Tastes, Structure and Solution Properties of D-Glucono-1,5-lactone

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Abstract

D-glucono-1,5-lactone differs from D-glucopyranose only in that it has a C=O group instead of CHOH group at carbon atom number one. The molecule therefore possesses an intact 3,4α-glycol group and is sweet. However, it autohydrolyses in water solution at room temperature, forming D-gluconic acid and D-glucono-1,4-lactone. As the solution pH falls it becomes sweet-sour and eventually almost completely sour as the generated hydronium ions dominate both the solution properties and the taste perceptions elicited. It is shown that the ratio of generated hydronium ions to unchanged lactone accords with anticipated taste quality during the first 28 min of autohydrolysis. Changes in both apparent specific volume and apparent isentropic compressibility illustrate increasing solute–solvent interaction and increasing disturbance of water structure during the course of autohydrolysis. These changes are consistent with the concurrent sweet to sour change, but do not explain the weak bitterness which also accompanies them. **Chem. Senses 22: 53–65, 1997.**

Introduction

The autohydrolysis of D-glucono-1,5-lactone incurs concurrent structural and 'sapophore' changes in the solute. Such changes are measurable by solution property and psychophysical parameters which should be mutually interpretable (Shamil *et al.*, 1987). The molecule is therefore unique as a model for exploring the mechanisms of taste chemoreception. This paper reports changes in the taste qualities in relation to solution composition and solute behaviour of D-glucono-1,5-lactone over the course of time.

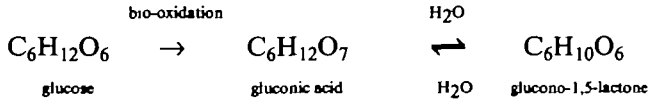
D-Glucono-1,5-lactone (also known as glucono-delta-lactone or GDL) is an interesting sapid molecule with a 'glucophore' or AH,B system (Shallenberger and Acree, 1967) identifiable at its 3,4α-glycol function. It differs from D-glucopyranose only in the absence of the hemiacetal centre, which does not, in any case, affect its taste (Birch *et al.*, 1986). The molecule therefore predictably tastes sweet, but even when crystals are placed within the mouth, the initial sweetness is soon accompanied by a sour sensation. The lactone is autohydrolysed by either water or oral fluid, producing D-gluconic acid, which is a mild acidulant.

In the organism, GDL is a normal metabolite of glucose degradation by the pentose phosphate pathway. The

FAO/WHO Joint Expert Committee on Food Additives (JECFA, 1986) has given GDL a non-specified Acceptable Daily Intake. GDL has also been given its GRAS status in 1986 by the Food and Drug Administration (FDA) in the USA (Anon., 1989).

Gluconic acid, its salts and lactones are mild, non-corrosive, non-toxic compounds. Their ability to form chelates with metal ions in caustic solutions make them important to industry. Their physiological compatibility means that they can be ingested without risk (Rajakylae, 1981) and thus are ideal molecules for taste research.

GDL is an internal ester of gluconic acid. It is produced by the fermentation of glucose, followed by crystallization of the lactone.



Because the taste sensation of aqueous solutions of GDL is complex, initially sweet and bitter, changing to sour over time, GDL provides a means of studying the relationships among chemical variables and taste quality. Accordingly, solution property measurements were carried out on solutions of GDL in water over time, and the intensities of their taste qualities determined.

Materials and methods

Chemical analyses

Chemicals used in solution measurements and psychophysical studies were reagent grade and were obtained from BDH, Lutterworth, Leicestershire, UK. Glucono-1,5-lactone was obtained from Sigma Chemical Co., Poole, Dorset, UK. Water used for solution studies was HPLC grade. Solutions were made up w/w and all measurements were carried out at 20°C. Analyses were duplicated to increase reliability. As no differences were found between analyses, the results of the first replicate are reported.

Optical rotations were measured using an automatic digital polarimeter (POLAAR 20, Optical Activity Ltd, Huntingdon, Cambridgeshire) at 589.3 nm. Specific rotations ($[\alpha]_D°$) were calculated using the following equation:

$$[\alpha]_D = 100\alpha/cL \quad (1)$$

where α = observed rotation (°); c = concentration of the solution (%); and L = length of polarimeter tube (dm).

Density and sound velocity values were determined using an Anton Paar Density Sound Analyser (DSA 48) from Paar Scientific Ltd, Raynes Park, London, UK. Temperature was maintained at 20 ± 0.1°C. The density of the sample was measured from the period of oscillation of an oscillating U-tube. The sound velocity was calculated from the propagation speed of ultrasonic pulses in a known distance within the sample in the measuring cell. The instrument was calibrated using air and distilled water. Density and sound velocity measurements were accurate to $\pm 1 \times 10^{-4}$ g/cm^3 and ±1 m/s respectively.

Apparent molar volumes (ϕ_v cm^3/mol) and apparent specific volumes (ASV cm^3/g) were calculated from density values using equations (2) and (3):

$$\phi_v = 1000(d_0 - d) / mdd_0 + M_2/d \quad (2)$$

where d_0 = density of water at one temperature (g/cm^3); d = density of solution at the same temperature (g/cm^3); m = molality of the solution (mol/kg of water); and M_2 = molecular weight of solute (g/mol):

$$\text{ASV} = \phi_v/M_2 \quad (3)$$

Isentropic apparent molar compressibilities ($K_{\phi(s)}$ cm^3/mol.bar) were calculated from both density and sound velocity values using the equation:

$$K_{\phi(s)} = 1000(\beta_s - \beta_{so})/md + \beta_s\phi_v \quad (4)$$

where β_s = isentropic compressibility coefficient of solution (bar^{-1}); and β_{so} = isentropic compressibility coefficient of water (bar^{-1}).

Isentropic compressibility coefficients were calculated from:

$$\beta_s = 100/u^2d \quad (5)$$

where u = sound velocity of solution (m/s).

Partial molar volume, partial specific volume and isentropic partial molar compressibility values were obtained at infinite dilution, by extrapolating the best fit to the curve to zero concentration.

Sensory analyses

Experiment I : changes in taste qualities of GDL over time

Subjects

Ten men and five women, ranging in age from 20 to 60 years (median age = 22 years), served as volunteer assessors.

Procedure

The subjects were screened for sensitivity to sweet (fructose), sour (citric acid), salty (sodium chloride) and bitter (quinine sulphate) materials, and their ability to identify these qualities. Each person was given samples of the tastants in increasing concentrations. They reported when a taste different from water was detected, and when the quality was identifiable. All subjects showed normal sensitivity. Some confusion between sour and bitter was found. When this occurred, the subjects were given practice in identifying those qualities. A week later, screening for the ability to discriminate taste intensities was done. For this the subjects were asked to rank three concentrations presented in random order, of each of the sweet, sour and bitter materials, and identify their qualities. All subjects did this without error.

A 10 % solution of GDL was tasted at 2, 7, 14, 21 and 28 min after introduction of bottled water (Highland Spring Ltd, Scotland) to the crystals. Samples were 10 ml and all material was spat out after tasting. The mouth was rinsed with bottled water before and after each tasting. Sweetness, sourness and bitterness were evaluated at different sessions, each a week apart, and then the series was replicated, again with weekly session. Thus a total of six test sessions were held. Three per cent fructose, 0.25% citric acid and 0.003% quinine sulphate were given as reference materials prior to the tasting of GDL in the respective sessions. For the reference materials and each tasting of GDL, the magnitude estimates of the sweetness, sourness or bitterness was indicated by panellists by marking an open-ended line; each estimate was made with respect to the reference solution.

Experiment II: estimates of the sweetness of GDL using tip of the tongue

The intensities reported by the panellists in experiment I were undoubtedly affected by mixture suppression, the reduction of intensity of tastes produced when two or more taste qualities are experienced simultaneously (e.g. Pangborn, 1961). Therefore the sweetness of GDL should be determined under conditions designed to minimize mixture suppression. Pre-exposure to one of the two tastants in a mixture can release the suppression resulting from that tastant (Kroeze, 1979; Lawless, 1979). Pilot work was conducted by holding citric acid in the mouth prior to tasting GDL to determine if its sourness would adapt sufficiently to remove the effects of mixture suppression. However, evidence of potentially harmful effects of keeping the acid in the mouth indicated this technique could not be used with assessors, and another method was sought.

Generally, the intensity of sourness and bitterness, relative to sweetness, of the appropriate tastants should be less when only the anterior tongue is stimulated than with whole mouth stimulation (Collings, 1974; Pfaffman *et al.*, 1969; Sandick and Cardello, 1981). Pilot tastings of GDL supported this expectation. Accordingly, to reduce the possible effects of mixture suppression, the sweetness of GDL was determined when only the anterior of the tongue was stimulated.

Subjects

Fifteen men and four women, with a median age of 24 years (range = 20–35) served as panellists. None had prior laboratory experience in the scaling of taste intensities.

Procedure

The method of magnitude estimation was described to the participants who then practised estimating the intensity of the hue of five samples of coloured water. When all understood the method and were comfortable making magnitude estimations, they were given labelled 25 ml samples of sweet (5% glucose), sour (0.1% citric acid), salty (0.5% sodium chloride) and bitter-tasting (0.003% quinine sulphate) solutions to remind them of those tastes. The samples were presented in 30 ml plastic cups (clear medicine cups, ART 1030, Roundstone Catering Equipment Limited, Wiltshire, UK). The assessors were told to extend their tongue, press the lips around it and then insert the tongue into the cup in order to taste the solution. They were told to note the tastes while the tongue was in the solution, and to ignore any tastes experienced during the subsequent water rinse. All subsequent tastings were also done in this manner.

The participants were then given a modulus solution of 8% glucose and told to report the number that best represented the intensity of its sweetness. Any other taste, should there be one, was to be ignored. Every 2.5 min

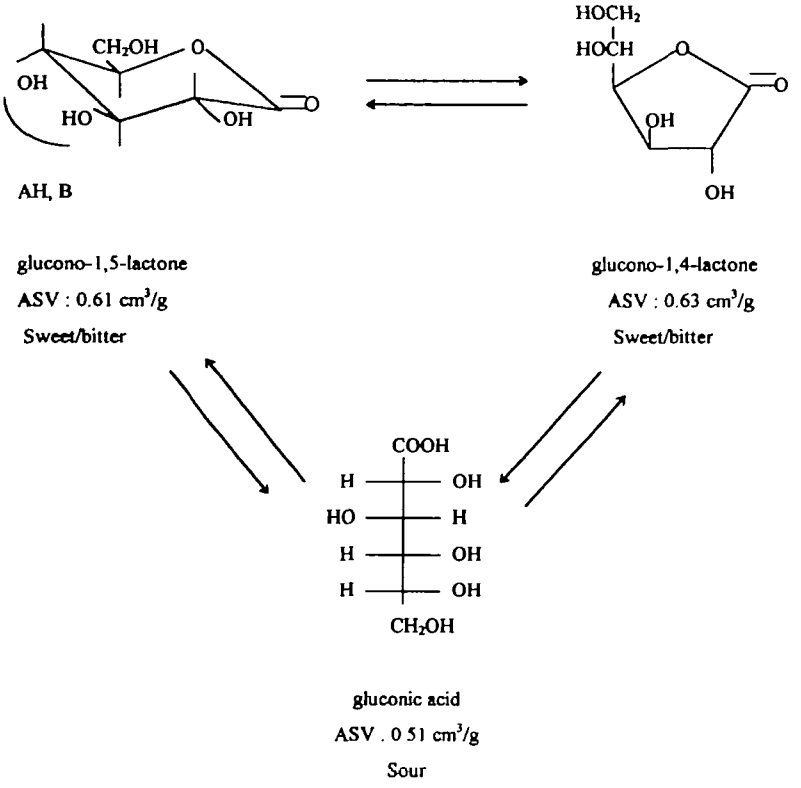


Figure 1 D-Glucono-1, 5-lactone, D-gluconic acid and D-glucono-1, 4-lactone system.

thereafter, one of 17 samples was tasted and the intensity of its sweetness estimated. Every third sample was a modulus and so identified. The panellists were told that its intensity should be rated independently of their earlier experiences with the modulus, and that the intensities of the other samples should be rated relative to the last modulus tasted. The two samples presented after the modulus presentations were randomly selected (without replacement) from four concentrations of glucose (4.0, 7.0, 10.0, 13.0%), citric acid (0.05, 0.1, 0.2, 0.3%) and 10% GDL solutions at ages 2, 7, 12 and 17 min since mixed with water. Because of constraints due to the age-times of the GDL solutions, they could be given only on the 1st, 5th, 11th, 13th and 17th presentations following the initial modulus sample. The mouth was rinsed before and after each tasting. All material was spat out.

Results and discussion

Chemical analyses

GDL is a molecule which changes both in structure and taste in solution over the course of time. The changing chemical features of the GDL molecule are measured as solution properties and these provide vital information about its hydrostatic packing characteristics within the three-dimensional hydrogen-bonded structure of water. These chemical changes were then related to the change in tastes as determined by psychophysical determinations.

On dissolution in water, glucono-1,5-lactone rapidly autohydrolyses to gluconic acid (Figure 1) which in turn eventually generates glucono-1,4-lactone. The concentration of the acid increases to a maximum at ~3 h. This corresponds to 87% of total solute as gluconic acid. When gluconic acid is dissolved, on the other hand, it lactonizes to the 1,4- and 1,5-forms. The higher the acid concentration, the greater is the rate of conversion of the acid to glucono-1,5-lactone and to glucono-1,4-lactone. The latter is a furanose form of lactone. Conversion of the acid into the furanose form (1,4-lactone) is more favoured than into the pyranose structure (1,5-lactone). Depending on the pH of the solution, direct interconversion of the lactones also occurs (Jermyn, 1960 ; Takahashi and Mitsumoto, 1963).

The pK_a of gluconic acid has been determined to be 3.60 (K_a = 2.51 × 10^{-4}) at 20°C. Gluconic acid ionizes in solution to gluconate ions and hydronium ions (Figure 2)

Autohydrolysis of GDL is slow at room temperature, with

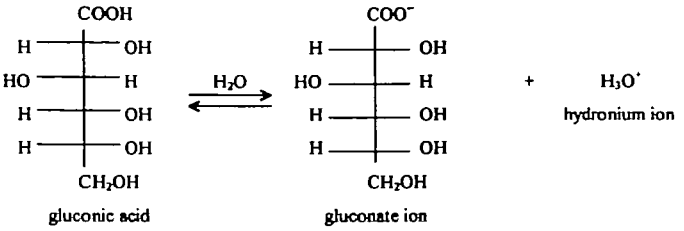


Figure 2 Ionization of gluconic acid.

the formation of ~83% gluconic acid (a small proportion of which is ionized), leaving 12% glucono-1,5-lactone and 5% glucono-1,4-lactone after 3 days. The equilibrium proportions of the constituents vary with temperature, concentration, solvent and pH (Isbell and Frush, 1963). The reaction is fastest in the first 3 h of hydrolysis as shown by HPLC (Combes and Birch, 1988) and by optical rotation plots (Figure 3).

Solution measurements carried out on a 10% solution of GDL clearly show the changes taking place in the solution.

Density values initially increase as GDL (mol. wt 178.14 g/mol) is converted to gluconic acid (mol. wt 196.16 g/mol), then decrease again as glucono-1,4-lactone is formed (Figure 4). Figure 4 also shows a similar trend in sound velocity values with a rise as the number of ions in solution increases.

Molar and specific volumes and compressibilities were then calculated from the density and sound velocity data at different times during the initial hydrolysis of GDL (Table 1).

Apparent molar volume (ϕ_v) and apparent specific volume (ASV) are expected to increase as the pyranose ring structure of GDL is converted to the acyclic form of gluconic acid. Since the free acid form of gluconic acid fits poorly with water structure, we would expect the packing efficiency of the molecules in water to be reduced as the concentration of undissociated gluconic acid rises in solution. However, dissociation of the free acid to ions would increase interaction with water and both ϕ_v and ASV might be expected to decrease. This phenomenon probably dominates in the solution, therefore an overall decrease in ϕ_v and ASV is observed. This latter point directly relates to the steady increase in sour taste with H^+ since the free hydronium ions are the principal originators of that taste quality (Ganzevles and Kroeze, 1987). ASVs fall from 0.609 cm^3/g (the sweet range) to 0.582 cm^3/g during the first 1.5 h of hydrolysis as the solution becomes more acidic (Figure 5).

Initial hydrolysis takes place so rapidly in solution that, while the solute is being dissolved, some conversion into the acid form has already taken place. Therefore the ASV of the solution at the start is estimated by extrapolating the initial part of the ASV versus time curve to time = 0. This suggests that the ASV of GDL (0.61 cm^3/g) lies in the sweet range despite the fact that the molecule tastes sweet/bitter by the time that the solution is tasted (Shamil *et al.*, 1987).

Isentropic apparent molar compressibilities ($K_{\phi(s)}$) can be regarded as the extent to which water of hydration around the solute molecule can be compressed (Galema and Hoiland, 1991). The following model briefly explains the concept of compressibility:

Large positive $K_{\phi(s)}$ ↓ Large negative $K_{\phi(s)}$

- Water ($K_{\phi(s)} = +8.17 \times 10^{-4}$ cm^3/mol.bar)
- Apolar, hydrophobic solutes
- Carbohydrates (intermediate, negative $K_{\phi(s)}$ values)
- Ions ($K_{\phi(s)} = -30$ to -50×10^{-4} cm^3/mol.bar)

Solutions become less compressible as $K_{\phi(s)}$ values become more negative. Assuming that the solutes themselves are incompressible, it follows that water is most compatible with itself and has a large positive $K_{\phi(s)}$ value. Moving down the model, compatibility of the solute with water structure decreases. In the case of carbohydrate molecules, the water structure is slightly disturbed by the hydrogen-bonded network around the solute which firmly holds the water around the solute, therefore $K_{\phi(s)}$ decreases. Ions, with their electrostrictive forces, cause water structure to collapse around the solute. This water of hydration is tightly held to the ions, reducing compressibility even further. Compatibility with water is thus reduced (even though interaction of the solute molecules with water molecules is increased), and water structure is disturbed.

Change in isentropic apparent molar compressibility and hence the structure of water is easily explained in the case of GDL (Figure 6). The lactone itself has an intermediate $K_{\phi(s)}$ value of $\sim -1.3 \times 10^{-3}$ cm^3/mol.bar. As gluconic acid is formed and the proportion of ions in solution increases, a fall in isentropic apparent molar compressibility is observed from -1.307×10^{-3} to -2.072×10^{-3} cm^3/mol.bar from 3 to 105 min of hydrolysis, indicating the disturbance of water structure caused by the ions. This results in a corresponding decrease in isentropic apparent specific compressibility from -7.337×10^{-6} to -1.155×10^{-5} cm^3/g.bar.

The whole concept of compressibility fits in well with that

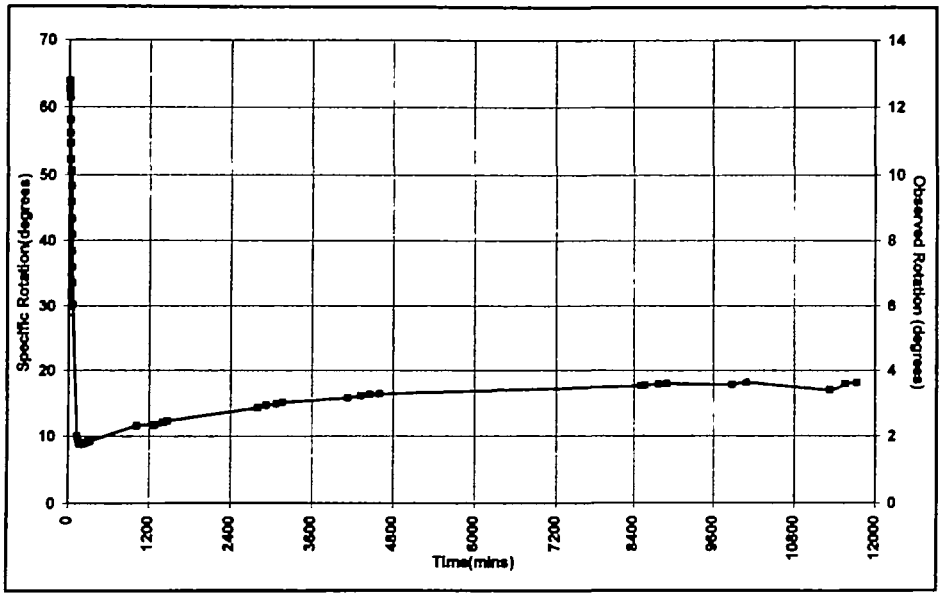


Figure 3 Plots of observed and specific rotations of GDL solution over time.

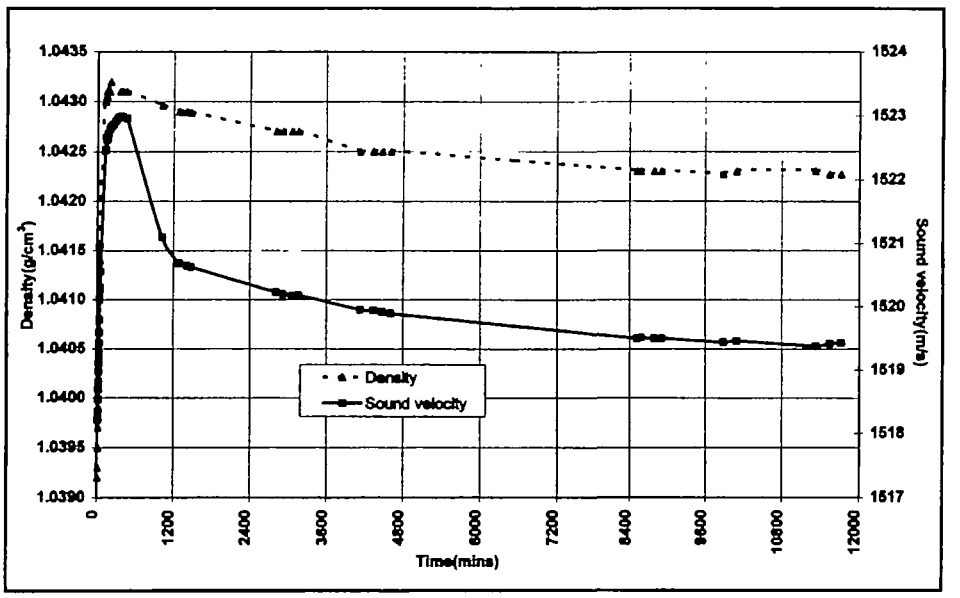


Figure 4 Plots of density and sound velocity of GDL solution over time.

of apparent molar and apparent specific volumes (Figure 7). Low $K_{\phi(s)}$, low ϕ_v and low ASV (as in the case of ionic structures which impart saltiness and/or sourness) all show that the solute molecule is heavily hydrated, therefore hydrophilic. This in turn implies that there is extensive hydrogen bonding and associated electrostrictive forces between solute and solvent molecules, and therefore the molecule can be more easily and rapidly transported to the receptors. It can also, for that matter, be transported to deeper layers of the lingual epithelium where the appropriate receptors lie (Birch *et al.*, 1993). There is physiological evidence that salt and sour receptor sites lie deeper in the lingual epithelium than sweet and bitter ones (Green and Frankmann, 1987).

Table 1 Solution property measurements on GDL solution

Time (min)	Average mol. wt (g/mol)	Density (g/cm^3)	Sound velocity (m/s)	Apparent molar volume (cm^3/mol)	Apparent specific volume (cm^3/g)	10^3 × Isentropic apparent molar compressibility (cm^3/mol.bar)	10^6× Isentropic apparent specific compressibility (cm^3/g.bar)
3	178.15	1.0389	1518.00	108.55	0.609	−1.307	−7.337
7	178.15	1.0390	1518.14	108.39	0.608	−1.332	−7.479
14	178.17	1.0393	1518.34	107.92	0.606	−1.388	−7.792
21	178.20	1.0397	1518.81	107.28	0.602	−1.481	−8.314
28	178.23	1.0401	1519.10	106.66	0.598	−1.558	−8.740
35	178.30	1.0405	1519.59	106.07	0.595	−1.650	−9.255
42	178.37	1.0408	1519.97	105.64	0.592	−1.730	−9.463
49	178.45	1.0411	1520.45	105.23	0.590	−1.798	−10.08
63	178.78	1.0416	1521.03	104.74	0.586	−1.900	−10.63
77	178.97	1.0419	1521.20	104.44	0.584	−1.945	−10.87
91	179.32	1.0422	1521.59	104.30	0.582	−2.004	−11.17
105	179.43	1.0425	1521.98	103.91	0.579	−2.072	−11.55

Initial glucono-1,5-lactone concentration = 0.6264 mol/l.

Sensory analyses

Experiment 1: Changes in taste qualities of GDL over time

The magnitude estimates were normalized for each subject separately for each session by dividing the estimates for GDL by that given for that session's reference material. Thus, the normalized estimate of the intensity of a GDL solution was proportional to the appropriate reference solution. A normalized estimate of 1.0 indicates the intensity was equal to that of the reference solution for the individual; an estimate of 1.5 indicates that the intensity was one and a half times more intense than the reference solution, etc.

Separate repeated-measures ANOVAs were performed for the estimates of sweetness, sourness and bitterness of GDL averaged over the two replications. Source tables for these analyses are given in Table 2, and plots of the mean estimates are shown in Figure 8. There were significant changes in the perceived intensity of sweetness, sourness and bitterness as age of GDL increased. For sweetness, the intensity decreased about one-third as GDL aged from 2 to 28 min. For the other two tastes, intensity increased ~4.5 times for sourness and 2-fold for bitterness, from that at age 2 min to age 28 min. Many of the panellists commented on the sourness of the GDL solution as being extremely unpleasant after 21–28 min.

Although the reference solutions were chosen to be approximately equal on the basis of pilot tasting by the authors, equality cannot be assumed for the individuals in the panel, and meaningful comparisons of intensity cannot be made between the taste qualities. However valid comparisons of the overall direction of change can be made.

The sweetness intensity of glucono-1,5-lactone throughout the first experiment was found to be lower than the sweetness of the 3% fructose reference supplied. The intensity of bitterness at time = 25 min was the same as that of the 0.003% quinine sulphate. For sourness, GDL time = 17 min had the same intensity as that of the 0.25% citric acid, i.e. a mean intensity response of 1.0 (Figure 8). These figures may well understate the intensity experienced if mixture suppression were absent.

Experiment II: Estimates of the sweetness of GDL using tip of the tongue

Each individual's estimates were normalized by multiplying them by the quotient of 10.0 divided by the mean of the estimates for the moduli. Thus the sample estimates were all represented relative to each individual's estimate of 8% glucose.

While tasting with only the anterior tongue reduced the sourness of the taste of the GDL, some sourness remained. Comments by participants and inspection of the data suggested that people differed in their ability to discriminate sweet and sour tastes with tongue immersion. Accordingly, for the analyses the participants were classified on the basis

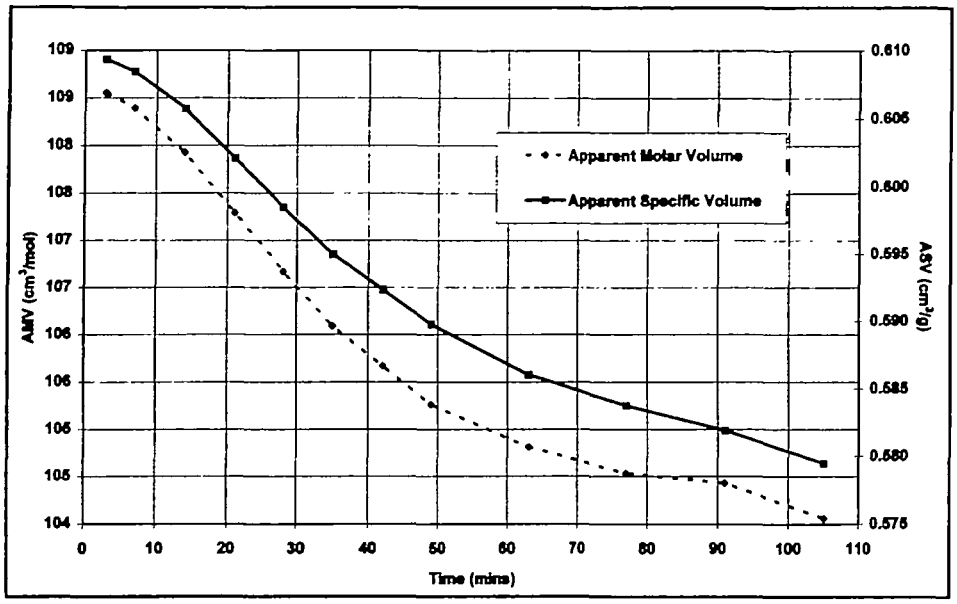


Figure 5 Plots of apparent molar and apparent specific volumes of GDL solution over time.

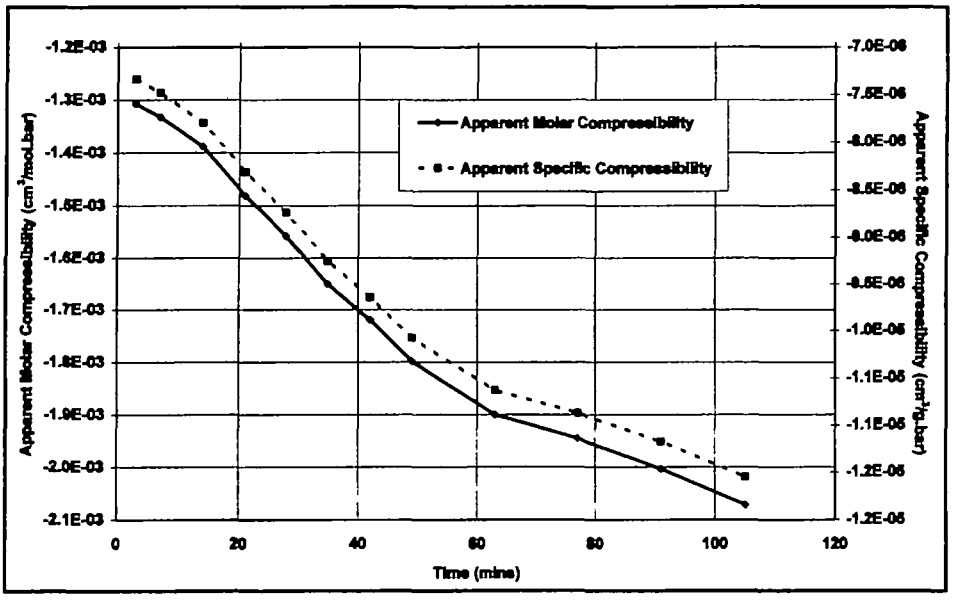


Figure 6 Plots of isentropic apparent molar and apparent specific compressibilities of GDL solution over time.

of the ratio of their mean estimate of the sweetness of glucose to that of citric acid. Those who reported the relative sweetness of glucose to be >4 times that of citric acid were classed as relative discriminators (n = 10), and the remaining as relative non-discriminators (n = 9). Thus the data were analysed by separate split-plot ANOVAs for each tastant. The main effects were age of GDL solution and discrimination class; for citric acid and glucose they were concentration and discrimination class. Table 3 presents the source tables for the analyses. Figure 9(a) shows the relative sweetness intensities of GDL as a function of age of GDL solution, grouped by discrimination class, and Figure 9(b,c) shows the sweetness intensities of glucose and citric acid as a function of concentration, with participants grouped by relative discrimination of sweetness in all three figures.

For the estimates of the sweetness of GDL, a significant

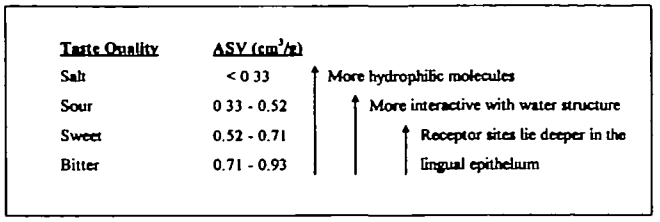


Figure 7 Apparent specific volumes and taste quality. (Source: Shamil *et al.*, 1987.)

interaction of age of solution and discrimination class was found. For those individuals classed as discriminators, the sweetness of GDL decreased as the GDL solution aged, and the concentration of the lactone decreased. However, the non-discriminators, who were apparently confusing sourness with sweetness, reported a general increase in intensity as the solution aged, and the concentration of gluconic acid and thus H^+ increased.

For the estimates of sweetness of glucose, there were no differences between the discriminators and non-discriminators; both showed very similar increases in sweetness as concentration of glucose increased. For the estimates of the sweetness intensity of citric acid there was no interaction of concentration of solution and classification, but, as expected, there was a significant main effect for classification. This difference in results for glucose and citric acid supports the interpretation that the classification into discriminators and non-discriminators was indeed based on a difference in response to a non-sweet quality; when only sweetness was involved, the groups did not differ.

Since all three sweetness functions were obtained from the same test sessions using a common modulus, comparisons can be made between them. The linear regression function for glucose, using log-transformed data from the 19 panellists, was used to determine the glucose concentration producing the sweetness of a 2 min old GDL solution. The function was found to be $y = 0.6368x + 0.4451$, $r^2 = 0.96$, where y is the log magnitude estimate of sweetness and x the log concentration of glucose (% w/v). Thus the sweetness of the 2 min old GDL was about that of 3% glucose.

Glucono-1,5-lactone and glucono-1,4-lactone have ASVs of 0.61 and 0.63 cm^3/g respectively and they both taste sweet/bitter (Shamil *et al.*, 1987). They possess the AH,B sites on their molecules which is believed to cause sweetness (Figure 1). The bitterness may originate in the C=O group which is always present in solution but increasing

Table 2 ANOVA source table, experiment I

Source of variation	df	MS	*F*	*P*
Sweetness				
Individuals	15	2.134		
Age of GDL	4	0.461	5.53	<0.001
Residual	60	0.083		
Sourness				
Individuals	15	1.655		
Age of GDL	4	9.600	65.39	<0.001
Residual	60	0.147		
Bitterness				
Individuals	15	2.362		
Age of GDL	4	1.434	8.04	<0.001
Residual	60	0.178		

concentration of gluconic acid may also elevate bitterness over the course of time.

Gluconic acid has an ASV of 0.51 cm^3/g and this clearly places it in the sour range of taste quality (Figure 7). It dissociates in solution ($pK_a = 3.60$) (Figure 2). As the pH of the solution falls during autohydrolysis (Figure 12), the intensity of sourness increases. Even though a fall in pH from 3.82 to 2.5 causes the proportion of dissociation of gluconic acid in solution to fall from 62.4 to 7.2% after 105 min, there is still a large concentration of the acid already in solution which supplies the necessary hydrogen ion concentration to maintain sourness (Table 4).

The concentration of lactone at time = 28 min (Table 4) suggests that there are enough molecules in solution to cause sweetness; ~99.5 % of the solution is the lactone. But the significant fall in sweet sensation reported by panellists is probably the result of mixture suppression by the sourness of gluconic acid. Hydrogen ion concentration increased by a factor of five from 2 to 28 min and this explains the exponential increase in sourness intensity. The relatively weak intensity of sourness at the beginning of hydrolysis was possibly due to the hydrogen ion concentration at time = 2 min (~1.5×10^{-4} M) being below the threshold for sourness.

Regression analyses at times 2, 7, 14, 21 and 28 min show that pH and H^+ concentration are highly correlated to percentage sourness (Table 5). We can therefore assume that sourness is mainly due to protons in solution.

Regression analyses show that the sweetness of GDL follows the molar concentration of lactone, and sourness follows the concentration of H^+. For experiment I, ψ =

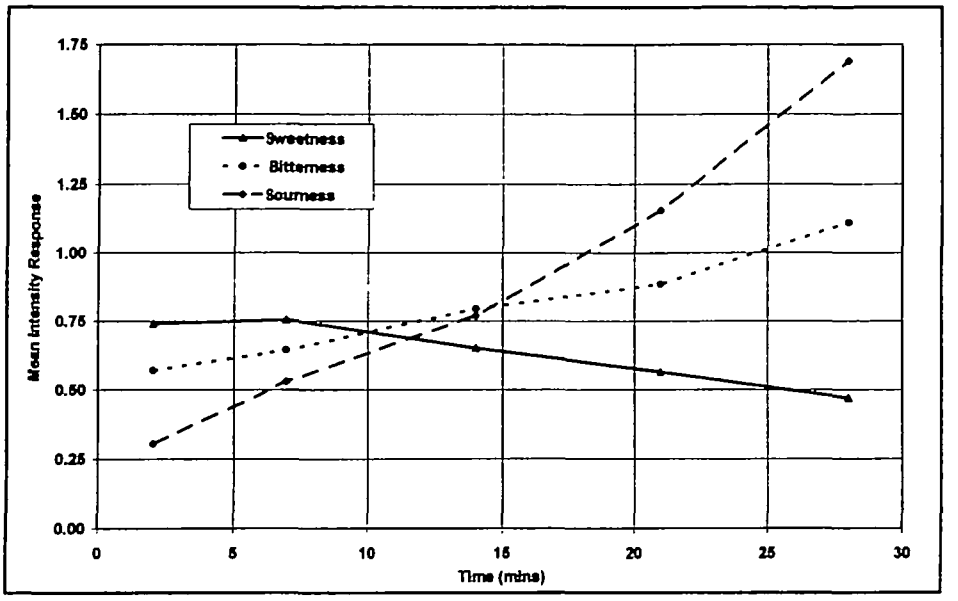


Figure 8 Experiment I (whole mouth tasting): mean intensity estimates for the sweetness, bitterness and sourness of GDL solution over time

Table 3 ANOVA source table

Source of variation	df	MS	*F*	*P*
GDL				
Individuals	18	119.587		
Discrimination class	1	541.156	5.709	<0.03
Individual within class	17	94.789		
GDL age	3	7.355	0.478	NS
GDL age × discrimination class	3	44.698	2.906	<0.05
GDL age within class	51	15.379		
Glucose				
Individuals	18	19.363		
Discrimination class	1	13.914	0.707	NS
Individual within class	17	19.684		
Glucose concentration	3	216.262	12.929	<0.001
Glucose concentration × discrimination class	3	14.143	0.846	NS
Glucose concentration within class	51	16.727		
Citric acid				
Individuals	18	41.107		
Discrimination class	1	421.402	22.491	<0.001
Individual within class	17	18.736		
Citric acid concentration	3	27.962	1.916	NS
Citric acid concentration × discrimination class	3	26.143	1.791	NS
Citric acid concentration within class	51	14.593		

100.07[lactone] – 55.61, $r^2 = 0.98$, and for the discriminators in experiment II: $\psi = 2155.4$[lactone] – 1208.7, $r^2 = 0.95$. The regression of sourness intensity judgements in experiment I over H^+ concentration produced $\psi = 2077.3[H^+] + 0.006$, $r^2 = 0.96$. The difference in slopes for the two sweetness functions is likely to be due to differences in procedure (whole mouth versus anterior tongue tasting) and in effects of mixture suppression. The slope was steeper for experiment II, where suppression was expected to be reduced by localisation of stimulus than in experiment I. This is the

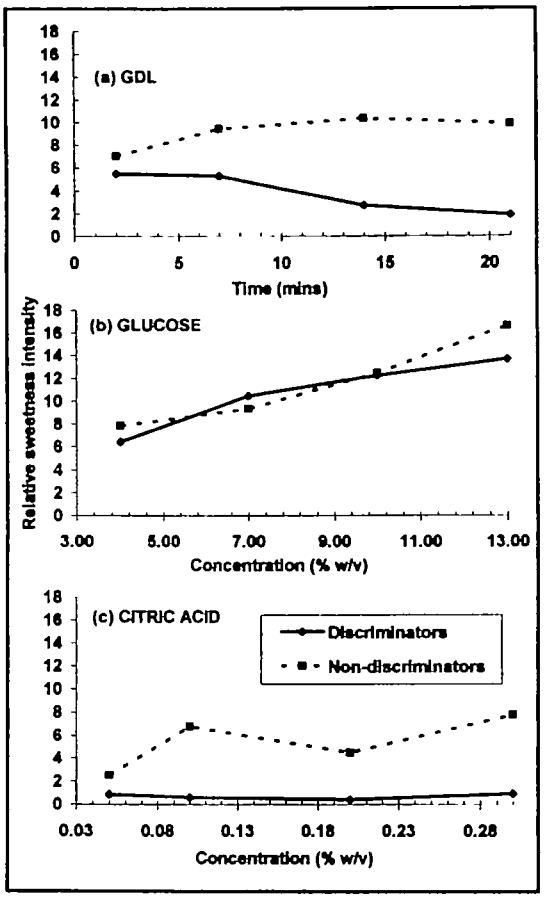


Figure 9 Experiment II (tip of the tongue tasting) : Sweetness intensities, relative to 8% glucose, for GDL, glucose and citric acid, grouped by subjects' discrimination of 'sweetness' vs sourness of citric acid. For GDL the results are shown as a function of age of solution. Table 4 gives the relevant concentrations of lactone and gluconic acid. For glucose and citric acid the results are shown directly as a function of concentration.

Table 5 Relationship between pH and hydrogen ion concentration to relative sourness of GDL solution

Time (min)	Mean sourness	pH[a]	$[H^+]^b$ (mol/l)
2	0.31	3.84	1.445×10^{-4}
7	0.53	3.69	2.042×10^{-4}
14	0.77	3.36	4.365×10^{-4}
21	1.16	3.23	5.888×10^{-4}
28	1.69	3.12	7.586×10^{-4}

[a] $r^2 = 0.9345$.

[b] $r^2 = 0.9802$.

expected result of reduced mixture suppression since suppression is related to concentration.

Conclusion

The solution chemistry of D-glucono-1,5-lactone and its hydrolysis products was explored in terms of solute hydration, hydrostatic packing characteristics and compressibilities. These parameters allowed the interpretation of some of the taste changes which accompany the autohydrolysis of the molecule. The autohydrolysis of D-glucono-1,5-lactone is accompanied by a marked increase in sourness, which is explained by the increase in hydronium ion concentration. The small, but significant drop in sweetness cannot, however, be attributed to any significant

Table 4 pH and concentration of constituents as GDL autohydrolyses over time

Time (min)	pH	$[H^+]$ (mol/l)	[Gluconic acid] total (mol/l)	[Lactone] as GDL (mol/l)	% Lactone remaining	% acid dissociated
3	3.82	1.514E-04	2.426E-04	0.626	99.96	62.40
7	3.69	2.042E-04	3.701E-04	0.626	99.94	55.16
14	3.36	4.365E-04	1.195E-03	0.625	99.81	36.53
21	3.23	5.888E-04	1.969E-03	0.624	99.69	29.90
28	3.12	7.586E-04	3.049E-03	0.623	99.51	24.88
35	2.97	1.072E-03	5.642E-03	0.621	99.10	18.99
42	2.89	1.288E-03	7.895E-03	0.618	98.74	16.32
49	2.82	1.514E-03	1.063E-02	0.616	98.30	14.23
63	2.65	2.239E-03	6.042E-01	0.604	96.46	10.09
77	2.59	2.570E-03	2.887E-02	0.597	95.39	8.90
91	2.51	3.090E-03	4.111E-02	0.585	93.44	7.52
105	2.49	3.236E-03	4.492E-02	0.581	92.83	7.20

Initial glucono-1,5-lactone concentration = 0.6264 mol/l.

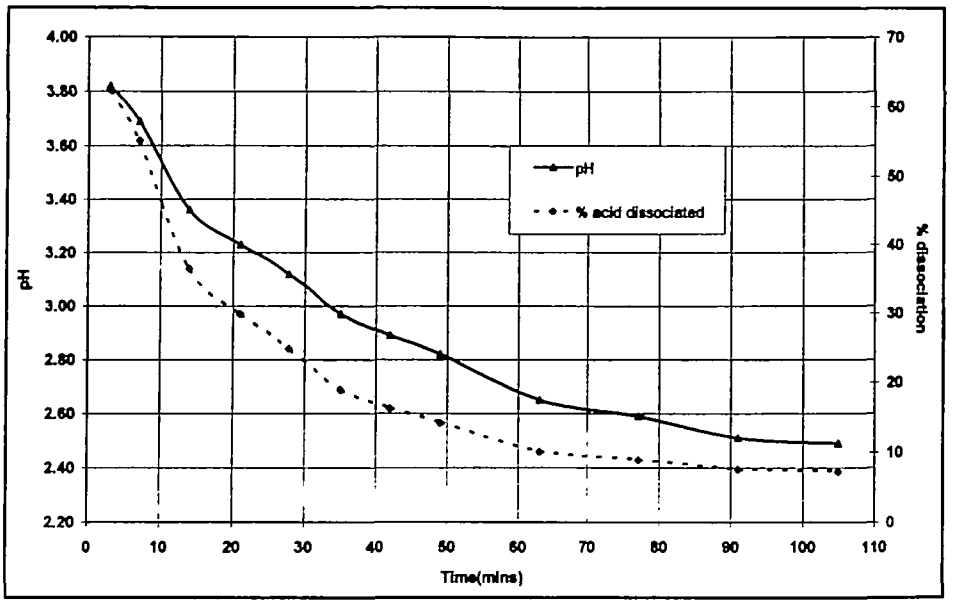


Figure 10 Change in pH and percentage dissociation of gluconic acid with time

change in lactone concentration but is presumably caused by mixture suppression. The results of this experiment conceptualize the use of combined solution effects for explaining the taste qualities of sweeteners. For example, molecules such as sodium saccharin and acesulfame K present combined ionic solutes to the receptors which might be interpretable in a similar manner to D-glucono-1,5-lactone. Such sweeteners are now under investigation.

ACKNOWLEDGEMENTS

We thank the European Community (EC-AIR PL-94-2107) and the BBSRC (45/F02510) for funding in support of this work.

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Received on May 14, 1996; accepted on July 24, 1996