Effect of Salivary Components on Volatile Partitioning from Solutions

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Partition of fourteen volatile compounds, representing the diverse physicochemical properties of aroma compounds, was measured by static equilibrium headspace in solutions containing the components of artificial saliva, either singly or in mixtures. Comparison of a bovine salivary mucin and pig gastric mucin showed no significant difference in partition behavior of the volatiles, so gastric mucin was used. Mucin viscosity changed with pH, but binding of volatile compounds did not show a marked dependence on pH. All combinations of the salivary components were tested for their effect on partition. Three types of behavior were noted. Partition of some compounds was unaffected by mucin, and with other compounds mucin decreased partition, whereas another group showed a decrease with mucin that was affected by the presence of salivary salts and sugar. When volatiles or sugar were added to a mucin solution, the final headspace concentration depended on the order of addition, indicating some competition for binding. These solute−mucin effects are discussed in relation to mucin structure and behavior in solution.

Keywords: *APIMS; sucrose; solute; interactions; mucin; artificial saliva*

INTRODUCTION

The mechanisms by which flavor compounds are released from foods, so that they can reach the gustatory and olfactory receptors in the mouth and the nose, respectively, have been discussed by several authors (*1*−*3*). For volatile compounds, the process can be summarized in terms of (a) initial release from the food into the saliva phase in mouth, (b) partitioning from the saliva to the air phase in the mouth, and (c) dilution and transport from the air phase in the mouth to the airways of the nose. This paper focuses on (b), the saliva−air partition step.

Because saliva is a hypotonic fluid, it contains the usual electrolytes of the body fluids: with sodium, calcium, potassium, chloride, phosphate, and bicarbonate being the principal ions (*4*). The bicarbonate concentration of saliva is highly dependent upon the type of salivary gland from which it originates, the nature of the stimulation, and the flow rate. As a result of a concomitant increase in bicarbonate concentration, the salivary pH rises with increasing rates of secretion. Saliva pH can range from 6.2 to 7.4, with the higher pH exhibited upon increased secretion (*4*). Besides the salts, saliva also contains proteins: the glycoprotein mucin, which is the major component responsible for the viscosity of saliva, and α-amylase, which has potential for starch hydrolysis.

As the collection of suitable volumes of human saliva for experimental purposes is tedious and unpleasant, several authors have formulated "artificial saliva" solutions, which use salt and protein concentrations typical of those found in the population (*5*−*8*). When formulating artificial saliva, the protein components of these mixtures (amylase and mucin) are often substituted by those from nonhuman sources, which are more abundant and therefore more economical than the human variants. A comparison of artificial and natural saliva by van Ruth and Roozen (*9*) showed no significant differences in the aroma release of six compounds from these solutions. When the two salivas were used to study release from a real food (dehydrated bell peppers) in a model mouth system, again, no significant difference was noted between artificial saliva (containing either porcine or human α-amylase) and the human saliva (*9*). Therefore, it appears that the formulation of artificial saliva using cheaper, substitute protein sources is an acceptable alternative to using human saliva.

When volatiles are released from food into the saliva phase, interactions may occur either between the volatile compounds and small-molecular-weight solutes (salt or sugar) and/or between proteins and volatile compounds in the liquid phase. It is well established that small-molecular-weight solutes in the aqueous phase can significantly affect volatile partition between the liquid and gas phases (*10*, *11*). The relationship has been expressed previously both in thermodynamic terms (*12*) and as a quantitative structure property relationship (*13*). Most of the work has studied the effect of solutes found in foods such as sugars, salts, and acid using simple aqueous solutions of the solutes. However, the components of saliva can also affect liquid−air partition. Research by van Ruth et al. studied volatile release from dehydrated bell pepper using various solutions to examine the effects of salivary components on release (*14*). These effects were studied in a device that simulated mouth conditions, and the concentrations of volatiles released into the headspace were measured. Under these conditions, there was no difference in volatile release when a solution of salivary salts or water was used, suggesting the salts had no effect on release.

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Adding α-amylase to the salt and mucin solutions caused no further change in headspace concentration, leading to the conclusion that this enzyme had an insignificant effect on volatile release from saliva solutions in low-starch systems (*14*). In a later study, significant differences in aroma release from high-starch foods using artificial saliva with and without amylase were observed (*9*). This result was obtained in a model mouth system over a relatively long period of time, however, and may not reflect the situation that occurs in vivo.

Mucin was identified as the key component in saliva that affected flavor release (*14*). It was suggested that less volatile, more hydrophobic compounds were more likely to be affected by the presence of mucal proteins although the peak areas of a wide range of volatile compounds decreased significantly in the mucin-containing saliva. The flavor−protein interactions were particularly significant in the case of aldehydes, which have been shown by other authors to bind either reversibly or irreversibly to proteins (*9*, *14*−*18*). Binding of volatiles to proteins in aqueous solutions has been reported for the dairy protein *β*-lactoglobulin (*19*) and various other food proteins (*20*). The effect of pH on the degree of binding to some proteins has also been shown (*21*).

This paper focuses on the interaction of volatile compounds with mucin and salivary salts. The rationale for this choice is as follows. Changes in partition behavior mediated by solutes depend on the molar concentration of the solute (*12*), and salivary salts are present at sufficient concentration to exert a significant effect for some compounds. Although the work of van Ruth et al. reported no significant changes for salivary salts, they used dynamic headspace techniques to investigate partition in a complex biological system (*14*). There may be subtle effects with some volatile compounds, and the use of simple aqueous solutions and a static equilibrium headspace analysis will show any partition effects clearly. Mucin, in contrast, cannot contribute through this mechanism as its molecular weight is about 4 orders of magnitude higher than those of simple salts and its molar concentration is correspondingly small. Mucin causes changes in partition because it can bind some compounds, reducing their effective concentration in solution and, therefore, their concentration in the headspace. Mucins possess similar structures across species and the structure of pig gastric mucin in solution is typical, consisting of a long extended protein chain with oligosaccharide side chains attached (*22*). It is these charged side chains that interact with solutes (e.g., salts and sugars) by modifying the charge repulsion between each mucus glycoprotein molecule (*22*). This charge modification alters the amount of "free space" between the mucin molecules and may therefore affect the interactions of mucins with volatile compounds.

The aim of this paper is to study the effects described above using a range of volatile compounds representing different degrees of polarity. The volatiles were chosen to cover a wide range of log *P* values, the octanol−water partition coefficient . Log *P* has previously been shown to be an important parameter in release of volatile compounds from solutions (*13*, *23*). The effects of sugar and salivary components (and their order of addition) will be investigated in terms of volatile partition and their effects on mucin conformation.

Table 1. Volatile Compounds, their Properties, and Concentrations Used

volatile compound	molecular weight	log *P*[a]	concentration used (mg/L)
2-methyl butanol	88.15	1.345	8.2
benzaldehyde	106.12	1.722	7.3
cymene	134.22	3.708	6.0
decanal	156.27	2.823	4.9
decanol	158.28	3.32	4.9
diacetyl	86.09	−1.021	9.8
dimethyl cyclohexanone	126.20	2.653	6.2
dimethyl pyrazine	108.14	0.722	24.8
ethyl hexanoate	144.21	2.023	6.1
heptanal	114.19	1.634	5.0
heptyl acetate	158.24	2.42	6.1
linalool	154.25	2.517	6.1
menthone	154.25	3.148	6.3
methyl acetate	74.08	−0.136	9.3

[a] Log *P* values were calculated using the atom-typing method of Ghose et al (*35*).

Table 2. Components of Artificial Saliva Solutions

solute	concentration required
calcium chloride dihydrate	7.5 mmol
sodium chloride	37.5 mmol
sodium bicarbonate	15.5 mmol
potassium phosphate dibasic trihydrate	15 mmol
potassium chloride	15 mmol
pig gastric mucin (or BSM)	0.2%

MATERIALS AND METHODS

Volatile compounds (>99% pure; Table 1) were obtained from Sigma-Aldrich (Gillingham, U.K.) and were used without further treatment. Sucrose and salts (analytical-reagent grade) were obtained from Fischer Scientific U.K. Ltd. (Loughborough, U.K.). Pig gastric mucin (PGM; partially purified Type III) and bovine submaxillary mucin (BSM; Type I-S) were obtained from Sigma-Aldrich.

Sample Preparation. Solutions of volatile compounds were made up with appropriate concentrations of volatiles so that the gas-phase concentrations produced similar ion intensities when analyzed by APcIMS (Table 1). Batches of solutions, each containing just two volatile compounds, were prepared to avoid any solute interactions. These solutions were shaken vigorously for 3 h on a SF1 flask shaker (Stuart Scientific, Redhill, U.K.) at ∼550 oscillations/min to ensure solubilization.

Sucrose solutions were prepared at a concentration of 34% (w/w). Aliquots (0.5 mL) were taken from the batch solutions of volatile compounds and added to the aqueous sucrose solutions to give final volatile concentrations of 1−25 mg L^{-1}. The samples were mixed for 24 h on a SRT2 roller bed (Stuart Scientific) and then equilibrated for up to 2 h at 25 °C in the sampling vessels prior to analysis.

Artificial saliva was made up using the components listed in Table 2. Solutions containing just mucin or salt were also prepared along with mixtures of mucin and salt. All solutions were adjusted to pH 7.00 (± 0.01). Where saliva plus sugar solutions were needed, the volatile was added to the sucrose solution and mixed thoroughly before the mucin solutions were added. Where this was the case, solutions of sugar and salivary components were made up at double strength so that the required concentrations were obtained on mixing.

Table 3 shows the order of addition for a series of solutions used to study interactions. For instance, water−mucin−volatile was added to a flask and equilibrated for 3 h before adding sugar; this was compared with water−mucin−sugar, which was also equilibrated for 3 h before adding volatile. These solutions were allowed to equilibrate by rolling the solution on a SRT2 roller bed (Stuart Scientific) at 4 °C for 4 h (to minimize volatilization of the aroma compounds) and then equilibrating to 25 °C before static equilibrium headspace analysis.

Table 3. Treatments to Investigate the Effect of Sequential Addition of Volatile and 34% Sugar to Aqueous or Artifical Saliva Solutions, on Final Headspace Concentration of Volatile Compounds

	initial solution	incubation at 22 °C	secondary addition
control	200 mL of H_2O 0.4 mL of volatile		
treatment 1	100 mL of H_2O 0.4 mL of volatile	3 h	100 mL of sugar solution
treatment 2	100 mL of H_2O 100 mL of sugar solution	3 h	0.4 mL of volatile
treatment 3	100 mL of artificial saliva 0.4 mL of volatile	3 h	100 mL of sugar solution
treatment 4	100 mL of artificial saliva 100 mL of sugar solution	3 h	0.4 mL of volatile

Static Equilibrium Headspace Analysis. Portions of the final solutions (50 mL) were placed in sealed glass Schott bottles (Fischer Scientific, Loughborough Leicestershire, U.K.) and equilibrated at 25 °C until a static equilibrium was attained: a maximum of 2 h for the volatiles used here. The headspace (73 mL) above each solution was sampled for approximately 30 s at a flow rate of 10 mL/min using a heated (120 °C) gas-phase interface, MS Nose, into the APcIMS (atmospheric pressure chemical ionization mass spectrometry) source (MicroMass, Manchester, U.K.). There, the volatile compounds were ionized by a 4-kV corona discharge (cone voltage 21 V) before they were sampled into the high vacuum region of the mass spectrometer. This resulted in minimal dilution of the headspace due to the small volume sampled. The ion trace showed an almost instantaneous increase as headspace was sampled, followed by a plateau value, which was maintained for the 30 s period of sampling. The plateau values for three replicates were obtained, and headspace concentrations were expressed as the ion current for the $[M+H]^+$ ion. Because each solution contained only two volatiles, it was facile to identify the characteristic $[M+H]^+$ ion of each component. At the concentrations used, there was no ion suppression (*24*).

Capillary Viscometry. The viscosity of each solution (2 mL) was measured in an Ostwald-type capillary viscometer (Schott Gerate Automatic Viscometer System 400). Each sample was equilibrated in the water bath for 15 min at 25 °C prior to the collection of 10 replicate measurements. Viscosity was expressed directly or as relative viscosity using the relationship

$$\eta_{rel} = \eta/\eta_0$$

where η_{rel} is the relative viscosity, η is the viscosity of the solution (e.g., sugar + water), and η_0 is the viscosity of the solvent (e.g., water). η_{rel} was converted to Poise by multiplying by the viscosity of the solvent at the relevant temperature. In the first instance the solvent was water; the viscosity of water is 0.8904 cP at 25 °C (*25*).

RESULTS AND DISCUSSION

Type of Mucin Used and Effect of Solution pH. Preliminary studies compared the effect of two different mucins, bovine submaxillary mucin (BSM) and pig gastric mucin (PGM), on the partitioning of four different volatiles between the air and liquid phase using static equilibrium headspace analysis. By its nature, static equilibrium headspace measures interactions that occur between molecules in the liquid phase (for example, solute−volatile and protein−volatile) but is not affected by viscosity, so that comparison can be made between systems of different physical properties. Although submaxillary mucins are believed to consist of a lower overall proportion of carbohydrate and shorter carbohydrate chains, compared to that of other mucins, they do have an overall homology with mucins like PGM in terms of assembly and conformation (*26*). There was no significant difference in the effect of BSM and PGM (as measured by headspace) on the volatile compounds tested (Figure 1) showing that the cheaper PGM was a suitable substitute for the more expensive BSM, in terms of the effect on volatile release. This common behavior between BSM and PGM is supported by the results of van Ruth and Roozen (*9*) who found no significant difference in volatile release between artificial saliva (containing porcine mucin) and human saliva that was collected from volunteers.

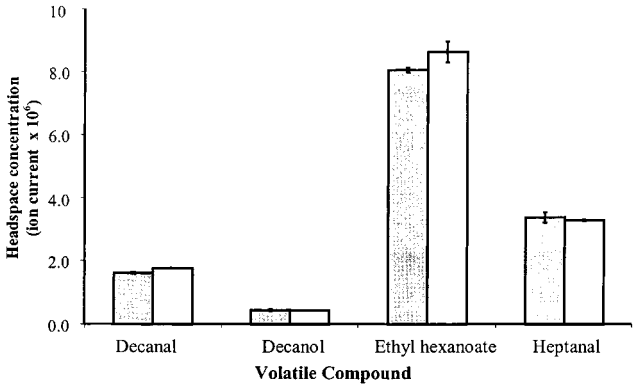

Figure 1. Equilibrium headspace concentrations of volatile compounds above bovine submaxillary mucin (grey) and porcine gastric mucin (white).

The comparative effects of PGM and BSM on volatile release reported in Figure 1 were obtained at pH 7.01 (± 0.01). Changing the pH of the solution over the pH range expected in saliva (Figure 2) did not cause significant changes in headspace concentrations. However, changing the pH of the PGM solution caused notable changes in the viscosity of the solutions. These were quantified (Figure 3) by carrying out viscosity measurements in water and in the presence of sucrose (18%) at a concentration likely to be found in the mouth during consumption of certain foods (*27*). In other experiments (Table 3) a higher concentration (34%) of sucrose was used to study headspace changes; but, for the viscosity experiments, a lower sucrose concentration was used because of problems with air bubbles forming in the more viscous solutions containing mucin and 34% sucrose. Figure 3 shows that as pH changed across 0.8 units, from pH 6.2 to 7.0, the relative viscosity decreased by 14% in water and 23% when sucrose was present. Mucin has an overall negative charge due to the sulfate groups on the oligosaccharide side chains, and as the pH increases the charge interaction between mucin moieties will change, changing the "space" between them and, therefore, the relative viscosity.

Effect of Sugar and Salivary Components on Air−Solution Partition. Initially, the effect of salivary components on the static equilibrium headspace above solutions of the 14 volatiles was measured in the presence and absence of sucrose (34%, a concentration likely to be found in mouth (*27*) when consuming high-sugar foods). Although all possible combinations of salivary components were tested, Table 4 presents those that showed significant changes. In Table 4, the equilibrium headspace concentrations are expressed relative to water, so that values <1 denote retention of the compound in the liquid phase, whereas a value >1 represents a "salting out" effect. Table 4 is arranged to show the three types of behavior noted among the fourteen compounds tested. One group showed very

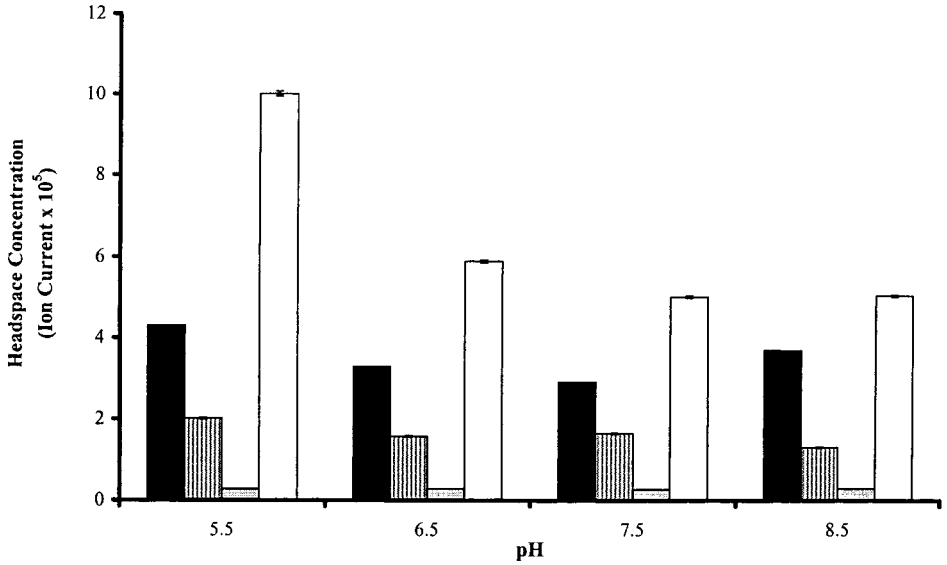

Figure 2. Effect of pH of PGM solution (without salt) on equilibrium headspace concentrations of volatile compounds: decanal (black), decanol (vertical lines), dimethyl pyrazine (grey), and heptanal (white).

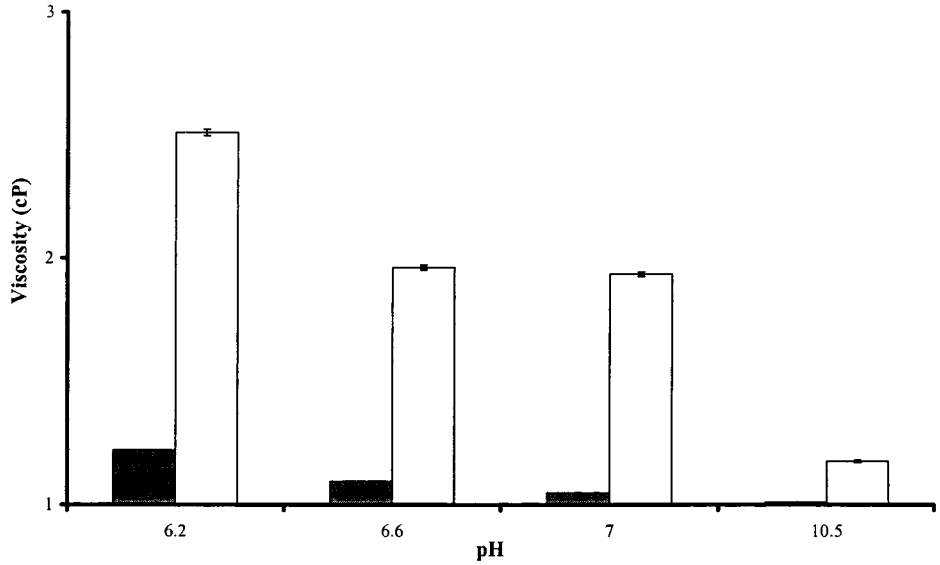

Figure 3. Effect of pH on the relative viscosity of PGM (without salt) in an aqueous solution (grey) and in an 18% sucrose solution (open).

similar relative headspace values for sugar and salt solutions with and without mucin. The behavior here was driven by the solutes, not mucin. The second group (cymene, decanal, decanol, and heptanal) showed a decrease in relative headspace on addition of mucin, but that change was unaffected by solutes. Behavior in this group seems to be driven entirely by mucin. The third group (benzaldehyde, diacetyl, ethyl hexanoate, and heptyl acetate) showed changes with mucin that were affected by the presence and type of solute present. Here, behavior seems to be due to interactions between mucin and the solutes.

Potential explanations for these behaviors are now presented. For the group 1 compounds, it can be assumed that there is no binding between these compounds and mucin, so that their behavior can be explained by the solutes alone. For the group 2 compounds, strong binding of the compounds to the mucin took place to such an extent that the effect of the solutes was insignificant. For this group, headspace was reduced to 10−50% of the control (aqueous solution) values. Group 3 showed differences, especially between the mucin and mucin−salt solutions. The differences between the sugar and sugar−salt−mucin were not so marked. This could be because the mucin has a finite number of binding sites which are preferentially taken up by the sugar rather than by the volatiles. Because volatile binding sites occupy only a few mg kg^{-1} of the sugar, this leaves a sufficient excess of sugar in solution to exert its characteristic salting-out effect on the aroma compounds. This is quite interesting: in that food proteins, generally, can bind specific aroma compounds (*18, 21, 28*), yet very few authors have focused on the effect of salivary proteins as a subject, particularly their effects on a range of volatile compounds (*9, 14*). It was expected that aldehydes would bind to mucin, as it is well-known that proteins can bind aldehydes to form Schiff bases (*15, 29*). However, the two aldehydes in this set of compounds behave in the same way as decanol and cymene, suggesting that there are either two binding mechanisms for these compounds to mucin or that the Schiff's base explanation for aldehyde binding

Table 4. Relative Headspace Volatile Concentration above Solutions of the 14 Volatiles in the Presence of Sugar (Su), Mucin (Mu), or Mucin−Salivary Salts (Sa), or Mucin with Salivary Salts and Sugar (see Table 1 for compositions)

compound[a]	+ Su	+ Mu	+ Mu + Sa	+ Mu + Sa + Su
		group 1		
2-methyl butanol	1.76	0.96	0.96	1.70
dimethyl cyclohexanone	1.44	0.96	0.96	1.50
dimethyl pyrazine	1.26	0.96	0.97	1.15
linalool	1.92	1.07	1.07	1.97
menthone	1.16	0.97	0.95	1.22
methyl acetate	1.84	0.94	0.93	1.79
		group 2		
cymene	0.87	0.50	0.55	0.57
decanal	1.00	0.16	0.16	0.16
decanol	1.00	0.48	0.48	0.48
heptanal	1.00	0.10	0.10	0.10
		group 3		
benzaldehyde	1.23	0.46	0.04	1.14
ethyl hexanoate	1.19	0.58	0.00	1.31
diacetyl	1.60	0.84	0.49	1.54
heptyl acetate	0.87	0.67	0.05	0.86

[a] Group 1 compounds are unaffected by the presence of mucin, group 2 compounds are affected by mucin, and group 3 compounds are affected by both mucin and the solute. Relative headspace values: water = 1; values < 1 show a decrease in headspace and values > 1 show an increase in headspace relative to water.Values are taken from the average of 3 replicates for each volatile compound in each sample with an average overall coefficient of variation of 8% (± 4%).

is incorrect in this particular case with the four compounds sharing a common binding mechanism. Most other interactions between proteins and aroma compounds are through hydrophobic interactions and tend to be reversible (*18*).

Most of the published data describe the effect of individual salts (derived from food) on headspace concentrations of volatile compounds (*12*, *30*), but there is less information on the combination of salts found in saliva, apart from the contributions of van Ruth et al. (*14*). For the most part, the mixture of salivary salts caused a small increase in headspace concentration of aroma compounds, compared to that of the water control (data not shown). However, the salivary salts seem to cause a modification of the interactions between the aroma compound and the protein (*18*). The ionic strength of the solutions plays a role in the conformational state of mucin; therefore, it is suggested that the mucin has a different conformation in the mucin only and mucin + salt salivas. The addition of salt to the solution may cause a charge-shielding effect on the charged areas of the mucin backbone. This process is likely to neutralize the mucin backbone and reduce the charge repulsion between glycoproteins and allow neighboring mucins to entwine, resulting in an increase in self-aggregation.

These interactions between salts and mucin should cause changes in viscosity, and the values for solutions containing solutes and mucin are shown in Figure 4. The viscosity of water at 25 °C is 0.8904 cP, and addition of the salivary salts (Table 2) increased the viscosity by just 0.01 cP, whereas the addition of 18% (w/w) sucrose increased the viscosity to 1.73 cP. The addition of salt and sugar at these concentrations gave an additive increase in viscosity, but in the mucin-containing solutions a different pattern was observed. The 0.2% PGM solution had a viscosity of 1.05 cP, which increased to 1.22 cP on addition of salivary salts and to 1.93cP on addition of 18% sugar. The addition of salt and sugar together increased the viscosity to 2.24 cP (an extra 0.14 cP above the additive viscosities). These results strongly suggest that addition of salt to the mucin solution causes restructuring of the hydration shell around the molecule which also directly affects the hydration of the sucrose in the vicinity of the mucin. This may modify the number of binding sites that the mucin has available and may also result in formation of hydrophobic inclusion sites that can trap volatiles within the solution structure.

Effect of Sequential Addition of Sugar and Salivary Components. The data in Table 4 showed the effect of mucin and solutes on compound partition. To study this effect further, solutions were prepared in which the salt and mucin were added sequentially (Table 3) to investigate the hypothesis that the effect was due to competition for binding sites between sugar and the volatiles. This was tested by adding the

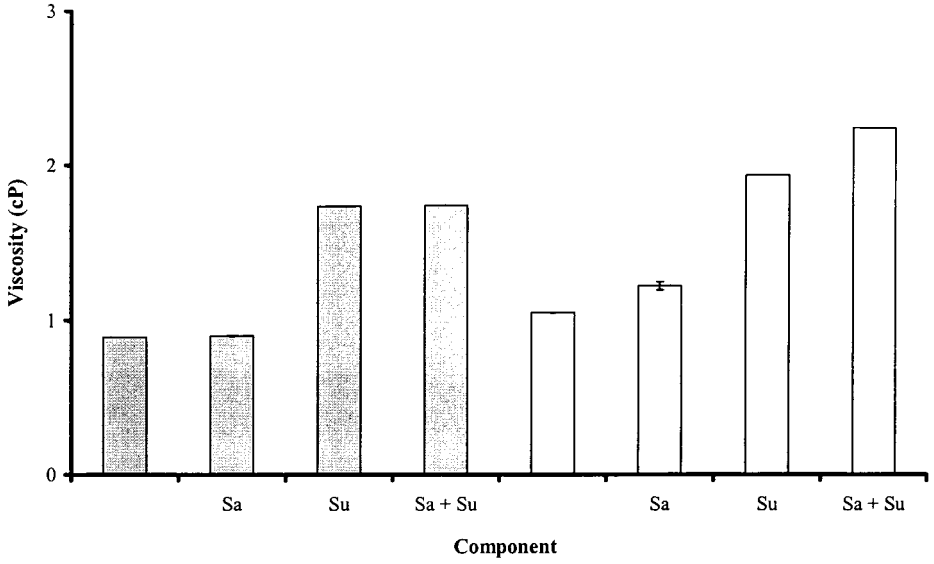

Figure 4. Viscosity of aqueous solutions (grey) and mucin solutions (open bars) containing the salivary salts and sugar (alone and in mixtures) at 25 °C and pH 7.00. Sugar concentration was 18%; actual salt and mucin concentrations are as detailed in Table 2.

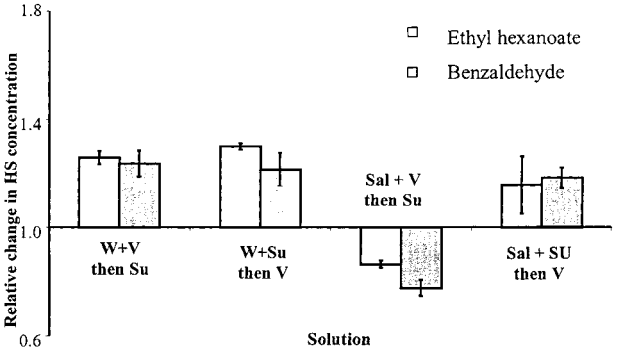

Figure 5. Effect of order of addition on equilibrium headspace change, relative to water, for two group 3 compounds that bind to mucin. Water, W; volatile, V ; 34% sugar, Su; saliva, Sal (see Table 3 for treatment details).

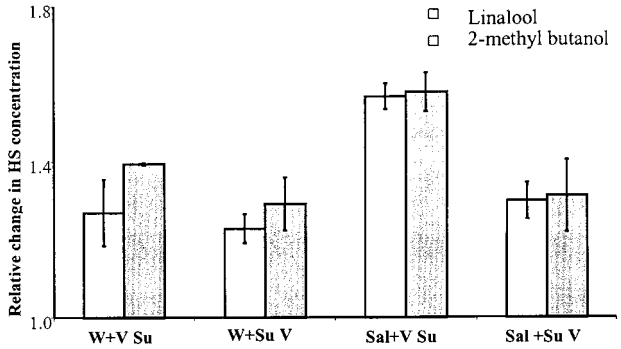

Figure 6. Effect of order of addition on equilibrium headspace change, relative to water, for two group 1 compounds that do not bind to mucin. Water, W; volatile, V; 34% sugar, Su; saliva, Sal (see Table 3 for treatment details).

components in different orders, allowing an equilibration period between additions, and then measuring the equilibrium headspace. It was also expected that the effect of adding a sugar solution to a batch volatile solution would give the same headspace concentration as adding a portion of a batch volatile solution to a sugar solution.

Figures 5 and 6 show the changes in headspace concentrations of four treatments expressed relative to the headspace concentration above water. Figure 5 plots the behavior of two group 3 compounds: ethyl hexanoate and benzaldehyde. In the aqueous solutions (treatments 1 and 2) the order of addition was irrelevant, but with the mucin solution (treatments 3 and 4), adding the saliva and volatile, then the sugar, caused a decrease in headspace concentration, whereas mixing saliva and sugar, then the volatile, caused an increase in headspace concentration compared to that of the water control. The behavior in treatment 3 is interpreted as strong binding of the volatile to the mucin, an effect which is not completely reversed by addition of sugar. In treatment 4, sugar either blocks the binding sites or changes the conformation of the mucin so that the volatile compounds cannot bind to the same extent and the headspace is similar to the aqueous behavior in treatments 1 and 2. Nawar also found that the order in which solutions are made up can affect the headspace concentration of volatile compounds (*31*), although, in this case, the differences were found between solutions prepared by dissolving solid sugar or mixing a sugar solution into an aqueous solution.

Figure 6 shows the same experiment but this time using Group 1 compounds (linalool and 2-methylbutanol), which had shown no mucin binding in an earlier experiment (Table 4). Again, in the aqueous systems there was little difference between treatments 1 and 2 but a difference between treatments 3 and 4, with a reduced headspace concentration when volatiles were added last. This suggests that, although some compounds do not bind to mucin, they can still be affected by its presence in solution. In the case of treatment 3, addition of the volatile to mucin first, with subsequent addition of sugar, seemed to increase the headspace concentrations of the two alcohols much more than expected. It appeared that the addition of sugar displaced the volatile compounds into the headspace as it bound to the mucin. In the case of adding sugar solution to the mucin before the volatile is added, it may be that the mucin binds to the sugar and forms a carbohydrate-like structure in the solution that is similar to the structure formed by sugar in water. Some sugar molecules can fit into the structure of water with hydrogen bonding; however, when the glycoprotein is also present different types of bonding are introduced. The presence of sugars has been shown to stabilize the solution structure of proteins (*32*−*34*). Thus, it is likely that treatments 3 and 4 involve the addition of volatiles to solutions in which the glycoprotein adopts different conformations because of this stabilization.

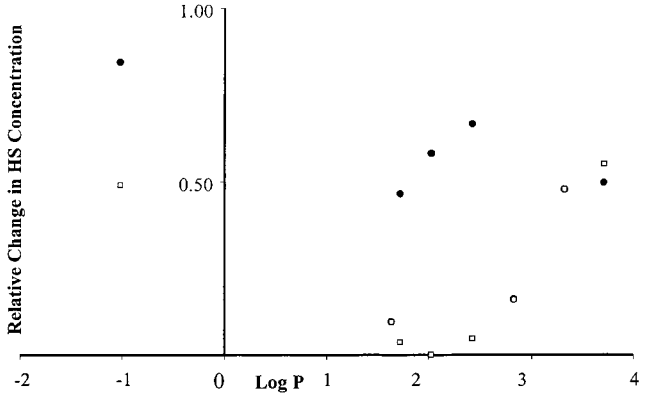

Figure 7. Relationship between hydrophobicity of the volatile compounds and their relative headspace concentration above mucin (●) and mucin−salt (□) solutions.

In an attempt to explain this behavior further, the hydrophobicity (log *P*) of the compounds and their relative headspace concentrations in salt and mucin (Figure 7) solutions were plotted. There was no correlation between log *P* and the relative headspace values for the salt and mucin solutions, but there was a good negative correlation for relative headspace above the sugar solutions (Figure 8). Log *P* has previously been shown, using an empirical modeling approach, to be an important parameter in the prediction of the effect of sucrose concentration on aroma release (*13*). Further experiments are required to determine the exact nature of the binding, although estimation of the number of binding sites per mole is not possible because of the polydisperse nature of mucin (both in terms of the number of basic units and the extent of glycosylation (*22*)). Empirical modeling may elucidate some of the important physicochemical factors involved in the binding of aroma volatiles to mucin, where it is not necessary to know the mechanisms involved (*13*).

The results presented above show that salivary components can affect the partition of volatile compounds between the liquid and gas phases. New data

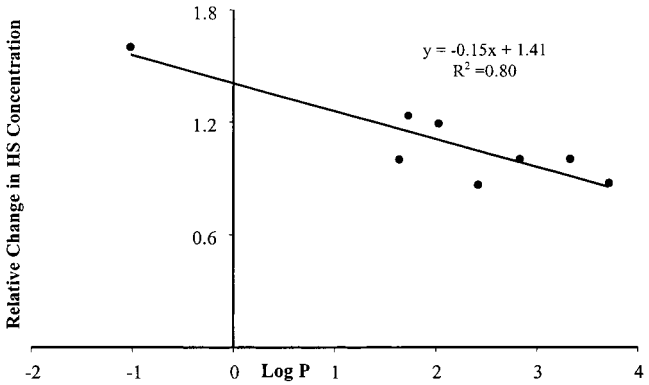

Figure 8. Relationship between hydrophobicity of the volatile compounds and their relative headspace concentration above sucrose solutions.

on the known solute and protein binding effects have been presented; and the fact that order of addition can change partition is of potential interest for in vivo flavor release and flavor perception. It should be emphasized that these data were obtained in a model system at equilibrium which can never imitate all of the complex operations performed by a mouth. Further work is needed to describe in more detail the phenomena observed, and to determine whether they are significant in vivo.

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