Chem. Senses 27: 67–72, 2002

Enhancement of Sucrose Sweetness with Soluble Starch in Humans

Norikazu Kanemaru, Shuitsu Harada and Yasuo Kasahara

Department of Oral Physiology, Kagoshima University Dental School, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

Correspondence to be sent to: S. Harada, Department of Oral Physiology, Kagoshima University Dental School, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan. e-mail: harada@phy.hal.kagoshima-u.ac.jp

Abstract

The effect of soluble starch (acid-modified starch) on taste intensity was investigated in human subjects. Different concentrations of sucrose (Suc), six sweeteners, NaCl, quinine–HCl (QHCl) and citric acid (Cit) were dissolved in either distilled water (DW; standard) or starch solution (test solution). The solutions were presented to naive subjects and each subject was requested to taste and compare the sweetness intensity between the standard and test solutions based on a scale ranging from +3 (enhanced) to –3 (inhibited). A greater sweetness intensity occurred with Suc at different concentration (0.1–1.0 M) dissolved in soluble starch (0.125% to 4.0%) than with Suc in DW. Similarly, five other different products of soluble starch at 0.25 and 4.0% resulted in enhancement of sweetness for 0.3 and 1.0 M Suc. With the sole exception of the taste of 0.3 M Suc, sweet enhancement did not occur with 0.43 M fructose, 0.82 M glucose, 0.82 M sorbitol, 0.0037 M aspartame, 0.0042 M saccharin-Na or 0.016 M cyclamate. Neither the saltiness of NaCl (0.01–0.3 M), the bitterness of QHCl (0.00003–0.001 M) nor the sourness of Cit (0.0003–0.01 M) were affected by the soluble starch. These results suggest that the taste enhancing effects of soluble starch on Suc sweetness might depend not only on the taste transduction mechanism, but also on the molecular interaction between Suc and soluble starch.

Introduction

The modulation of sweet taste has been observed in humans (Birch, 1999). The selective inhibition of sweetness, a type of negative modulation, is induced by gymnemic acid (Bartoshuk *et al.*, 1969), hodulcin (Kennedy *et al.*, 1988; Kolodny and Kennedy, 1988) and ziziphin (Kennedy and Halpern, 1980). These inhibitory modulations of sweet taste were explained by direct action on the receptor molecule responsible for the sweet sensation. In contrast, positive modulation on binary mixtures of intense sweeteners has also been reported (Wells, 1989; Schiffman *et al.*, 1995; Hutteau *et al.*, 1998). Although the precise mechanisms that produce synergism among the sweeteners are not known, it is suggested that multiple receptors and multiple transduction mechanisms may be involved (Schiffman and Erickson, 1993; Schiffman *et al.*, 1995).

Recently, we observed an enhancing effect of 2% soluble starch on the sucrose neural response in the rat greater superficial petrosal nerve (unpublished data). Also, we found that sweetness intensity of sucrose is enhanced by acid-modified starch (soluble starch) (Kanemaru *et al.*, 2000). The properties of the enhancement by soluble starch may be different from those reported previously, since soluble starch itself does not produce a sweet sensation at relatively low concentrations. Therefore, the present experiments were designed to assess the characteristics of the enhancement of the synergism produced by soluble starch on sweet taste sensation in humans.

Materials and methods

Subjects

The subjects were 131 male and 51 female Kagoshima University Dental School students aged 20–36 years (mean 23.9 ± 0.3 SE). All of the subjects were healthy and of Asian ancestry.

Stimuli

Stimulus solutions were made with reagent grade chemicals (Nacalai Tesque Inc.) in distilled water (DW) or in soluble-starch solutions where DW was the solvent. Chemicals included sucrose (Suc), maltose (Mal), lactose (Lac), fructose (Fru), glucose (Glu), sorbitol (Sorb), aspartame (Aspar), saccharin-Na (Sacc), cyclamate (Cyclo), NaCl, citric acid (Cit), and quinine–HCl (QHCl).

The soluble starches were different reagent grade products from different companies: Nacalai nos 321-22 (N-22), 321-26 (N-26) and 321-31 (N-31); Merck no. 1.01252 (Mer); and Wako nos 191-03985 (W-L) and 195-03961 (W-H, high-grade compound for biochemical research). The soluble-starch solution was gelatinized by heating to 80°C for a few minutes.

The stimuli were: (i) 0.1, 0.3 and 1.0 M Suc dissolved in DW and in DW along with W-H soluble starch at 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0%; (ii) 0.3 and 1.0 M Suc dissolved in DW and 0.25 and 4.0% N-22, N-26, N-31, Mer, W-L and W-H soluble-starch solution; (iii) 0.3 M Suc, 0.67 M Mal, 1.0 M Lac, 0.43 M Fru, 0.82 M Glu, 0.82 M Sorb, 0.0037 M Aspar, 0.0042 M Sacc and 0.016 M Cyclo dissolved in W-H or W-L solution at 0.25 and 4.0%; and (iv) NaCl (0.01–0.3 M), QHCl (0.00003–0.001 M) and Cit (0.003–0.01 M) dissolved in 0.25 and 4.0% W-H solution. The soluble-starch solutions were adjusted to pH values of between 5.3 and 5.6 by titration with 0.3 M tris(hydroxymethyl)-aminomethane (TRIS). The TRIS solution was tasteless even at 90 mM, the maximum concentration resulted in the titration.

Viscosity for 0.1, 0.3 and 1.0 M Suc dissolved in DW and 0.25 and 4.0% of W-H was measured five times with a Viscotester (RION, VT-03) and each mean ± SE calculated.

Test solutions were prepared daily and presented at 22 ± 1°C, i.e. room temperature.

Procedure

The solutions were presented to naive subjects and each subject was asked to taste and compare the sweetness intensity between the standard (DW solution) and test (soluble-starch) solutions based on a scale ranging from +3 (enhanced) to –3 (inhibited).

Data analysis

Differences in the effect of soluble starch on sweetness intensity were analysed by ANOVA, and a *post hoc* multiple comparison (Bonferroni/Dunn) was used for testing the statistical significance ($P < 0.05$) of the difference in sweetness between each possible stimulus pair.

Results

Taste of soluble starch solution

Soluble starch (W-H) solutions at concentrations <0.5% produced no taste in 55.8–65.4% of the subjects, and at concentrations <1.0% in 28.8–38.5% of subjects (Table 1). The percentage of subjects who perceived the W-H solution as slightly sweet ranged from 3.8 to 13.5% at concentrations ranging from 0.125 to 4.0%. The percentage of subjects who perceived the taste of the W-H solution as other than sweetness ranged between 28.9 and 55.8%, although the sensations were weak. No difference was observed between males and females.

Enhancement of Suc sweetness by soluble starch

The sweetness intensity of 0.1 M Suc increased with an increase in W-H concentration, while that of 0.3 and 1.0 M sucrose reached a peak at 0.25%, then decreased and increased again with an increase in W-H concentration ranging from 0.125 to 4.0% (Figure 1). A similar concentration–intensity curve with two phases was obtained for 1.0 M sucrose in W-L solution (n = 43). The sweetness intensities of 0.1 M Suc with 4.0% W-H, 0.3 M Suc with 0.25% W-H and 1.0 M Suc with 4.0% W-H were significantly greater than that of the Suc alone (Bonferroni/Dunn, $P < 0.05$). Also, the W-L solution at 0.25 and 4.0% significantly (Bonferroni/Dunn, $P < 0.05$) enhanced the sweetness of 1.0 M Suc. No statistical difference was observed between the sensitivity of males and females.

The viscosity of each solution is listed in Table 2. The viscosity of Suc in DW increased with increasing Suc concentration, reaching 6.95 ± 0.11 mPa·S at 1.0 M. The viscosity of 4.0% W-H in DW was almost half that of 1.0 M Suc in

Table 1 Percentages of answers by subjects (n = 52) tasting soluble starch (W-H) solutions at different concentrations, ranging from 0.125 to 4.0%

Taste	Concentration (%)					
	0.125	0.25	0.5	1.0	2.0	4.0
No taste	59.6	65.4	55.8	38.5	28.8	34.6
Slightly sweet	11.5	3.8	11.5	13.5	13.5	9.6
Slightly bitter	17.3	13.5	13.5	17.3	26.9	19.2
Slightly sour	11.5	9.6	9.6	23.1	11.5	17.3
Slightly astringent	1.9	3.8	5.8	1.9	3.8	4.7
Powder-like	0.0	1.9	1.9	1.9	3.8	3.8
Other taste	3.8	5.8	7.7	13.5	19.2	19.2

Subjects were requested to express their perceived taste of given solutions as precisely as possible. Answers may overlap more than one taste. 'Other taste' includes 'metallic', 'vegetable', or 'inexpressible' tastes.

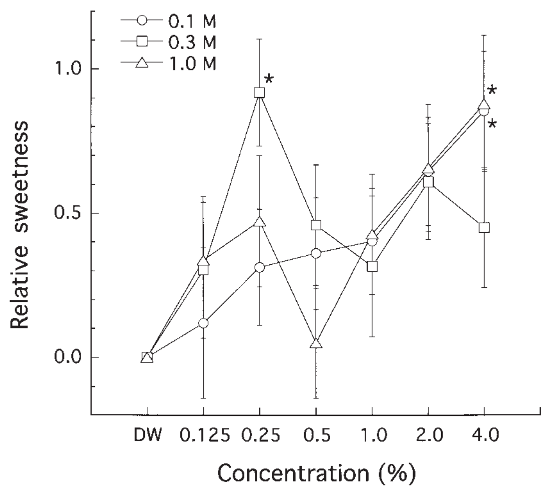

Figure 1 Enhancement of relative sweetness (see Materials and methods) of Suc at 0.1 (n = 31), 0.3 (n = 32) and 1.0 M (n = 30) by soluble starch (W-H) at different concentrations, ranging from 0.125 to 4.0%. Error bars show standard error of the mean. Asterisks indicate a statistically significant enhancement (Bonferroni/Dunn, $P < 0.05$).

Table 2 Viscosity (mPa·s) for 0.1, 0.3 and 1.0 M Suc containing 0.25 or 4.0% soluble starch (W-H)

W-H	DW	Suc		
		0.1 M	0.3 M	1.0 M
DW	2.35 ± 0.09	2.78 ± 0.06	3.36 ± 0.10	6.95 ± 0.11
0.25%	2.62 ± 0.04	3.03 ± 0.09	3.49 ± 0.07	7.19 ± 0.26
4.0%	3.94 ± 0.04	4.44 ± 0.15	4.89 ± 0.17	11.5 ± 0.47

DW. The viscosity of the solution of 1.0 M Suc in 4.0% W-H was the highest (11.5 ± 0.47 mPa·S), 1.65 times higher than that of 1.0 M Suc in DW.

Effects of different soluble starches

To determine properties of sweetness enhancement, subjects were divided into two groups based on the effects of W-H: (i) a ‘sweetness-enhanced’ group (relative sweetness > 0) and (ii) a ‘no sweetness effect’ or ‘inhibited’ group (relative sweetness 0). In the sweetness-enhanced group, five additional products of soluble starch at 0.25 and 4% significantly enhanced sweetness of Suc at 0.3 or 1.0 M (Figure 2A). Suc sweetness was significantly enhanced (Bonferroni/Dunn, $P < 0.05$) by soluble N-22 starch at 4.0% for 0.3 and 1.0 M Suc, 0.25% N-31 for 1.0 M Suc and 4.0% Me for 0.3 M Suc. No significant effects of soluble starch were observed in the non-enhanced group (Figure 2B).

Effects of soluble starch on sweetness of different sweeteners

To determine properties of sweetness enhancement, subjects were divided into two groups based on the effects of W-H: (i) a ‘sweetness-enhanced’ group (relative sweetness > 0) and (ii) a ‘no sweetness effect’ or ‘inhibited’ group (relative sweetness 0). In both groups, there were no significant effects of the addition of soluble starch (W-H and W-L) at 0.25 (Figure 3A,B) and 4.0% (Figure 4A,B) to six sweeteners with similar sweetnesses relative to that of 0.3 M sweetener alone.

Effects of soluble starch on the four basic tastes

As Figure 5 (NaCl, QHCl and Cit), there was no effect of soluble starch (W-H) at 0.25 or 4.0% on the intensity of saltiness (0.01–0.3 M NaCl), bitterness (0.00003–0.001 M QHCl) or sourness (0.0003–0.01 M Cit).

Discussion

The treatment of starch with acid, without substantially changing the granular form, results in modified starch with properties that have commercial value in food and technological industries. Although soluble starch has the same granular appearance, similar birefringence and essentially the same insolubility in cold water as the parent starch, soluble starch can be expected to exhibit a higher ratio of cold- to hot-paste viscosity, higher osmotic pressure and increased solubility in water at temperatures just below the gelatinization temperature (Rohwer and Klem, 1984).

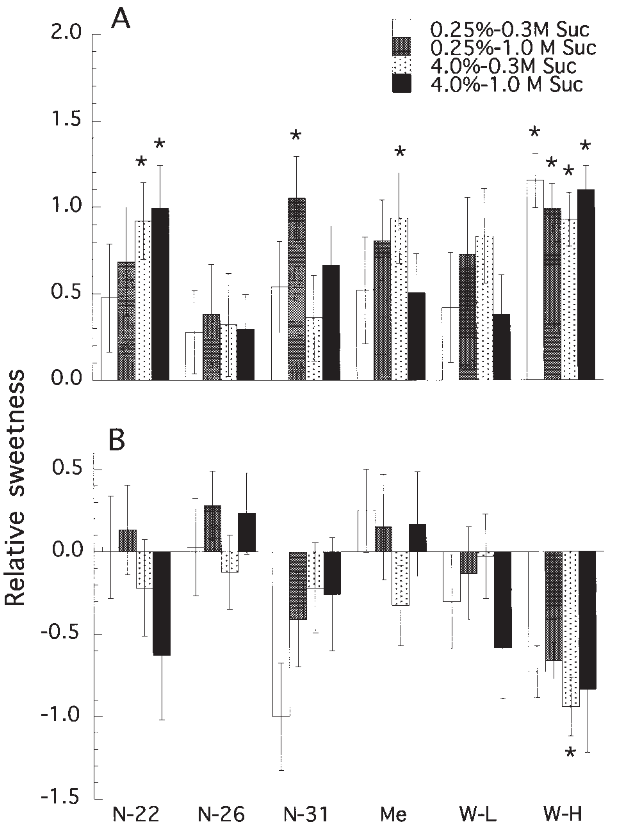

Figure 2 Effects of six different soluble starch at 0.25 and 4.0% on 0.3 or 1.0 M Suc sweetness. Subjects were divided into two groups, depending on whether the sweetness of sucrose (Suc) was enhanced or not by the soluble starch (W-H). **(A)** Enhanced group (ANOVA; 0.25% with 0.3 M Suc, $n = 19$, $P = 0.0154$, $F = 2.765$, d.f. = 6; 0.25% with 1.0 M Suc, $n = 15$, $P = 0.0168$, $F = 2.764$, d.f. = 6; 4.0% with 0.3 M Suc, $n = 18$, $P = 0.0019$, $F = 3.779$, d.f. = 6; 4.0% with 1.0 M Suc, $n = 27$). **(B)** Non-enhanced group (0.25% with 0.3 M Suc, $n = 21$; 0.25% with 1.0 M Suc, $n = 21$; 4.0% with 0.3 M Suc, $n = 22$; 4.0% with 1.0 M Suc, $n = 11$, $P < 0.0001$, $F = 5.034$, d.f. = 6). Error bars show standard error of the mean. Asterisks indicate statistically significant enhancement (Bonferroni/Dunn, $P < 0.05$).

Soluble starch inhibited responses to different sugars in the labellar sugar receptor cell of the blowfly (Hara, 1983). In experiments using affinity electrophoresis, the addition of soluble starch into the running polyacrylamide gel resulted in a competitive inhibition of the pyranose site of the labellar sugar receptor in the blowfly (Ozaki, 1988). These results suggest that soluble starch has an affinity to the sugar receptive protein of the labellum of the blowfly. If this is also applicable for human taste mechanisms, the addition of soluble starch in a sugar solution may competitively inhibit the sweet taste of the sugar. However, we have demonstrated previously that soluble starch (W-L) at relatively weak concentrations ranging between 0.125 and 4.0% enhanced the sweetness of 1.0 M Suc (Kanemaru *et al*., 2000). This

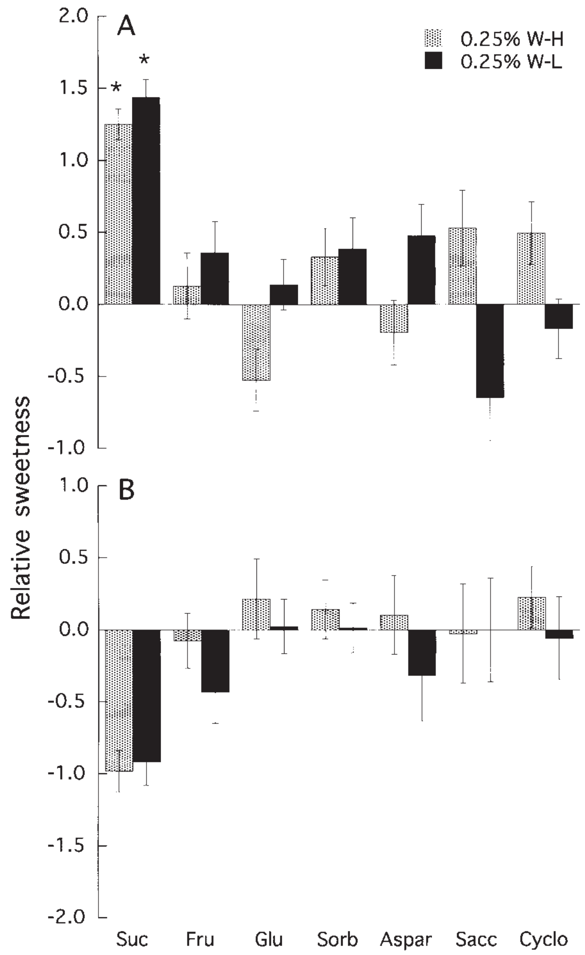

Figure 3 Effects of soluble starch (W-H and W-L) at 0.25% on the perceived sweetness of seven sweeteners. **(A)** enhanced group (W-H, n = 37; W-L, n = 37). **(B)** Non-enhanced group (W-H, n = 22; W-L, n = 22). Error bars show standard error of the mean. Asterisks indicate a statistically significant enhancement (Bonferroni/Dunn, $P < 0.05$).

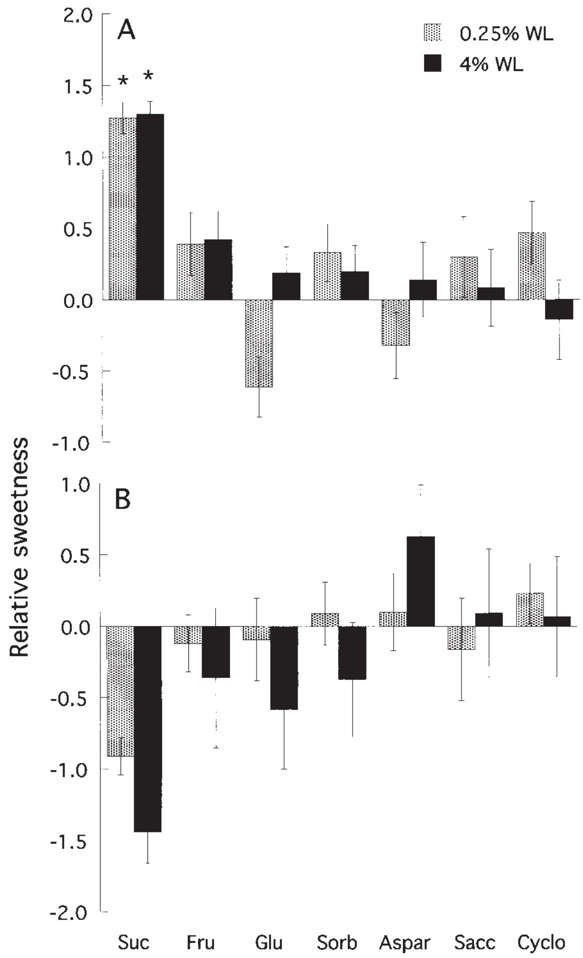

Figure 4 Effects of soluble starch (W-L) at 0.25 and 4.0% on the perceived sweetness of seven sweeteners. **(A)** enhanced group (0.25%, n = 33; 4.0%, n = 33). **(B)** Non-enhanced group (0.25%, n = 19; 4.0%, n = 19). Error bars show standard error of the mean. Asterisks indicate a statistically significant enhancement (Bonferroni/Dunn, $P < 0.05$).

enhancement effect of Suc sweetness with soluble starch was confirmed in the present experiment for 0.1, 0.3 and 1.0 M Suc in 0.125–4.0% soluble starch (W-H). Furthermore, five different products of soluble starch at relatively weak concentrations (0.25 and 4.0%) in 0.3 and 1.0 M Suc resulted in the enhancement of sweetness. Therefore, soluble starch apparently plays a different role in the perception of sweetness in humans from that in the blowfly.

The synergistic effects of Suc in enhancing the sweetness of various sweeteners (Schiffman *et al.*, 1995; Hutteau *et al.*, 1998) have been previously reported, but the mechanisms were unknown. It was, however, suggested that multiple receptors as well as multiple transduction mechanisms played a role in the synergy (Schiffman *et al.*, 1995). In the present experiments, only 3.8–13.5% of the subjects perceived a slightly sweet taste with soluble starch and the perceived sweetness was extremely low compared to that for Suc. Since the synergistic enhancement in sweetness by soluble starch was only observed with Suc, the mechanism may be different from that observed in the experiments on binary mixtures of sweet compounds (Schiffman *et al.*, 1995).

Gymnemic acid abolished both the sweet sensation and the chorda tympani nerve response to Suc and Sacc in human subjects (Diamant *et al.*, 1965). Similarly, gurmarin, a protein extracted from *Gymnema sylvestre*, significantly depressed the phasic taste responses to sugars and Sacc recorded from both the rat chorda tympani (Imoto *et al.*, 1991) and greater superficial petrosal nerve (Harada and Kasahara, 2000). Single fiber analysis of the chorda tympani response in monkeys revealed that across-fiber correlation coefficients between responses to Sacc and Suc were highly significant in macaque (Sato *et al.*, 1975) and cynomolgus monkey (Sato *et al.*, 1994). In conditioned taste aversion qualities in hamsters (Frank, 1973) and in mice (Ninomiya and Funakoshi, 1989), Suc and Sacc showed similar patterns of sensation across four tastes. All of the previous reports suggested that the sweeteners tested in the

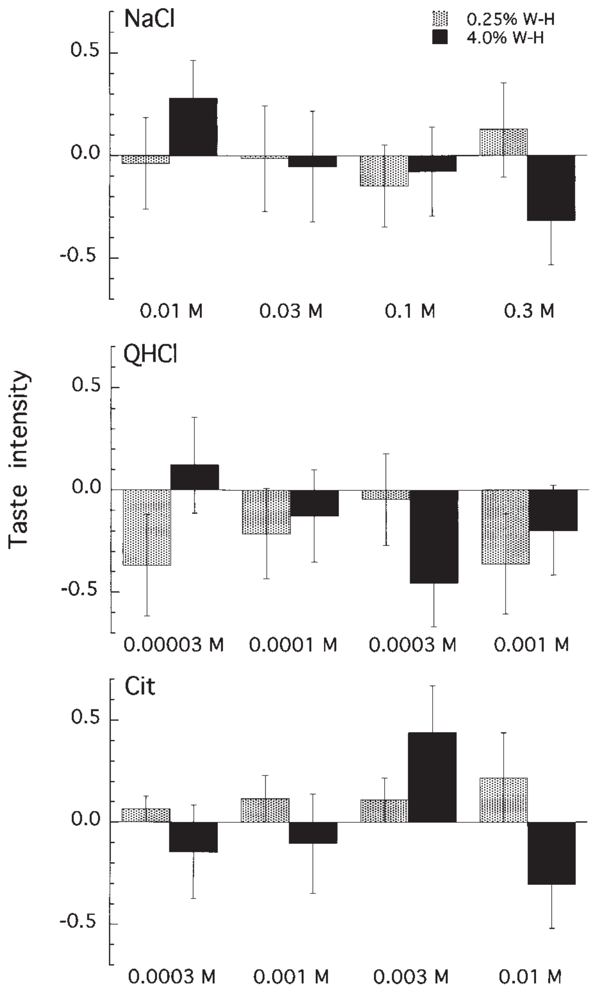

Figure 5 Effects of soluble starch (W-H) at 0.25 or 4.0% on the taste to NaCl (0.01–0.3 M), QHCl (0.00003–0.001 M) and Cit (0.003–0.01 M). For NaCl, $n = 30$; for QHCl and Cit, $n = 31$. Error bars show standard error of the mean.

present experiments, especially Suc, Sacc and Fru, share the same transduction mechanism(s). The enhancement of sweetness by soluble starch, however, was observed solely for Suc among the tested sweeteners. Therefore, the synergistic effects of soluble starch on the taste of Suc are difficult to explain by a direct activation of sweet receptor protein(s).

It is proposed that the interaction between a sweet compound and a receptor protein occurs at two electronegative atoms, A and B, separated by 2.5–4.0 Å resulting in intermolecular hydrogen bonding between A-H and B in each of the molecules (Shallenberger and Acree, 1967). A third structural feature (X) relative to the A-H/B features may be involved in dispersion bonding with an appropriate molecular structure, suggesting that the X may be related to the intensity of the sweetness (Kier, 1972). Considering these hypotheses, one possible mechanism of synergism by soluble starch may be that Suc selectively combined with the soluble starch, offering a new X site in the soluble starch that resulted in the synergistic effect on the sweetness of the mixture of starch and Suc. A change in structure in the parent starch, suggested by the different physical properties of soluble starch compared with the parent starch (Rohwer and Klem, 1984), is consistent with this hypothesis. In contrast, the enhancement effect was not observed for 0.3 and 1.0 M Suc dissolved in any of the four products of refined starch—wheat, potato A and B, and corn (Kanemaru *et al*., 2000).

Another possible explanation for the synergism of Suc sweetness by soluble starch is that the sweetness of Suc may emerge due to the masking of other tastes. However, this explanation is not consistent with a single receptor mechanism for sweet substances, since the tastes of the other sweeteners tested were not affected by the soluble starch. Also, the increase in viscosity of the tested taste solution due to the addition of the soluble starch was not a factor in the resulting synergism, since viscosity lowers the perceived sweetness of both Sacc and Suc (Arabie and Moskowitz, 1971) and increases the threshold values of primary tastes (Paulus and Hass, 1980).

In conclusion, the present results suggest that the taste-enhancing effects of soluble starch on Suc sweetness might depend not only on the taste transduction mechanisms, but also on the molecular interaction between Suc and soluble starch.

Acknowledgements

We thank Drs Mamiko Ozaki and John Caprio for valuable comments on the manuscript.

References

Arabie, P. and **Moskowitz, H.R.** (1971) *The effects of viscosity upon perceived sweetness.* Percept. Psychophys., 9, 410–412.

Bartoshuk, L.M., Dateo, G.P., Vandenbelt, D.J., Buttrick, R.L. and **Long, J.L.** (1969) *Effects of* Gymnema sylvestre *and* Synsepalum dulcificum *on taste in man.* In Pfaffmann, C. (ed.), Olfaction and Taste III. Rockfeller University Press, New York, pp. 436–444.

Birch, G.G. (1999) *Modulation of sweet taste.* BioFactors, 9, 73–80.

Diamant, H., Oakely, B., Strom, L., Wells, C. and **Zotterman, Y.** (1965) *A comparison of neural and psychophysical responses to taste stimuli in man.* Acta Physiol. Scand., 64, 67–74.

Frank, M. (1973) *An analysis of hamster afferent taste nerve response functions.* J. Gen. Physiol., 61, 588–618.

Hara, M. (1983) *Competition of polysaccharides with sugars for the pyranose and the furanose sites in the labellar sugar receptor cell of the blowfly,* Phormia regina. J. Insect Physiol., 29, 113–118.

Harada, S. and **Kasahara, Y.** (2000) *Inhibitory effect of gurmarin on palatal taste responses to amino acids in the rat.* Am. J. Physiol., 278, R1513–R1517.

Hutteau, F., Mathlouthi, M., Portmann, M.O. and **Kilcast, D.** (1998) *Physicochemical and psychophysical characteristics of binary mixtures of bulk and intense sweeteners.* Food Chem., 63, 9–16.

Imoto, T., Miyasaka, A., Ishima, R. and **Akasaka, K.** (1991) *A novel peptide isolated from the leaves of* Gymnema sylvestre*. I. Character-*

ization and its suppressive effect on the neural responses to sweet taste stimuli in the rat. Comp. Biochem. Physiol., 100A, 309–314.

Kanemaru, N., Harada, S. and **Kasahara, Y.** (2000) *Enhancement of sweetness with soluble starch.* Chem. Senses, 25, 234.

Kennedy, L.M. and **Halpern, B.P.** (1980) *A biphasic model for action of the gymnemic acids and ziziphins on taste receptor cell membranes.* Chem. Senses, 5, 149–158.

Kennedy, L.M., Saul, L.R., Sefecka, R. and **Stevens, D.A.** (1988) *Hodulcin: selective sweetness-reducing principle from* Hovenia dulcis *leaves.* Chem. Senses, 13, 529–543.

Kier, L.B. (1972) *A molecular theory of sweet taste*. J. Pharm. Sci., 61, 1394–1397.

Kolodny, D.E. and **Kennedy, L.M.** (1988) *A model system for receptor cell studies with the taste modifier, hodulcin.* Chem. Senses, 13, 545–557.

Ninomiya, Y. and **Funakoshi, M.** (1989) *Behavioural discrimination between glutamate and the four basic taste substances in mice.* Comp. Biochem. Physiol., 92A, 365–370.

Ozaki, M. (1988) *A possible sugar receptor protein found in the labellum of the blowfly,* Phormia regina. Zool. Sci., 5, 281–290.

Paulus, K. and **Hass, E.** (1980) *The influence of solvent viscosity on the threshold values of primary tastes.* Chem. Senses, 5, 23–32.

Rohwer, R.G. and **Klem, R.E.** (1984*) Acid-modified starch: production and uses.* In Whistler, R.L., Bemiller, J.N. and Paschall, E.F. (eds), Starch: Chemistry and Technology. Academic Press, New York, pp. 529–541.

Sato, M., Ogawa, H. and **Yamashita, S.** (1975) *Response properties of macaque monkey chorda tympani fibers.* J. Gen. Physiol., 66, 781–810.

Sato, M., Ogawa, H. and **Yamashita, S.** (1994) *Gustatory responsiveness of chorda tympani fibers in the cynomolgus monkey.* Chem. Senses, 19, 381–400.

Schiffman, S.S. and **Erickson, R.P.** (1993) *Psychophysics: insights into transduction mechanisms and neural coding.* In Simon, S.A. and Roper, S.D. (eds), Mechanisms of Taste Transduction. CRC Press, Boca Raton, FL, pp. 397–424.

Schiffman, S.S., Booth, B.J., Carr, B.T., Losee, M.L., Sattely-Miller, E.A. and **Graham, B.G.** (1995) *Investigation of synergism in binary mixtures of sweeteners.* Brain Res. Bull., 38, 105–120.

Shallenberger, R.S. and **Acree, T.E.** (1967*) Molecular theory of sweet taste.* Nature, 216, 480–482.

Wells, A.G. (1989) *The use of intense sweeteners in soft drinks.* In Grenby, T.H. (ed.), Progress in Sweeteners. Elsevier Applied Science, London, pp. 169–214.

Accepted September 27, 2001