Polysaccharide food matrices for controlling the release, retention and perception of flavours

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Abstract

Polysaccharides have many roles across both the food and pharmaceutics industries. They are commonly used to enhance viscosity, stabilise emulsions and to add bulk to food products. In the pharmaceutics industry, they are also utilised for their mucoadhesive nature. Mucoadhesive polysaccharides can facilitate retention of active ingredients at mucosal sites for a prolonged time and formulations can be designed to control their release and bioavailability. This study investigates how polysaccharides, with differing physicochemical properties (e.g. functional groups and molecular weight), affect the release and perception of flavour compounds from films. Polysaccharide films were prepared using either high or low viscosity carboxymethyl cellulose, pullulan or hydroxypropyl methylcellulose. Glucose, vanillin or a combination of both was also added to the films to assess the effect of flavour release and perception over time. The films were assessed for glucose release *in vitro*, swelling and disintegration times, and mucoadhesive ability. Results show that flavour release and perception depend on the polysaccharide matrix properties; this includes how quickly the films dissolves, the rate of release of

tastant compounds, and the mucoadhesive strength of the polysaccharide. A higher viscosity and slower disintegration time resulted in slower release of glucose *in vitro* and flavour perception *in vivo*.

Key words: polysaccharides, flavour, controlled, release, mucoadhesion

1. Introduction

Flavour perception requires the release of taste and aroma compounds from the food matrix and the subsequent transport of those compounds to the respective receptors. This process is dependent on many factors including the properties of the compound, the components of the food matrix constituents, food structure, how it is manipulated in the mouth and the physiological conditions of the mouth, nose and throat during consumption of the food. Furthermore, the onset and duration of flavour delivery is dependent on factors such as partitioning, mass transport and diffusion. These factors play varying roles and combined, result in a characteristic flavour profile for a food.

Typically, the polysaccharides, proteins and fats present in liquid food systems determine the structure. The influence of these large molecules on smaller molecules, such as aroma and tastant compounds, has been investigated with various studies concluding that viscosity changes (Hollowood, Linforth, & Taylor, 2002; Izutsu, Taneya, Kikuchi, & Sone, 1981; Kokini, Bistany, Poole, & Stier, 1982; Secouard, Malhiac, Grisel, & Decroix, 2003; Stevenson & Mahmut, 2011) and physical entrapment of compounds (Keršiene, Adams, Dubra, Kimpe, & Leskauskaite, 2008; Kora, Souchon,

Latrille, Martin, & Marin, 2004; Kuo & Lee, 2014) together explain perceptual differences (S. L. Cook, Bull, Methven, Parker, & Khutoryanskiy, 2017). These studies tend to focus on the matrix structure and the release characteristics when contemplating changes in perception.

Chemical interactions between the flavour compounds and the food matrix is also important (Heilig, Heimpel, Sonne, Schieberle, & Hinrichs, 2016; Rodríguez-Bencomo et al., 2011; Scherf, Pflaum, Koehler, & Hofmann, 2015). Factors such as charge of the flavour compound and other food constituents will influence interactions between the two. For example, sodium is positively charged and will therefore interact with negatively charged polysaccharides, such as carboxymethyl cellulose, affecting the ions availability to elicit a salt taste (Scherf et al., 2015). Retention of flavour compounds in the matrix will obviously decrease their perception, as they will not reach the respective receptors to be perceived and risk being swallowed in the food bolus before triggering perception. However, if the matrix also adheres to the oral mucosa then fewer tastant molecules may be swallowed allowing for release of the flavour over time.

Many studies have investigated the impact on aroma release when reducing fat in foods (Arancibia, Jublot, Costell, & Bayarri, 2011; Bayarri, Taylor, & Hort, 2006). They have found, in general that aroma retention in the matrix of a high fat food will increase as the P (partition coefficient of a molecule between a lipophillic and an aqueous phase, usually octanol and water, respectively) of the aroma compound increases. This means it will favour

being in the fatty matrix over partitioning into the aqueous saliva. Hydrophilic compounds (log P equal to or less than zero) on the other hand tend to be less dependent on changing fat levels (Arancibia, Castro, Jublot, Costell, & Bayarri, 2015; Arancibia et al., 2011). In low fat systems, the release of hydrophobic aromas will be faster leading to an unbalanced flavour profile.

More recently, interactions between food components and the oral and nasal mucosa have been investigated. Specifically, interactions between flavour molecules and the oral mucosa may explain persistence of aromas in certain foods (Esteban-Fernández, Rocha-Alcubilla, Muñoz-González, Moreno-Arribas, & Pozo-Bayón, 2016; Sánchez-López, Ziere, Martins, Zimmermann, & Yeretzian, 2016). Furthermore, interactions between food matrices and the oral mucosa have been of interest with regard to negative sensory characteristics of dairy products (Bull et al., 2015; Hilal Y et al., 2015; Withers, Cook, Methven, Godney, & Khutoryanskiy, 2013) and the impact of fat reduction on perception of foods (De Hoog, Prinz, Huntjens, Dresselhuis, & Van Aken, 2006; Dresselhuis, van Aken, de Hoog, & Martien, 2008).

Many polysaccharides are mucoadhesive, meaning they adhere to mucosal surfaces in the body via intermolecular forces (hydrogen bonding, electrostatic attraction, hydrophobic interactions and covalent bonds) and physical penetration and entanglement of polymer chains (Andrews, Laverty, & Jones, 2009; Huang, Leobandung, Foss, & Peppas, 2000; Jabbari, Wisniewski, & Peppas, 1993). Though this phenomenon has been of interest and well utilised in the pharmaceutics field for decades, the importance in the food

industry is beginning to gain interest (Bull et al., 2015; S. L. Cook, Bull, et al., 2017; S. L. Cook, Woods, Methven, Parker, & Khutoryanskiy, 2018; Gibbins & Carpenter, 2013; Hilal Y et al., 2015; Malone, Appelqvist, & Norton, 2003; Withers et al., 2013).

Mucoadhesive polymers can retain and control the release of active pharmaceutical ingredients (APIs) at mucosal surfaces including those in the oral cavity (Andrews et al., 2009). The mechanisms of mucoadhesion have been described in the literature numerous times (Peppas & Huang, 2004; Shaikh, Singh, Garland, Woolfson, & Donnelly, 2011; Smart, 2005, 2014). The physicochemical interactions depend on the polymeric substance (e.g ionic groups, chain length), the state of hydration of the polymer, the mucosal secretions (e.g. pH, thickness, mucin concentration) and the epithelial structure and morphology (e.g. roughness and presence of micro cracks). The fact that mucoadhesive polymers can retain small molecules at mucosal surfaces and control their release will be important for the food industry to consider as these frequently used polysaccharides may also retain tastant and aroma molecules in a similar way (S. L. Cook, Woods, et al., 2018).

Many polysaccharides used in the food industry that are also mucoadhesive include, but are not limited to; carboxymethyl cellulose (Yehia, El-Gazayerly, & Basalious, 2008, 2009), sodium alginate (Juliano, Gavini, Cossu, Bonferoni, & Giunchedi, 2004; Richardson, Dettmar, Hampson, & Melia, 2004) and pectin (Kaur & Kaur, 2012; Thirawong, Nunthanid, Puttipipatkhachorn, & Sriamornsak, 2007). Buccal films are a formulation type made by dissolving a

polymer in a solvent, adding the API and evaporating the solvent to leave a thin film of polymer matrix containing the API (Gherman, Zavastin, Ochiuz, Biliuta, & Coseri, 2016; Kaur & Kaur, 2012; Satishbabu & Srinivasan, 2008; Semalty, Semalty, Kumar, & Juyal, 2008). Buccal films can be designed to release API over differing periods of time.

The only study investigating the effect of mucoadhesive polysaccharides on flavour retention and perception was within an aqueous system. Also from our group, our findings suggest that sodium ions are retained in the mouth for longer when mucoadhesive polysaccharide is used as a thickener compared to non-mucoadhesive matrices (S. L. Cook, Woods, et al., 2018). This current study is concerned with the effect of mucoadhesive polysaccharides on flavour perception from a solid food system (films). Various food grade polysaccharides that differ in their chemical and physical properties were used to assess the effect on release, retention and perception of flavours from polysaccharide films.

Polysaccharides were cast into films containing glucose and/or vanillin. These were based on films usually made for pharmaceutical applications. The mucoadhesive properties, swelling ratio, dissolution rate, film thickness, water activity and temporal sensory perception were assessed. Whilst this study takes those factors into consideration, a further interaction between the food matrix and the oral anatomy, mucoadhesion, is investigated. The aim for this study was to assess the differences in flavour release from different polysaccharide matrices in a solid state. It was hypothesised that films made

with more viscous, slower dissolving polysaccharides will reduce the intensity but prolong the perception of flavours over time. Furthermore, the mucoadhesive properties of the matrices were assessed and related to flavour delivery. This study, therefore, provides a foundation of understanding of the mechanisms by which mucoadhesive ingredients can alter the perception of flavour over time, which may help in the development of reformulated products.

2. Methods

2.1. Materials

Four different polysaccharides were chosen for this study due to their differing chemical properties (Table 1). Pullulan (PUL) (Hayashibara nagase europa group, Düsseldorf, Germany) was chosen as a non-ionic, low viscosity and fast dissolving film former. Hydroxypropyl methyl cellulose (HPMC) (product code METHOCEL K4M, Dow The Chemical Company, Staines, UK) was chosen as a high viscosity, non-ionic film former. Two carboxymethyl cellulose products were used, one low molecular weight (LCMC) (product code AKUCELL AF 0305, AkzoNoble, Amsterdam, The Netherlands) and one high molecular weight (HCMC) (product code WALOCEL 4500, Dow The Chemical Company, Staines, UK). Carboxymethyl cellulose was chosen as it is well known for its mucoadhesive properties due to its ionic nature and high viscosity.

Table 1. Polysaccharide characteristics

Sample	Molecular weight (Da)	Sodium content (% w/v)	Degree of substitution	Viscosity of 2% (w/v) solution at 25°C (mPa.s)
PUL	250, 000	<0	N/A	11
LCMC	140, 000	15.4 *	0.8	450
HPMC	300, 000	<0	1.8 methoxyl 0.13 hydroxypropyl	4500
HCMC	950, 000	8.7	0.8	5200

All data provided by the respective manufacturer except those indicated by *.

* Sodium content determined by flame photometry

2.2. Samples

Films were prepared by dissolving polysaccharides in deionised water (2% w/v) with glucose, vanillin (Sigma- Aldrich, St. Louis, Missouri, United States) or glucose and vanillin (Table 2). The solution (30g) was weighed into circular petri dishes (90 mm) and placed in an oven at 65°C for 20 hours. Once the films were dry they were removed from the petri dish and cut into squares (approx. $1cm^2$). Glucose containing films weighed 100 mg and the aroma only films 30 mg. This was to ensure that each sample contained the same amount of polysaccharide. The water activity (a_w) of the films was measured after the drying process using a HygroLab C1 Bench-Top Water Activity Monitor.

195 Table 2. Final concentrations of ingredients in each type of film

Film type	Polysaccharide (%)	Glucose (%w/v)	Vanillin (%)
Sweet	30	70	-
Vanilla	99.1	-	0.9
Sweet and Vanilla	29.5	69.4	0.9

196

197 *2.3. Artificial saliva*

198 Artificial saliva (AS) was used for all *in vitro* experiments to emulate conditions
199 in the mouth. This was adapted from Madsen *et al.* (2013) and consisted of
200 0.21 g/L $NaHCO_3$, 0.43 g/L NaCl, 0.75 g/L KCl, 0.22 g/L $CaCl_2 \cdot 2H_2O$, 0.91 g/L
201 $NaH_2PO_4 \cdot 2H_2O$ dispersed in deionized water. For the mucoadhesion
202 experiment 2.5 g/L pig gastric mucin (PGM) type II (Sigma- Aldrich, St. Louis,
203 Missouri, United States) was also added. The pH of the AS was adjusted to
204 6.8 and kept at 37 °C during experiments and at 4 °C when not in use.

205

206 *2.4. Swelling and disintegration*

207 Swelling studies were carried out in an incubator set to 37 °C. Each film was
208 placed on to netting and fully submerged in a petri dish with 40 mL of AS. At
209 set time periods the sample was removed from the AS, excess water was
210 carefully absorbed with tissue paper and the film on the netting was weighed.
211 This process was repeated until the weight had returned to that of the netting
212 alone. Each type of film was tested 6 times with duplicate batch repeats. Film
213 thickness was measured before these experiments with a micrometer. The

maximum swelling ratio was determined by dividing the weight of the film at set time points with the original weight of the film.

2.5. Dissolution

Each film containing glucose was placed onto netting and carefully submerged into an individual beaker with 200 mL AS. The solution was stirred by a magnetic stirrer bar at a constant rate throughout the experiment. At set time points 1 mL aliquots of the AS medium were removed and put into labelled Eppendorfs for analysis. The glucose in the samples was quantified spectrophotometrically using an Amplex Red, glucose oxidase kit following the advised protocol (Fisher Scientific, Loughborough, UK). Each sample was tested 6 times with duplicate batch repeats. The time taken to release 50 and 100 % of the glucose was calculated from the results.

2.6. In vitro mucoadhesion

Adhesion experiments were carried out using a texture analyser (TA) with a 10mm cylindrical probe (on a TA-XT plus, Stable Micro Systems, UK). Porcine tongues were collected from a local butcher (P D Jennings, Hurst, UK) less than 24 hours after slaughter. They were stored on ice whilst the majority of muscle and connective tissue was removed leaving a thin section of the surface mucosa. These sections were stored at -20°C until required when they were thawed in the fridge for 3 hours before use.

Each area of the tongue was cut into 1 cm^2 sections and secured on the bottom platform of the TA. The film sample to be tested was stuck to the

probe with double-sided sticky tape. Before each experiment, the tongue tissue section was conditioned with 100µL of AS and incubated at 37°C. The contact time between the probe and the tissue was 60 seconds before pulling apart with a removal speed of 1mm/s.

2.7. In vivo retention

The study was given a favourable opinion for conduct by the University of Reading, School of Chemistry, Food and Pharmacy (study number 27/15). Five volunteers (3 males and 2 females, age range 23-30) were asked to place a film sample on their tongue and keep it between the tongue and roof of their mouth for the duration of the experiment. They were instructed to treat the film like a hard candy with some manipulation by the tongue. The experiment was timed and volunteers were asked to note the time (s) when the film began to adhere, when the adherence ceased and when the film dissolved. They were also asked where in the mouth the film adhered to. Adherence was noted as an inability to move the film with their tongue.

2.8. Sensory perception

Time intensity; profiling involves trained sensory panellists continuously recording the intensity of one or two attributes over a specified time. This enables perception to be captured during consumption and can be summarised as parameters such as onset, persistence and duration. Over a period of three weeks, 8 trained panellists from the University of Reading Sensory Science Centre panel scored each of the film samples in duplicate. There were 12 samples in total. For each polysaccharide, films were made

with either glucose alone, vanillin alone or glucose with vanillin. Each week was used for one set of polysaccharide films. For example, in week 1 the glucose only films were scored, in a balanced order, for sweetness over time.

Training took place before each scoring week to familiarise the panel to the samples and the time intensity protocol. Each film was presented to the panel and a discussion of the different flavour release behaviours for each of them took place. During these sessions, the panel were given 3 standards for both glucose and vanillin. Glucose standards were 8%, 4% and 2%, and aroma samples were 0.02%, 0.01%, and 0.005%. The panellists decided where these standards scored on the line scale with their strongest standard representing 100 on a standard 100-point scale. These standards were given to the panellists at the start of each scoring sessions to re-familiarise them with the standard intensities.

Panellists were trained on single and dual attribute time intensity scoring using Compusense@hand software (Ontario, Canada) and feedback was given to those who were not showing good reproducibility. The time intensity test lasted for 5 minutes, which was the agreed amount of time that the panellists could concentrate for without fatigue or boredom. The attributes scored were sweet for glucose only films, vanilla for aroma only films and both sweet and vanilla for the combined films. Panellists were also trained on how to manipulate the sample in the mouth. They were asked to gently rub the film between the tongue and roof of the mouth to facilitate flavour release.

288 Panellists were instructed to treat each sample the same way to avoid biasing
289 release.
290
291 Each week the panellists were given a training session on the first day
292 followed by two days of scoring the samples. Four samples were served
293 monadically, in a petri dish, in a balanced order with individual blinding codes
294 each day with the duplicate being served on a consecutive scoring day.
295 Panellists were provided with isolated sensory booths, computers with
296 Compusense Software and warm water for palate cleansing. There was a 2-
297 minute delay between samples to allow for palate cleansing. Time intensity
298 curves were produced for each panellist and each sample in duplicate.
299
300 *2.9. Statistical analysis*
301 One way or two way repeated measures ANOVA (rmANOVA) was used for
302 the appropriate test. Bonferroni or Tukey's HSD corrections were used on
303 pairwise analysis to account for multiple comparisons, at a significance level
304 of $p \leq 0.05$.
305
306 3. Results & Discussion
307 *3.1. Film characteristics*
308 A range of standard methods were used to characterise the polymeric films
309 (Morales & McConville, 2011; Nair et al., 2013). Each film was measured for
310 thickness, water activity (a_w), glucose release, and swelling / disintegration
311 times (Table 3).
312

The thickness of the films varied between the different polysaccharides and between the films with and without glucose. The order of film thickness was HPMC>HCMC>LCMC>PUL. This is not surprising as HPMC and HCMC were higher viscosity grades than LCMC and PUL and therefore will occupy more space, retain more water and form thicker films. Glucose films were thicker than those without glucose, which was expected, as the glucose was in addition to the polysaccharides. The thickness of a film will impact the dissolution rate as a thicker film will have a smaller surface area to volume ratio and this can slow water uptake from the surrounding medium. This will impact mucoadhesion as hydration of the dosage form is integral for polymer - mucin interactions to occur.

PUL and LCMC films fully dissolved after a similar time; however, LCMC films swelled more before beginning to disintegrate (Table 3 & Figure S1). This is because LCMC is more viscous than PUL (table 1) and possesses ionic groups, which interact strongly with water molecules due to the higher osmotic pressure induced by the high entropy of the counter-ions. LCMC and HCMC films swelled considerably more than the non-ionic, PUL and HPMC films with relation to their disintegration time. The carboxymethyl cellulose films absorbed more water, forming a swollen gel-like layer, before beginning to degrade. HCMC samples took the longest time to dissolve and swelled the most due to their high viscosity. All films without glucose had higher swelling ratios than their glucose containing counterparts and took longer to dissolve. This is because the small, highly hydrophillic glucose molecules contained within the film matrix will quickly dissolve into the surrounding medium,

leaving pores for the water molecules to enter, effectively increasing the surface area of the film.

The glucose release from the films followed a similar pattern to the dissolution rates. PUL and LCMC released glucose fully after 7.0 and 7.8 min respectively, followed by HPMC (186 min) and then HCMC (300 min). HPMC quickly released 50% of the total glucose in the film over a mean of 14 minutes. This fast initial release is most likely due to crystallisation of the glucose molecules on the outside of the film. This was visually observed, as these films were cloudy with a fine powder covering them. Furthermore, the HPMC samples took a long time to fully dissolve, most likely due to the high viscosity network it forms which will slow permeation of water molecules. The HCMC released the glucose at a constant rate. The HCMC films swelled considerably so the swollen, surface of the film contained loosely associated polymer chains, which would then allow the glucose molecules to diffuse out and dissolve in the surrounding medium. The increased surface area caused by the high swelling degree of the HCMC films may facilitate glucose release, however, the thick gel layer covering the outer surface of the film may also decrease diffusion by physical entrapment. Additionally, the thick gel layer may prevent matrix disintegration and affect subsequent water uptake when unperturbed (Rodriguez, Bruneau, Barra, Alfonso, & Doelker, 2000). HPMC did not swell substantially but took a long time to dissolve, therefore the glucose molecules would essentially be trapped in the film matrix until is started to erode.

363 Table 3. Characteristics of films

Polymer	Glucose content (%)	a_w (mean)	Thickness (mm)	Dissolution time (min)	Max swelling ratio	50% glucose release (min)	100% glucose release (min)
PUL	-	0.451 ^{a}	0.071 ^{a}	5 ^{a}	5.8 ^{a}	-	-
LCMC	-	0.486 ^{b}	0.094 $^{a, b}$	4 ^{a}	11.6 ^{a}	-	-
HPMC	-	0.478 ^{b}	0.148 ^{b}	147 ^{b}	11.6 ^{a}	-	-
HCMC	-	0.474 ^{b}	0.104 $^{a, b}$	357 ^{c}	34.9 ^{b}	-	-
PUL	70	0.502 ^{b}	0.281 ^{a}	5 ^{a}	1.8 ^{a}	3.2 ^{a}	7.0 ^{a}
LCMC	70	0.491 ^{b}	0.369 $^{a, b}$	5 ^{a}	3.4 ^{b}	3.3 ^{a}	7.8 ^{a}
HPMC	70	0.460 ^{a}	0.429 ^{b}	153 ^{b}	4.4 ^{b}	14.4 ^{b}	186.1 ^{b}
HCMC	70	0.496 ^{b}	0.360 $^{a, b}$	210 ^{c}	16.0 ^{c}	150.0 ^{c}	300.0 ^{c}

364 Films are separated into those without glucose and those with glucose. Each
365 value is the mean of 6 replications for the measured parameters (2 batch
366 repeats). Mean values within a column and film group not sharing the same
367 letter were significantly different from each other at $p \leq 0.05$ using Tukey's
368 HSD correction.

369

370 It was expected that changes in flavour perception over time would be
371 influenced by the parameters measured (Table 3). For example, it was
372 hypothesised that PUL films would result in a high intensity flavour that
373 decreased in intensity quickly as they dissolved faster and released glucose
374 quickly. Conversely, it was expected that as the HCMC would slow the

release of glucose and aroma and therefore reduce the initial intensity of flavour but prolong the sensation over time. The results gained from this study are in concordance with the authors expectations.

3.2. Mucoadhesion in vitro

Two values were obtained from the TA experiments; the maximum force required to separate the probe from the tongue (peak force of detachment) and the area under the curve (total work of adhesion). The mean values for peak force of attachment and total work of adhesion decreased in order of LCMC, HCMC, PUL and HPMC for films without glucose and LCMC, PUL, HCMC and HPMC for films with. In films both with and without glucose the LCMC film was significantly more mucoadhesive than the HPMC film (Figure 1a & b). The films without glucose required a significantly higher force to separate the film from the tissue suggesting a stronger adhesive joint (Figure 1a). This is not surprising as the glucose content was high and therefore the relative amount of polymer in contact with the tissue was smaller. The HPMC films with glucose exerted the lowest total work of adhesion and peak force of detachment (Figure 1). This is probably due to the non-ionic nature of HPMC along with the large molecule size and slow swelling (Table 3 & Figure S1).

Mucoadhesion of solid polymeric substances is dependent on the hydration of the formulation, which will create a polymeric mesh enabling the interactions between polymer and mucin chains. The mucin used in the artificial saliva were PGM purchased from Sigma-Aldrich, which is dehydrated and potentially denatured due to production processes (Kocevar-Nared, Kristl, & Smid-

Korbar, 1997). Therefore, the interactions that may occur with salivary mucin may not be represented by this commercial mucin. Furthermore, an adhesive joint is formed due to the viscous gel formed between the film and the moist mucosal surface. However, over-hydration of the film will lead to a slippery mucilage being formed and will result in an adhesive joint failure. The swelling ability of a polymeric substance is important for establishing a mucoadhesive bond as this enables polymer chains to be available to interact with the mucosa.

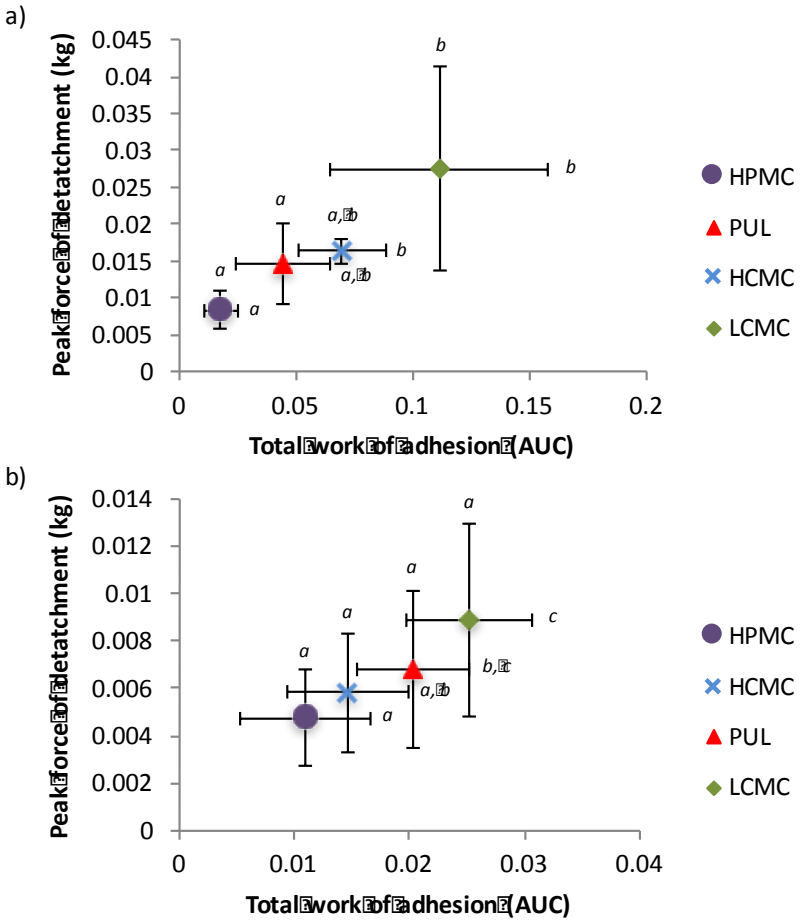
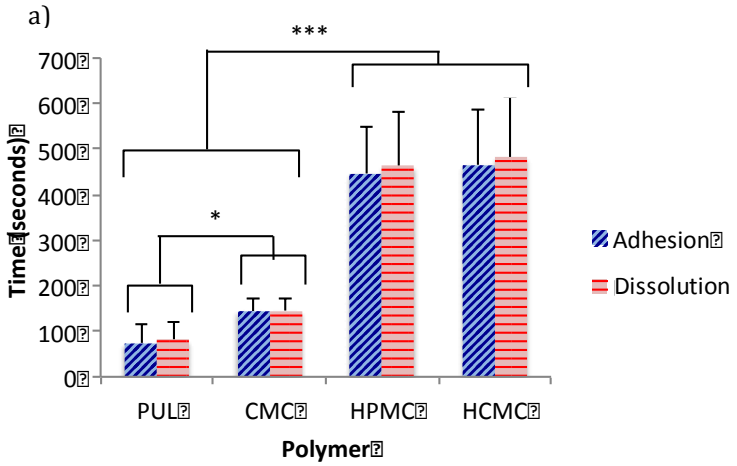


Figure 1. Total work of adhesion against the peak force of detachment for films a) without glucose and b) with glucose. Results determined by texture analysis. Data points are means of 6 measurements and error bars are SD. Superscript letters represent statistically different groupings ($p<0.05$). Letters

413 on top of the data point refer to the y axis and those to the right hand side
414 refer to the x axis.
415
416 *3.3. Mucoadhesion in vivo*
417 *In vivo* mucoadhesion experiments were carried out with 5 panellists that were
418 asked to record the following: where the film stuck, for how long and when it
419 dissolved. All films, except for HPMC with glucose, were reported to adhere
420 for the duration of the time that the film was in the mouth (Figure 2a & b).
421 Adherence was mainly to the roof of the mouth but also the tongue. The time
422 that the films took to dissolve reflected the *in vitro* dissolution (Table 3) as
423 PUL and LCMC took the least amount of time to dissolve followed by HPMC
424 then HCMC. For films without glucose, HPMC and HCMC films did not differ
425 in time for dissolution *in vivo* (Figure 2a) despite the difference in the *in vitro*
426 test. This is probably due to the participants manipulating the film with their
427 tongue during these experiments, thereby exerting mechanical stress on the
428 film. Therefore, as the HCMC swells and takes up water to produce a gel–like
429 layer, the tongue pressure will remove it and therefore speed up the time of
430 erosion.
431



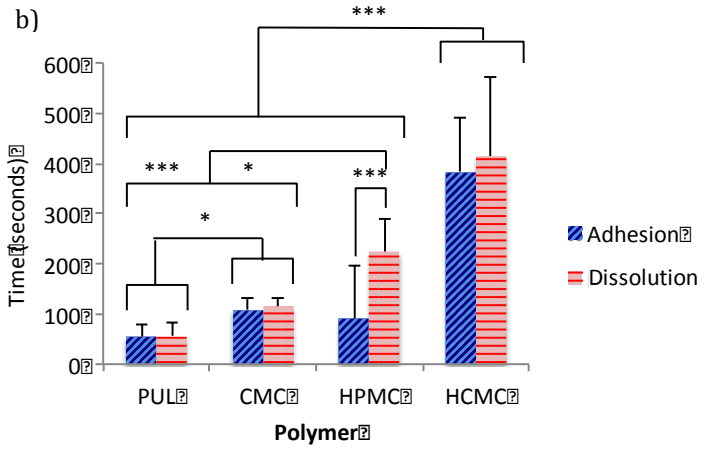


Figure 2. *In vivo* mucoadhesion of a) polymer films without glucose and b) polymer films with glucose. Each bar represents the mean of 10 separate data points, error bars represent standard deviation. N= 5 in duplicate. * = $p<0.05$, *** = $p<0.001$.

The HPMC films with glucose were reported to adhere for a significantly shorter time than it took to dissolve and 3 out of 5 of the panellists reported that the film did not adhere at all (Figure 2b). This reflects the *in vitro* tensile experiments where HPMC was concluded to be significantly less adhesive than the other films. Contrary to these *in vitro* tensile experiments, HPMC films without glucose were mucoadhesive in the *in vivo* experiments, with all

445 panellists reporting adherence after an initial delay. There are two
446 explanations to this. Firstly, AS was used in the *in vitro* experiments, which
447 contained Sigma-Aldrich PGM as opposed to human salivary mucin. This may
448 affect interactions between the polysaccharide matrix and the saliva due to
449 differences in denaturation states and response to pH. For example, mucin
450 chains must be flexible and uncoiled enough to allow interpenetration with
451 polymer chains. Secondly, the hydration of the oral cavity *in vivo* may be
452 different to that which was on the porcine tongue in the *in vitro* experiments.
453 This may have led to a stronger adhesion *in vivo*, as the film did not become
454 overhydrated.
455
456 The PUL film dissolving and adherence time was significantly quicker for
457 LCMC films in these experiments. The PUL films dissolved on average at 81
458 seconds compared to 145 seconds for the LCMC films during these
459 experiments. This is in contrast to the results obtained from the *in vitro*
460 dissolution tests (table 3) where they were not significantly different. This
461 difference was expected to have an impact on flavour release from LCMC
462 films compared to PUL. Film thickness is the most likely explanation for the
463 differences observed, LCMC films were thicker than PUL and therefore, when
464 in contact with the moist mucosal surface, will take longer to take up water. To
465 properly assess the impact of polysaccharide type on dissolution times, the
466 thickness of the films would need to be matched.
467
468 *3.4. Perception of tastant and aroma from films over time changes depending*
469 *on polysaccharide used*

Panellists produced time intensity curves for each sample and repeat. They continuously scored either sweetness or vanilla, or both attributes at the same time, over the course of 5 minutes using an unstructured line scale. Various parameters were extrapolated from the curves including the area under the curve (AUC), time to maximum intensity (T_{max}), maximum intensity (I_{max}), duration of perception, and incline and decline angles (Figure S2). One-way rmANOVA was used for each parameter

3.4.1. Glucose only films

Time intensity curves were averaged across all panellists and both replicates (Figure 3). The mean sweetness AUC and I_{max} values for the films decreased in order of PUL >LCMC >HPMC >HCMC with the reverse order for T_{max} (Table 4) where PUL was significantly higher than HCMC and higher for all other films for I_{max}. This suggests a fast onset of intensity for PUL and LCMC, which is supported by their larger incline angles compared to HPMC and HCMC. Furthermore, PUL and LCMC decline angles were also larger than the other two film types suggesting a quicker rate of decline. These results were expected as *in vitro* results (Table 3) show that PUL and LCMC films were faster dissolving and release glucose quicker than HPMC and HCMC films (table 3). Although the total duration of perception was not significantly different between the films, there was a trend that HPMC and HCMC films prolonged the flavour perception compared to PUL and LCMC (see “duration” in table 4).

494 Regarding mucoadhesion, the HPMC films containing glucose were found to
495 have poor adhesive abilities (Figures 1 & 2). In the perception experiments
496 panellists were asked not to swallow these films and, therefore, the perception
497 may have been artificially prolonged due to consciously keeping the film in the
498 mouth. During normal consumption in a real food system the material would
499 be chewed into a bolus and, without mucoadhesive ability, it may well be
500 swallowed with the food bolus thereby negating any further release. On the
501 other hand, HCMC films showed strong adhesion (Figures 1 & 2) and
502 therefore would be more likely to adhere to the oral cavity for longer,
503 prolonging the release.
504
505 *3.4.2. Vanillin only films*
506 For films containing the polysaccharide and vanillin the mean scores for I_{max}
507 decreased in order of PUL>LCMC>HPMC>HCMC (Table 4, Figure 3b).
508 Where PUL was significantly higher than HCMC. T_{max} and AUC were not
509 dependent on polysaccharide type. The duration of perception was longest in
510 the HPMC samples followed by HCMC. This suggests that although the total
511 intensity of perception was the same for each film, the aroma was delivered at
512 a slightly lower intensity for longer in the HPMC and HCMC samples. This is
513 supported by the decline angles being larger for PUL and LCMC samples
514 suggesting the intensity decreased more quickly in these films.
515
516 To date, the only studies investigating aroma release and perception in food
517 thickened with polysaccharides are in liquid and semi-solid foods. These
518 studies have found confounding results with regard to interactions between

519 aroma molecules and the food matrix. Arancibia et al. (2011) found that
520 thickener type affected total aroma release from dairy desserts with CMC
521 thickened samples reducing the cumulative release of hydrophobic aroma
522 (linalool) compared to starch. Furthermore, a follow up study by Arancibia,
523 Castro, Jublot, Costell, & Bayarri (2015) found that thickener type affected
524 both hydrophilic aroma (cis-3-hexen-1-ol) and hydrophobic (linalool) aroma.
525 The CMC thickened dairy desserts reduced the release of both aromas,
526 though it had more of an impact on the hydrophilic compound. Cook, Linforth,
527 et al., (2003) on the other hand found that in-nose measurements of
528 hydrophobic aroma release were not dependent on thickener type or on an
529 increase in viscosity. These studies exemplify the complex behaviour of
530 aroma release and its dependence on the food matrix.
531
532 In this current study, vanillin, a slightly hydrophobic molecule with a log P of
533 1.2, was used as the aroma. Perception results show that films made with
534 slow dissolving polysaccharides (HPMC and HCMC) reduced the I_{max} but
535 prolonged the duration of perception. Perception results for the aroma only
536 films were not as distinguishable as the films containing glucose. This may be
537 because the panel found scoring the aroma only films particularly difficult as
538 they contained no tastant along with the aroma, which does not normally
539 occur in food products.
540
541 *3.4.3. Glucose & vanillin films*
542 Dual attribute time intensity was used to simultaneously and continuously
543 monitor sweetness and vanilla attributes over 5 minutes. Results for the

sweetness attribute were similar for the dual attribute and single attribute tests (Table 4, Figure 3c and d). The AUC and I_{max} were highest for PUL and lowest for HCMC. HPMC and HCMC took longer to reach T_{max} compared to PUL and LCMC.

The AUC for the vanilla attribute did not significantly differ with the different polysaccharides (Table 4). HPMC and HCMC had reduced I_{max} and increased T_{max} results compared to PUL and LCMC. The total duration of perception was striking in these films with the HCMC averaging 53 seconds longer than PUL. HPMC also increased the duration significantly compared to PUL and LCMC. Although not statistically significant, LCMC followed the trend of prolonging the perception compared to PUL. The incline angles for HPMC and HCMC were, again, smaller than PUL and CMC suggesting a slower rate of onset.

These results suggest that PUL films give a quick burst of flavour that declines quickly. LCMC films are almost as quick to release as PUL but take somewhat longer to reach I_{max}. HPMC has a slower onset to reach I_{max} and the perception continues for longer than LCMC and PUL. Finally, HCMC films have the slowest onset with a steady release over time. This is particularly evident for the vanilla attribute, which prolongs the perception for longer than the faster dissolving films.

Although from this perception data HPMC films appear to give a sustained, medium level intensity of flavour, this formulation was not particularly

569 mucoadhesive and, therefore, it would most likely be swallowed along with the
570 bolus in a real food system. Participants were instructed not to chew or
571 swallow the film and many suggested that this would have been possible if
572 they were eating normally. However, the other formulations were firmly
573 adhered to the roof or tongue tissue and would not be easily swallowed.
574
575 Table 4. Parameters from time intensity results.

Film type	Attribute	Polymer	AUC	I_{max}	T_{max} (secs)	Duration (secs)	Incline angle (°)	Decline angle (°)
Glucose	Sweet	PUL	8410 *b*	91 *d*	22 *a*	200 *a*	73 *b*	30 *b*
		LCMC	7468 *a, b*	75 *c*	48 *b*	201 *a*	58 *b*	27 *b*
		HPMC	7126 *b*	54 *b*	61 *b*	231 *a*	38 *a*	20 *a*
		HCMC	4834 *a*	31 *a*	88 *b*	249 *a*	34 *a*	11 *a*
Aroma	Vanilla	PUL	7291 *a*	68 *b*	41 *a*	196 *a*	57 *a*	25 *b, c*
		LCMC	7154 *a*	59 *a, b*	40 *a*	195 *a*	50 *a*	28 *c*
		HPMC	7622 *a*	53 *a, b*	50 *a*	264 *b*	47 *a*	14 *a*
		HCMC	6176 *a*	51 *a*	38 *a*	230 *a,b*	54 *a*	19 *a, b*
Aroma and Glucose	Sweet	PUL	9154 *b, c*	92 *d*	25 *a*	221 *a*	73 *c*	28 *b, c*
		LCMC	9295 *c*	82 *c*	32 *a*	224 *a*	64 *b*	27 *c*
		HPMC	6661 *a, b*	50 *b*	64 *b*	245 *a*	41 *a*	17 *a,b*
		HCMC	5864 *a*	36 *b*	64 *b*	266 *a*	34 *a*	12 *a*
	Vanilla	PUL	9499 *a*	87 *b*	29 *a*	239 *a*	67 *b*	21 *a*
		LCMC	10957 *a*	82 *b*	35 *a*	254 *a, b*	67 *b*	23 *a*
		HPMC	10081 *a*	56 *a*	54 *a, b*	276 *b*	46 *a*	14 *a*
		HCMC	10770 *a*	54 *a*	73 *b*	292 *b*	43 *a*	16 *a*

8 panellists scored each sample in duplicate therefore each result is the mean of 16 separate results. Statistical analysis was done for each attribute separately comparing the different polysaccharides. Different letters represent significantly different groupings for each set of data.

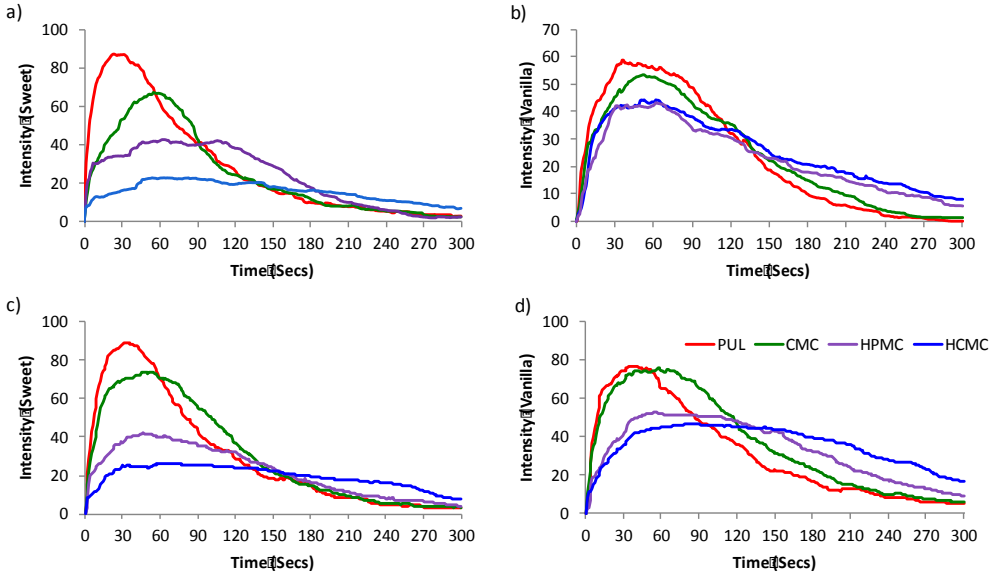


Figure 3. A panel of 8 trained panellists scored different polysaccharide films in duplicate for either sweetness of vanilla perception over time. Time intensity curves for a) glucose only films, b) vanillin only films were produced from single attribute time intensity tests. Dual attribute time intensity tests produced the curves for glucose and vanillin films in c) and d).

3.5. Comparing perception results to in vivo dissolution

During the in vivo experiments where participants were asked to record the adhesion and dissolution of the films, PUL was reported to dissolve after an average of 57 seconds. When comparing these timings to the perception data it is clear that perception of flavour is continuing after the film has completely dissolved (Table 4 & Figure 3). There are two explanations for this. Firstly, the glucose and aroma molecules may still be present at the respective receptors,

thereby initiating a response. Secondly, as the intensity of sweetness was very high, an adaptation type response could occur where the sweet signal is switched on for a longer time even after the stimulus has gone.

The physiological differences between participants were not collected for the in vivo mucoadhesion nor the sensory perception experiments. Factors such as salivary flow and constituents varies between individuals (Fenoli-Palomares et al., 2004) and will therefore impact the mucoadhesive strength and rate of film dissolution. Despite not adding these covariates in analysis, there were still significant results gained from the experiments.

3.6. Comparisons between different film types

Time intensity results were compared between 5 panellists who were consistent for both experiments. The AUC for the vanilla attribute differed between films with and without glucose (Figure 4). Significant increases in the total perception intensity (AUC) of vanilla were observed for LCMC, HPMC and HCMC films containing vanillin plus glucose compared to those without glucose.

During single attribute time intensity, the attribute is scored horizontally but during dual attribute, one must be scored vertically. The vanilla attribute was scored vertically in the dual attribute tests, which may have affected the results. Duizer, Bloom, & Findlay, (1995) investigated this issue and found that scoring an attribute vertically lead to approximately 13% increase in

scores. However, as the increase is more substantial it is unlikely this is the only factor.

A more likely explanation is that the presence of glucose in the films enhanced the aroma through cross modality (D. J. Cook et al., 2003; Niimi, Eddy, Overington, Heenan, et al., 2014; Niimi, Eddy, Overington, Silcock, et al., 2014). T_{max} was also significantly ($p < 0.05$) increased for vanillin in the HCMC films going from 26 to 89 seconds (Figure S3). This suggests that when glucose was present the perception of aroma had a slower onset, which lasted for longer and was sustained.

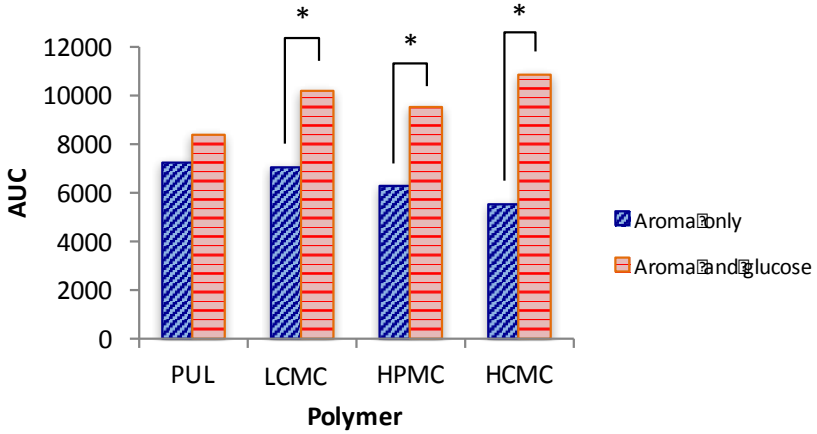


Figure 4. Comparisons of the area under the curve for the vanilla attribute of films with and without glucose. * denotes significant differences $p = <0.05$ using Bonferroni correction.

4. Conclusions

This study has shown that polysaccharides affect the retention, release and perception of flavour compounds, dependant on the physicochemical properties of the polysaccharide matrix. The viscosity and swelling ability of

637 the polysaccharide influences the release of flavour molecules from the
638 matrix. This in turn has an impact on the flavour perception. Fast dissolving
639 polysaccharides resulted in a quick burst of flavour at high intensity that
640 tapered more quickly whereas slow dissolving films gave a slower onset and a
641 more consistent release over time. The mucoadhesive ability of the films will
642 influence how long the matrix stays in the mouth whilst releasing the flavour
643 compounds before being swallowed. Furthermore, in line with previous
644 literature, this study shows that aroma intensity is dependent on the
645 perception of a congruent tastant, giving more evidence for cross modal
646 interactions.
647
648 The mucoadhesive nature of some of the polysaccharides tested will have an
649 effect on flavour delivery over time as those that adhere to the oral cavity will
650 continue to release flavour whilst those that are not mucoadhesive will be
651 swallowed. This study investigated flavour release from very simple food
652 matrices, polysaccharide films; of course in a real food there will be many
653 other food components that could affect flavour release. However, this study
654 provides some fundamental understanding of how different polysaccharide
655 matrices affect flavour release. Results from this study can be used to inform
656 the food industry of the impact that the addition of these polysaccharides can
657 have on temporal flavour perception. Possible applications include topical
658 coatings, confectionary, low fat and low sugar foods. However, there is a need
659 for further research into this area to understand the full impact on the
660 organoleptic properties of foods.
661

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687 References

688 Andrews, G. P., Laverty, T. P., & Jones, D. S. (2009). Mucoadhesive
689 polymeric platforms for controlled drug delivery. *European Journal of*
690 *Pharmaceutics and Biopharmaceutics : Official Journal of*
691 *Arbeitsgemeinschaft Für Pharmazeutische Verfahrenstechnik e.V*, *71*(3),
692 505–18. http://doi.org/10.1016/j.ejpb.2008.09.028
693 Arancibia, C., Castro, C., Jublot, L., Costell, E., & Bayarri, S. (2015). Colour,
694 rheology, flavour release and sensory perception of dairy desserts.
695 Influence of thickener and fat content. *LWT - Food Science and*
696 *Technology*, *62*(1), 408–416. http://doi.org/10.1016/j.lwt.2014.08.024
697 Arancibia, C., Jublot, L., Costell, E., & Bayarri, S. (2011). Flavor release and
698 sensory characteristics of o/w emulsions. Influence of composition,
699 microstructure and rheological behavior. *Food Research International*,
700 *44*(6), 1632–1641. http://doi.org/10.1016/j.foodres.2011.04.049
701 Bayarri, S., Taylor, A. J., & Hort, J. (2006). The Role of Fat in Flavor
702 Perception: Effect of Partition and Viscosity in Model Emulsions. *Journal*
703 *of Agricultural and Food Chemistry*, *54*, 8862–8868.
704 Bull, S. P., Hong, Y., Khutoryanskiy, V. V., Parker, J. K., Faka, M., & Methven,
705 L. (2015). Whey protein mouth drying influenced by thermal denaturation.
706 *Food Quality and Preference*, *56*, 233–240.
707 http://doi.org/10.1016/j.foodqual.2016.03.008
708 Cook, D. J., Linforth, R. S. T., & Taylor, A. J. (2003). Effects of hydrocolloid
709 thickeners on the perception of savory flavors. *Journal of Agricultural and*
710 *Food Chemistry*, *51*(10), 3067–3072. http://doi.org/10.1021/jf0211581
711 Cook, S. L., Bull, S. P., Methven, L., Parker, J. K., & Khutoryanskiy, V. V.

712 (2017). Mucoadhesion: A food perspective. *Food Hydrocolloids*, *72*, 281–
713 296.
714 Cook, S. L., Woods, S., Methven, L., Parker, J. K., & Khutoryanskiy, V. V.
715 (2018). Mucoadhesive polysaccharides modulate sodium retention,
716 release and taste perception. *Food Chemistry*, *240*, 482–489.
717 http://doi.org/10.1016/j.foodchem.2017.07.134
718 De Hoog, E. H. A., Prinz, J. F., Huntjens, L., Dresselhuis, D. M., & Van Aken,
719 G. A. (2006). Lubrication of oral surfaces by food emulsions: The
720 importance of surface characteristics. *Journal of Food Science*, *71*(7).
721 http://doi.org/10.1111/j.1750-3841.2006.00140.x
722 Dresselhuis, D. M., van Aken, G. A., de Hoog, E. H. A., & Martien, A. C. S.
723 (2008). Direct observation of adhesion and spreading of emulsion
724 droplets at solid surfaces. *Soft Matter*, *4*(4), 1079–1085.
725 http://doi.org/10.1039/b800106e
726 Duizer, L. M., Bloom, K., & Findlay, C. J. (1995). The effect of line orientation
727 on the recording of time-intensity perception of sweetener solutions. *Food*
728 *Quality and Preference*, *6*(2), 121–126. http://doi.org/10.1016/0950-
729 3293(94)00021-M
730 Esteban-Fernández, A., Rocha-Alcubilla, N., Muñoz-González, C., Moreno-
731 Arribas, M. V., & Pozo-Bayón, M. Á. (2016). Intra-oral adsorption and
732 release of aroma compounds following in-mouth wine exposure. *Food*
733 *Chemistry*, *205*, 280–288. http://doi.org/10.1016/j.foodchem.2016.03.030
734 Fenoli-Palomares, C., Munoz-Montagud, J. V, Sanchiz, V., Herreros, B.,
735 Hernandez, V., Minguez, M., & Benages, A. (2004). Unstimulated salivary
736 flow rate, pH and buffer capacity of saliva in healthy volunteers. *Revista*

Española de Enfermedades Digestivas, *96*(11), 773–783. Retrieved from http://scielo.isciii.es/pdf/diges/v96n11/original4.pdf

Gherman, S., Zavastin, D., Ochiuz, L., Biliuta, G., & Coseri, S. (2016). Enalapril maleate loaded pullulan film for mucoadhesive buccal drug delivery applications. *Cellulose Chemistry and Technology*, *2*(3), 507–17. http://doi.org/10.1517/17425247.2.3.507

Gibbins, H. L., & Carpenter, G. H. (2013). Alternative mechanisms of astringency - What is the role of saliva? *Journal of Texture Studies*, *44*(5), 364–375. http://doi.org/10.1111/jtxs.12022

Heilig, A., Heimpel, K., Sonne, A., Schieberle, P., & Hinrichs, J. (2016). An approach to adapt aroma in fat-free yoghurt systems: Modelling and transfer to pilot scale. *International Dairy Journal*, *56*, 101–107. http://doi.org/10.1016/j.idairyj.2016.01.011

Hilal Y, Ç., Gudjónsdóttir, M., Meier, S., Duus, J. Ø., Lee, S., & Chronakis, I. S. (2015). Spectroscopic studies of the interactions between beta-lactoglobulin and bovine submaxillary mucin. *Food Hydrocolloids*. http://doi.org/10.1016/j.foodhyd.2015.04.026

Hollowood, T. A., Linforth, R. S. T., & Taylor, A. J. (2002). The effect of viscosity on the perception of flavour. *Chemical Senses*, *27*(7), 583–591. http://doi.org/10.1093/chemse/27.7.583

Huang, Y., Leobandung, W., Foss, A., & Peppas, N. A. (2000). Molecular aspects of muco- and bioadhesion: Tethered structures and site-specific surfaces Yanbin. *Journal of Controlled Release*, *65*(1–2), 63–71. http://doi.org/10.1016/S0168-3659(99)00233-3

Izutsu, T., Taneya, S., Kikuchi, E., & Sone, T. (1981). Effect of viscosity on

762 perceived sweetness intensity of sweetened sodium
763 carboxymethylcellulose solutions. *Journal of Texture Studies*, *12*(2), 259–
764 273.
765 Jabbari, E., Wisniewski, N., & Peppas, N. A. (1993). Evidence of
766 mucoadhesion by chain interpenetration at a poly (acrylic acid)/mucin
767 interface using ATR-FTIR spectroscopy. *Journal of Controlled Release*.
768 http://doi.org/10.1016/0168-3659(93)90109-I
769 Juliano, C., Gavini, E., Cossu, M., Bonferoni, M. C., & Giunchedi, P. (2004).
770 Mucoadhesive alginate matrices containing sodium carboxymethyl starch
771 for buccal delivery: in vitro and in vivo studies. *Journal of Drug Delivery*
772 *Science and Technology*, *14*(2), 159–163. http://doi.org/10.1016/S1773-
773 2247(04)50029-1
774 Kaur, A., & Kaur, G. (2012). Mucoadhesive buccal patches based on
775 interpolymer complexes of chitosan-pectin for delivery of carvedilol. *Saudi*
776 *Pharmaceutical Journal*, *20*(1), 21–27.
777 http://doi.org/10.1016/j.jsps.2011.04.005
778 Keršiene, M., Adams, A., Dubra, A., Kimpe, N. De, & Leskauskaite, D. (2008).
779 Interactions between flavour release and rheological properties in model
780 custard desserts: Effect of starch concentration and milk fat. *Food*
781 *Chemistry*, *108*(4), 1183–1191.
782 http://doi.org/10.1016/j.foodchem.2007.11.011
783 Kocevar-Nared, J., Kristl, J., & Smid-Korbar, J. (1997). Comparative
784 rheological investigation of crude gastric mucin and natural gastric
785 mucus. *Biomaterials*, *18*(9), 677–81. Retrieved from
786 http://www.ncbi.nlm.nih.gov/pubmed/9151999

Kokini, J. L., Bistany, K., Poole, M., & Stier, E. (1982). Use of mass transfer theory to predict viscosity-sweetness interactions of fructose and sucrose solutions containing tomato solids. *J. Texture Studies*, *13*, 187–200.

Kora, E. P., Souchon, I., Latrille, E., Martin, N., & Marin, M. (2004). Composition rather than viscosity modifies the aroma compound retention of flavored stirred yogurt. *Journal of Agricultural and Food Chemistry*, *52*(10), 3048–56. http://doi.org/10.1021/jf034597o

Kuo, W.-Y., & Lee, Y. (2014). Effect of Food Matrix on Saltiness Perception-Implications for Sodium Reduction. *Comprehensive Reviews in Food Science and Food Safety*, *13*(5), 906–923. http://doi.org/10.1111/1541-4337.12094

Madsen, K. D., Sander, C., Baldursdottir, S., Pedersen, A. M. L., & Jacobsen, J. (2013). Development of an ex vivo retention model simulating bioadhesion in the oral cavity using human saliva and physiologically relevant irrigation media. *International Journal of Pharmaceutics*, *448*(2), 373–81. http://doi.org/10.1016/j.ijpharm.2013.03.031

Malone, M. E., Appelqvist, I. A. M., & Norton, I. T. (2003). Oral behaviour of food hydrocolloids and emulsions. Part 1. Lubrication and deposition considerations. *Food Hydrocolloids*, *17*(6), 763–773. http://doi.org/10.1016/S0268-005X(03)00097-3

Morales, J. O., & McConville, J. T. (2011). Manufacture and characterization of mucoadhesive buccal films. *European Journal of Pharmaceutics and Biopharmaceutics : Official Journal of Arbeitsgemeinschaft Für Pharmazeutische Verfahrenstechnik e.V*, *77*(2), 187–99. http://doi.org/10.1016/j.ejpb.2010.11.023

812 Nair, A. B., Kumria, R., Harsha, S., Attimarad, M., Al-Dhubiab, B. E., &
813 Alhaider, I. A. (2013). In vitro techniques to evaluate buccal films. *Journal*
814 *of Controlled Release*, *166*(1), 10–21.
815 http://doi.org/10.1016/j.jconrel.2012.11.019
816 Niimi, J., Eddy, A. I., Overington, A. R., Heenan, S. P., Silcock, P., Bremer, P.
817 J., & Delahunty, C. M. (2014). Aroma-taste interactions between a model
818 cheese aroma and five basic tastes in solution. *Food Quality and*
819 *Preference*, *31*(1), 1–9. http://doi.org/10.1016/j.foodqual.2013.05.017
820 Niimi, J., Eddy, A. I., Overington, A. R., Silcock, P., Bremer, P. J., &
821 Delahunty, C. M. (2014). Cross-modal interaction between cheese taste
822 and aroma. *International Dairy Journal*, *39*(2), 222–228.
823 http://doi.org/10.1016/j.idairyj.2014.07.002
824 Peppas, N., & Huang, Y. (2004). Nanoscale technology of mucoadhesive
825 interactions. *Advanced Drug Delivery Reviews*, *56*(11), 1675–1687.
826 http://doi.org/10.1016/j.addr.2004.03.001
827 Richardson, J. C., Dettmar, P. W., Hampson, F. C., & Melia, C. D. (2004).
828 Oesophageal bioadhesion of sodium alginate suspensions: particle
829 swelling and mucosal retention. *European Journal of Pharmaceutical*
830 *Sciences : Official Journal of the European Federation for Pharmaceutical*
831 *Sciences*, *23*(1), 49–56. http://doi.org/10.1016/j.ejps.2004.05.001
832 Rodríguez-Bencomo, J. J., Muñoz-González, C., Andújar-Ortiz, I., Martín-
833 Álvarez, P. J., Moreno-Arribas, M. V., & Pozo-Bayón, M. Á. (2011).
834 Assessment of the effect of the non-volatile wine matrix on the volatility of
835 typical wine aroma compounds by headspace solid phase
836 microextraction/gas chromatography analysis. *Journal of the Science of*

Food and Agriculture, *91*(13), 2484–94. http://doi.org/10.1002/jsfa.4494

Rodriguez, C. F., Bruneau, N., Barra, J., Alfonso, D., & Doelker, E. (2000). Hydrophilic cellulose derivatives as drug delivery carriers: influence of the substitution type on the properties of compressed matrix tablets. Retrieved from http://books.google.com/books?id=paEzHRHlXE4C&pgis=1

Sánchez-López, J. A., Ziere, A., Martins, S. I. F. S., Zimmermann, R., & Yeretzian, C. (2016). Persistence of aroma volatiles in the oral and nasal cavities: real-time monitoring of decay rate in air exhaled through the nose and mouth. *Journal of Breath Research*, *10*(3), 36005. http://doi.org/10.1088/1752-7155/10/3/036005

Satishbabu, B. K., & Srinivasan, B. P. (2008). Preparation and evaluation of buccoadhesive films of atenolol. *Indian Journal of Pharmaceutical Sciences*. http://doi.org/10.4103/0250-474X.41451

Scherf, K. A., Pflaum, T., Koehler, P., & Hofmann, T. (2015). Salt taste perception in hydrocolloid systems is affected by sodium ion release and mechanosensory–gustatory cross-modal interactions. *Food Hydrocolloids*, *51*, 486–494. http://doi.org/10.1016/j.foodhyd.2015.05.043

Secouard, S., Malhiac, C., Grisel, M., & Decroix, B. (2003). Release of limonene from polysaccharide matrices: Viscosity and synergy effect. *Food Chemistry*, *82*(2), 227–234. http://doi.org/10.1016/S0308-8146(02)00518-6

Semalty, M., Semalty, A., Kumar, G., & Juyal, V. (2008). Development of Mucoadhesive Buccal Films of Glipizide. *International Journal of Pharmaceutical Sciences and Nanotechnology*, *1*(2), 184–190.

862 Shaikh, R., Singh, T. R. R., Garland, M. J., Woolfson, A. D., & Donnelly, R. F.
863 (2011). Mucoadhesive drug delivery systems. *Journal of Pharmacy &*
864 *Bioallied Sciences*, *3*(1), 89–100. http://doi.org/10.4103/0975-7406.76478
865 Smart, J. D. (2005). The basics and underlying mechanisms of
866 mucoadhesion. *Advanced Drug Delivery Reviews*, *57*(11), 1556–68.
867 http://doi.org/10.1016/j.addr.2005.07.001
868 Smart, J. D. (2014). Theories of Mucoadhesion - Mucoadhesive Materials and
869 Drug Delivery Systems. In V. V. Khutoryanskiy (Ed.), . London: Jon Wiley
870 & Sons Ltd.
871 Stevenson, R. J., & Mahmut, M. K. (2011). Experience dependent changes in
872 odour-viscosity perception. *Acta Psychologica*, *136*(1), 60–66.
873 http://doi.org/10.1016/j.actpsy.2010.10.001
874 Thirawong, N., Nunthanid, J., Puttipipatkhachorn, S., & Sriamornsak, P.
875 (2007). Mucoadhesive properties of various pectins on gastrointestinal
876 mucosa: an in vitro evaluation using texture analyzer. *European Journal*
877 *of Pharmaceutics and Biopharmaceutics : Official Journal of*
878 *Arbeitsgemeinschaft Für Pharmazeutische Verfahrenstechnik e.V*, *67*(1),
879 132–40. http://doi.org/10.1016/j.ejpb.2007.01.010
880 Withers, C. A., Cook, M. T., Methven, L., Godney, M. A., & Khutoryanskiy, V.
881 V. (2013). Investigation of milk proteins binding to the oral mucosa. *Food*
882 *& Function*. http://doi.org/10.1039/C3FO60291E
883 Yehia, S. A., El-Gazayerly, O. N., & Basalious, E. B. (2008). Design and in
884 vitro/in vivo evaluation of novel mucoadhesive buccal discs of an
885 antifungal drug: relationship between swelling, erosion, and drug release.
886 *AAPS PharmSciTech*, *9*(4), 1207–1217. http://doi.org/10.1208/s12249-

008-9166-1

Yehia, S. A., El-Gazayerly, O. N., & Basalious, E. B. (2009). Fluconazole mucoadhesive buccal films: in vitro/in vivo performance. *Current Drug Delivery*, *6*(1), 17–27. http://doi.org/10.2174/156720109787048195