetener acesulfame K, different committees have based the overall NOAEL on data from the dog or the rat, yielding ADIs of 9 and 15 mg/kg body weight per day, respectively.

As it is most conventional in animal studies to administer food additives at fixed levels in the diet, expression of the NOAEL in mg/kg body weight is achieved either by reference to detailed food consumption data or more commonly by reference to conversion tables (Lehman, 1954). For example, in the rat, a NOAEL of 3% is equivalent to 1500 mg/kg body weight, whereas in the mature beagle dog it is equivalent to 750 mg/kg body weight.

In order to extrapolate the animal-derived NOAEL to humans in the form of an ADI, some form of safety factor is used. The extrapolation of the NOAEL to humans on the basis of body weight (BW^1) has been considered by some as too simplistic and the use of $BW^{0.75}$ has been advocated (Feron *et al.*, 1989), though such an approach has not gained widespread support (Lu, 1989). The use of a safety factor is intended to provide an adequate margin of safety for the consumer by assuming that the human being is more sensitive (10 times) than the test animal and that the difference in sensitivity within the human population is within a (ten-fold) range. Thus, the safety factor traditionally employed is 100. In some circumstances the value can be lower (e.g. if good, reassuring human data are available or if the additive is metabolised to normal body constituents), and in other situations it can be higher (e.g. when the mechanism of toxicity is unclear and/or when additional studies are considered necessary). In the latter situation, the

allocation of the ADI would probably be on a temporary basis until sufficient new data to allay the concerns raised during the initial evaluation became available. This type of evaluation of saccharin by the UK regulatory authorities resulted in late 1990 in a doubling of the ADI to 5mg/kg body weight per day and recommendations on labelling and advice to diabetics concerning the reduction or saccharin intake (MAFF, 1990). Hence,

$$\text{ADI} = \frac{\text{NOAEL}}{\text{Safety factor (normally 100)}} = 0\text{--}x \text{ (mg/kg body weight per day)}$$

In the case of some additives, it is not considered necessary to set a numerical ADI, the intense protein sweetener thaumatin and all of the bulk sweeteners falling into this category (see Codex Alimentarius, section 6). In other cases, setting an ADI for a breakdown product and/or impurity may be considered essential. This has occurred in the case of the diketopiperazine (DKP) derivative of aspartame which can be present as an impurity in aspartame, and is also formed as a degradation product during processing and storage of aspartame-containing products.

11.3.1.4 Regulatory procedures. For a new sweetener, regulatory authorities would on the basis of their own information and that provided by the petitioner, make a series of detailed projections of intake levels by different population groups, especially those likely to contain high consumers such as children, adolescents and young women, and compare such values with the ADI. If the 90th or 95th percentile intake estimates were well below the ADI, it is likely that all applications in the petition would be allowed. If the projected intakes were close to or greater than the ADI, then use of the sweetener would not be allowed, or more likely, only limited applications would be approved. In the case of sweeteners already in use, ADI values are compared with consumption data obtained by various survey techniques. Any evidence for the exceeding of specific ADIs on a long-term basis would trigger a review and possibly lead to restrictions on use. Following a 1988 U.K. sweetener survey, which indicated that saccharin intake in some high consumers such as diabetics exceeds the ADI of 2.5 mg/kg body weight per day (MAFF, 1989a), an appraisal of the situation was undertaken by two advisory committees (Food Advisory Committee—FAC, and Committee on Toxicity—COT).

After the successful scientific evaluation of a new sweetener, the steps leading to eventual legal approval differ considerably from one country to another. There may well be a detailed report or press notice outlining the assessment and inviting comments on the proposal to approve the sweetener. Bearing in mind the current sensitivity of government ministers, their advisers and civil servants to criticism on food safety, any major new initiative such as the approval of a new sweetener would be undertaken

only when no significant objections were likely to emerge in the public comment period. Prior evaluations by other authorities or organisations would be important considerations.

11.3.2 US approach

11.3.2.1 Legal background. The Food and Drug Administration (FDA), now part of the Department of Health and Human Services, is the primary US agency concerned with the regulation of food additives. The Food, Drug and Cosmetic Act (FD and C Act) of 1938 is the principal legislation controlling food standards and safety. The Food Additives Amendment passed by Congress in 1958 fundamentally changed the regulation of intentional additives in three ways. Firstly, it placed the burden of proof of safety on the petitioner, rather than requiring FDA to demonstrate that a food substance was unsafe. Secondly, recognising the impossibility of proving absolute safety, the Amendment indicates that 'safe' means 'reasonable certainty' that no harm will result from the intended use of the food additive—known as the 'general safety standard'. Thirdly

Table 11.2. Legal status of sweeteners in the United States.

Sweetener	Comments
Acesulfame K	Approved as a food additive, non-nutritive sweetener (21 CFR Part 172.800)
Aspartame	Approved as a food additive (21 CFR Part 172.804)
Cyclamate	Prohibited from use in human food (21 CFR Part 189.135); FAP for reapproval filed Nov 12, 1982
Neo-DHC	Not permitted in human food (use not petitioned)
Saccharin	Permitted on an interim basis (21 CFR Part 180.37)
Thaumatococin	FEMA (Flavour Extract Manufacturers Association) GRAS for some dry uses e.g. chewing gum
Hydrogenated glucose syrup	Not permitted; GRAS affirmation petition filed Feb 27, 1984
Maltitol	Not permitted; GRAS affirmation petition filed Dec 23, 1986
Lactitol	Not permitted; FAP filed Aug 19, 1983
Mannitol	Permitted on an interim basis (21 CFR Part 180.25)
Palatinin (isomalt)	Not permitted (use not petitioned as of Dec 31, 1989)
Sorbitol	Approved as a food substance affirmed as GRAS (21 CFR Part 184.1835)
Xylitol	Approved as food additive for special dietary uses (21 CFR Part 172.395)
Alitame	Not approved; FAP filed Sep 29, 1986
Stevioside	Not approved (use not petitioned).
Sucralose	Not approved; FAP filed May 8, 1987

for carcinogens, the Delaney Clause specified that 'no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal'.

Regulations enforced by the FDA are contained in Chapter 21 of the Code of Federal Regulations (21 CFR). In these regulations, food substances fall into three basic categories:

- (a) food additives, direct and indirect
- (b) substances generally recognised as safe (GRAS)
- (c) prior sanctioned food ingredients

Sweeteners are regulated primarily as additives, sorbitol being affirmed as GRAS, and cyclamate and its derivatives being prohibited from use in human food (Table 11.2).

The use of sweeteners in the United States is further controlled by provisions on foods for special dietary use (21 CFR Part 105), among which is stated:

The use of an artificial sweetener in a food, except when specifically and solely used for achieving a physical characteristic in the food which cannot be achieved with sugar or other nutritive sweetener, shall be considered a use for regulation of the intake of calories and available carbohydrate, or for use in the diets of diabetics and is therefore a special dietary use.

In practical terms, this means that the use of intense sweeteners is restricted to those applications in which a dietary claim can be substantiated, such as that of 'reduced calorie' where an energy reduction of at least one third is required in comparison with the regular (sugar-containing) food.

11.3.2.2 Food additive petitions. Food additive petitions (FAPs) are reviewed by the Center for Food Safety and Applied Nutrition within the FDA. Petitions must contain the technical, safety and environmental information specified in 21 CFR Part 171.1. When the FDA has determined that a petition is complete, which may take several months after receipt, a notice of filing is published in Federal Register. From the date of filing, virtually all data in the petition are available for public disclosure and comment. A petitioner may at any time make amendments to the petition or withdraw the petition without prejudice. In theory, FDA has up to 180 days in which to review the petition and make a decision on acceptance or rejection. However, any substantive amendment by the petitioner allows FDA to re-start the 180-day time limitation. If FDA asks the petitioner in writing for further explanations or information, the petition is put on 'hold' and is described as 'held to be inadequate' until the petitioner provides a satisfactory response at which time the time limitation begins to run anew. The prolongation of the approval process by these kinds of events is well illustrated by the case of the non-nutritive sweetener acesulfame K. The petition was filed on October 15, 1982 and approval occurred on July 28, 1988 (Federal Register, 1988).

General recognition of safety for food ingredients 'may be based only on the views of experts qualified by scientific training and experience to

evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food.' [21 CFR Part 170.30(a)].

An application for GRAS status based on scientific procedures,

... shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. [21 CFR Part 170.30(b)].

The scientific data submitted to FDA must be collected together in a GRAS affirmation petition, the format, content and procedures for which are indicated in 21 CFR Part 170.35(c). No environmental impact evaluation is required. Several bulk sweeteners are currently the subject of GRAS affirmation petitions (Table 11.2).

11.3.2.3 Toxicological evaluation. Written guidelines for petitioners are available from FDA on a range of topics, the most important concerning toxicological testing. Issued in 1982, these guidelines (Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food) have come to be known as the FDA Red Book (FDA, 1982). The Red Book serves two essential purposes. Firstly, it provides a system for determining the extent of toxicological testing required for an additive, and secondly it provides guidelines for conducting toxicological tests and standards of performance for evaluating such tests.

The determination of the amount of toxicological testing required is based on the 'level of concern' of a food or colour additive. The Red Book defines three levels of concern assessed primarily on the basis of anticipated human exposure to the substance, and to a lesser extent on the substance's chemical structure. The concern level may be increased or decreased as more information becomes available concerning toxic properties or exposure levels. These considerations are largely academic for most sweeteners since they are likely to be assigned to concern level III which attracts the most extensive series of toxicological tests.

Although FDA emphasises that the Red Book is a guide rather than an absolute standard for the extent of toxicological testing, significant deviations would be unexpected bearing in mind the public accountability of the FDA and the openness of the evaluation system. The Red Book has been criticised for undervaluing the role of metabolic and pharmacokinetic studies (called ADME studies in the Red Book) by not including such studies in the mandatory test packages and not explicitly providing a mechanism for the results of ADME studies to influence the extent of toxicological testing (MacGibbon, 1983). For example, many regulatory bodies would probably not require a fully comprehensive set of studies for an additive that could be shown to be completely converted to normal food

or body constituents before absorption from the gastrointestinal tract. Since first publication of the Red Book, FDA has produced a series of papers extending and reinforcing the concepts of their 'priority-based assessment system' (Rulis *et al.*, 1984; Rulis and Hattan, 1985; Hattan and Rulis, 1986; Rulis, 1987).

11.3.2.4 Safety assessment. In common with international practice, FDA derives ADIs for (non-carcinogenic) food additives by applying a safety factor to the 'highest no-effect level' (HNEL), equivalent to the NOAEL. The safety factor employed is usually 100 but can be more or less depending on the circumstances. For example, based on metabolic and clinical data, a safety factor of 80 was considered appropriate for aspartame. If the resulting value of the ADI is significantly greater than the expected human exposure, then approval is likely. In the case of the recently approved acesulfame K, FDA determined the 90th percentile estimated daily intake in the dry-food applications requested to be 1.6 mg/kg body weight per day which is considerably less than the FDA ADI of 15 mg/kg body weight per day.

In the case of food additives that induce tumours in experimental animals, the FDA is prohibited by the Delaney Clause from allowing such substances to be added to the food supply. About 10 years ago when the bladder carcinogenicity of saccharin in the male rat was confirmed by a Canadian study, FDA felt it had no option but to recommend prohibition of saccharin for food use. It was only by intervention of Congress (Saccharin Study and Labelling Act), spurred by massive public opinion, that saccharin remained available on an interim basis (21 CFR Part 180.37).

Concerning the bulk sweeteners, only sorbitol, xylitol and mannitol are permitted in the United States. Xylitol is permitted in foods for special dietary use (21 CFR Part 172.395) and sorbitol has been affirmed as GRAS (21 CFR Part 184.1835). Mannitol is permitted on an interim basis for various applications (21 CFR Part 180.25). GRAS affirmation petitions have been filed for hydrogenated glucose syrup (February 27, 1984) and maltitol (December, 23 1986). A food additive petition has been filed to clear the use of lactitol as a reduced calorie sweetening agent (August 19, 1983) (Table 11.2).

11.3.3 European Economic Community (EEC) approach

11.3.3.1 Background. Bound by the Treaty of Rome, the twelve member states of the EEC, Belgium, Denmark, Ireland, Germany, Greece, Italy, Luxembourg, The Netherlands, Portugal, Spain and United Kingdom, are pledged to eventual economic, monetary and political unity. In order to develop a united market within its boundaries to enable unhindered

circulation of commodities, the EEC is seeking to achieve harmonisation of the national legislation of its member states. To this end, the EEC draws up regulations and directives. EEC regulations are applicable to all member states and are binding. In practice in the food sector, regulations apply only to wine and certain agricultural products. EEC directives apply to manufactured goods and require that all member states adopt national laws to implement the objectives of the directives.

The European Commission is the civil service of the EEC and the administrator of Community policy. The Commission is headed by 14 Commissioners and is divided into 20 sections, each one called a Directorate General (DG). The brief of DG III concerns the internal market and industries and includes consideration of trade barriers and foodstuffs. The Council of Ministers is the supreme decision making body of the EEC. It consists of one member from each member state and, depending on the subject to be decided, governments are free to send any minister they choose. Other important EEC institutions are the Committee of Permanent Representatives (COREPER), the Economic and Social Committee (ECOSOC), the European Parliament and the Court of Justice.

Food law harmonisation is being carried out in the EEC through directives. 'Horizontal' directives concern food additives and contaminants, food labelling, materials and articles in contact with food and food for particular nutritional uses. 'Vertical' directives lay down compositional standards for food products such as cocoa and chocolate, coffee and chicory, jams, sugars, honey and mineral waters.

EEC food legislation is developed in three main phases:

- (i) drafting of a proposal (EEC Commission)
- (ii) assessment of the proposal (ECOSOC and European Parliament)
- (iii) approval of the proposal (Council of Ministers)

When a food-related directive is planned, the Commission forms a sub-group or working party of experts usually drawn from the food and/or health ministries of the member states. These sub-groups carry out the initial drafting of the proposals for a directive.

11.3.3.2 Submissions to the EEC. In order to be accepted for review by the EEC, a new additive requires evidence on technological need and on safety. A case of need must first be made to the Commission by one or more of the following:

- national food authorities of the member states
- industry, through an appropriate EC industry association such as Confédération des Industries Agro-Alimentaires (CIAA)
- EEC institutions (usually European Parliament or ECOSOC)

- other interest groups (consumer associations, trade unions, academic experts, specialist committees)

The case of need must be accepted by the Advisory Committee for Food (ACF) on which all non-government interests are represented (consumers, industry, trade unions and agriculture) before the Commission is willing to turn to its advisory committee on matters of food safety, the Scientific Committee for Food (SCF). Detailed guidelines on the content and format of submissions to the SCF are available (CEC, 1989a). Both a summary dossier and a main dossier are required providing all of the technical and safety data normally required for a food additive as well as information justifying the use of the additive. No data on environmental impact are currently required.

11.3.3.3 Toxicological evaluation. The SCF, which provides impartial advice on food safety, is composed of 15–20 eminent scientists (mainly toxicologists and nutritionists) from all of the member states. In 1980, the SCF published *Guidelines for the Safety Assessment of Food Additives* (CEC, 1980) which need to be used in conjunction with the submission guidelines (CEC, 1989a). The approach described in the Guidelines is by no means as codified as that described in the FDA Red Book, particularly in regard to the determination of the extent of toxicological testing required for a particular additive. However, the SCF employs the normal concepts of the NOAEL, safety factors and ADIs in its evaluation of toxicological data, reporting ADIs as permanent or temporary, and using the term ‘acceptable’ in those cases where it is considered unnecessary for a numerical ADI to be set. The SCF’s opinion is normally expressed in the form of a report. A report on sweeteners was issued in 1985 (CEC, 1985) which was updated in 1989 (CEC, 1989b). Table 11.3 summarises the conclusions of SCF’s review of sweeteners as indicated in these two reports.

Following a successful review of an additive by the SCF, there remains a considerable number of administrative steps, including a review by the ACF, before the additive is included in a directive. All additives that are approved by the EEC for use in foods are allocated code numbers, ‘E’ numbers. These E numbers, which are also used in Australia, serve a useful purpose in food additive control such as in food labelling. The E numbers allocated so far to sweeteners are shown in Table 11.3.

11.3.3.4 The Single European Market. In view of the sluggish progress in achieving harmonisation of legislation, in 1985 the Commission produced a White Paper advocating the completion of the internal market by 1992. Subsequently, the Single European Act which came into operation on July 1, 1987 gave legal force to this notion by insertion of Article 100A in the EEC Treaty (Gray, 1990).

Table 11.3. EEC evaluation of sweeteners (CEC, 1985, 1989b; CEC, 1990a).

Sweetener	Evaluation (ADIs in mg/kg body weight per day)	E number
Acesulfame K	ADI=9	E 950
Aspartame	ADI=40 ^a	E 951
Cyclamate	ADI=11 (temporary)	E 952
Neo-DHC	ADI=5	E 959
Saccharin	ADI=2.5 (temporary)	E 954
Thaumatococin	Acceptable	E 957
Maltitol and maltitol syrup	Acceptable ^b	E 965
Lactitol	Acceptable ^b	E 966
Mannitol	Acceptable ^b	E 421
Isomalt	Acceptable ^b	E 953
Sorbitol and sorbitol syrup	Acceptable ^b	E 420
Xylitol	Acceptable ^b	E 967
Alitame	Under evaluation by SCF	—
Stevioside	Not toxicologically acceptable	—
Sucralose	Not toxicologically acceptable	—

^aADI=7.5 for diketopiperazine (DKP).

^bLaxation may be observed at high intakes. Consumption of the order of 20 g/person per day of polyols is unlikely to cause laxative symptoms.

The Commission took the view that this general policy would be particularly appropriate for foodstuffs. In November 1985, a streamlining of EC food legislation was recommended particularly in respect of the vertical (compositional) directives and simplified decision making, but at the same time ensuring:

- public health protection
- provision of adequate information and economic protection to consumers
- fair trading
- effective public inspection

The new approach on compositional standards draws to a large extent on the Cassis de Dijon judgement of the European Court which reinforced the principle that a product lawfully manufactured and marketed in one member state should be allowed entry to other member states unless there are health reasons for restriction.

In the context of food additives, on December 21, 1988 the Council adopted the proposal, for a 'framework directive' on additives. The main principle of this directive is that an additive may be used in food only if its use has been expressly authorised (i.e. on a 'positive list'). As indicated in

the Framework Directive, food additives can be approved only provided that:

- (1) there can be demonstrated a reasonable technological need and the purpose cannot be achieved by other means which are economically and technologically practicable
- (2) they present no hazard to the health of the consumer at the level of use proposed, so far as can be judged on the scientific evidence available
- (3) they do not mislead the consumer

So far, only about half of the additives concerned have been regulated by the EEC and so positive lists are being drawn up for remaining categories (including sweeteners). Harmonisation of additive regulations under the Framework Directive will involve the cooperation procedure which is designed to lead to a common position being adopted by the Council of Ministers (by qualified majority voting) in time to enable legislation to be enacted to meet the 1992 deadline. This will lead to a comprehensive Directive on all food additives, existing food additive directives being revoked. Proposals from the EC Commission have in fact concerned two directives on food additives: one on sweeteners and the other on 'certain food additives' (preservatives, antioxidants and miscellaneous additives). Commission proposals for a third directive, on colours, are expected by the end of 1990. In the proposed Sweeteners Directive (CEC, 1990a), sweeteners are defined as 'food additives which are used to impart a sweet taste to foodstuffs', but exclude 'foodstuffs with sweetening properties such as mono-disaccharides and honey'. The sweeteners included in the positive list of sweeteners are those in Table 11.3 for which E numbers are shown. Detailed conditions of use are proposed: the bulk sweeteners are limited only by good manufacturing practice (*quantum satis*) whereas the intense sweeteners are subject to individual maximum use levels in a fairly restricted range of products including soft drinks, desserts, cereals, edible ices and confectionery.

11.3.4 Codex Alimentarius

11.3.4.1 Background. Following a special conference on food additives in 1955 organised jointly by the World Health Organization (WHO) and Food and Agriculture Organization (FAO), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was founded. JECFA established general principles governing the use of food additives (WHO/FAO, 1957) and describing the data necessary to evaluate the safety of a food additive (WHO/FAO, 1958). Since that time, JECFA has met annually to evaluate food additives in terms of safety, to set purity criteria, and to revise and

update guidelines. Experts are appointed to JECFA by WHO (primarily in relation to toxicological evaluations) and by FAO (primarily in relation to setting specifications).

The Codex Alimentarius Commission (CAC) was established in 1962 in order to implement the Joint FAO/WHO Food Standards Programme in which most countries of the world now participate. The purpose of the programme is:

to protect the health of consumers and to ensure fair practices in the food trade; to promote coordination of all food standards work undertaken by international governmental and non-governmental organisations; to determine priorities and initiate and guide the preparation of draft standards through and with the aid of appropriate organisations; to finalise standards and after acceptance by governments publish them in a Codex Alimentarius either as regional or world-wide standards. (CAC, 1981).

The Codex Alimentarius is intended to guide and promote the elaboration and establishment of definitions and requirements for foods, including food additives, 'to assist in their harmonisation and in doing so to facilitate international trade' (CAC, 1981). The CAC Programme has been only partially successful in the harmonisation of international food standards and considerable non-tariff barriers to trade still exist. A recent special review of the position has recommended a reappraisal of CAC procedures and better compliance with standards and JECFA evaluations by member nations (Denner, 1989).

An important part of the work of the CAC is conducted by the Codex Committee on Food Additives and Contaminants (CCFAC) which meets annually in the Netherlands. The CCFAC uses JECFA as its adviser on all matters relating to the safety of food additives and contaminants. The Working Group on Priorities of the CCFAC each year establishes a priority list of food additives and contaminants for evaluation by JECFA.

Beginning in 1980, all JECFA activities related to the safety of food additives and contaminants were administratively gradually incorporated into the International Programme on Chemical Safety (IPCS). The IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organization and the World Health Organization with the main objective of carrying out and disseminating evaluations of the effects of chemicals on human health and the quality of the environment. The major impact of the IPCS has been its role in updating the criteria used by JECFA in the safety evaluation of chemicals, culminating in 1987 in the publication of *Principles for the Safety Assessment of Food Additives and Contaminants in Food* (IPCS, 1987).

11.3.4.2 Toxicological evaluation. The principles used by JECFA in evaluating the safety of food additives (IPCS, 1987) are broadly similar to those used by FDA, SCF and national committees. Like the SCF, but unlike FDA, JECFA does not have a formalised procedure for assessing

testing requirements. The principal factors taken into account are

- exposure
- chemical structure
- metabolic and pharmacokinetic data

Nevertheless, for a sweetener with widespread predicted usage, JECFA is likely to be equally as demanding as either FDA or SCF in terms of toxicity data.

JECFA evaluates additives in a conventional way, allocating ADIs normally using a safety factor of 100. As well as a full ADI, JECFA can award a temporary ADI in which case a larger safety factor (e.g. 200, as for saccharin) is normally used and additional data are requested to be submitted in 2 years' time. In some cases, JECFA considers that the allocation of a numerical ADI is unnecessary (as is the case for most of the bulk sweeteners) and allocates for such substances 'ADI not specified'. JECFA defines this term to mean that 'on the basis of the available data (chemical, biochemical, toxicological and other) the total daily intake of the substance, arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the committee, represent a hazard to health'. Specifications drawn up by JECFA are either 'final' or 'tentative' in which case further information is requested, normally to be submitted within 2 years.

Table 11.4. JECFA sweetener evaluations.

Sweetener	Evaluation (ADIs in mg/kg body weight per day)	Comments
Acesulfame K	ADI=15	a
Aspartame	ADI=40	ADI=7.5 for DKP
Cyclamate	ADI=11 (temporary)	—
Neo-DHC	Not yet evaluated	—
Saccharin	ADI=2.5 (temporary)	—
Thaumatococin	ADI not specified	—
Hydrogenated glucose syrup	ADI not specified	b
Lactitol	ADI not specified	b
Mannitol	ADI not specified	b
Isomalt	ADI not specified	b
Sorbitol	ADI not specified	b
Xylitol	ADI not specified	b
Alitame	Not yet evaluated	—
Stevioside	Not yet evaluated	—
Trichlorogalactosucrose (sucralose)	ADI=15	Additional data desirable

^aADI =9 prior to 1990

^bComment by Committee that excessive consumption of polyols leads to laxation.

Table 11.4 summarises the safety evaluations made by JECFA on both intense and bulk sweeteners.

11.4 Bulk sweeteners: special considerations

All currently approved bulk sweeteners are polyols, being mono- or disaccharide sugar alcohols (sorbitol, mannitol, xylitol and lactitol) or mixtures of sugar alcohols (isomalt and hydrogenated glucose syrup). Bulk sweeteners have to be used at quite high levels (i.e. in the percent range) in a variety of food products including confectionery, chewing gum, jams, jellies, desserts and baked goods, in order to accomplish their technological effects. Consequently, it is not possible to achieve anything like a 100-fold ratio between the NOAEL in laboratory animals and the possible human intake which could reach several tens of grams. In practice, bulk sweeteners have been subjected to a range of conventional toxicological studies, the maximum doses in long-term studies being 10–30% of the diet.

Owing to some extent to their poor absorption from the small intestine, most of the bulk sweeteners produce similar effects in rats at the high doses used. Certain kidney and adrenal changes resulting from changes in calcium uptake and excretion were observed uniquely in the rat. It is considered that these changes, which probably result from gross dietary imbalance leading to metabolic and physiological disturbances, are not relevant to humans. In humans, as a consequence of their incomplete absorption, bulk sweeteners can cause laxation and flatulence. The amounts of the various bulk sweeteners required to cause laxation vary from one sweetener to another and depend upon whether the dose is spread over a number of meals or consumed all at once, whether the recipient has fasted or not, and on individual differences in susceptibility. The evidence suggests that xylitol, sorbitol, mannitol, hydrogenated glucose syrup and isomalt cause largely 'osmotic diarrhoea' which results from osmosis across the intestinal wall owing to presence in the lumen of unabsorbed bulk sweetener and its metabolites. On the other hand, lactitol causes significant 'fermentative diarrhoea' brought about by the action of the large gut microflora to produce volatile fatty acids and intestinal gases.

Overall, on the basis of a variety of factors such as natural occurrence, metabolic conversion to normal body constituents, long history of food (and clinical) use and extensive human studies, most committees and regulatory authorities have considered that bulk sweeteners are safe for human consumption even though the normal safety margin between experimental animal and consumer intake cannot be achieved. The SCF has stated that laxation may be observed, but that an intake of up to 20 g/day of polyols is unlikely to cause symptoms (SCF, 1985, 1989). In the United States the label of foods whose consumption may result in a daily

intake of 50 g of sorbitol or 20 g of mannitol must bear the statement, 'Excess consumption may have a laxative effect' (21 CFR Parts 184.1835 and 180.25). JECFA has cautioned on the laxative effect of high doses of polyols, but no numerical recommendations have been made on maximum intakes.

A further consequence of the generally poor absorption of bulk sweeteners is that of reduced energy value in comparison to sucrose. A recent review of this topic (Voedingsraad, 1987) suggests that three main factors determine the energy value of a particular sugar alcohol:

- extent of absorption in the small intestine
- extent of utilisation of absorbed fraction
- energy value of fermentation products generated in the large intestine

The energy value (E_w) of a bulk sweetener can be calculated from

$$E_w = [(A \times B) + (1 - A) \times 0.5] \times 16.5 \times R_e \text{ kJ/g}$$

where for the particular bulk sweetener, A = the fraction absorbed from the small intestine B = the fraction utilised by the body after absorption from the small intestine, R_e = the ratio of the energy content of the bulk sweetener concerned compared with that of sucrose, 0.5 = the proportion of utilisable energy available from the large intestine and 16.5 = the energy content of sucrose in kJ/g.

Using this method, the energy values for six bulk sweeteners, shown in Table 11.5, have been determined. The results should be regarded as only preliminary. Although the assessment of energy values for bulk sweeteners and bulking agents is still an area of active research, in drawing up its Directive on Nutrition Labelling for Foodstuffs, the EC Commission has decided, for convenience, to adopt a single value of 10 kJ/g for all sugar alcohols (CEC, 1990b).

11.5 Sweetener intake assessments

A critical part of the evaluation of any new sweetener is the comparison of the ADI with the projected consumer intake, and for a sweetener already approved and established in the food supply, regulatory authorities may feel the need to monitor actual consumption to ensure that the ADI is not being exceeded especially by any special population subgroups.

In order to make projections of the likely consumer intakes of a new sweetener, various approaches are possible depending largely upon the quality and quantity of the food consumption data that are available in

Table 11.5. Energy values of bulk sweeteners^a

Bulk sweetener	Energy value kJ/g (kcal/g)
Xylitol	15 (3.6)
Sorbitol	12.5 (3.0)
Mannitol	8 (1.9)
Maltitol	12 (2.9)
Isomalt	10 (2.4)
Lactitol	8.5 (2.0)

^aVoedingsraad (1987).

the country in question. Some fairly simple estimates can be made based on *per capita* sugar disappearance, *per capita* commodity disappearance or sugar or commodity consumption (IPCS, 1987). More sophisticated intake estimates can be made which encompass various age/sex groups, special sub-groups, all persons and consumers only, 50th, 90th and 95th percentiles, etc. For example the Market Research Corporation of America (MRCA) maintains a large continuously updated database on portion size and frequency of eating for a wide range of commodities.

Post-market surveillance is now being carried out quite frequently on sweeteners, an example being a study conducted on aspartame in Canada (Lauer and Kirkpatrick, 1989). Over 5000 households in 10 Canadian cities completed 7-day diaries for amounts of aspartame-containing food that were consumed. The respondents were segmented by sex and age group and results were reported separately for aspartame users and the total population. The study was in fact conducted twice, in a 'cold weather wave' (CWW) from February to April 1987 and in a 'warm weather wave' (WWW) from July to September 1987. In the CWW, the mean and 90th percentile all-persons intakes in eaters only were 3.79 and 5.54 mg/kg body weight per day, respectively, and in the WWW were 4.05 and 5.87 mg/kg body weight per day. Intakes in diabetics and persons following a weight loss diet were comparable with those for the general population. The aspartame intake data obtained from this study were compared with estimates of consumption made by three methods typically used in the estimation of food additive intakes as part of the petition process (Table 11.6). Whereas actual whole population average intakes were around 0.6 mg/kg body weight per day, estimated intakes ranged from 3.2 to 11 mg/kg body weight per day. This provides good evidence for what has often been suspected to be the case that the intake calculation methods used by regulators almost invariably lead to a significant over-estimate of food additive consumption. Of the four highest aspartame consumers noted in the Canadian study, average intakes ranged from 12 to 29 mg/kg body weight per day. It is interesting that three of these four high consumers were aged 6 or under, and that a 4-year-old male exceeded the Canadian

Table 11.6 Estimated and actual aspartame intakes (Lauer and Kirkpatrick, 1989).

Procedure	Intake (all persons mean intake in mg/kg body weight per day)
Estimated values	
Approach 1 ^a	11
Approach 2 ^b	4
Approach 3 ^c	3.2–7.4
Actual intake values	
Cold weather wave (CWW)	0.56
Warm weather wave (WWW)	0.60

^aBased on per capita disappearance of sugar.

^bBased on per capita disappearance of foodstuffs eligible to contain aspartame and typical use levels in those products.

^cBased on 24-h Nutrition Canada food consumption data of foods eligible to contain aspartame and concentrations of aspartame in those foodstuffs.

ADI for aspartame (40 mg/kg body weight per day) on two occasions (42 and 43 mg/kg body weight per day).

11.6 The ADI concept

Although over the 30 years of its use, the ADI has become increasingly accepted across the world as a tool for the evaluation and regulatory control of food additives, many valid criticisms can be made concerning the concept of the ADI and its underlying components, the NOAEL and the safety factor (Kroes, 1989).

- The allocation of the NOAEL is dependent on the actual doses used in the toxicity studies, which, in hindsight, may not have been chosen to give the highest NOAEL. For example, a chronic rat study run at dose levels of 0.3, 1.0 and 3.0% may have produced marginal effects at the highest dose giving an NOAEL of 1.0%. In hindsight, running the study at doses of 1, 2 and 4% would probably have produced a NOAEL of 2.0%.
- The determination of the NOAEL can, in spite of detailed guidelines, be relatively subjective and vary from one committee to another, the difference between the FDA and initial SCF/JECFA evaluations of acesulfame K being a good example.
- The NOAEL value (and hence the ADI) is based upon the average additive intake during a chronic study. In the conventional constant-concentration dietary 2-year rat feeding study, however, the high food consumption during the initial stages to support the rapidly growing animal yields a high intake of the additive. Over such a

study, the intake of an additive decreases from about 250 to 70% of the average value. In the human situation therefore, concern over young children slightly exceeding additive ADIs (as in the case of aspartame in the previous section) is probably misplaced as the safety margin in relation to the test animal intake is not likely to have been compromised.

- Present-day toxicity studies in which large numbers of animals are used and more parameters than ever before are measured, and in which special biochemical and ultrastructural elements may be included, will almost certainly produce lower NOAELs than studies conducted one or two decades ago.
- In striving to show some high-dose toxic effect for additives that are essentially non-toxic, physiological disturbances may result but may be difficult to distinguish from non-specific effects such as growth rate reduction. In these circumstances, it may be better to use lower doses and lower safety factors.
- Use of a more or less standard safety factor takes no account of dose-response relationships. Use of a lower safety factor for steep dose-response effects would seem appropriate. Good animal and human metabolic and pharmacokinetic data are now available on most new food additives. The use of blood level data would allow good interspecies comparisons to be made and would enable an appropriate rather than an arbitrary safety factor to be chosen.
- The normal safety of 100 (10×10) is probably too conservative since the two variants contributing to this value (inter and intra species sensitivity) are not necessarily related. Moreover, the safety factors for many safely consumed nutrients, such as vitamins, are considerably less than 100, and may be less than 10.
- The ADI concept implies that there is a threshold of risk, rather than a continuum. This can be very misleading in the regulatory and public affairs contexts.
- The ADI concept falls down badly for macro ingredients such as bulk sugars, bulking aids and novel foods.

Indeed the penetrating observations made by Leon Golberg in 1971 remain just as true today: 'Extrapolation of animal results to man has remained essentially guesswork. A mirage of safety evaluation has been created on whose illusory green pastures browse three sacred cows—the no-effect level, the safety factor and the acceptable daily intake.'

The development of approaches that are more scientific than the ADI concept is no doubt possible; just developing a system of allocating safety factors on a more rational basis would be a major innovation. However, it seems doubtful that, in the short-term, regulatory authorities will be willing to accept many changes to a system that has, by and large,

managed to satisfy the needs and aspirations of a broad spectrum of the public including political and consumer groups, industry and workers' groups, etc. A major research programme would be necessary in order to understand the biological basis of dose-response relationships and to make more reliable inter species extrapolations. The results could form the basis of the development and validation of alternative approaches, all of which seems unlikely to occur in the prevailing regulatory climate.

A normal human consumes no more than 25 g solid food and 100 ml fluid per kg body weight per day.

Assume a sweetener has an ADI of a mg/kg body weight per day. To ensure that the ADI is not exceeded, the concentration limits would be

- in solid food, $1000/25a = 40a$ mg/kg
- In beverages, $1000/100a = 10a$ mg/kg

If the predominant use of the sweetener were in soft drinks, acknowledging that the maximum consumption of soft drinks is 25 ml/kg per day, the concentration limit in this situation would be $40a$ mg/l.

If used in both food and beverages, the ADI would be proportioned b in food and $1-b$ in beverages. Assume also that only a fraction c of food and a fraction d of beverages will contain the sweetener, leading to the following concentration limits:

- for solid foods, $40ab/c$
- for beverages, $10a(1-b)/d$

Usually half of the ADI is allocated to solid foods and beverages, but a larger fraction for beverages may be more appropriate for most sweeteners.

Figure 11.1. The Danish budget method (Hansen, 1966).

Therefore, it seems that even with all of its uncertainties and arbitrariness, the ADI is here to stay. Apparently somewhat reluctantly, the U.K. authorities recently announced that they were falling into line with most other agencies around the world by adopting an ADI evaluation system (MAFF, 1989b). Further regulatory developments such as the use of the Danish Budget Method for calculating use levels (Figure 11.1) could have a significant impact on the market penetration of various intense sweeteners. As illustrated in Table 11.7, using a typical scenario for the use of intense sweeteners in food and drinks, it can be predicted by the Budget Method that the only sweetener, when used alone, that would have the possibility of providing an adequate level of sweetness (normally around 10% sugar equivalent in soft drinks) would be aspartame. The maximum use levels proposed in the EC Sweeteners Directive (shown for desserts and soft drinks in Table 11.7) for sweeteners other than aspartame are generally higher than these predicted levels but even so not enough to allow one sweetener to produce an adequate level of sweeteners in such a major application as soft drinks. Although binary and possibly ternary sweetener mixtures

could be used to exploit sweetness synergies (e.g. aspartame/acesulfame K, saccharin/cyclamate), aspartame seems set to achieve a dominant position.

Table 11.7. Comparison of sweetener use levels calculated by the Danish budget method with proposed levels in EC Sweeteners Directive.

Sweetener	ADI in mg/kg body weight per day	Maximum use level in ppm ^b		Sweetness factor	Sugar equivalent(%)	
		Foods	Liquids		Foods	Liquids
Acesulfame K	9	360 (350)	270 (350)	150	5.4 (5.3)	4.1 (5.3)
Aspartame	40	1600 (1000)	1200 (600)	200	32 (20)	24 (12)
Cyclamate	11	440 (250)	330 (400)	30	1.3 (0.8)	1.0 (1.2)
Saccharin	2.5	100 (100)	75 (80)	350	3.5 (3.5)	2.6 (2.8)

^aAssumptions: each sweetener is used 10% in foods and 90% in liquids; 10% of all foods and 30% of all liquids contain each sweetener. From Figure 11.1, $b=0.1$, $c=0.1$, $d=0.3$. Hence, sweetener concentration in foods is $40a$ and in liquids is $30a$ where a is the ADI value.

^bFigures in parentheses indicate maximum use levels proposed in EC Sweeteners Directive for sugar-free desserts and soft drinks respectively.

11.8 Future trends

Following the 1970s, a decade during which the food use of cyclamate was prohibited in many countries and in which saccharin narrowly avoided the same fate, the 1980s have witnessed the emergence of several new sweeteners and the partial restoration of confidence in the two older sweeteners. More exacting and extensive toxicological testing programmes combined with often unrealistically high public expectations of food additive safety, have made the path to regulatory approval for new sweeteners awesomely difficult.

In Europe the establishment of the Single European Market in 1992 is expected to be a watershed for sweeteners. Once the positive list of EC sweeteners has been established, it will be far from easy to demonstrate the technical superiority of a new sweetener over existing ones when used singly or in combination. Technological need as well as safety-in-use considerations will no doubt prevent all but the most exceptional new sweeteners from gaining access to the market place.

Criticised by toxicologists, but loved by regulators, the ADI seems here to stay for intense sweeteners, which, amongst other things, will help

aspartame consolidate its already dominant commercial position.

Overall, although the 1990s is expected to be characterised by an expansion of sweetener markets (Frost and Sullivan, 1988), there is unlikely to be any respite from increasingly demanding regulatory requirements.

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