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In-Mouth Amylase Activity Can Reduce Perception of Saltiness in Starch-Thickened Foods

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Sensory scores for saltiness and thickness obtained for savory liquids thickened with starches or the nonstarch hydrocolloid hydroxypropylmethyl cellulose (HPMC) were correlated with the panelists' amylase activity. Although higher enzyme activities were linked to lower thickness scores for systems thickened by starch, they were also associated with a decreased taste perception, particularly for starches retaining a granular structure after gelatinization (wheat and modified waxy maize). Microscopic evidence showed that the enzyme can disrupt such structures, and this is associated with a decreased mixing efficiency with water and consequently a reduced transport of tastant (sodium) to the saliva (aqueous) phase and to the taste buds. This explains the lower saltiness scores for subjects with higher amylase activity, even if they are associated with a lower perceived thickness.

KEYWORDS: Starch; taste; salt; amylase; saliva; mixing

INTRODUCTION

Reduction of salt (NaCl) levels in foods is an important public health objective. Hypertension which is an important risk factor for stroke and heart diseases, has been shown to be related to salt (NaCl) intake (*1*, *2*). As a consequence of this, there is pressure to reduce salt levels in the diet. For example, the United Kingdom Government's Food Standards Agency has very recently given food manufacturers salt reduction targets for a number of products (*3*). There is no known alternative material to salt that gives the same taste (*4*), and therefore it is important to ensure that the perception of saltiness is maximized for a given salt level.

In liquid foods, it is widely believed that taste perception decreases with increased viscosity (*5*−*8*) and that therefore higher levels of salt will be required for optimum taste perception in high viscosity compared with low viscosity foods. It is well recognized that increasing viscosity with nonstarch polysaccharides such as guar gum and hydroxypropylmethyl cellulose reduces taste and flavor perception (*9*, *10*). Following an original suggestion of Baines and Morris (*9*), we have recently pursued the hypothesis that the reduction in perception is not fundamentally related to viscosity but to a reduction in the efficiency of mixing between the liquid food and saliva. When mixing is poor, salt will be retained in the food rather than distributed to the salt receptors on the tongue. Thus salt will be swallowed within the food before the time required to equilibrate with the saliva is reached. Aqueous polysaccharide solutions at concentrations above the coil overlap (c^*) concentration will mix relatively slowly with water or saliva because of entanglements between interpenetrating polymer chains. Such an interpretation explains why it is often possible to generate a master curve by plotting taste and flavor perception against c/c^* where c is the polymer concentration in solution (*11*). A reduction in perception is observed when c/c^* becomes greater than 1.

The most widely used thickeners in foods are starch based (*12*). In this case, it has recently become clear that perceived saltiness is often inhibited to a much lesser degree at high viscosities than when nonstarch polysaccharide thickeners are used (*13*). It was suggested that this was due to the fact that in contrast to solutions of polysaccharides, a dispersion of swollen starch granules mixes very efficiently with saliva, allowing salt to reach the receptors in the mouth rapidly.

On heating a suspension of starch in water, the starch granule will swell partly as a result of the disruption of the ordered amylopectin regions in the granule (*14*), with initially little or no loss of granule integrity and only limited release of the constituent polysaccharides from the granule. This results in a system where the origin of viscosity is not a solution of the constituent polysaccharides of starch, amylose and amylopectin, but rather a suspension of swollen granules. Because waxy maize starch contains no amylose the swollen granules disrupt easily when subjected to heat and shear. To avoid this, waxy

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maize starch granules are often strengthened by chemical crosslinking or physical modification by heating (*15*). We have recently demonstrated that, if starch is in the form of swollen granules, the perception of salt is substantially improved at high viscosities compared with systems consisting of disrupted granules or polysaccharide solutions (*13*).

Saliva contains the enzyme α-amylase, capable of hydrolyzing bonds within amylose and amylopectin, and we and others have shown that amylase at levels found in the mouth can substantially lower starch viscosity in seconds (*16*, *17*). Therefore, the enzyme-induced changes in starch structure within the mouth may be relevant to taste perception. This work addresses the effect of differences in salivary amylase activity on perception of saltiness in viscous systems as well as on the solution structure and mixing behavior, to elucidate the possible effect of the enzyme on taste perception.

MATERIALS AND METHODS

Materials. Three different types of starch, unmodified wheat and waxy maize starch (Sigma, Poole-Dorset, UK) and physically modified waxy maize starch (National Starch, UK), were used. Hydroxypropylmethyl Cellulose (K4M) was supplied by Methocel, Dow Company (Germany). Basil flavoring was supplied by Firmenich SA (Switzerland). Bottled water (Brecon Carreg still water), food coloring (Supercook red coloring), and salt (Coop table salt) were purchased in local supermarkets. Potassium chloride and porcine pancreatic α-amylase were obtained from Sigma (Poole-Dorset, UK)

Preparation of Starch Pastes. Aqueous salt solutions (85mmol·L^{-1}) were heated to 40 °C. Starch was then added and thoroughly mixed with an overhead paddle stirrer. The dispersion was continuously heated to 95 °C and kept at this temperature for 2.5 min, to allow gelatinization.

The paste was then allowed to cool to 80 °C and the basil flavoring (0.05%) added thoroughly by manual stirring. The flavored paste was poured into a vacuum flask and transported to a water bath at 65 °C until required for sensory assessments. Samples were assessed between 3 and 6 h after preparation.

Hydroxypropylmethyl Cellulose Solution Preparation. The salt solution (85 mmol·L^{-1}) was heated to 65−70 °C. HPMC was added gradually until it dispersed completely. The solution was then cooled slowly while being stirred. When cooled, the flavoring was stirred in. The paste was then kept overnight at 4 °C in a Schott bottle to allow full hydration of the hydrocolloid and then warmed to 65 °C in a water bath before use. Samples were assessed between 3 and 6 h after preparation.

In this case, as for starch systems, sodium or potassium chloride was used for sensory analysis and ion measurements, respectively. In all cases, concentrations of the different thickeners were adjusted in order to reach equivalent viscosities at 50 s^{-1}. Details of these concentrations are described in **Table 1**.

Sensory Evaluation. Panelists ($n = 14$, aged 35 to 66, 1 male) were selected from the University of Nottingham external panel on the basis of their ability to discriminate between samples varying in viscosity and salt concentration. These experienced panelists received further training in the assessment of thickness and saltiness using a magnitude estimation scale. A system thickened by wheat starch (5% w/v concentration, viscosity at 50 s^{-1} = 100 mPa/s) was chosen as a fixed reference and was allocated a value of 100 for both attributes.

Panelists assessed saltiness and thickness, defined as the force to squeeze the sample between the tongue and the palate, on separate 10 mL samples. Great care was taken to ensure that panelists were presented with samples at the same temperature (63 ± 2 °C). Five samples were randomly selected for each session and were assessed in duplicate. The order of presentation was balanced within a session. Plain crackers, bottled water, and diluted lime cordial were used between each sample as palate cleansers.

Determination of Panelists' Salivary Amylase Activity. During a separate session, panelists were asked to chew a Salivette swab (Sarstedt, Germany) for 1.5 min and to collect their saliva. Prior to this session, they were asked not to consume any food or liquids other than water for at least 1 h before arrival. Moreover, they were instructed to thoroughly rinse their mouth by drinking a glass of water immediately before the collection started. The Salivette was then centrifuged for 5 min at 3000 rpm. The supernatant was collected and diluted to the appropriate level in order to obtain results within the range of the calibration data. The dilution (1 mL) was added to a freshly prepared wheat starch sample (8%), and viscosity changes were recorded during 5 min using a Rapid Visco Analyzer (Newport Scientific, Australia). The time to halve the viscosity ($t_{1/2}$) was calculated and compared with calibration values obtained with known dilutions of pancreatic porcine α-amylase (8 to 80 U·mL^{-1}, 1 unit being defined as the amount of enzyme necessary to liberate 1 mg of maltose from starch in 3 min at pH 6.9 at 20 °C) added to the wheat starch pastes. All measurements (calibration and diluted saliva) were done at least in duplicates.

Table 1. Concentrations (% w/w) Used for the Preparation of the Different Starch Pastes and HPMC Solutions for the Different Targets Viscosities at 50 s^{-1} (mPa/s)

	target viscosity				
	80	180	280	380	480
wheat	4.90	5.60	6.15	6.45	6.70
waxy maize	1.81	2.30	2.90	3.40	3.80
modified waxy maize	2.70	3.05	3.45	3.80	4.20
HPMC	0.80	1.30	1.50	1.80	1.90

Statistical Analysis. Pearson's correlation coefficients were calculated between the scores obtained for the saltiness and thickness attributes during the assessment of the basil-flavored pastes and the individuals' salivary amylase activity using the software SPSS for Windows. As the sensory scores presented a log-normal distribution, correlations were calculated between amylase activity and the logarithm of the magnitude estimation scores.

Mixing Efficiency and Potassium Release. Viscous pastes (480 mPa/s at 50 s^{-1}) from each thickener were prepared and colored using a red food coloring. Portions (5 mL) were carefully introduced in 20 mL of distilled water or amylase solutions. The system was mixed for about 3 s with a spoon.

The amount of potassium present in the water phase was measured using K^+-specific electrodes (Microelectrodes Inc., Bedford, MA) 10 s after mixing, and photographs were taken. Experiments were done in triplicate.

Microscopic Observations. After preparation, 2 mL of a viscous starch paste were mixed with 1 mL of an amylase solution (50 and 100U·mL^{-1}) or water. Zinc chloride (1 mL at 200 mmol·L^{-1}) was added 10 s later to inhibit the enzyme (*18*). Lugol (2 mL) was added to stain the samples. An aliquot (25 μL) of the mix was deposited on a microscope glass slide, and a cover slide was added. Excess liquid was removed. Observations were made using a bright field triocular compound light microscope (Leitz Diaplan) with a magnification of ×250.

RESULTS AND DISCUSSION

Amylase activity was measured in each panelist's saliva, following a method based on the potential of the enzyme to reduce viscosity of a starch system, and adapted from a protocol developed for the determination of amylase activity in flours (*19*). The results showed a large variation among individuals, with values varying between 38 and 1325U·mL^{-1} and an average of 603 U·mL^{-1}, although standard deviations for an individual did not exceed 17%. Such variations have been observed in the past (*20*) and are the result of genetic differences. Correlation coefficients were then calculated between the individuals' scores for thickness and saltiness during sensory evaluation of the products and their salivary amylase activity and are presented in **Table 2**. The systems were thickened by wheat starch and physically modified waxy maize starch, both of which have a swollen granule structure in solution, native

Table 2. Pearson's Correlation Coefficients between Salivary Amylase Activity and Sensory Scores Given by the Panelists[a]

	thickener type	
	saltiness	thickness
HPMC	0.11ns	0.09ns
waxy maize starch	–0.20*	–0.28***
modified waxy maize starch	–0.43***	–0.19*
wheat starch	–0.25***	–0.14ns

[a] ns: not significant at a level of 5%, $p > 0.05$; *: significant at a level of 5%; ***: significant at a level of 1%.

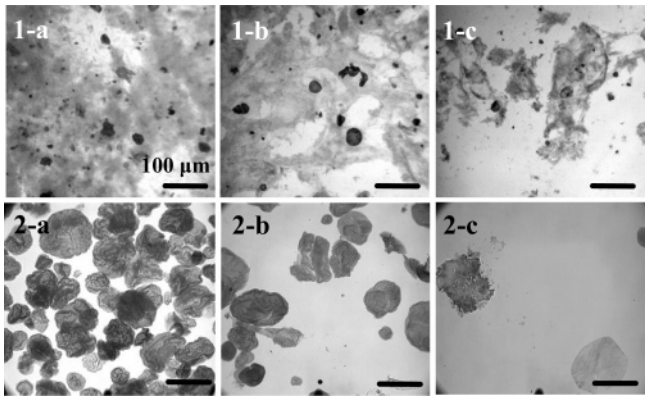

Figure 1. Structure of starch suspensions after exposure to water and amylase. Waxy maize starch pastes, unmodified (1) and physically modified (2), were exposed for 10 s to (a) water (b) 50 U·mL^{-1} amylase and (c) 100 U·mL^{-1} amylase.

waxy maize starch where the granules are disrupted, or a solution of the nonstarch polysaccharide hydroxypropylmethyl cellulose (HPMC).

The effect of amylase on the structures of the waxy maize and modified waxy maize starches are compared in **Figure 1**. For the modified waxy maize starch, there is a reduction in the number of granules as a result of amylase activity, and the iodine straining clearly revealed that the enzyme released polysaccharide to the nongranular phase. This is seen in **Figure 1** as a darkening of this phase.

For all four thickeners the initial viscosity ranged from 80 to 480 mPa/s measured at 60 °C at a shear rate of 50s^{-1}, conditions reported to be representative of those encountered in the mouth (*21*). As expected, there was no significant correlation for the systems thickened by the nonstarch polysaccharide HPMC since it was not affected by the enzyme. A significant and negative correlation was found between α-amylase activity and thickness scores for the unmodified and modified waxy maize starches. Our previous in-vitro viscosity measurements show that viscosity reduction on amylase addition is more rapid for the native waxy maize starch (*16*), and the in vivo sensory panel results are consistent with this (see **Table 2**).

For the starch systems, there is a negative correlation between perceived saltiness and amylase activity with a much higher level of significance for the granular wheat and modified waxy maize starches. The correlation coefficients were obtained from a data set of 140 observations (14 panelists, 5 concentrations, 2 replicates).

Amylase activity will reduce starch viscosity (*16*, *17*). Thus in contrast to what is commonly believed, a decrease in viscosity can be associated with a decrease rather than an increase in taste perception. Previous work has shown that a reduced taste perception can be explained by a mixing restriction between the product and saliva (*9*, *13*). Consequently it is a possibility

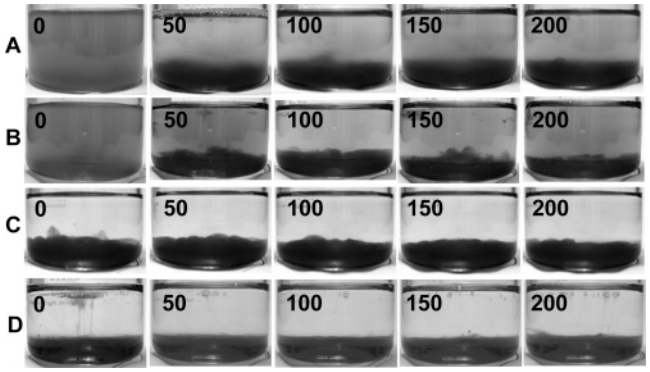

Figure 2. Mixing efficiency of viscous systems with solutions containing an increasing amount of α-amylase. Viscous samples (480 mPa/s at 50 s^{-1}) of different starches (wheat, A; modified waxy maize, B; waxy maize, C; HPMC, D) were stirred into distilled water or solutions containing 50, 100, 150, and 200 U·mL^{-1} of amylase and photographed after 1 min.

that the observed decrease in salt perception with increasing amylase activity is a consequence of a reduced mixing efficiency. This could be the result of entanglement within the polymeric phase released from the granule into solution following amylase attack as shown in **Figure 1.** In the case of waxy maize starch, no granules are observed, even without enzyme treatment. In that case, adding α-amylase leads to an apparent decrease in the density of the amylopectin network.

In vitro amylase was shown to disrupt the structure of granular starches. It is believed that systems composed of a solution of macromolecules mix less efficiently with saliva than granular suspensions. An enzymatic disruption would therefore lead to a lower level of mixing between the sample and an aqueous phase. This decrease in mixing efficiency can be visualized from a simple experiment where 5 mL of the thickened solutions containing a red food dye is mixed rapidly by hand with 20 mL of water or amylase solution for about 3 s. **Figure 2** shows these systems photographed 1 min after mixing had ceased. In the absence of amylase, mixing is very efficient for the modified waxy maize and wheat starch systems, whereas the other extreme is shown for the HPMC-thickened material. On standing quiescent for several hours, this sample eventually became homogeneous as a result of diffusion processes. For the initially granular starch pastes (wheat and modified waxy maize starch), the presence of amylase led to the observation of two visually distinct phases after mixing. We interpret this as a consequence of the polymeric material released by the enzyme as mixing poorly and forming the lower phase as a result of higher densities, with the upper phase containing the remaining granular material which mixes more efficiently. In the case of waxy maize starch, no drastic effect of α-amylase on mixing was observed. For this system, the enzyme does not, in that case, change the structure of the sample to a large extent, in that the granules were already disrupted. The mixing capability is therefore not reduced additionally.

To determine if inefficient mixing could result in a reduction in the rate of release of ions to receptors, potassium chloride was added to the thickened solutions, and concentrations of K^+ were measured close to the top surface of the solution/ dispersion 10 s after mixing. Potassium rather than sodium chloride was used in this case since the enzyme preparation contained high levels of Na^+ which would interfere with the measurement of sodium from added NaCl.

Figure 3 presents the concentration of potassium in the aqueous phase after mixing for the HPMC and starch systems at the different levels of amylase. Overall, for the four systems

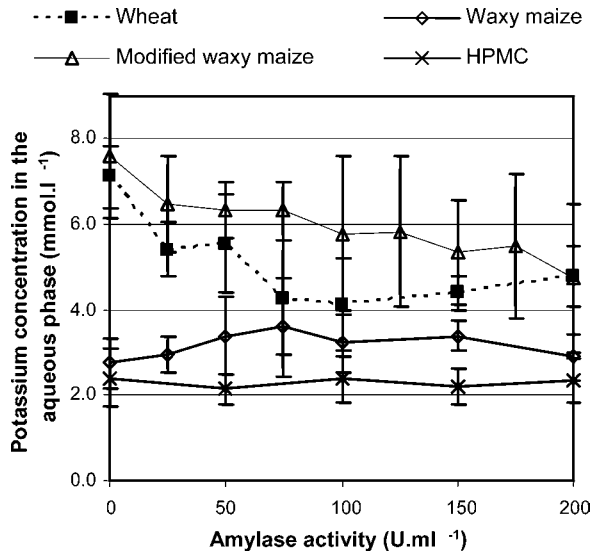

Figure 3. Effect of amylase activity on potassium concentration in the aqueous phase after mixing of the four different viscous systems (480 mPa/s at 50 s^{-1}).

the lowest K^+ concentration was measured in the poor mixing HPMC system, followed by the native waxy maize starch. We have previously reported that HPMC-thickened systems followed by native waxy maize starch have the poorest salt perception and the two granular starches have the highest salt perception (*13*) which is consistent with these ion concentration measurements. Increasing amylase level reduces the measured K^+ concentration for the two initially granular starches. In the case of HPMC and waxy maize systems, the enzyme has little effect on the ion movement to the aqueous phase after mixing. This is consistent with the visual observations of the mixing efficiency with water/amylase solutions, where it was noticed that α-amylase mainly affected the mixing pattern of the granular starches, with little or no effect for HPMC and waxy maize starch. This shows that mixing efficiency strongly affects ion transport between the viscous matrix and water or saliva. If K^+ ions were uniformly distributed as a result of mixing, the measured concentration would be 17 mmol/L. The maximum value observed (crosslinked waxy maize starch no amylase) was less than half of this. This shows that even with this starch, perfect mixing was not achieved. This is not surprising because mixing time was only a few seconds, as the experiment was designed to mimic the short times liquid foods are held in the mouth prior to swallowing.

Efficiency of mixing with saliva may affect mouthfeel as well as taste perception. For example in very early work, it was shown that solutions thickened with maize starch or high amylose starch were regarded as having a pleasant mouthfeel compared with waxy maize or nonstarch polysaccharide-thickened systems which were generally regarded as slimy and difficult to swallow (*22*). We interpret this as a consequence of the presence of strands of a poorly mixed high viscosity phase persisting in the mouth. Other workers have shown the importance of in-mouth mixing on taste, flavor, and texture attributes by modifying normal mastication patterns of semisolid foods (*23*).

In summary, the presence of amylase in the saliva can affect the paste structure in the mouth for granular (wheat and modified waxy maize) and nongranular starches (waxy maize starch) in different ways as summarized in **Figure 4**. For the granular starches this affects mixing efficiency with and reduces ion movement into the salivary phase and hence the taste buds. This

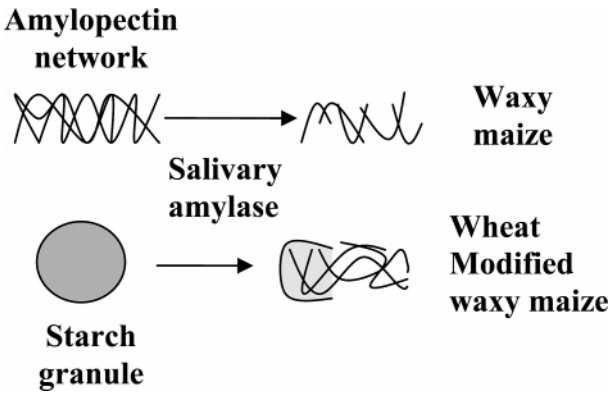

Figure 4. Summary of the proposed mechanisms for the effect of salivary α-amylase on the starches studied.

explains the negative correlation between enzyme activity and sensory scores for saltiness during sensory evaluation (**Table 2**). In vitro, the effect of the enzyme was lower in the case of the waxy maize starch, reflecting the absence of granular structure in the system (**Figure 4**). This is consistent with the sensory data, where a lower correlation coefficient was obtained between the same parameters. These simple experiments provide strong support for the hypothesis that salt perception is not only determined by the Na^+ concentration in the aqueous phase but also by the structure of the food product. For starch-containing products the structure within the mouth will vary between individuals, partly because of the wide variation in in-mouth amylase activity.

This work, together with previous reported work (*13*), shows that a reduction in mixing efficiency rather than an increase in viscosity is the explanation for the frequently reported decrease in taste intensity with increasing thickener concentration. It confirms the hypothesis that for liquid-thickened products, an important reason why starch or starch-containing flours are preferred thickeners compared with nonstarch polysaccharides is that they mix efficiently with saliva, provided the granular structure is retained. This not only gives rise to higher taste perception but to good mouthfeel as well. Ease of disruption of granular structure will depend on the type of starch as well as the degree of shear and the heating rates used during processing. Thus granules of native waxy maize starch which have not been reinforced by chemical crosslinking or physical modification will be disrupted readily, giving rise to poor salt perception and mouthfeel, as shown in an earlier work.

In addition to manipulation of solution structure through selection of thickeners and control of processing it is possible that salt perception could be improved by inhibition of amylase activity through the manipulation of pH or other aspects of the ionic environment. The rheological properties responsible for the different mixing behaviors between isoviscous solutions of nonstarch polysaccharides and suspensions of swollen starches are of interest. It seems likely that extensional rather than shear properties are the most relevant and will be studied in the future. Further research of interest is the extension of this work to more complex systems containing biopolymer mixtures that can phase separate as well as food emulsions. For the latter, it has been shown that salt perception is not simply dependent on ion concentration in solution but on the structure of the emulsion (*24*). We believe that this could be understood in terms of differences in mixing behavior resulting in different rates of ion release to the receptors.

This observation that the level of salt incorporation in starch-thickened foods for optimum taste and flavor will depend on

the in-mouth amylase activity of the consumer raises the possibility of developing products with different salt levels to optimize taste perception for consumer groups with different amylase activities.

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