Pure & Appl. Chem., Vol. 69, No. 4, pp. 685–692, 1997.
Printed in Great Britain.

Importance of molar volumes and related parameters in sweet taste chemoreception

Gordon G. Birch, Sneha Parke, Rachel Siertsema and Jane M. Westwell

Department of Food Science and Technology, The University of Reading, Reading, Berks, UK

Abstract: A major new chemical approach to the study of sweet taste chemorecpetion now centres on the role of water. Interaction of sweet stimulus with receptor requires a molecular "fit" which in turn demands specific volume requirements of the stimulus in water and probably also in the biophase. Apparent specific volume, rather than partial specific volume, defines taste quality at normal tasting concentrations and sweet taste quality is largely confined to the range 0.51-0.71 cm^3g^{-1} with the "ideal" quality of sugar and sugar alcohol sweetness being about 0.60-0.64 cm^3g^{-1}. Specific volumes define hydrostatic packing of sweet molecules among water molecules whereas the related intrinsic viscosities define their hydrodynamic behaviour. Both are related to "characteristic volumes" which are partial specific volumes at absolute zero.

A further solution characteristic is the partial molar isentropic compressibility (K_{2s}) which defines the compactness of the hydration layer around sweet molecules. This parameter is much more sensitive to structural differences between sweeteners than is partial molar volume and it best represents compatibility with water structure. The K_{2s} of D-galactose, for example, is -2.08 x $10^{-3}cm^3mol^{-1}bar^{-1}$ while that of the conformationally analogous D-glucose is $-1.76x10^{-3}cm^3mol^{-1}bar^{-1}$. D-galactose is therefore less compatible with water than D-glucose and half as sweet.

These studies will help to elucidate the mechanistic differences between sweeteners and their mode of interaction with water and flavours in foods.

INTRODUCTION

Why is the volume of any molecule important in a biological context? The answer to this question is self-evident in metabolic pathways involving specific enzymic catalysis and particularly in the field of chemoreception. Molecular volume will affect first the mass fraction of a stimulus interacting with a limited population of receptors, second the accessibility of the stimulus and, third, its steric fit and activation efficiency with the receptor. In sweet taste chemoreception there is a need to know how sweet receptors work at the initial peripheral level. Quantitative structure-activity relationships (QSARs) within the plethora of molecules which all elicit the sweet sensation will help to illuminate the receptor mechanism and molecular volumes may be essential for the derivation of such QSARs.

Molecular volumes of stimulus molecules are related to other important physical parameters such as hydrophobicity, compressibility and surface tension. The last two are in a sense opposite forces as compressibility represents the openness of structured molecules while surface tension reflects the cohesion between them, resulting in a compacted structure.

Sweet taste chemoreception is only one example of thousands of different stimulus-receptor processes known to biologists and McGowan and Mellors (ref.1) have shown how important molecular volumes are in the efficacy of any drug action. It is generally possible to relate enzyme inhibition to molecular volume of specific inhibitors and Wright (ref.2) has suggested that the Stevens exponents of a wide range of odorants are related to their parachors. Beck (ref.3) may have been one of the first to propose that sweet taste was related to "molecular contraction", (i.e. sum of the atomic volumes in a sweet molecule divided by the molecular volume). However, one of the first workers to utilise volume parameters systematically was Spillane (ref.4) in the field of sulphamate sweeteners. In a range of molecules of general structure R-CH(NH)SO_3^-, it was possible to predict that sweetness was confined to height and width constraints in the R group, and this excellent work illustrated the value of structure-activity relationships within one chemical class of sweeteners. The problem with sweetness, however, is that it is a single qualitative response elicited by structurally different types of molecule. How then can we explain that sodium chloride (partial molar volume 15.3cm^3mol^{-1}) and thaumatin (partial molar volume ca 15,000 cm^3mol^{-1}) are both sweet? Clearly if there is a common sweet receptor involved, there must be a mechanism common to all known sweeteners logically involving weak receptor forces such as hydrogen-bonding (ref.5). The common medium for such a mechanism is of course water and the equilibrium solution properties of one of the most important classes of sweeteners (small carbohydrates) have already been outlined (ref.6).

ROLE OF WATER

Water is clearly important in sweet taste because no molecule can be tasted unless it is soluble and transportable to the receptors via oral fluid. After dissolution, solutes become more or less hydrated and the nature of the hydration governs the size and shape of the solute (ref.6). This, for example, will stabilise most simple monosaccharides as pyranoses in the $^4C_1$, or $_4C^1$ conformation and it has been proposed that the hydration layer around simple salts, such as sodium chloride, explains their sweetness which is only observable at low concentration (ref.7). What effects ensue from the hydration layer? Is the hydrated sweet solute an effectively enlarged molecule? The answer to these questions lies in the array of solution measurements (ref.6) that can be obtained in sapid solutions and, in particular, partial and apparent molar volumes determined in water. The latter are determined at normal tasting concentrations and are therefore relevant to behavioural studies. However, they are usually larger than partial molar volumes (ϕ_v^0) due to a contribution from solute-solute interaction according to the standard equation:- $\phi_v = \phi_v^0 + mS_v$

where ϕ_v = Apparent molar volume
m = Molality of solution
S_v = Slope in cm^3kgmol^{-2}

Partial and apparent molar volumes are hydrostatic packing measurements.
They may be regarded as solvent-generated surface volumes but they really represent the effective volume of a molecule after interaction with water structure. The measurements obtained are therefore a resultant of displacement of water molecules and disturbance of water structure. Polar molecules (such as sugars or salts) interact with water structure much more than do hydrophobic molecules. It therefore turns out that the most heavily hydrated molecules have smaller apparent molar volumes than unhydrated molecules. Partial molar volumes are apparent molar volumes at infinite dilution (i.e. free from solute-solute effects). McGowan and Mellors have extended the concept further to "characteristic volumes" (Vx) (ref.1) which are partial molar volumes at absolute zero. Most organic compounds have characteristic volumes which are smaller than their partial molar volumes. Sugars (and many other types of sweetener) do not, because of the multiple sites of interaction with water structure. Interaction generates electrostrictive forces causing collapse of water structure around polar molecules and hence low experimental values for molar volumes. It is therefore common to find a particular molecule with a partial molar volume smaller than that of an analogous molecule of higher molecular weight. Examples of this are ethanol (55.08 cm^3mol^{-1}) and 1,2-ethane diol (54.63 cm^3mol^{-1}) (ref.7), the latter constituting the archetypal AH,B system. However, molecules of vastly different molecular weight will have partial molar volumes which are related to their molar mass and it is necessary to evaluate their relative hydrostatic packing characteristics by comparisons on a mass basis.

For this the apparent specific volume(ASV) is the most convenient parameter and is obtained by dividing ϕ_v by the molecular weight. ASVs are clearly important in sweet taste investigations as they allow direct comparisons of mass fractions of solutes, stimulating a finite population of receptors. More profoundly, since they reflect the degree of interaction with surrounding water molecules, they may indicate degree of accessibility to receptor sites.

Water is the medium for all sweet taste chemoreception processes. However, the hydrostatic molar and specific volumes of sweet molecules are not the only parameters of importance. Intrinsic viscosity ([η]) is a hydrodynamic solution volume which is relevant to the kinetics of mouth movement during tasting. For molecules of similar molecular weight or chemical class it is interesting to compare intrinsic viscosities with apparent specific volumes. Sugars, and their derivatives, for example, fit within a narrow range for both parameters ([η]=2.27-2.61cm^3g^{-1}; ASV=0.60-0.69cm^3g^{-1}) whereas amino acids cover a much wider range ([η]=1.29-4.20cm^3g^{-1}; ASV=0.562-0.712cm^3g^{-1}). These differences between the two chemical classes of sweeteners may be related to the fact that sugars elicit a pure sweet response whereas the sweetness of amino acids is often contaminated with other taste qualities (refs.8 and 9). It is also noticeable that intrinsic viscosity is always greater than ASV for any particular molecule and the difference between the two parameters is usually much greater for an amino acid than for a sugar molecule. All such experimental volume measurements are indicative of the effective size of sweet molecules in the aqueous environment of receptors. It is interesting to compare them with theoretically computed molar volumes.

COMPUTED AND EXPERIMENTAL VOLUMES

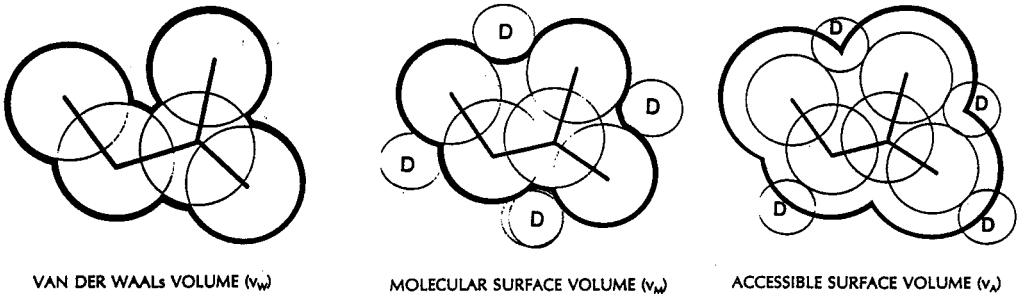

Fig. 1 Computer Generated Volumes

Figure 1 shows how van der Waal's volumes of sweet molecules can be compared with GEPOL-generated volumes, based on a mechanical interaction of the solvent molecules, (represented as spheres) and the sweet solute. Such volumes take no account of the electrostrictive effects between solute and water molecules embodied in the experimental apparent molar volume measurements but they nevertheless correlate very well with them (ref.10). Table 1 lists the equations relating apparent molar volume (ϕ_v) to molecular surface volume (Vm) for carbohydrates, amino acids and sulphamates, all of which show correlation coefficients better than 0.95. Although calculations based on molecular graphics are valuable they are not sufficiently sophisticated to include the likelihood of hydrogen bond formation or indeed encounter with water molecules around the different regions of the solute molecule. Astley et al (ref.11) have therefore used molecular dynamics simulations to obtain radial distribution functions of water molecules viewed from varying sites on the solute molecule. These calculations not only yield valuable information about hydration layer volume but also help to identify the hydration centre of sweet solutes and to localise putative glucophores as already proposed by Lichtenthaler and Immel (ref.12). They are essential for evaluating the dimensions of sweet receptor clefts as already proposed by Suami and Hough (ref.13) and they help to quantify fragmental contributions to volume which may not be revealed by experimental measurements.

TABLE 1. Equations relating apparent molar volume (ϕ_v) to molecular surface volume (V_m) for carbohydrates, amino acids and sulphamates. (refs. 10,27).

Sweetener	Equation
Carbohydrates	$\phi_v = 0.750(\pm.025)V_m - 1.83\ (\pm 4.4)$
Amino Acids	$\phi_v = 0.811\ (\pm.045)V_m - 11.4\ (\pm 5.8)$
Sulphamates	$\phi_v = 0.689\ (\pm.054)V_m + 17.6\ (\pm 7.9)$

APPARENT SPECIFIC VOLUME AND TASTE QUALITY

Table 2 lists some ranges of apparent specific volumes in relation to, not only sweetness, but all four basic taste qualities with examples.

TABLE 2. Taste quality and apparent specific volume (ASV) (ref. 14)

Taste Quality	ASV range cm^3g^{-1}	Examples
Salt	0.1 - 0.3	NaCl(0.295)
Sour	0.3 - 0.5	Phosphoric acid(0.456)
Sweet	0.5 - 0.7	Glucose (0.615)
Bitter	0.7 - 0.9	Caffeine (0.717)

The parameter is evidently a broad determinant of taste quality (ref.14) and the entire human taste range is more or less confined to the range 0.1-0.9cm^3g^{-1}. Although there may be exceptions to the general trend shown in Table 2, it allows the correct prediction of taste quality in molecules with several potential taste features (i.e. "multisapophoric molecules"). An example of the latter is gluconic acid which has both sweet (polyhydroxy character) and sour (carboxyl group) sapophores but its apparent specific volume (0.51cm^3g^{-1}) places it in the sour range which is indeed its taste. It is also interesting to note that sugars fit into the middle of the sweet range (mainly 0.60-0.64 cm^3g^{-1}) which accounts for their pure sweet taste quality and that of the related sugar alcohols. However, the apparent specific volumes of the sugar alcohols are usually about 10% higher than those of the parent sugars which indicates less effective hydrostatic packing. This must be due to the loss of cyclic structure rather than the additional hydroxylic group because scyllo-inositol has an apparent specific volume about 10% lower than the hexoses (ref.6). What do the results in Table 2 imply? The most likely interpretation is that those molecules that interact most strongly with water structure (i.e. good hydrostatic packing and low apparent specific volumes) are conveyed by the water to the deepest layers of the lingual epithelium. In other words the receptors, or ion-channels for the salt and sour stimuli, are deepest in the epithelium whereas the sweet and bitter receptors are in more shallow locations. This logical proposal is not yet supported by any anatomical evidence. However, it is known that sweet taste receptors are easily damaged by proteinases (ref.15) and that sweet and bitter sensations are more affected by temperature than are salty and sour (refs.16&17).

PARTIAL MOLAR COMPRESSIBILITY OF SOLUTE AND COMPATIBILITY WITH WATER STRUCTURE

Although apparent molar and specific volumes indicate how well a sweet molecule may be interacting with water structure, it does not follow that a molecule which reacts strongly with water is compatible with water structure. Rather a molecule with a low apparent specific volume may be disturbing water structure by generating electrostrictive forces. A better indicator of water-compatibility is the partial molar compressibility (ref. 18,19,20,21) which may be regarded as a measurement of the compactness of the hydration layer surrounding the solute. Galema and Hoiland (ref.18) have shown that the

partial molar isentropic compressibility is related to the configurations of hydroxyl groups in pyranose structures and their interaction with surrounding water structure. Galactose for example with its 2 equatorial-4 axial configuration suffers a poor "fit" with next nearest oxygen neighbours of surrounding water and this results in a very low partial molar isentropic compressibility -(20.4x10^{-4}cm^{3}mol^{-1}bar^{-1}). Although most hexopyranoses have slightly negative compressibility, they are not as low (i.e. large negative) as galactose. Ions are of course even lower in compressibility due to the compactness of their hydration layers. Sodium saccharin, having an ionic structure, has a partial molar compressibility (-25-30x10^{-4}cm^{3}mol^{-1}bar^{-1}) which is even lower than galactose and this may constitute a useful distinguishing characteristic when comparing sugars with intense sweeteners. Water itself has a positive compressibility (+8.17x10^{-4}cm^{3}mol^{-1} bar^{-1}). All such compressibility measurements are small in magnitude compared to partial molar volumes. However, they are more sensitive measurements for comparing sweetener structures.

FRAGMENTAL CONTRIBUTIONS TO PARTIAL MOLAR VOLUMES

The fragmental contributions to partial molar volumes can be obtained by comparing values of molecules in homologous series or molecular analogues. However, the values obtained depend on the molecular environment of the fragments involved and hence the amount of electrostriction which those fragments experience. The -CH_3 group, for example, contributes only about 14.5cm^{3}mol^{-1} in a polar environment but 22.6 - 26.5cm^3 mol^{-1}, in an apolar environment (refs.7,22). Similarly an -OH group (despite the higher molar mass) contributes only 0.50cm^{3}mol^{-1} (ref.22) because of the hydrogen bonding forces which it engenders.

TABLE 3. Fragmental contributions to partial molar volumes (refs. 7,22).

Molecular Fragment	Molar Contribution (cm^{3}mol^{-1})
-CH_3 (apolar environment)	22.6-26.5
-CH_3 (polar environment)	12-13
-CH_2- (acyclic)	15.9
-CH_2- (cyclic)	14.2
-CH_2OH (apolar environment)	28.2
-CHOH (polar environment)	17.0
-OH (polar environment)	0.50
Ionisation of carboxylic acids (mono carboxylic acids from butanoic upwards)	-14.2

Table 3 lists the fragmental contributions of various groups to partial molar volumes, including the contribution due to ionisation which is all negative. The difference between cyclic -CH_2 (16.9cm^{3}mol^{-1}) and acyclic -CH_2 (15.9cm^{3}mol^{-1}) is noteworthy and corroborates the known hydrostatic packing efficiency of 6-membered rings such as inositol. Cyclohexanol has a ϕ_v value of 103.5cm^{3}mol^{-1} whereas 1-hexanol has ϕ_v=118.7cm^{3}mol^{-1}. Thus the advantage of the cyclic structure is more easily lost on insertion of a cyclic -CH_2 group. In relation to this point the ϕ_v values of D-glucose and 2-deoxy-D-glucose are the same (110cm^{3}mol^{-1}). Of particular importance for sugar sweetness is the contribution of -CHOH, obtained by substituting -CH_2OH for H in a pentopyranose. The result can be obtained accurately only by comparing analogues (glucose with xylose, galactose with arabinose etc) and it is 17.0cm^{3}mol^{-1} (ref.7,23) among sugars or corresponding polyols. A corollary of this calculation is that the primary alcohol group of hexoses cannot be interfering with the interplay of hydrogen bonds among the secondary hydroxyls and the latter are the main contributors to the sweetness of sugars (ref.24) and the intense sweetness of chlorinated sugars (ref.25).

MOLAR VOLUMES OF GLUCOSE SYRUPS

Glucose syrups constitute homologous groups of molecules which are used in large quantities for sweetening foods. A particular syrup consists of a band of molecules of specified average molecular weight but with one predominant linkage, i.e. 1,4-α-D-glucopyranosidic. As the average molecular weight of a range of syrups increases, so does the apparent molar volume (measured at a normal tasting concentration). However the apparent specific volume decreases over the same range. In

other words, a unit mass of solute of large molecules packs better into water structure than does a unit mass of smaller molecules, presumably because the larger molecules have greater numbers of well-ordered hydration sites (ref.26). Confirmation of this well-ordered solution effect is given by ^{1}H NMR pulse relaxation data (T_2 - values) (ref.26,27).

TABLE 4. Apparent molar volumes (ϕ_v), ^{1}H NMR spin-spin relaxation times (T_2) and taste effects of 30% w/v glucose syrups (refs. 20,26).

Average Degree of Polymerisation of syrup	Average Molar Concn.	ϕ_v (cm^3mol^{-1})	T_2 (s)	Sweetness Intensity smurf units	Sweetness Persistence (s)
8.3	0.219	833	0.427	15.8	32.8
4.8	0.380	482	0.538	22.7	35.6
2.6	0.675	272	0.750	41.5	45.7
1.6	1.073	173	0.962	52.0	53.7
1.0	1.665	113	1.139	63.2	66.2

Table 4 lists some solution parameters and sweet taste effects of glucose syrups. There is clearly a trend in the solution properties which accord with both molar mass and hydration sites and this trend may confer an advantage on the larger molecules in terms of accession to and activation of receptor sites. Thus, although it is well known that high molecular weight glucose syrups are not as sweet as low molecular weight syrups on a mass basis, the reverse may be true on a molar basis and Kearsley et al. (ref.28) have already reported threshold data which substantiate this sweetness advantage in the larger molecules.

VOLUMES AND COMPRESSIBILITIES OF MOLECULES WHICH CHANGE DURING TASTING

Some molecules change their solution characteristics during the tasting process and the best known example of this is the mutarotation of sugars which, in many pyranoses, results in no measurable change in apparent molar volume (ref.19), presumably because the anomeric centre does not interact much with water structure. An exception to this conclusion is β-D-fructopyranose, which however, suffers a 28% conversion to the furanose form during muturotation, and this change is accompanied by a commensurate drop in sweetness. Table 5 lists some changes in apparent molar volume and apparent isentropic compressibility of sugars during muturotation of sugars. The latter parameter is much more sensitive to the mutarotation change. However, apart from fructose there are no convincing reports of sweetness changes during mutarotation.

TABLE 5. Mutarotational effects, on rotations, $[\alpha]^D$, apparent molar volumes, (ϕ_v), and apparent molar compressibilities, [$K\phi_s$] of sugars.

Sugar	$[\alpha]_D$		ϕ_v		10^3. $K\phi_s$	
	t=0-7 min	t=70 min	t=0-7min	t=70min	t=0-7min	t=70min
L-Arabinose	+172	+107	93.21	93.21	-1.632	-1.690
D-Xylose	+74.0	+23.5	94.94	95.51	-1.060	-1.144
D-Galactose	+140	+94.8	110.3	110.1	-1.769	-1.798
D-Glucose	+83.6	+64.1	112.0	112.2	-1.566	-1.530

An interesting molecule for exploring taste change whilst tasting is D-glucono-1,5-lactone (Fig.2). This molecule is analogous to D-glucopyranose and has an AH, B glucophore assignable to the same

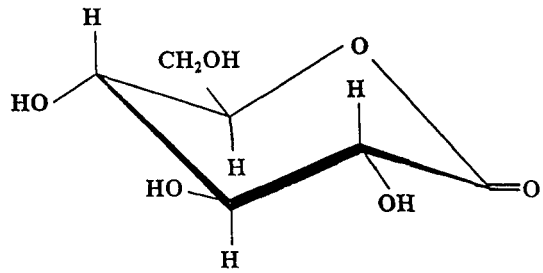

Fig. 2 D-glucono-1,5-lactone
ASV : 0.61 cm^3/g

3,4 α-glycol group. It is therefore sweet but rapidly autohydrolyses to D-gluconic acid which is sour (ref.21). The taste change is accompanied by an appropriate change in apparent specific volume from 0.61 cm^3g^{-1} to 0.57cm^3g^{-1} at equilibrium and the entire course of the reaction can be mapped by solution changes which define the change in taste. Apparent isentropic molar compressibilities are also good indicators of the change in this molecule but the equilibrium mixture is complicated, involving 1,4 -lactone, 1,5 -lactone and both dissociated and undissociated forms of the free gluconic acid. In analysing multisapophoric solutions such as this it is therefore a challenge to disentangle the perceptual attributes from the molecular volume changes.

The solution compressibility may be viewed as an opposing concept to surface tension which is a force of cohesion between the molecules (ref.1). This has led to the apparent parachors [P] which are defined by the formula

$$[P] = \phi_v.\gamma^{1/4} \qquad \text{where } \gamma = \text{surface tension}$$

Parachors are really molar volumes when surface tension is maintained at unity; in addition to their use in odour chemoreception (ref.2) they have been used in attempts to relate sweetness of sugars to solution effects (ref.30) and to derive structure activity relationships in peptide sweeteners (ref.31).

SYNERGY IN RELATION TO MOLAR VOLUMES

There is no doubt that synergy exists in mixtures of certain sweeteners (refs. 32 and 33) and one explanation of this is that there are different sweet receptors offering a greater occupation capacity to selected mixtures. However, the synergy may be explainable by the hydration characteristics of the solutes (ref.34) and hence the accession to a receptor may be favoured by a low apparent molar volume even to the extent that one sweet solute of a binary mixture is totally dominant. Evidence in favour of this latter idea has already been offered (ref.32).

MULTIPOINT ATTACHMENT AND IMPLICATION FOR THE VOLUME OF THE SWEET RECEPTOR SITE

Nofre and Tinti (refs. 35,36) have synthesised a number of highly potent sweeteners on the basis of their theory of multipoint attachment of sweet solute to sweet receptor. This theory has been elaborated to involve fifteen points of attachment (ref.36) and now defines the sweet receptor so specifically that it seems highly probable that it is unique. Evidence from both primate (ref.37) and mouse (ref.38) behaviour studies is consistent with the proposal of a single sweet receptor though there may be two genes involved, one conferring general sweet taste response and the other conferring a greater degree of sweet taste acuity. The primate studies have led to the idea of an "aspartame pocket" in the sweet receptor which is only effective in catarrhine species including homo sapiens (ref.37). Since the approximate values of the distances between the 15 recognition sites of the human sweetness receptor have now been estimated using CPK models (ref.36), we are close to estimating its effective volume from the internal topology.

CONCLUSION

Although the experimental and computational studies of molar volumes have already proved useful

exclusive mechanism for eliciting the taste response. Ion channels may be generated by some taste stimuli (ref.39) and Naim et al (ref.40) have shown that some taste substances are direct activators of G-proteins involved in transduction mechanisms. Nevertheless molar volumes have already been established as indicators of sweet taste quality and intensity and, more profoundly as quantifiers of water interaction in the initial stages of sweet taste chemoreception.

ACKNOWLEDGEMENT

We thank the BBSRC (Grant No. 45/FO2510) and the European Commission (Grant No. PL94-2107) for kind support.

REFERENCES

1. J. C. McGowan and A. Mellors. *Molecular Volumes in Chemistry and Biology*, pp 259, Ellis Horwood, New York (1986).
2. R. H. Wright. *Chem. Senses* 7, 211-213 (1982).
3. G. Beck. *Chem. Ztg.*, **46**, 18-22 (1943).
4. W. J. Spillane and G. McGlinchey. *J.Pharm. Sci.*, **70**, 933-935 (1981).
5. R. S. Shallenberger. *Taste Chemistry*, pp. 613. Blackie CH, London (1993).
6. F. Franks. *Pure Appl. Chem.* **59**, 1189-1202 (1987).
7. H. Hoiland. In *Thermodynamic Data for Biochemistry and Biotechnology* (H.-J. Hinz, ed.), 1-44, Springer Verlag, Berlin (1986).
8. S. E. Kemp, G. G. Birch, M. Mathlouthi and M. O. Portmann. *J. Sci. Fd. Agric.*, **51**, 97-107 (1990).
9. G. G. Birch and S. E. Kemp. *Chem. Senses*, **14**, 249-258 (1989).
10. W. J. Spillane, G. G. Birch, M. G. B. Drew and I. Bartolo. *J. Chem. Soc. Perkin Trans.*, **2**, 497-503 (1992).
11. T. Astley, G. G. Birch, M. G. B. Drew, P. M. Rodger and G. R. H. Wilden. *Fd. Chem.*, **56**, 231-240 (1996).
12. F. W. Lichtenthaler and S. Immel. In *Sweet Taste Chemoreception* (M. Mathlouthi, J. A. Kanters and G. G. Birch, eds.), 21-54. Elsevier A S (London) (1993).
13. T. Suami, L. Hough, T. Machinami and N. Watanabe. *Fd. Chem.*, **56**, 275-281 (1996).
14. S. Shamil, G. G. Birch, M. Mathlouthi and M. N. Clifford. *Chem. Senses*, **12**, 397-409 (1987).
15. Y. Hiji and J. Ito. *Comp. Biochem Physiol.*, **58**, 109-113 (1977).
16. B. G. Green and S. P. Frankmann. *Chem. Senses*, **12**, 609-619 (1987).
17. S. P. Frankmann and B. G. Green. *Chem. Senses*, **12**, 656 (1987).
18. S. A. Galema and H. Hoiland. *J. Phys. Chem.*, **95**, 5321-5326 (1991).
19. A. Cesaro. In *Thermodynamic Data for Biochemistry and Biotechnology* (H.-J. Hinz, ed.), 177-207, Springer-Verlag (Berlin) (1986).
20. S.A. Galema, E. Howard, J. F. N. Engberts and J. Raul Grigera. *Carbohydrate Res.*, **265**, 215-225 (1994).
21. G. G. Birch, S. Parke, R. Siertsema and J. Westwell. *Fd. Chem.*,**56**, 223-230 (1996).
22. S. Shamil, G. G. Birch, M. Dinovi and R. Rafka. *Fd. Chem.*, **32**, 171-188 (1989).
23. G. G. Birch, R. Karim, A. Lopez and G. Morini. In *Sweet Taste Chemoreception* (M. Mathlouthi, J. A. Kanters and G. G. Birch, eds), 127-140, Elsevier A S (London) (1993).
24. G. G. Birch and S. Shamil. *J. Chem. Soc. (Farad. Trans)*, **184**, 2635-2640 (1988).
25. L. Hough and S. P. Phadnis. *Nature*, **263**, 800 (1976).
26. G. G. Birch and R. Karim. *J. Sci. Fd. Agric.*, **58**, 563-568 (1992).
27. G. G. Birch, R. Karim and A. Lopez. *Fd. Quality and Preference*, **5**, 87-93 (1994).
28. M. W. Kearsley, S. Z. Dziedzic, G. G. Birch and P. D. Smith. *Starke*, **32**, 244-247 (1980).
29. G. G. Birch, S. Shamil and Z. Shepherd. *Experientia*, **42**, 1232-1233 (1988).
30. S. Shamil, G. G. Birch and S. Njoroge. *Chem. Senses*, **13**, 457-461 (1988).
31. H. Van der Heijden, H. Van der Wel and H. G. Peer. *Chem. Senses*, **10**, 73-88 (1985).
32. S. L. Munton and G. G. Birch. *J. theor. Biol.*, **112**, 539-551 (1985).
33. S. S. Schiffmann, B. J. Booth, B. T. Carr, M. L. Losee, E. A. Sattely-Miller and B. G. Graham. *Brain Res. Bull.*, **38**, 105-120 (1995).
34. G. G. Birch. *Chem. Ind.*, **1**, 13-15 (1983).
35. C. Nofre and J. M. Tinti. In *Sweet Taste Chemoreception* (M. Mathlouthi, J. A. Kanters and G. G. Birch, eds.),205-236, Elsevier A S (London) (1993).
36. C. Nofre and J. M. Tinti. *Fd. Chem.*, **56**, 263-274 (1996).
37. D. Glaser, J. M. Tinti and C. Nofre. *Chem. Senses*, **20**, 573-584 (1995).
38. I. E. Lush, N. Homigold, P. King and J. P. Stoye. *Genet. Res. Camb.*, **66**, 167-174 (1995).
39. J. G. Brand and A. M. Feigin. *Fd. Chem.*, **56**, 199-207 (1996).
40. M. Naim, R. Seifer, B. Nurnberg, L. Grumbaum and G. Schultz. *Biochem. J.*, **297**, 451-454 (1994).