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

Sarah L. Cook a, Lisa Methven b, Jane K. Parker b, Vitaliy V. Khutoryanskiy* a

a Department of Pharmacy, University of Reading, Whiteknights, PO box 224, Reading, Berks, RG6 6AD, United Kingdom
b Department of Food and Nutrition Sciences, University of Reading, Whiteknights, Reading, Berks, RG6 6AD, United Kingdom

* Correspondence to: Professor V Khutoryanskiy Department of Pharmacy, University of Reading, Whiteknights, Reading, Berks, RG6 6AD, United Kingdom. v.khutoryanskiy@reading.ac.uk

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

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

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. 1cm²). 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 (aₜ) of the films was measured after the drying process using a HygroLab C1 Bench-Top Water Activity Monitor.
Table 2. Final concentrations of ingredients in each type of film

<table>
<thead>
<tr>
<th>Film type</th>
<th>Polysaccharide (%)</th>
<th>Glucose (% w/v)</th>
<th>Vanillin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet</td>
<td>30</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>Vanilla</td>
<td>99.1</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>Sweet and Vanilla</td>
<td>29.5</td>
<td>69.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

2.3. Artificial saliva

Artificial saliva (AS) was used for all *in vitro* experiments to emulate conditions in the mouth. This was adapted from Madsen *et al.* (2013) and consisted of 0.21 g/L NaHCO₃, 0.43 g/L NaCl, 0.75 g/L KCl, 0.22 g/L CaCl₂·2H₂O, 0.91 g/L NaH₂PO₄·2H₂O dispersed in deionized water. For the mucoadhesion experiment 2.5 g/L pig gastric mucin (PGM) type II (Sigma-Aldrich, St. Louis, Missouri, United States) was also added. The pH of the AS was adjusted to 6.8 and kept at 37 °C during experiments and at 4 °C when not in use.

2.4. Swelling and disintegration

Swelling studies were carried out in an incubator set to 37 °C. Each film was placed on to netting and fully submerged in a petri dish with 40 mL of AS. At set time periods the sample was removed from the AS, excess water was carefully absorbed with tissue paper and the film on the netting was weighed. This process was repeated until the weight had returned to that of the netting alone. Each type of film was tested 6 times with duplicate batch repeats. Film 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 cm² 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 100\(\mu\)L 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.
Panellists were instructed to treat each sample the same way to avoid biasing release. Each week the panellists were given a training session on the first day followed by two days of scoring the samples. Four samples were served monadically, in a petri dish, in a balanced order with individual blinding codes each day with the duplicate being served on a consecutive scoring day. Panellists were provided with isolated sensory booths, computers with Compusense Software and warm water for palate cleansing. There was a 2-minute delay between samples to allow for palate cleansing. Time intensity curves were produced for each panellist and each sample in duplicate.

2.9. Statistical analysis
One way or two way repeated measures ANOVA (rmANOVA) was used for the appropriate test. Bonferroni or Tukey’s HSD corrections were used on pairwise analysis to account for multiple comparisons, at a significance level of $p \leq 0.05$.

3. Results & Discussion
3.1. Film characteristics
A range of standard methods were used to characterise the polymeric films (Morales & McConville, 2011; Nair et al., 2013). Each film was measured for thickness, water activity ($a_w$), glucose release, and swelling / disintegration times (Table 3).
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 hydrophilic 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 it started to erode.
Table 3. Characteristics of films

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Glucose content (%)</th>
<th>aw (mean) (mm)</th>
<th>Thickness (mm)</th>
<th>Dissolution time (min)</th>
<th>Max swelling ratio</th>
<th>50% glucose release (min)</th>
<th>100% glucose release (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUL</td>
<td>-</td>
<td>0.451 (a)</td>
<td>0.071 (a)</td>
<td>5 (a)</td>
<td>5.8 (a)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LCMC</td>
<td>-</td>
<td>0.486 (b)</td>
<td>0.094 (a,b)</td>
<td>4 (a)</td>
<td>11.6 (a)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HPMC</td>
<td>-</td>
<td>0.478 (b)</td>
<td>0.148 (b)</td>
<td>147 (b)</td>
<td>11.6 (a)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HCMC</td>
<td>-</td>
<td>0.474 (b)</td>
<td>0.104 (b)</td>
<td>357 (c)</td>
<td>34.9 (b)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

It was expected that changes in flavour perception over time would be influenced by the parameters measured (Table 3). For example, it was hypothesised that PUL films would result in a high intensity flavour that decreased in intensity quickly as they dissolved faster and released glucose 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
on top of the data point refer to the y axis and those to the right hand side refer to the x axis.

3.3. Mucoadhesion in vivo

In vivo mucoadhesion experiments were carried out with 5 panellists that were asked to record the following: where the film stuck, for how long and when it dissolved. All films, except for HPMC with glucose, were reported to adhere for the duration of the time that the film was in the mouth (Figure 2a & b). Adherence was mainly to the roof of the mouth but also the tongue. The time that the films took to dissolve reflected the in vitro dissolution (Table 3) as PUL and LCMC took the least amount of time to dissolve followed by HPMC then HCMC. For films without glucose, HPMC and HCMC films did not differ in time for dissolution in vivo (Figure 2a) despite the difference in the in vitro test. This is probably due to the participants manipulating the film with their tongue during these experiments, thereby exerting mechanical stress on the film. Therefore, as the HCMC swells and takes up water to produce a gel–like layer, the tongue pressure will remove it and therefore speed up the time of erosion.
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
panellists reporting adherence after an initial delay. There are two explanations to this. Firstly, AS was used in the in vitro experiments, which contained Sigma-Aldrich PGM as opposed to human salivary mucin. This may affect interactions between the polysaccharide matrix and the saliva due to differences in denaturation states and response to pH. For example, mucin chains must be flexible and uncoiled enough to allow interpenetration with polymer chains. Secondly, the hydration of the oral cavity in vivo may be different to that which was on the porcine tongue in the in vitro experiments. This may have led to a stronger adhesion in vivo, as the film did not become overhydrated.

The PUL film dissolving and adherence time was significantly quicker for LCMC films in these experiments. The PUL films dissolved on average at 81 seconds compared to 145 seconds for the LCMC films during these experiments. This is in contrast to the results obtained from the in vitro dissolution tests (table 3) where they were not significantly different. This difference was expected to have an impact on flavour release from LCMC films compared to PUL. Film thickness is the most likely explanation for the differences observed, LCMC films were thicker than PUL and therefore, when in contact with the moist mucosal surface, will take longer to take up water. To properly assess the impact of polysaccharide type on dissolution times, the thickness of the films would need to be matched.

3.4. Perception of tastant and aroma from films over time changes depending 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 ($T_{\text{max}}$), maximum intensity ($I_{\text{max}}$), 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 $I_{\text{max}}$ values for the films decreased in order of PUL > LCMC > HPMC > HCMC with the reverse order for $T_{\text{max}}$ (Table 4) where PUL was significantly higher than HCMC and higher for all other films for $I_{\text{max}}$. 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).
Regarding mucoadhesion, the HPMC films containing glucose were found to have poor adhesive abilities (Figures 1 & 2). In the perception experiments panellists were asked not to swallow these films and, therefore, the perception may have been artificially prolonged due to consciously keeping the film in the mouth. During normal consumption in a real food system the material would be chewed into a bolus and, without mucoadhesive ability, it may well be swallowed with the food bolus thereby negating any further release. On the other hand, HCMC films showed strong adhesion (Figures 1 & 2) and therefore would be more likely to adhere to the oral cavity for longer, prolonging the release.

3.4.2. Vanillin only films

For films containing the polysaccharide and vanillin the mean scores for $I_{\text{max}}$ decreased in order of PUL $>$ LCMC $>$ HPMC $>$ HCMC (Table 4, Figure 3b). Where PUL was significantly higher than HCMC. $T_{\text{max}}$ and AUC were not dependent on polysaccharide type. The duration of perception was longest in the HPMC samples followed by HCMC. This suggests that although the total intensity of perception was the same for each film, the aroma was delivered at a slightly lower intensity for longer in the HPMC and HCMC samples. This is supported by the decline angles being larger for PUL and LCMC samples suggesting the intensity decreased more quickly in these films.

To date, the only studies investigating aroma release and perception in food thickened with polysaccharides are in liquid and semi-solid foods. These studies have found confounding results with regard to interactions between
aroma molecules and the food matrix. Arancibia et al. (2011) found that thickener type affected total aroma release from dairy desserts with CMC thickened samples reducing the cumulative release of hydrophobic aroma (linalool) compared to starch. Furthermore, a follow up study by Arancibia, Castro, Jublot, Costell, & Bayarri (2015) found that thickener type affected both hydrophilic aroma (cis-3-hexen-1-ol) and hydrophobic (linalool) aroma. The CMC thickened dairy desserts reduced the release of both aromas, though it had more of an impact on the hydrophilic compound. Cook, Linforth, et al., (2003) on the other hand found that in-nose measurements of hydrophobic aroma release were not dependent on thickener type or on an increase in viscosity. These studies exemplify the complex behaviour of aroma release and its dependence on the food matrix.

In this current study, vanillin, a slightly hydrophobic molecule with a log P of 1.2, was used as the aroma. Perception results show that films made with slow dissolving polysaccharides (HPMC and HCMC) reduced the I_{max} but prolonged the duration of perception. Perception results for the aroma only films were not as distinguishable as the films containing glucose. This may be because the panel found scoring the aroma only films particularly difficult as they contained no tastant along with the aroma, which does not normally occur in food products.

3.4.3. Glucose & vanillin films

Dual attribute time intensity was used to simultaneously and continuously 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 $I_{\text{max}}$ were highest for PUL and lowest for HCMC. HPMC and HCMC took longer to reach $T_{\text{max}}$ 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 $I_{\text{max}}$ and increased $T_{\text{max}}$ 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 $I_{\text{max}}$. HPMC has a slower onset to reach $I_{\text{max}}$ 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
mucoadhesive and, therefore, it would most likely be swallowed along with the bolus in a real food system. Participants were instructed not to chew or swallow the film and many suggested that this would have been possible if they were eating normally. However, the other formulations were firmly adhered to the roof or tongue tissue and would not be easily swallowed.

### Table 4. Parameters from time intensity results.

<table>
<thead>
<tr>
<th>Film type</th>
<th>Attribute</th>
<th>Polymer</th>
<th>AUC</th>
<th>(I_{\max})</th>
<th>(T_{\max})</th>
<th>Duration</th>
<th>Incline angle</th>
<th>Decline angle</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Sweet</td>
<td>PUL</td>
<td>8410</td>
<td>b</td>
<td>91</td>
<td>22</td>
<td>a</td>
<td>73</td>
</tr>
<tr>
<td>Aroma</td>
<td>Vanilla</td>
<td>LCMC</td>
<td>7468</td>
<td>a, b, c</td>
<td>75</td>
<td>48</td>
<td>b</td>
<td>201</td>
</tr>
<tr>
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<td>Vanilla</td>
<td>HPMC</td>
<td>7126</td>
<td>b</td>
<td>54</td>
<td>61</td>
<td>b</td>
<td>231</td>
</tr>
<tr>
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<td>HCMC</td>
<td>4834</td>
<td>a</td>
<td>31</td>
<td>88</td>
<td>b</td>
<td>249</td>
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<td>PUL</td>
<td>7291</td>
<td>a</td>
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<td>41</td>
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<tr>
<td>Aroma</td>
<td>Vanilla</td>
<td>LCMC</td>
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<td>a</td>
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<td>40</td>
<td>a</td>
<td>195</td>
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<tr>
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<td>HPMC</td>
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<td>264</td>
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<tr>
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<td>HCMC</td>
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<td>38</td>
<td>a</td>
<td>230</td>
</tr>
<tr>
<td>Sweet</td>
<td>Aroma</td>
<td>PUL</td>
<td>9154</td>
<td>b, c</td>
<td>92</td>
<td>25</td>
<td>a</td>
<td>221</td>
</tr>
<tr>
<td>Sweet</td>
<td>Aroma</td>
<td>LCMC</td>
<td>9295</td>
<td>c</td>
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<td>32</td>
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<td>224</td>
</tr>
<tr>
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<td>Aroma</td>
<td>HPMC</td>
<td>6661</td>
<td>a, b</td>
<td>50</td>
<td>64</td>
<td>b</td>
<td>245</td>
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<tr>
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<td>Aroma</td>
<td>HCMC</td>
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<td>a</td>
<td>36</td>
<td>64</td>
<td>b</td>
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</tr>
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<td>54</td>
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<td>54</td>
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</table>
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](image_url)

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
the polysaccharide influences the release of flavour molecules from the matrix. This in turn has an impact on the flavour perception. Fast dissolving polysaccharides resulted in a quick burst of flavour at high intensity that tapered more quickly whereas slow dissolving films gave a slower onset and a more consistent release over time. The mucoadhesive ability of the films will influence how long the matrix stays in the mouth whilst releasing the flavour compounds before being swallowed. Furthermore, in line with previous literature, this study shows that aroma intensity is dependent on the perception of a congruent tastant, giving more evidence for cross modal interactions.

The mucoadhesive nature of some of the polysaccharides tested will have an effect on flavour delivery over time as those that adhere to the oral cavity will continue to release flavour whilst those that are not mucoadhesive will be swallowed. This study investigated flavour release from very simple food matrices, polysaccharide films; of course in a real food there will be many other food components that could affect flavour release. However, this study provides some fundamental understanding of how different polysaccharide matrices affect flavour release. Results from this study can be used to inform the food industry of the impact that the addition of these polysaccharides can have on temporal flavour perception. Possible applications include topical coatings, confectionary, low fat and low sugar foods. However, there is a need for further research into this area to understand the full impact on the organoleptic properties of foods.
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References


Cook, S. L., Bull, S. P., Methven, L., Parker, J. K., & Khutoryanskiy, V. V.


http://doi.org/10.1111/j.1750-3841.2006.00140.x


http://doi.org/10.1039/b800106e


http://doi.org/10.1016/0168-3659(93)90109-I


http://doi.org/10.1016/j.jsps.2011.04.005


http://doi.org/10.1016/j.foodchem.2007.11.011


