University of Reading

An exploration of the role of mucoadhesives in food

Doctor of Philosophy

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Sarah Cook

Signed.....

Abstract

Mucoadhesives are used to enhance drug permeability and retention at mucosal membranes in the body. This is achieved by the adherence of a pharmaceutical dosage form to a mucosal membrane through interactions between a mucoadhesive material and the mucosa. Many polysaccharides (PSs) used in the food industry as thickeners, emulsifiers, stabilisers and fat replacers are also used as mucoadhesives in the pharmaceutical industry. This overlap of use has provoked an interest in utilising these PSs to modulate the organoleptic properties of food.

This work aimed to elucidate the role of mucoadhesion in the organoleptic properties of simple food systems. Mucoadhesive PSs were either in an aqueous solution or cast into films containing flavour compounds. A novel method for assessing mucoadhesion for liquid formulations was developed using fluorescence microscopy with labelled and unlabelled PS. Time intensity and progressive profiling sensory experiments were employed to assess the impact of mucoadhesives on flavour perception in liquid and solid model food matrices. A range of *in vitro* tests were used to assess various properties of PS films loaded with flavourings such as texture analysis, dissolution and swelling ratios in order to explain perception results. Finally, a selected mucoadhesive (carboxymethyl cellulose) was incorporated into popcorn seasoning and sensory perception changes were assessed.

This body of work describes method development to assess the mucoadhesive strength of PSs, shows that mucoadhesive PSs in solution prolong the retention of

a model tastant in the oral cavity and controls delivery and thus perception of flavour from solid polymeric materials, and describes an attempt to incorporate a mucoadhesive PSs in a snack food formulation.

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Abbreviations

API	Active pharmaceutical ingredient
AS	Artificial saliva
AUC	Area under the curve (time-intensity)
c*	Coil overlap concentration
CMC	Carboxymethyl cellulose
DW	Deionised water
HCMC	High viscosity carboxymethyl cellulose
HPMC	Hydroxypropylmethyl cellulose
LCMC	Low viscosity carboxymethyl cellulose
LMEP	Low methylester pectin
MUC5B	High-molecular-weight mucins
MUC7	Low-molecular-weight mucins
η*	Complex viscosity
PGA	Propylene glycol alginate
PS	Polysaccharide
PTS	Proline, threonine, serine
PUL	Pullulan
rmANOVA	Repeated measures analysis of varience
SA	Sodium alginate
WO_{50}	Wash off 50% of the sample
XG	Xanthan gum

Publications arising from this work

S.L. Smith, M.T. Cook, J.K. Parker, L. Methven, V.V.Khutoryanskiy, A role for mucoadhesion in avour enhancement and retention, in A.J. Taylor and D.S. Mottram (Eds), Flavour Science: Proceedings of the XIV Weurman Flavour Research Symposium, Packington, Leicestershire, 2015, pp. 365-368.

M.T. Cook, S.L. Smith, Khutoryanskiy, V.V, Novel glycopolymer hydrogels as mucosa-mimetic materials to reduce animal testing, Chem. Comm. 51 (2015) 14447–14450.

S.L. Cook, S.P. Bull, L. Methven, J.K. Parker, V.V. Khutoryanskiy, Mucoadhesion: A food perspective, Food Hydrocoll. 72 (2017) 281–296.

S.L. Cook, S. Wood, L. Methven, J.K. Parker, V.V. Khutoryanskiy, Mucoadhesive polysaccharides modulate sodium retention, release and taste perception, Food Chem. 240 (2018) 482-489.

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Chapter 1: General introduction & literature review

1.1. Introduction

Polysaccharides (PSs) are commonly used in the food industry as thickeners, gelling agents, stabilisers, emulsifiers, and binders. They are most commonly used in liquid or semi-solid dairy products, meat products, sauces and confectionary [1]. The impact to the structure and sensory perception of a food product when adding PSs is gathering interest [2–6]. The fact that many of these PSs are mucoadhesive has rarely been reported in the literature as an influencing phenomenon to consider when investigating the results obtained with regards to flavour release and sensory perception.

Mucoadhesion has attracted a lot of attention in pharmaceutical research and the pharmaceutical industry, and is therefore well defined and effectively utilised within these fields. In the simplest terms, mucoadhesion is the adhesion of a polymeric material to a mucosal membrane in the body. The polymeric material, containing an active pharmaceutical ingredient (API), adheres to a target mucosa for an extended period of time compared to the API itself, thereby prolonging the API residence on mucosal surfaces, increasing permeation and thus bioavailability for certain APIs [7].

The recognition and consequent extensive research of mucoadhesion in the pharmaceutical field has led to an excellent understanding of the mechanical, chemical and physical factors involved. This understanding has subsequently advanced the development of dosage forms, improving the delivery and efficacy of APIs. However, the ability of mucoadhesives to retain small molecules at mucosal surfaces may prove important to the food industry.

It was intended that the work contained in this PhD thesis would enhance the knowledge and understanding of how mucoadhesion may influence organoleptic properties of food. More specifically, experiments were designed to investigate the impact on dynamic flavour perception that mucoadhesive PS have on model food systems. Furthermore, the mucoadhesive nature of the PS was investigated and experiments were designed to measure their ability to retain flavour molecules in a similar way to APIs. The project was a BBSRC case studentship, jointly funded by McCormick, UK Ltd, who produces spices, seasonings and flavourings.

The thesis is divided into 8 chapters, 5 of which describe the primary research conducted to test the hypotheses outlined at the end of this chapter. Chapter 1.2 provides an explanation of mucoadhesion, an overview of the literature regarding PS, mucoadhesion and flavour perception, and the overall aims and objectives of this body of work. Chapter 2 gives the chemical characteristics of the PS used throughout the thesis and some theory behind the rheology used in research chapters. Chapters 3 to 5 describe work done to elucidate the basic impact of mucoadhesives in model liquid food. Chapter 6 describes the move to working with solid PS matrices. Chapter 7 describes an attempt to apply these fundamental findings to a real food product.

1.2. Literature review on mucoadhesion, polysaccharides and their impact on food products

1.2.1. Mucoadhesion

The importance and interest in developing mucoadhesive formulations has increased as more challenging drugs, such as peptides, proteins and oligosaccharides have been discovered and synthesised. These types of therapeutics are challenging for various reasons, such as their poor solubility, limited uptake, fast breakdown or short half-life. Furthermore, it may be necessary for certain drugs to bypass first pass metabolism and therefore alternative routes such as sublingual administration is sought. The systemic absorption of APIs through diffusion or transport across mucosal surfaces may be enhanced by the addition of mucoadhesives. This enhancement is termed polymer-mediated enhancement of API delivery. These controlled release formulations have been researched for many years and subsequently employed in a variety of pharmaceutical applications [7–14].

Mucoadhesives can be utilised in drug formulations to deliver APIs to a variety of target mucosal tissues. These include: the nasal route via sprays, gels and pumps; vaginal or urethral routes using suppositories, pessaries, vaginal rods and gels; and the oral route via buccal and sublingual patches, tablets and gels. One of the most commercially recognised formulations containing mucoadhesives is Gaviscon Liquid®. This product contains sodium alginate, a mucoadhesive PS, which gels in the presence of Ca2+ ions. Due to its mucoadhesive and gel forming abilities, this formulation is used to treat heart burn by coating the esophageal walls with the viscous, mucoadhesive gel, protecting it against the acid rising from the stomach [15]. The oral route for drug delivery includes targeting formulations

to the buccal tissue in the mouth as well as the rest of the gastrointestinal tract (GI), including the oesophagus, stomach, small and large intestine. Each of these routes of administration has different mucosal structures and a different secretory mucus composition, which will affect the mucoadhesive's strength of the dosage form.

1.2.1.1. Oral cavity mucosa

The anatomy and histology of the human oral cavity has been described extensively in the literature [16]. Important for mucoadhesion, the oral mucosa is the moist membrane lining all surfaces of the oral cavity except for the teeth (Image 1.1.). There are three different kinds of oral mucosa, each with characteristics that reflect their role in mastication and speech. The masticatory mucosa is keratinised and covers the gingiva and hard palate. As the name suggests, this mucosa is responsible for masticatory processes and must be tough as it is at risk of abrasions and potential infection from pathogen-harbouring food. The rest of the oral cavity is covered with soft, non-keratinised epithelium, called the lining mucosa. The dorsal of the tongue is an exception to this, possessing a specialised mucosa with characteristics of both masticatory and lining mucosae. The mucosal surfaces are covered in a layer of mucosal secretion, which, in the oral cavity, is the saliva. This is a relatively thin covering compared to other areas of the body, between 1 and 100 μm thick [17–19]. This mucosal secretion serves many roles similar to other secretions in the body as it protects tissues against mechanical and pathogenic stress. In addition to these roles, it serves many specialised roles necessary for speech, mastication, bolus formation and deglutition [20].

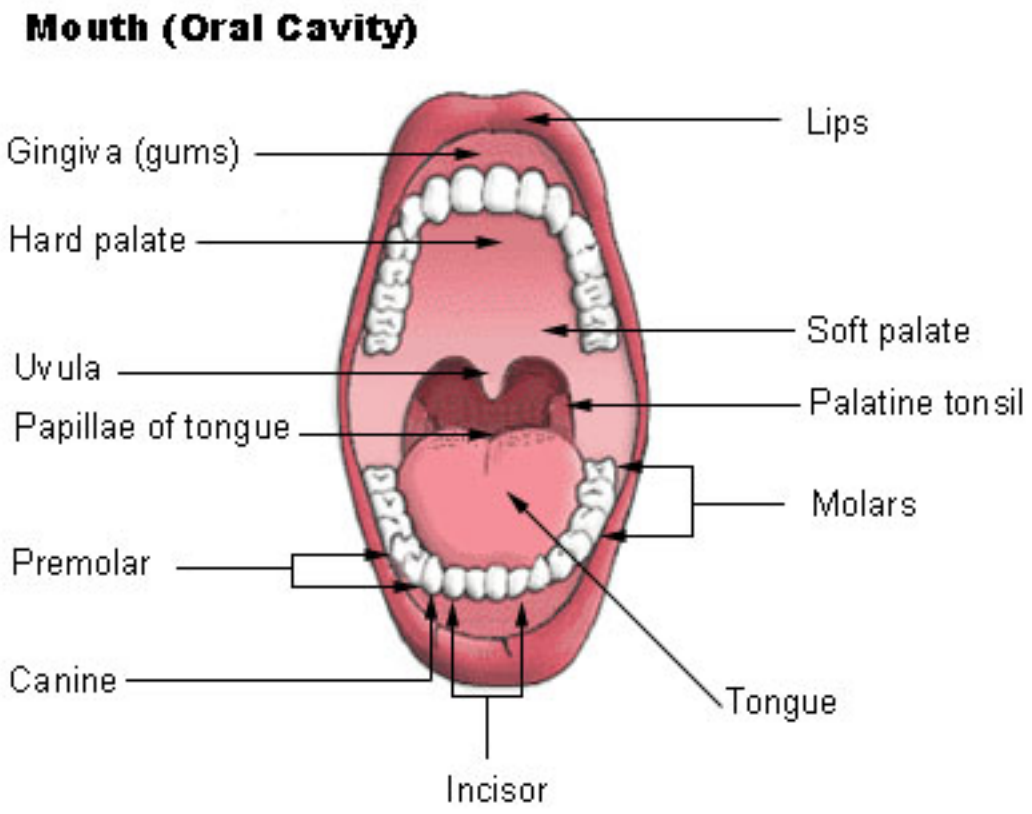

Image 1.1. Cross section of oral structures.

Saliva is a highly aqueous viscoelastic material consisting of around 95% water with the remainder comprising salts and proteins. Mucins are large glycoproteins of particular importance in establishing mucoadhesion, comprising approximately 1.2 mg/mL in healthy individuals [21]. Mucins are responsible for the highly viscoelastic nature of all mucosal secretions due to the formation of aqueous, gel-like networks. This viscoelasticity is important, serving as a barrier to foreign substances, slowing diffusion and inhibiting large molecules from penetrating. However, with regard to mucoadhesion, polymer chains that can penetrate into this mucus layer can interact with the mucin resulting in a continuous network of polymer and mucin interactions, strengthening a mucoadhesive joint.

Mucins exist as both secretions in the saliva, as well as transmembrane mucins on epithelial cells, which are exposed to the oral cavity. Mucins are integral for the lubrication of the oral cavity, due to their water retaining capacity, enabling all the usual functions of mastication, swallowing and speech. Mucins enable saliva to serve many functions including: acting as a diffusion barrier for nutrients, pathogens and drugs; hydration of the underlying epithelia; and protection from chemical and mechanical damage [20,22,23].

The molecular weights of mucins range from 500 kDa to 20 MDa, however they have a tendency to aggregate and form large supramolecules, driven by hydrophobic interactions of nonpolar groups and the hydrogen bonding of sugar units [24]. Generally speaking, all mucins are derived from a similar structure and will, to a certain degree, serve the same function of protecting the delicate underlying tissues. However, there is large heterogeneity and diversity between the complex structures of mucins [25] influenced by the variation of the environments to which they are exposed, such as pH.

Mucins found in the oral cavity can be divided into high-molecular-weight (MUC5B) and low-molecular-weight (MUC7) fractions [26,27]. MUC5B mucins are produced by all salivary glands except the parotid gland [22] and has similar characteristics to mucin in other mucosal secretions in the body [22]. The MUC5B mucins are one of the major mucins present in saliva and are associated with the gel-like formation of saliva, which is attributed to entanglements of these mucin molecules with one another [26,28]. The interactions thought to be important for this gel formation include hydrophobic interactions between the hydrophobic regions of the core proteins [29], van der Waals and hydrogen bonds between

oligosaccharide side chains and calcium-mediated crosslinks [30]. MUC7 mucins are thought to be uniquely found in salivary secretions [22], produced by the submandibular, sublingual and palatine glands [31].

The protein core of mucin is glycosylated by many oligosaccharide side chains covalently linked in areas of clustered proline, threonine and serine (PTS) amino acids [27]. These highly branched oligosaccharides contribute up to 80% of the dry weight of mucin. There is heterogeneity within and between mucin types and the saccharides that glycosylate them, with MUC5B possessing a more diverse range than MUC7 [27]. The O-linked chains are initiated with N-acetylgalactosamine with up to 20 more residues extending from this. The large variations of sugar units that may be attached include N-acetylglucosamine, N-acetylgalactosamine and other glucose, galactose and fructose derived residues [25]. The chains are terminated with sialic acid, sulfonic acid, or l-fructose residues, with the first two possessing a net negative charge at neutral pH [32,33]. This is important for the adhesion of positively charge polymers that can form ionic bons with the mucin but also for negatively charged polymers as it will facilitate polymer and mucin chain uncoiling due to electrostatic repulsion. Recent studies have confirmed the presence of both types of salivary mucins in the mucosal pellicle that lines the oral epithelia [34,35]. The pellicle is a biological film adhered to the epithelial cells which serves to protect the underlying tissue from abrasions and plays a role in bacterial colonisation [36].

1.2.1.2. Theories of mucoadhesion

Mucoadhesion occurs due to a range of physicochemical interactions between the polymeric material and the mucosal environment. The properties of the

environment, such as the pH and flow rate of the mucosal secretion, will determine polymer-mucin interactions. Generally, there are two stages considered to be essential in establishing mucoadhesion [25]. Firstly, the initial intimate contact between the polymeric material and the mucosal surface is required. Secondly, the consolidation period can ensue which reinforces the mucoadhesive bonding. There are six main theories of mucoadhesion, which have been proposed and evaluated in the literature [37–43]. These include: adsorption, wetting, electronic, diffusion, dehydration and mechanical theories (Figure 2). These theories can be thought of as complementary, describing different phenomena that occur simultaneously or at different stages of the process, which facilitate mucoadhesion.

- Wetting theory is concerned with polymer spread and ability to swell on the wet mucosal surface. A higher affinity to spread on the mucosa results in stronger mucoadhesion. Typically, the wetting phenomena are important for liquid mucoadhesives.
- Dehydration theory describes the process where a material capable of gelling is brought into contact with a moist mucosal membrane. The movement of water from the mucus gel to the water-absorbing material reaches equilibrium and facilitates an adhesive joint. An example of this is the water uptake by a solid dosage form containing a hydrophilic polymer, such as poly(acrylic acid), when placed on a moist surface. Once in contact with the wet mucosa, the dosage form will rapidly dehydrate the surface and adhesion will occur [41].
- Diffusion theory considers the entanglement of polymer and mucin chains due to interpenetration, allowing for further primary and potentially secondary bonds to form, strengthening the adhesion [41,43].

- Adsorption theory considers interactions between the mucosal surface and polymer; including Van der Waals forces, hydrogen bonds, and hydrophobic interactions [42]. These non-covalent interactions are likely to form most interactions; however, covalent bonding is possible depending on the chemical properties of the polymer. Thiolated polymers can form disulphide bonds with cysteine groups in mucins via thiol exchange reactions, or the oxidation of free thiol groups [44]. The protein backbone of some mucins contains large regions high in cysteine residues and low in oligosaccharides, which provide a potential area for strong chemical bonds to occur [45].
- Electronic theory describes the transfer of electrons between the mucoadhesive and the mucus layer, resulting in the formation of a charged double layer at the interface of the mucin and polymer networks [37,38].
- Mechanical theory describes the effect of contact area on the interaction between the polymer and mucosal surface [9]. The effect of this will be particularly relevant in the oral cavity, which has a very thin layer of saliva in some areas; therefore, the mucoadhesive is more likely to contact the rough underlying tissue. Irregular surfaces and micro-cracks give a larger contact area and thus mucoadhesive strength. The papillae on the tongue provide a suitably rough surface and therefore greater surface area for penetration by mucoadhesives.

Buccal mucoadhesion is extensively researched in the pharmaceutical field [10,46] due to the ease of application and the ability to bypass the first-pass metabolism in drug delivery. Whilst buccal mucoadhesion is an important area to consider, regarding relevance to the food industry, adhesion occurring on the tongue may be more revealing. There has been much interest in the interactions of food

emulsions on the tongue including the adhesion of emulsion droplets and how this corresponds to the lubricating properties of the system. A body of work by Dresslehuis et al and Silletti et al have found that adhesion of emulsion droplets is dependent on the sensitivity of coalescence with a higher sensitivity resulting in a higher retention of fat in the mouth [47–50]. This body of work is important when considering the perception of fat in foods and when considering ways to reduce fat content whilst maintaining the lubricating mouthfeel.

Recent work suggesting that milk proteins bind to the tongue by mucoadhesive interactions has lead the authors to suggest that mucoadhesion plays a role in creating negative sensory attributes associated with milk products such as drying and astringency [51,52]. Conversely, this adhesion to the tongue may be useful for incorporating mucoadhesive polymers into food products to produce positive sensory results. An example of this application would be the utilisation of mucoadhesives to prolong the retention and consequent perception of tastants on the tongue.

1.2.1.3. Properties of mucoadhesives

The mucoadhesive strength of a polymeric material is dependent on various factors including the size and physicochemical properties of the polymer, and the environment in which it will reside. Polymer characteristics such as molecular weight and viscosity show positive correlation with mucoadhesive strength [53,54]. Bond formation between the polymer and mucin chains is dependent on the ability of the polymer to diffuse into the mucus layer, therefore, higher polymer flexibility results in better diffusion into the mucus network and consequently stronger mucoadhesion [39]. Along with flexibility, hydrogen-

bonding moieties, such as carboxylic acids, are essential for strong mucoadhesion, enabling interactions between the polymer and the mucin oligosaccharide hydroxyl groups [55].

The ionic charge of a polymer, which can influence the mucoadhesive strength, is dependent on the pH of the medium in which it resides, which varies among different mucosal environments. In the case of oral mucoadhesion the medium is saliva where the pH is typically between 7.0 and 7.5 or slightly acidic between 5.9 and 7 dependent on disease state [56]. Anionic polymers such as some PSs possessing carboxyl groups will be partially negatively-charged at a near neutral pH; their strong mucoadhesive properties is thought to be due to hydrogen bonding and Van der Waals forces [43]. Cationic polymers, such as chitosan, which possess amino functional groups (pKa ~ 6.5), are also strong mucoadhesives. Due to the relatively high pKa, chitosan forms a gel in acidic conditions, such as those found in the stomach. However, chitosan is insoluble at neutral pH, and therefore is suitable for oral delivery of APIs targeting the GI tract, as it is insoluble in saliva [57]. Neutral polymers such as starch or dextran generally exhibit poorer mucoadhesive properties compared to polyelectrolytes [8].

Thiolated polymers, which can be either cationic or anionic, form mucoadhesive bonds via disulphide bonding, therefore the concentration of thiolate ions is the key factor in forming mucoadhesive interactions. In situ cross-linking of thiomers could also contribute to their mucoadhesive properties, as disulphide bonds within the polymer, strengthening bonds made with the mucosa. Another important factor in determining thiomer mucoadhesive strength is the molecular mass of the polymer chains.

The concentration of polymer is an important consideration for optimum mucoadhesion. If the concentration is too low the interaction between polymer and mucin is unstable [43], whereas too high will result in the polymer network being impervious to the solvent resulting in a lack free polymer chains to diffuse into the mucus interface, due to the highly coiled and compact structure [10]. Hydration of the polymer chains within the mucus layer is influenced largely by the concentration and is required for the polymer to expand and form a network with the mucus to form a strong adhesive joint. Salivary flow and constituents can vary considerably between individuals [56] and therefore may explain some of the variability in mucoadhesion test results obtained in the literature. The hydration of the dosage form and the solutes in the solvent will impact mucoadhesive strength [58,59].

1.2.2. Mucoadhesives and food

The mucoadhesive properties of food ingredients may be important in explaining perceptual changes. Although mucoadhesion per se is seldom investigated as an influencing factor to explain outcomes reported within food and sensory science research, attributes such as mouthcoating, stickiness and creaminess are more than likely pertaining to this phenomenon. The phenomenon is becoming increasingly recognised and investigated in the literature [60,61] and has been implicated in considering the astringency of tannins [52,62] and drying nature of milk proteins [52,63]. Furthermore, Silletti and Dresselhuis have studied interactions between emulsions and the oral cavity with regard to the adhesive interactions [48,49,64,65].

1.2.2.1. Mucoadhesive polysaccharides

Many PSs substantially increase the viscosity of aqueous solutions. This property has made them an attractive ingredient to use in manufactured liquid and semi-solid products to add bulk and improve texture, stability, and appearance. These PSs include: carboxymethyl cellulose (CMC), pectin, alginate, xanthan gum (XG), guar gum and carrageenan. They come from a variety of sources and exhibit diverse chemical properties. Many of these PSs have been evaluated as mucoadhesives, and are reported extensively in pharmaceutical literature [66–70] and are utilised for their mucoadhesive capability in various pharmaceutical applications. Table 1.1 details commonly used PS characteristics and mucoadhesive strength based on the current literature.

Mucoadhesive strength is a continuum dependent on: the polymer chemistry and molecular weight; dosage form (e.g. particulates, tablets, films, liquids etc); other ingredients present in the formulation; and how it is measured. Studies investigating the best formulation for a mucoadhesive dosage form will often use a combination of polymers for optimum mucoadhesion, comparing many polymers in one study. Therefore, it is impossible to attribute a definitive value of mucoadhesive strength to any particular mucoadhesive, as the variables are seemingly infinite. Grabovac, Guggi, and Bernkop-Schurch (2005) published a study of the nineteen most commonly used mucoadhesive polymers and conducted a large study comparing the difference in small intestine mucoadhesive strength, giving a guide to the mucoadhesive strength of commonly used polymers for mucoadhesive formulations. Table 1.1. outlines the mucoadhesive strength and ranking of commonly used PS in the food industry based on the type of formulation tested. The studies referenced are restricted to those that have

investigated buccal or gingival mucoadhesion. As can be seen in table 1.1. there are a variety of PS that have been assessed for mucoadhesion, each with differing results depending on the formulation.

Table 1.1. Polysaccharides commonly used in the pharmaceutics and food industry for various applications. Details of the chemical properties and mucoadhesive strength, according to current literature.

Polysaccharide type	**Characteristics**	**Mucoadhesion studies**
Acacia gum	Also known as gum Arabic, a complex mixture of glycoproteins and polysaccharides.	Few studies to date have been produced with acacia gum; however one study found it to be a very weak mucoadhesive in a patch formulation [72].
Carboxymethyl cellulose (CMC)	An anionic polysaccharide produced by reacting alkali cellulose with sodium monochloroacetate. Comes in varying degrees of substitution of hydroxyl groups.	CMC has been the subject of many mucoadhesive studies as it is a good mucoadhesive in both solid [73–77], liquid [78] and gel [79,80] formulations.
Carrageenan	A linear sulphated PS that forms helical structures. The chain is made up of repeating units of galactose and 3,6-anhydrogalactose. The degree of sulphation can differ: it's denoted by the prefix (kappa, iota, lambda).	Carrageenan has been found to be moderately mucoadhesive [76] and a good mucoadhesive in combination with other polymers [130-133]. This PS with charged sulphur groups has potential to be a good mucoadhesive.

Carboxymethyl starch	An anionic derivative of starch with carboxylic group.	Ionic derivatives of starch have shown good mucoadhesion in solid form [74].
Chitosan	A cationic, linear polysaccharide composed of randomly linked D-glucosamine and N-acetyl-D-glucosamine. Made by treating chitin shells of crustaceans with alkaline substances.	Chitosan is one of the most extensively studied mucoadhesives and is a good mucoadhesive, particularly in solid form when studied for the oral cavity [77,81,82].
Guar gum	A non-ionic, branched polysaccharide composed of galactose and mannose sugars. Produced from the endosperm of guar beans.	Guar gum has been found to enhance the mucoadhesion of solid formulations when with a mixture of other mucoadhesive polymers [54]. Studies have found guar gum to range from being a relatively poor mucoadhesive [83] to exhibiting good mucoadhesion [84].
Gellan gum	Anionic polysaccharide made of repeating tetrasaccharide units of two D-glucose residues, one L-rhamnose and one D-glucuronic acid.	In solid form, gellan gum has been found to be a weak mucoadhesive in the oral cavity [85].
Hydroxyethyl cellulose (HEC)	A non-ionic polysaccharide made by reacting ethylene oxide with alkali cellulose.	In solid form HEC has been found to exert low mucoadhesive strength [73] but in gels exhibits

		moderate mucoadhesion [79,80].
Hydroxypropyl cellulose (HPC)	A non-ionic cellulose ether in which some hydroxyl groups in the repeating glucose units have been hydroypropylated using propylene oxide.	HPC has been found to show moderate mucoadhesive strength [75].
Hydroxypropylmethyl cellulose (HPMC)	A non-ionic cellulose ether in which some hydroxyl groups in the repeating glucose units have been replaced with hydroxyproply or methyl groups.	There are mixed results obtained for HPMC with some showing strong [84] to moderate mucoadhesion [73,75,77] in solid for and good [73] to weak mucoadhesive strength in gel form [80].
Pectin	An anionic hetero-polysaccharide rich in galacturonic acid. 80% of the carboxyl groups of galacturonic acid are esterified with methanol, however, this can be artificially manipulated to change the behavioural properties. Such as gelling in the presence of Ca^{2+} ions.	Pectin has been found to show good mucoadhesion in solid and liquid formulations [77,78,81,82]. The different degrees of esterification have all been shown to be relatively mucoadhesive [86].

Sodium alginate (SA)	An ionic polysaccharide found in cell walls of brown algae. It is a linear copolymer with homopolymeric blocks of mannuronate (M) and guluronate (G). This M:G ratio is important in determining the polymers properties. SA gels in the presence of Ca+ ions.	SA has been studied multiple times for its mucoadhesive abilities and is generally regarded as an excellent mucoadhesive in both solid [73,74,85] and liquid formulations [78].
Xanthan gum (XG)	An anionic polysaccharide composed of pentasaccharide repeat units of glucose, mannose and glucuronic acid.	Xanthan gum has mixed results with regard to its mucoadhesive strength with some studies of buccal patches showing poor mucoadhesion [69,87], whereas others found it was an excellent mucoadhesive in tablet form [83].

1.2.3. Polysaccharides as fat replacers

PSs are a popular ingredient in reduced fat products as they add bulk and increase viscosity, whilst contributing fewer calories than fat. Fat plays a significant role in the overall sensory experience and thus, satisfaction and acceptability of the food product. As well as structural impacts with regards to providing hydrophobic matrices, fat affects all sensory aspects of food including appearance, texture, mouthfeel and flavour profile. Fat is not only a source of flavour itself, but contributes to the temporal release and perception of flavours in the food matrix. Additionally, mounting evidence is suggesting that fatty acids should be regarded as the sixth basic taste [88]. Therefore, reducing fat content of a food will

undoubtedly alter these aspects, which must be characterised in order to rectify them. From here on the term “flavour” will refer to both taste (tastants) and aroma (volatile compounds) perception.

The food choice of consumers is influenced by many factors [89]; however, ultimately consumers select food because they like the taste and an important factor in this is a high quality, balanced flavour profile [90]. Consumers can become highly attuned to flavour imbalances, especially in familiar products, so maintaining a sensory balance is integral. Therefore, it is important to consider the impact to the food microstructure, flavour release and subsequent physiological perception of aroma and taste when attempting to develop lower fat alternatives with PSs. A lower fat content will reduce the binding of lipophilic aroma compounds to the food matrix, whilst the increase of water content to counterbalance the reduction of fat will relatively dilute tastants and more hydrophilic aromas, leading to alterations in flavour perception.

There are many examples of this change in flavour perception in the literature. Shamil, Wyeth, and Kilcast (1991) used a time intensity study to compare the sensory profiles of reduced-fat cheese and salad cream to their full-fat counterparts. They found that maximum intensity and total intensity perceived (area under the curve, AUC) of bitterness, sharpness and astringency was higher in reduced fat products. Saltiness on the other hand was reduced in the lower fat products. Since then other studies regarding salt perception and thickeners in low fat systems have shown similar results and is thought to be due to the relative dilution of hydrophilic compounds when fat is reduced [92]. More recent studies investigated the effects of different fat levels in oil in water emulsions and dairy

desserts on perception and flavour release *in vivo* [93,94]. Their findings show that the release and perception of a lipophilic aroma compound, linalool, was quicker when fat was reduced; whereas the release and perception of a more hydrophilic compound (cis-3-hexen-1-ol) was less effected by fat but depended on the thickness of the medium.

As fat content is reduced, rate of release of lipophilic aroma compounds is increased, which alters time intensity flavour perception [95]. Generally, reducing fat not only impacts the initial intensity of aroma but also the intensity over time, usually resulting in the former being initially higher and the latter diminished. Aroma perception in high fat foods is generally lower in intensity but sustained over a longer period of time, compared to an initial burst of intense aroma that rapidly disappears in lower fat counterparts. This change in aroma release can result in an unbalanced flavour profile; therefore, attempts at controlling the release of lipophilic flavour compounds have been made by encapsulation of these compounds [95,96]. Conflicting results have been found when investigating flavour release in regards to fat replacement with PS and some of these studies will be discussed.

Another barrier for the food industry to overcome regarding fat reduction is maintaining the creamy, fatty mouthfeel associated with higher fat products. This is a particularly difficult endeavour as it is not entirely certain what aspects of a food product are associated with the perception of these attributes but adhesion and spreading over oral surfaces is thought to be important [48,97]. Whilst there is a relationship between creaminess perception and viscosity in liquid and semi-solid food [98], there is mounting evidence that viscosity is not the only important

aspect [99–101]. Frictional forces between the food, saliva and oral mucosa may be equally as important. The lubrication of oral surfaces has been of great interest to many researchers in this field in an attempt to identify the mechanisms important for an enhanced perception of fattiness in lower fat products. Relevant to the mucoadhesion discussion, Dresslehuis et al. identified that the adhesion, spreading and coalescence of emulsion droplets on oral tissues is important in reducing the in-mouth frictional forces and thus enhances the lubricating properties [49,97,97,102]. As some mucoadhesive PS also enhance lubrication, a better understanding and employment of mucoadhesives may lead to better product design with respect to these properties [99,103].

1.2.4. Flavour modulated by mucoadhesives

The perception of flavour is complex, however, in the simplest terms it is a combination of the senses of smell and taste (Image 1.2) . Of course, there are other influencing factors on the perception of flavour, such as texture [104], temperature, health, memory and emotional states; however, the physiological interactions concern the mouth and nose. The release of aroma and taste compounds from food is initiated by the breakdown of the matrix upon mastication and dilution with the saliva. Therefore, flavour release and perception is largely dependent on the matrix with which these compounds reside and their interactions with the saliva and mucosa.

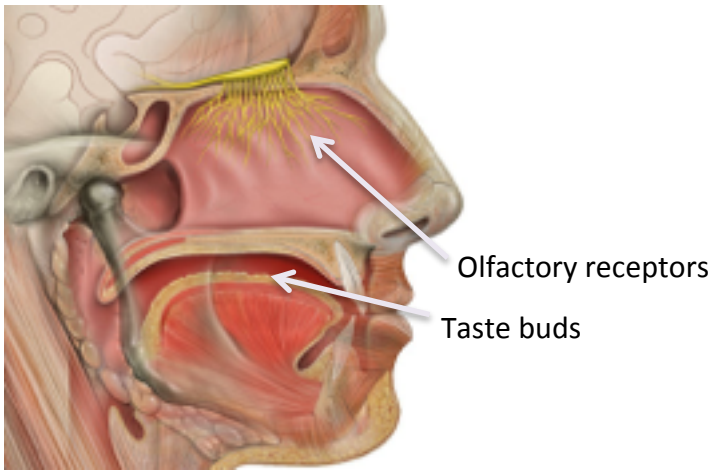

Image 1.2. Cross section showing the location of taste buds and olfactory receptors.

PS thickeners are known to alter perception and release of both tastants and aroma molecules [91]. Perception of tastants is primarily influenced by their ability to travel through the food matrix and saliva, diffusing into the taste bud lumen to activate taste receptor cells. Conversely, aroma compounds are released due to masticatory processes breaking up the food matrix allowing these compounds to escape and be mixed with the saliva. Depending on the hydrophobicity and volatility of these compounds they will travel to the nasal cavity upon swallowing, where aroma is perceived by the olfactory bulb via signals received from nerve endings in the nasal cavity, which are coated in olfactory mucosa. The eventual perception will, therefore, largely depend on the affinity of the aroma compound for the food matrix and saliva. In addition to these factors, aroma compounds themselves can adsorb directly to oral and pharyngeal mucosa [105,106] or to food residues adsorbed to the mucosa [61,107]. Furthermore, the expiration of breath after swallowing the food bolus facilitates the transport of these compounds retronasally to olfactory receptors. The perception of odours can occur for a prolonged period once the food has been swallowed [107]. This

mechanism is responsible for the aroma persistence of certain foods as opposed to the first aroma impression when the food is still in the mouth.

Flavour compounds vary significantly in their chemical structure and their target receptors. Tastants require access to taste buds, predominantly on the tongue, and aroma compounds need to be released from the food matrix in order to travel to the olfactory epithelium. The heterogeneity of these molecules, ranging from highly charged metal ions to polar hexose sugars to lipophilic aromatic rings, makes it impossible for a universal theory describing the matrix changes affecting their perception and release. For example, saltiness is perceived due to the direct uptake of sodium ions into sodium channels in taste bud receptor cells. As sodium ions are small and hydrophilic, they will reside in aqueous solutions and preferentially move to the saliva components during consumption of a high fat food, thereby increasing the perception. On the other hand, aroma molecules are volatile with a tendency to be lipophilic, so have lower affinity for saliva and mucosa. Therefore, during the consumption of high fat products, the aroma compound will reside with the food matrix and be released more slowly.

There are numerous studies investigating the influence of PS thickeners on viscosity, *in vitro* and *in vivo* flavour release, and sensory perception. The effect that any particular PS will have on a food will depend largely on the food matrix, the concentration (and thus viscosity) and state of the PS. Investigations into the adhesive nature of them rarely advance further than the assessment of attributes such as mouthcoating or stickiness. The vast amount of literature using an exhaustive combination of PSs, viscosity grades, concentrations and matrix constituents makes it difficult to draw any real conclusions of the effect of

mucoadhesion in these findings, as this aspect is seldom assessed or discussed. Table 1.2 outlines some of the studies that compare various PS thickeners and the effect they have on sensory perception of various aromas and tastants. This table is not exhaustive but is to illustrate the vast combinations of PS, flavours and food matrices studied in the literature.

Table 1.2. Effect of polysaccharide thickeners on sensory attributes.

Food matrix	**Polysaccharide(s) used**	**Effect on sensory perception**
Fermented whey drink [108]	Propylene glycol (PG) alginate CMC High-methoxy pectin XG	CMC and PG alginate ↑ sweetness and ↓ acidity and yoghurt attributes compared to other polysaccharides and control. Mouthcoating was most strongly associated with CMC
Custard dessert [109]	CMC with varying viscosity grades and concentrations used	Increasing concentration and viscosity ↓ sweetness perception and ↑ the in-nose total release and Imax of ethyl butyrate, ethyl 3-methylbutanoate, ethyl hexanoate compared to lower concentration of the same viscosity grade.
Gels with differing rigidities [2]	Pectin Gelatin	Increased gel rigidity ↓ in-nose release rates, perception of odour, strawberry flavour and sweetness but ↑ total release and intensity for hexanal, ethyl butanoate, ethyl 3-methylbutanoate and ethyl hexanoate. Pectin gels ↑ AUC and Imax compared to gelatin gels for all aromas.

Pastes with differing viscosities at a shear rate of 50 s^{-1} [110]	HPMC Starches: wheat, waxy maize, and modified waxy maize	HMPC ↓ salt and basil flavour perception compared to all starches. Waxy maize starch ↓ salt and basil flavour compared to other starches.
Dairy dessert containing carrageenan and starch [111]	Pectin - with differing Ca^{2+} reactivities	Perception of adhesiveness ↑ in desserts with pectin compared to control without. Sweetness and vanilla perception were unaltered.
Lemon flavoured dairy dessert [93,94,112]	CMC Modified starch	CMC ↓ linalool and cis-3-hexen-1-ol *in vivo* aroma release compared to samples thickened with starch but had a similar release to the fat only samples. CMC ↓ overall flavour and sweetness perception compared to starch samples.
Aqueous solutions with aspartame [113]	CMC SA	CMC ↓ sweetness perception of aspartame, particularly beyond c*. SA did not influence sweetness perception.
Sucrose solutions thickened with low, medium and high molecular weight PS [114]	Guar gum	Below c* there was no impact on sweetness or flavour perception but above this there was significant reduction in flavour intensity. No differences were found between the different molecular weights.

1.2.4.1. Effect on tastant perception

Malkki, Heinio, and Autio (1993) alluded to mucoadhesion as an explanation for their findings on flavour release and perception in PS thickened solutions. They compared three thickeners, CMC, oat gum and guar gum with respect to their

impact on sweetness and aroma perception over time. They found that oat gum was sweeter than CMC and guar gum solutions and proposed that adherence of the solution to the taste buds for longer could provide an explanation for the apparent increase in sweetness, although they did not carry out any experiments to test this hypothesis. The viscosities were matched at the shear rate of 50 s-1, which is considered to be the shear rate of the mouth, and oat gum showed the weakest shear thinning behaviour indicating that at lower shear rates, the viscosity would be lower than the other two samples. This could affect mass transfer of glucose molecules to the receptors; however, they do report that even the most viscous sample was sweeter than the least viscous CMC and guar samples. Interestingly they also found that oat gum solutions had the lowest aroma perception over time. This may suggest that the benefit obtained from adherence of the matrix at taste buds, prolonging tastant perception, may also reduce aroma release from the matrix of the food. However, there was no control used for aroma perception data so it is difficult to draw this conclusion as all PSs may have altered perception over time compared to the aroma compounds in water.

Hydroxypropylmethyl cellulose (HPMC) is a non-ionic, cellulose derivative and is a relatively weak mucoadhesive in the oral cavity compared to other PSs such as chitosan and CMC [77]. This PS is used in many studies as a viscosity modifier so that there is limited chemical interaction occurring between the PS and flavour compound. Studies have found that this thickener decreases the perception of saltiness, sweetness and aroma compounds in liquid systems due to the enhancement in viscosity [92,116]. These studies found that by increasing the concentration of HPMC, above a critical concentration, named the coil overlap concentration (c*), a decrease in perception of tastants and aromas was observed.

c* refers to the concentration above which PS molecules physically interact and overlap, and is determined by a sharp increase in viscosity after this point [114,117]. The authors propose that the reduction in taste intensity was due to entrapment of the compounds within the polymer network, slowing the mass transfer to taste buds and nasal receptors.

This effect could be advantageous in low fat systems where flavour is unbalanced. Taste-aroma interactions have been documented in the literature with the former usually enhancing the latter in congruent pairings [92,118–120]. This interaction could be taken advantage of by intelligently designing food, using mucoadhesives to change the temporal perception of flavour through controlled delivery of tastants.

1.2.4.1. Effect on aroma perception

Gallardo-Escamilla et al. (2007) investigated the sensory impact of various PSs in a fermented whey drink. The selected PSs were high methyl-ester pectin, propylene glycol alginate (PGA), CMC and XG (Table 2). The viscosities of the drinks were matched, although the authors recognise the high shear rate used to match viscosity may have affected results. They found that the presence of all PS reduced the overall typical yoghurt aroma released in headspace analysis compared to control, however, perception data showed only a significant decrease when thickened with PGA. The perception of acidity was decreased in all samples (except XG) compared to the control, and sweetness was perceived to be higher in the CMC and PGA samples [108]. Mucoadhesion may explain part of the results in this study, as the enhanced sweetness found by adding known mucoadhesives (pectin, CMC and alginate) could play an important role in prolonging the

residence of the sugar molecules in close proximity to the taste receptors. Bayarri, Chulia, and Costell (2010) also found that carrageenan enhanced the perception of sweetness and vanilla aroma intensity in model fat-reduced custards compared to a full-fat counterpart.

Cook et al. (2003) and Hollowood et al. (2002) investigated the impact of thickeners on flavour perception and coupled the experiments to *in vivo* release of aroma compounds. Their findings suggest that although flavour perception was decreased in thickened samples, the *in vivo* aroma release was not significantly reduced. The authors concluded that the perception decrease of aroma was due to aroma-taste interactions, where a decrease in the perception of saltiness or sweetness decreases the perception of the congruent aromas, even though the same amount of aroma may be delivered to the nasal cavity [92,116]. As with most studies of this nature, the sensory perception data collected is a static measurement. Although some measures were taken to control the time the panellists scored at, such as not holding the sample for longer than a few seconds, the panellist only scored once. This means that any change in delivery of the tastant or aroma compounds is not captured.

The decrease in tastant perception in solutions thickened with HPMC may be because it is non-ionic and therefore will not interact with the tastant compounds compared to ionic mucoadhesives such as CMC. Therefore, the salt and sugar molecules may favour partitioning into the salivary phase during mastication and be swallowed before activating taste receptors on the tongue that may be shielded by the viscous PS. Conversely, a mucoadhesive PS that is ionic may associate with the tastant and therefore retain it in close proximity to the mucosa.

There is abundant research in the field of viscosity, thickeners, and flavour perception and release. However, most studies investigating these parameters use a model thickener and do not necessarily consider the differences between thickener types in terms of mucoadhesion. Much like the differing strengths of mucoadhesion each thickener will possess, the interaction between the thickener and flavour molecules will differ. Therefore, it is difficult to draw conclusions about the role mucoadhesion plays in many of these studies, as the mucoadhesive strength of the thickeners is not measured. This oversight is a limitation as the mucoadhesive strength of the thickeners could be a factor in the difference in aroma release between different thickeners, which is only assessed as the sensory perception of adhesiveness [108,110,121,122]. These studies mentioned above and detailed in table 1.2 emphasize the complex relationship between thickener, flavour perception and flavour release.

To further complicate the picture, aroma adsorption to the oral mucosa has also been investigated for many years. A study by Hussein, Kachikian, and Pidel (1983) was one of the first to investigate the effect of aroma persistence after consumption. In this study, participants rinsed their mouths after 1 and 5 minutes post-consumption, and measured the amount of volatile left in the mouth. The authors found the most persistent aromas to be menthol and anethole; however, it was unclear whether the extraction technique was suitable to remove all volatile compounds, especially those adhered to the mucosa. More recently, Esteban-Fernandez et al. (2016) used intra-oral SPME/GC-MS to investigate wine "after-aroma". The authors found that the strength of the aroma-mucosa interactions was more important that the actual amount of aroma adsorbed.

1.2.5. Polysaccharides and texture

Trained sensory panels often describe the textural aspects of high fat foods as creamy, fatty, slippery, oily and smooth; dependent on the type of food. It can be difficult for panellists to distinguish between these types of words; partly due to the difficulty in classifying these perceptions by experimental means. Factors including rheology, tribology, colloidal behaviour and flavour all have an important influence.

As mentioned previously, fat serves many purposes in food with many textural cues that are difficult to mimic without it. Emulsions are designed with this in mind in an attempt to mimic the lubricating, thick and creamy properties that fat imparts [61,124,125]. Many studies highlight the importance of thin film rheology and tribology as well as bulk rheology when comparing thickeners to fuller fat systems. In order to understand perceived textural changes to food when incorporating mucoadhesives, it is vital to establish a way to characterise these changes. Malone et al. (2003) studied the adsorption of oil-in-water emulsions to a mucin-coated film. The authors found that the addition of mucoadhesive, chitosan, enhanced the affinity of the oil to the mucin film. The authors also note that the presence of chitosan resulted in an astringent mouthfeel when given to a trained sensory panel, which was attributed to chitosan binding to mucin molecules causing precipitation [99]. This is one of the few studies, which attempts to directly employ mucoadhesives as a way to modulate the organoleptic properties of food by texture modulation. There are, of course, many other studies investigating the textural aspects of liquid, semi- liquid and semi- solid foods, some of which specifically investigate the interaction between the food and mucosa [50,105,124–126]. Many of these refer to the specific interactions of

flavour molecules with the food matrix and oral anatomy, however, select studies have investigated the influence of hydrocolloids on the textural perception of emulsions [50,124,125,127].

Most of the studies regarding the effect of PS thickeners on texture are focused on liquid products as this is where they are most utilised [6,128,129]. However, the results from these studies, and the likely role of mucoadhesion in contributing to the changes in sensory perception, may generate interest in incorporating these mucoadhesives into dry food products. For many mucoadhesives, the solid form has the highest mucoadhesive strength, due to swelling and spreading behaviour upon contact with the moist mucosal surface of the oral cavity. This results in a strong, lubricating, adhesive joint.

To date, and to the best of the authors' knowledge, there aren't any studies specifically investigating the impact on flavour retention, release and perception of mucoadhesive PSs in liquid and solid food products. The work in this thesis was the first to assess the mucoadhesive strength of PSs in an aqueous solution on different areas of porcine tongue and related that to the effect of *in vivo* flavour perception with a sensory panel. It is also the first to incorporate mucoadhesive PSs into popcorn seasoning to assess the effect on flavour perception over time.

1.2.6. Concluding remarks

The understanding of mucoadhesion in food substances could have many impacts on the food industry, whether mucoadhesives are added as a functional ingredient, or whether native mucoadhesives in the food are manipulated to control sensory properties. By understanding the properties of mucoadhesive food components, a

higher level of control could be achieved in the texture and flavour of a food product. Mucoadhesion could also play a significant role in the future of low-fat foods utilising fat replacers.

In conclusion, mucoadhesion is an important consideration for food researchers and product developers and has the potential to be utilised in enhancing the organoleptic properties of foods. The impact of mucoadhesive ingredients on sensory perception is beginning to be elucidated, however further research in this area is required for a better understanding.

Chapter 1.3: Thesis aims, objectives and hypotheses

This PhD work investigates the impact of mucoadhesive PSs in food products considering the literature above.

1.3.1. Aims

The overall aims of the work were as follows:

- To develop a method to compare the retention of a range of PS formulations on tongue tissue.
- To assess changes that solutions thickened with mucoadhesive PSs have on tastant perception and *in vivo* retention.
- To investigate the role of mucoadhesive PSs in solid formulations to control the release flavours over time.
- To attempt an application for mucoadhesive PSs relevant to industrial sponsor of this project.

1.3.2. Objectives

The objectives to complete these aims were as follows:

- Development of an *ex vivo* retention model with porcine tongue tissue as the substrate to test a variety of PS materials for their retention ability.
- In vivo time intensity sensory perception testing of the taste of aqueous solutions containing sodium or glucose, with and without mucoadhesive.
- Collection of saliva at set time points after consumption to measure PS ability to retain tastants in the mouth.
- Time intensity sensory testing for PS films with tastant and aroma compounds.

- Characterisation of the PS films including swelling and disintegration studies, dissolution testing and *in vitro* mucoadhesion tests.
- Incorporation of mucoadhesive PSs into popcorn seasoning and assessing the impact on flavour perception.

1.3.3. Hypotheses

The hypotheses of this work are as follows:

- Ionic, viscous PS will be adhesive to oral tissues such as the tongue.
- Solutions thickened with mucoadhesive PSs will decrease the intensity of the perception of tastants but will prolong their residence and therefore, give sustained perception of them compared to water.
- Mucoadhesive PSs in aqueous solutions will retain tastants in the mouth for longer than less mucoadhesive PS and water.
- The properties of the PS such as swelling degree, dissolution speed and adhesiveness will impact the release and subsequent perception of flavours in solid formulations.

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Chapter 2: Mucoadhesive materials and their characterisation

In this chapter, the materials used throughout the thesis are discussed in detail and some background theory behind the rheology used to characterise PSs.

2.1. Structure, functionality and uses of polysaccharides

PSs are employed across many different industries as adhesives, thickeners, binders, film-formers, water-retention agents, lubricants, emulsifiers and stabilisers. Naturally-derived PSs commonly used include; gums, starches, cellulose derivatives, chitosan, pectins, alginates and pullulan. The structures of these PSs vary in complexity dependent on the type. For example, starch and cellulose are made up of glucose monomers connected by alpha and beta linkages, respectively. Whereas, alginates are copolymers of β-D-mannuronate (M) and its epimer α-L-guluronate (G) arranged in various combinations of homopolymeric blocks.

Depending on their chemical and physical properties, PSs are employed in a wide range of products. In the food industry, they are commonly used as viscosity enhancers in liquid and semi-solid products, particularly those with reduced fat contents, as a way to compensate for lost bulk. Furthermore, they are useful in dairy drinks and desserts with reduced fat contents to compensate for loss of viscosity and creaminess. Other common uses for carboxymethyl cellulose in particular are in bakery products to retain moisture and confectionary for structural properties.

In addition to the many other uses in the pharmaceutical industry, many PSs are used for their adhesive nature. Mucoadhesive PSs are those that adhere to mucosal

surfaces in the body for a prolonged period. As described in chapter 1, section 1.2. mucoadhesion is a well-characterised phenomenon and is used in pharmaceutics to control drug delivery at mucosal surfaces [1–4]. Due to the retention of such PSs at mucosal surfaces, they can be incorporated into formulations containing active pharmaceutical ingredients. The PSs chosen for experiments in this work were selected from those frequently used in the pharmaceutical industry, based on their mucoadhesive strength, viscosity enhancing abilities and their suitability for food use. The structural characteristics and functions of the PSs used will be reviewed below. Information provided by the manufacturers regarding the properties of the PS are detailed in table 2.1.

Table 2.1. Details of polysaccharides used in experiments.

Manufacturer	**Product code**	**Polysaccharide**	**[a]Mw (kDa)**	**Other**	**Apparent viscosity***
Herbstreith & Fox	CU 701	Pectin (LMEP)	54	[b]D.E 36% Galacturonic acid content 89%	-
Kimica Corporation	ALGIN I-5	Sodium alginate (SA)	250	[c]M:G ratio 1.1:0.9	1% solution 517 mPa.s
Dow	METHOCEL F450	Hydroxypropyl-methyl cellulose (HPMC)	300	methoxyl [d]D.S 1.8 hydroxypropyl molar substitution 0.13	2% solution 4584 cp
Akzonobel	AKUCELL AF 0305	Carboxymethyl cellulose (LCMC)	140	D.S 0.8	-

Dow	WALOCEL	Carboxymethyl cellulose (HCMC)	950	D.S 0.8	2% solution 5200 mPa.s
Hayashibara	PULLULAN	Pullulan (PUL)	250	-	2% solution 10 cp
Nutricia	Nutilis	Starch	-	Contains maltodextrin, modified starch (E1442), tara gum, xanthan gum, guar gum.	-

[a]molecular weight (Mw), [b]degree of esterification of pectin (D.E), [c]M = mannuronate, G = guluronate, [d]degree of substitution (D.S).

*Viscosity determined on Brookfield viscometers at a shear rate of 7 rad/s^{-1}.

All PS were kindly provided by the manufacturers stated apart from Nutilis, which was purchased from a local boots store. The characterisation data were provided by the manufacturers (Table 2.1.). Further characterisation was carried out, and is discussed in the appropriate chapters.

2.1.1. Sodium carboxymethyl cellulose

Sodium carboxymethyl cellulose (CMC) is a water-soluble derivative of cellulose. The glucose monomer units of cellulose are linked via glycosidic β – linkages. Each monomer has three hydroxyl groups that have the potential to be substituted by carboxymethyl groups. This is termed the degree of substitution (DS). Substitution is achieved during the manufacturing process by reacting cellulose with sodium monochloroacetate to substitute up to 3 of the hydroxyl groups with

carboxymethyl groups, forming CMC (Figure 2.1.). This process renders the polymer water-soluble by decreasing the amount of hydroxyl groups that can hydrogen bond with each other in a complex network, excluding water and making it insoluble.

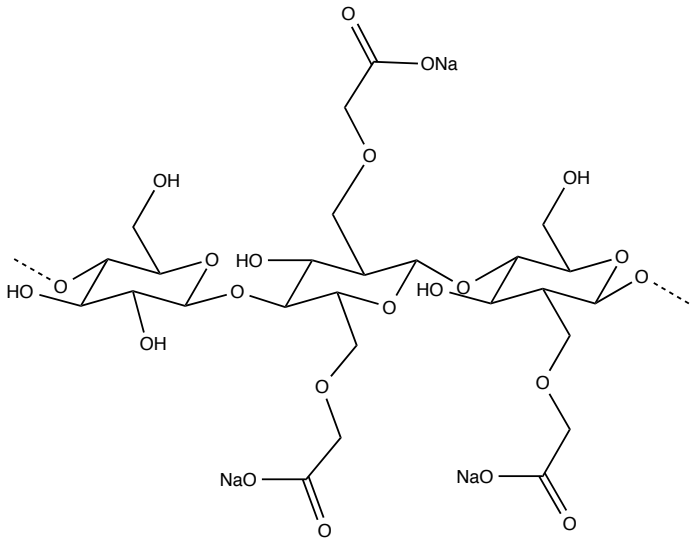

Figure 2.1. Sodium carboxymethyl cellulose structure.

CMC is a long chain, linear PS. The solution characteristics depend on the DS and molecular weight of the product. Reducing the DS of CMC will result in a decreased solubility and therefore the product swells and does not completely dissolve, leading to an enhanced viscosity. As with most PS, a larger molecular weight results in an increase in solution viscosity. Similarly, as the concentration of CMC is increased so is the viscosity. Neutralisation of carboxymethyl groups will also affect viscosity; therefore, the pH of the solution is important.

CMC is used for many applications in the food industry including cake mixes to retain moisture, confectionary to control sugar crystallisation, ice cream to control ice crystallisation, and pet foods for its gelling properties [5]. Furthermore, CMC has many applications in the pharmaceutics industry in a variety of drug

formulations. Properties that are useful include, but are not limited to, film forming, stabilising, binding, dispersing agent and adhesive. The mucoadhesive nature of CMC is well documented in the literature and is thought to be due to its high viscosity and ionic nature [6–9].

2.1.2. Hydroxypropylmethyl cellulose

Hydroxypropylmethyl cellulose (HPMC) is a non-ionic, water-soluble cellulose ether similar to CMC. The structure of HPMC is a cellulose backbone with ether linked methoxyl and hydroxypropyl side group substituents attached through ether linkages to the cellulose chain hydroxyl groups (Figure 2.2.). This modifcation is achieved by heating cellulose fibres with a sodium hydroxide, which in turn is treated with methyl chloride and propylene oxide to produce the methyl and hydroxpropyl ethers, respectively. HPMC products possess varying ratios of methyl and hydroxypropyl substitutions that affect solubility and thermal gelation temperature.

The HPMC used for experiments in this PhD thesis were obtained from DOW (product code F450)[10]. This product has an average degree of methoxyl substitution of 1.8. The average molar substitution (MS) of hydroxypropyl groups per mole of anhydroglucose for this product is 0.13. These substitutions render the cellulose ether water-soluble. The molecular weight of HPMC will affect the rheological behaviour but generally, HPMC exhibits pseudoplastic behaviour, although at very low shear, it will appear to be Newtonian. The apparent viscosity of solutions of HPMC is directly related to the molecular weight or chain length of the PS. The manufacturer determines apparent viscosity in water at 20 °C, with a

concentration of 2% of the product. The product F450 used for this work has a molecular weight of 300,000 Daltons.

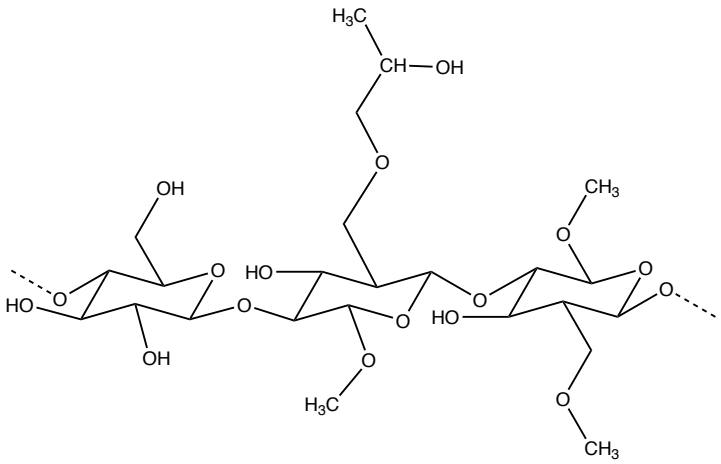

Figure 2.2. Hydroxypropyl methyl cellulose structure.

For pharmaceutical applications HPMC is regularly used as a film-forming agent, thickener, binder, sustained-release agent, emulsifying agent and suspending agent in a variety of dosage forms. HPMC is not generally regarded as a strong mucoadhesive on its own, as it is non-ionic, however, it is frequently used in mucoadhesive formulations for its film forming abilities and slow disintegration [8,11]. Furthermore, HPMC possesses hydrogen bonding groups and has/exhibits a high viscosity, which lends itself to mucoadhesion in the presence of moisture. Rheological factors such as viscosity, spreading on the mucosal surface and hydration leading to interpenetration of polymer chains where interactions can occur with mucins will facilitate this adhesion.

2.1.3. Pectin

Pectins are a complex group of heteropolysaccharides found in the primary cell walls and middle lamella of many plants. Most commonly, pectins are extracted from the peel of citrus fruit. Galacturonic acid units make up the majority of the

structure of pectin (Figure 2.3.) and the carboxylic acid groups of the uronic units can be free or associated with various counter-ions such as sodium, potassium, calcium, or ammonium, or naturally esterified with methanol. Pectins are polyelectrolytes so are sensitive to pH fluctuations. Pectins exhibit acidic pH in solutions due to the charged carboxylic groups [12].

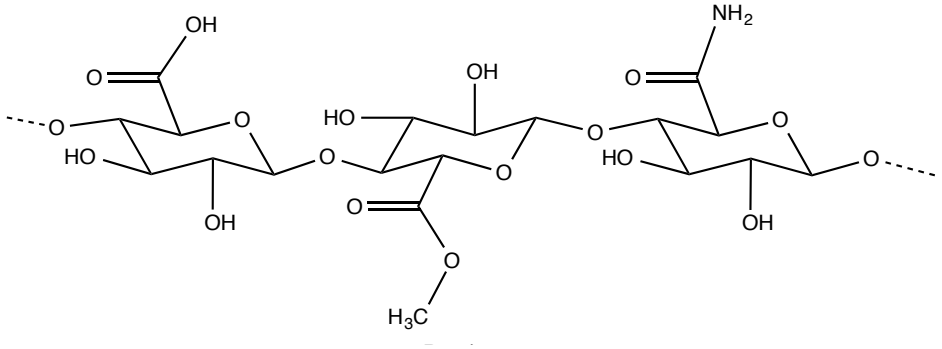

Figure 2.3. Pectin structure with various functional groups.

The galacturonic backbone of pectin has many neutral sugars branching from it at varying degrees. The galacturonic backbone is also interjected with rhamnose sugars at a substitution of between 1 and 4 %. Between 20 and 80% of these rhamnose units are substituted at C-4 with neutral and acidic oligosaccharide side chains, mainly consisting of arabinofuranosyl and galactopyranosyl residues [13].

The carboxylic acid functional groups on each galacturonic monomer unit can react with methanol, either naturally or during the manufacturing process, to produce different levels of methyl esterification. The degree of esterification (DE) is defined as the % of carboxylic groups that are esterified with methanol, and it influences the physical characteristics of pectin. Pectins with a DE above 50% are referred to as high methyl ester pectins (HMEP) and below this are low methyl ester pectins (LMEP). Pectin can also be treated with ammonia to product differing

degrees of amidation (DA) of the carboxylic acid groups; these are referred to as amidated pectins.

LMEP and amidated pectins exhibit increased gelling response in the presence of divalent cations such as calcium ions. Gelation is due to the formation of intermolecular junctions of homogalacturonic regions of different pectin chains with the divalent cation being the “egg” in the hypothetical “egg in box” theory [13]. Furthermore, amidated pectins show an enhanced resistance to precipitation when high concentrations of the cations are in solution, unlike other forms of pectin.

Pectins are used in a variety of applications in the food industry but most commonly they are used for their gelling abilities in high sugar systems such as jams and vegetarian jellies. Pectins can also be used in acidic fruit juices and protein beverages as a stabiliser. Besides the common uses in the food industry, pectins have proven useful in the pharmaceutics industry as a carrier for active ingredients in controlled release formulations [14] and in mucoadhesive formulations [15,16].

The mucoadhesive strength of different kinds of pectin has been investigated with some contradictory results. Some studies investigating specific interactions with mucins to colonic mucosa [17] using atomic force microscopy [18], have suggested that LMEP exhibits the strongest mucoadhesion compared to other types of pectin. However, Thirawong et al. (2007 & 2008) conducted texture analysis and rheological synergism tests and found HMEP formulations showed better potential as mucoadhesives. This discrepancy might be explained by the fact that these

studies use different methods to assess mucoadhesion with a range of *in vitro* and *in vivo* tests. Furthermore, it is likely that the type of mucin or tissue used to assess mucoadhesion will have a significant effect on the mucoadhesive strength.

At neutral pH, the free carboxylic groups of the LMEP will be negatively charged and the mucins will also be negatively charged. The charged groups may result in electrostatic repulsion of the polymer chains leading to uncoiling. This may then facilitate chain entanglements and bond formations. SEM images of pectin and mucin interactions by Liu et al. (2005) support this and the authors conclude that entanglement of PS chains with mucin chains is the dominant mode of interaction facilitating mucoadhesion.

2.1.4. Pullulan

Pullulan (PUL) is a water soluble, non-ionic, linear PS consisting of maltotriose residues. α 1- 4 Glycosidic bonds link the three glucose molecules that make up maltotriose and each maltotriose unit in turn is connected to another maltotriose via α 1- 6 linkages (Figure 2.4.). PUL is a fungal exopolysaccharide produced from starch by the *Aureobasidium pullulans*. The molecular weight of pullulan can be between 10 and 400 kDa [20] depending on the growth conditions. In water PUL quickly dissolves to form a stable, non-gelling, viscous solution. Solutions of PUL are Newtonian and the viscosity is relatively low compared to other PS.

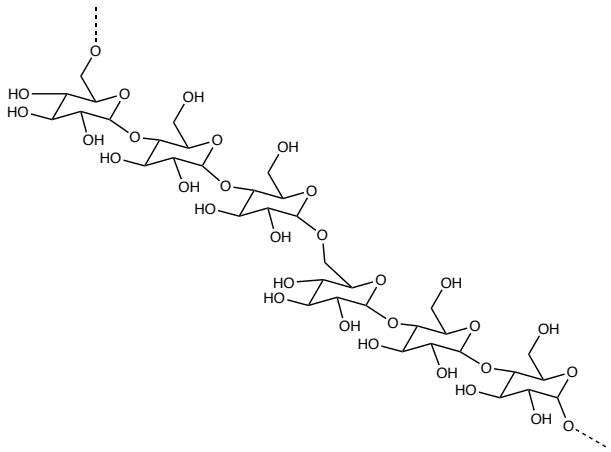

Figure 2.4. Pullulan structure.

PUL is used in the food industry due to its chemical properties and range of applications that can take advantage of these properties. PUL is tasteless and odorless and is therefore attractive for use in low fat products as a bulking and stabilizing agent. It is a soluble dietary fibre, has excellent water retention properties and provides an oxygen permeation barrier. Furthermore, PUL has good adhesive and film forming properties. These properties make PUL an attractive additive for the food industry and has been used for over 20 years in Japan [21].

Fast dissolving, mucoadhesive films made with PUL are used in the pharmaceutical industry to deliver drugs systemically whilst bypassing first pass metabolism and as alternatives to tablets and capsules, which can be difficult to swallow [22,23]. PUL based films can also be used to deliver active ingredients to treat halitosis causing bacteria combined with a range of flavorings at the same time. PUL is used in this way for Listerine Pocketpacks® as pullulan films are adhesive and fast dissolving. The films quickly dissolve in contact with saliva, releasing the flavourings and active ingredients to fight bad breath. More recently, PUL is being investigated for its applications in the biomedical field including targeted drug delivery, tissue engineering [24] and wound healing [21].

2.1.5. Sodium alginate

Sodium alginate (SA) is a naturally occurring, anionic PS extracted from brown algae (*Phaeophyceae*). It is a linear copolymer, consisting of two epimeric monomers, b-D-mannuronic acid (M) and a-L-guluronic acid (G) residues (figure 2.5.). The proportions and distribution of these epimers vary within each polymer chain with differing combinations of M- blocks, G- blocks and MG- blocks. The M:G ratio is important as only the G blocks are thought to be involved in intermolecular interactions between divalent cations (calcium), which results in gelation [25,26]. The viscosity of SA solutions increase as pH decreases due to protonation of the carboxylate groups resulting in hydrogen bonding between chains [27]. SA has many applications across lots of different fields including; food, pharmaceutics, bioengineering and cosmetics.

In the food industry alginates have various applications including; thickening, stabilising, film former, pre- and probiotic encapsulation [28] and aroma encapsulation [29,30]. As SA gels in the presence of calcium it has been a popular ingredient in fine dining for experimenting with textures and flavours. SA is a soluble fibre and has been investigated for satiety enhancing effects when consumed in a drink [31].

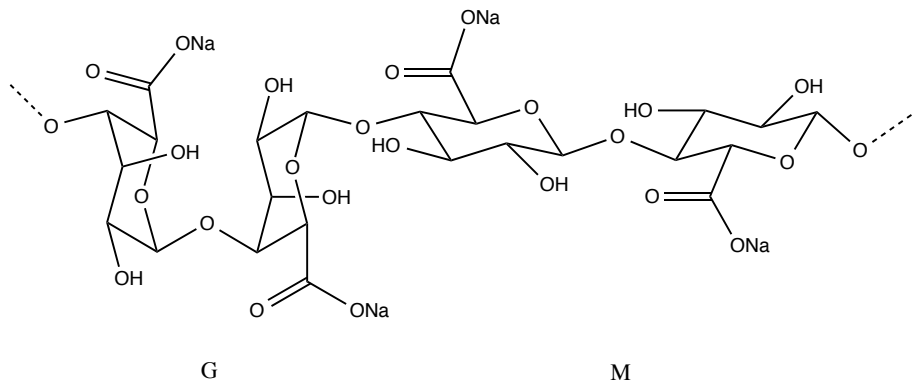

Figure 2.5. Sodium alginate structure.

In pharmaceutics, SA is one of the most commonly used, naturally occurring, mucoadhesive materials [8,32,33]. The mucoadhesive properties of SA have been exploited in a variety of formulations including; buccal discs, tablets and films [8,34,35], liquid formulations [36,37] and gels [38].

2.1.6. Modified maize starch

Starch is a plant PS stored in roots and seeds. Unlike the other PS mentioned in this chapter, starch provides energy as it is broken down into glucose units by the enzyme amylase. Starch is insoluble in cold water and will form swelled granules that create a temporary suspension as each granule absorbs water. There are many types of starch and modified starches, each possessing different properties and suited to different applications [39].

Starch is made up of two polymers, amylose and amylopectin. Amylose typically makes up about 25% of the PS and the amount of amylose in starch is responsible for its gelation ability and resistance to degradation by amylase. Amylose is a long, linear chain of α 1-4 linked glucose units (Figure 2.6.). Amylose usually forms stiff, left-handed helices in solution [40]. The majority of starch is made up with amylopectin molecules. Similarly to amylose, amylopectin is made up of many glucose molecules with α 1-4 glycosidic linkages, however, every 15-30 units there is a branching α 1-6 linkage [39]. This branching makes the amylopectin molecules highly branched and bushy.

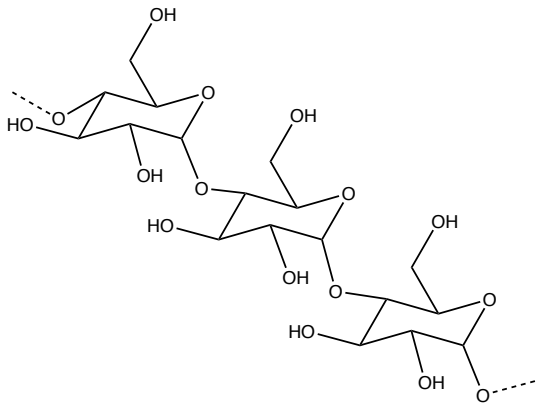

Figure 2.6. Starch structure.

Nutilis is a brand of modified maize starch that is resistant to amylase, meaning it does not breakdown into glucose units as readily. In solution, like most starches, nutilis starch granules swell and form heterogeneous, insoluble granules [41]. Modified starches are produced by chemically treating native starch to alter the physical properties. These changes will affect the stability, appearance and performance of the starch in food products. Nutilis is a product for adults with dysphagia. It is used to thicken liquids and resist amylase therefore aiding with swallowing.

Starches are frequently used in the pharmaceutical industry as diluents, binders and lubricants. Starch is not particularly useful as a mucoadhesive due to the granular nature in solution. However, various modified forms of starches such as carboxymethyl starch [33] and conjugated copolymers with poly acrylic acid [42] have been investigated for the use in mucoadhesive formulations. These starches will have enhanced water solubility due to the increased polarity of the polymer by the charged groups (carboxylic acid). Furthermore, the addition of a negative charge to it will enable interactions between the polymer and mucins via electrostatic interactions and hydrogen bonds.

2.2. Rheological characterisation of polysaccharide solutions

Throughout this work a rheometer was used to characterise the rheological aspects of the PS solutions used. A brief description of the theory behind the tests is described here. Rheology is used to assess the behaviour of a liquid, suspension or slurry under applied stress. It allows observation of the flow of a fluid that cannot be defined by a single value such as those obtained from a viscometer. The way a fluid responds to applied stress over dynamic parameters such as time, temperature and strain is elucidative and valuable for scientists. There are two types of rheometers commonly used, rotational rheometers are those that apply a shear strain or stress and extensional rheometers are those that apply extensional stress or strain are.

Rheology was used to match the viscosities of solutions used in this work. Viscosity is defined as the resistance of a material when placed shear or tensile stress. The resistance exerted by the material is a result of the inter-molecular friction of molecules in the fluid as they attempt to slide passes one another. The equation for viscosity is:

$$\eta = \delta / \gamma$$

Where η represents viscosity, δ represents the shear stress and γ represents strain.

Many polymers in solution exhibit viscoelastic behaviour, meaning they possess both viscous and elastic properties under stress. This viscoelastic behaviour of polymer samples can be characterised by observing the storage (G') and loss (G") moduli, which correspond to the solid- like (elastic) and liquid-like (viscous) contributions of a stress response in a fluid, respectively. When G'' is higher than G', tan (δ) is > 1, the sample is more viscous than elastic or more liquid like. The opposite is true when and G' is higher and δ is < 1, the sample is more solid like.

When δ =1 this is considered the crossover or gel point of fluid from a liquid-like substance to a more solid-like substance.

A Newtonian fluid is one that has linear strain to stress. Some PS in solution will exhibit Newtonian behaviour at very low shear and concentrations [43], however, most are non-Newtonian as their viscosity is shear dependant. The majority of non-Newtonian PS solutions are shear thinning (pseudoplastic) however, some cane be shear thickening (dilatant).

A rotational TA AR2000x rheometer was used for all experiments described in this body of work. Oscillatory rheology was used for all experiments to determine the viscosity of PS solutions. Oscillatory was chosen as opposed to rotational tests so that low shear viscosity could be understood where the internal structure of the sample was not destroyed. The basic principle of oscillatory rheometry is to induce a sinusoidal shear deformation in the sample and measure the resultant stress response. The test sample is placed between two parallel plates; the bottom plate remains stationary whilst the top one oscillates. The test material is subjected to oscillatory movements that are controlled by altering the frequency (speed of the turning plate) and amplitude (how far the turning plate moves) of the oscillation.

The complex viscosity (η*) of solutions was determined for solutions used in this work. η* is a dynamic measurement where viscosity is shear dependent. The complex viscosity of a fluid can be calculated by the following formula:

$$\eta^* = G^*/\omega$$

Where G* is the complex (dynamic) modulus and ω is the angular frequency. G* is calculated by the storage (G') and loss moduli (G"):

$$G^* = G' + iG''$$

Where i is the imaginary unit.

In order to test the PS solutions that were used for experiments in this work the linear viscoelastic region (LVR) for the highest concentration samples was determined, first. Within the LVR, the materials response is independent of the stress applied and the materials internal structure is maintained intact. The LVR is determined by performing an amplitude sweep at a set frequency and observing the G' and G" moduli. Within the LVR the G' and G" moduli are linear. Frequency sweeps were then carried out at a 1% strain (amplitude within the LVR) to obtain η* over a range of frequencies.

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Chapter 3: *In vitro* method for assessing the mucoadhesion of food grade polysaccharides on porcine tongue

3.1. Introduction

The adhesive nature of PSs has been known for some time [1]. Therefore, PSs are utilized in a plethora of different industries for this adhesive property including food, pharmaceutics, engineering and textile. The mucoadhesive nature of PSs is well defined for pharmaceutical applications, and countless studies have been performed to understand the mechanisms and uses of many different polymers [2–4]. However, those studies focus on mucosal surfaces that allow good drug absorption such as buccal, oesophageal, stomach and intestines. The tongue is not ordinarily used for drug absorption due to its epithelial structure and mechanical role during eating and speaking.

Many PSs that are known to be mucoadhesive are commonly used in the food industry, particularly in liquid and semi-solid foods, as viscosity modifiers, stabilizers, and emulsifiers. Only recently the role of mucoadhesion is becoming recognized as a factor that may contribute to sensory perception of foods containing such ingredients [5–8]. Polymer adhesion to the tongue mucosa has only been explored by Withers et al. (2013), who investigated the binding of milk proteins to the tongue epithelia, in an attempt to explain mouth drying attributes of milk products.

This chapter investigates the mucoadhesive properties of three commonly used PS; LCMC, LMEP and SA, on different areas of *ex vivo* porcine tongue. Whilst mucoadhesion tests have been carried out on other mucosal tissues in the oral

cavity, such as sublingual and buccal, the mucoadhesion of PSs to the dorsal of the tongue has not yet been investigated. The ability of mucoadhesive polymers to retain small molecules on these mucosal surfaces may be of interest to the food industry.

The tongue is the primary tissue responsible for taste sensations as this is where most taste receptor cells are located. Therefore, if a PS sample adheres to the tongue for a prolonged duration it may facilitate the retention of tastant molecules such as glucose and sodium chloride. This retention could result in changes in the rate of delivery and subsequently, the temporal perception of tastants.

There is no standardised way to measure the mucoadhesive strength of a material. However, there are a variety of *in vitro* and *in vivo* methods used in the literature to investigate various aspects of the adhesion process. *In vitro* methods range from the observation of physical interactions between polymer and mucin chains using techniques such as rheology [9–11], AFM [12] and BIACORE [13], to detachement measurements with a texture analyser [14,15]. The substrates used to test adhesion of polymers can vary from purified mucin [16,17] to mucosa-mimetic materials [18]. An important consideration for these *in vitro* methods is that the results of one test does not necessarily match with results from another [19].

Many studies use *ex vivo* tissue from animal models to more accurately represent *in vivo* adhesion. Mucoadhesion can be measured by placing a formulation in contact with the tissue, washing it with an eluent and collecting the wash off to measure the amount of active ingredient in the wash off [20,21]. *Ex vivo* tissue can also be used as a substrate for mucoadhesion testing using a texture analyser [22].

In this chapter, we have developed a dynamic retention method to measure the retention of liquid formulations by using fluorescence imaging. The objective for this chapter was to assess whether PSs in solutions were adhesive on different areas of porcine tongue by developing a method to evaluate the mucoadhesive strength of the PSs. Labelled and unlabelled PSs in solution were assessed to determine if chemical labelling of PSs is necessary. It was hypothesised that liquid mucoadhesives would adhere to the tongue dependent on the area being tested. For example, the front of the tongue contains many papillae that provide an increased surface area so according to the mechanical theory of mucoadhesion, this area should retain more solution. Furthermore, it was hypothesised that as viscosity increases so does the mucoadhesive strength.

3.2. Methods

3.2.1. Materials

LMEP, SA, and LCMC (detailed in Chapter 2) were the PSs used for these experiments. All other chemicals and reagents were purchased from Sigma Aldrich.

3.2.1.1 Sample preparation

PSs were dissolved in deionised water (DW) and left overnight in the fridge before testing took place to ensure full hydration of polymer chains and remove air bubbles. If the PS chains are not fully hydrated this will have a huge impact on how mucoadhesive it will be as hydration will free up the polymer chains enabling interaction with the mucin molecules. Ensuring there were no air bubbles was essential as the amount of PS used for each experiment was done by volume so bubbles would have introduced variability. LMEP samples were adjusted to pH 7 with NaOH to have the same pH as the SA and LCMC samples.

The concentrations of PSs used for retention tests are detailed in tables 3.2. and 3.3. The samples were matched for viscosity rather than concentration as viscosity is a major influencer of mucoadhesive strength with an increase in viscosity resulting in an increase of mucoadhesion. Concentration will also have an impact but for these experiments it was decided that viscosity would be more important. Each experiment in this chapter was repeated 3 times for each sample.

3.2.2. Rheology of polysaccharide solutions

PS samples were removed from the fridge at least an hour before the test to bring it up to room temperature. Three separate batches were made for each concentration and 3 analytical repeats were carried out so there was a total of 9 readings for each PS at each concentration. The solution was stirred before 600 µL was taken up with a plastic syringe and placed onto the bottom parallel plate of the rheometer. The rheometer was set to equilibrate the sample to temperature (37°C) before beginning sweeps. The linear viscoelastic region (LVR) was determined for the most viscous PS (Figure 3.1.). The LVR was determined by using an oscillatory test with a 40 mm parallel plate at a constant frequency of 1 Hz with a 400 µm gap. An appropriate strain of 1 % was chosen as this was well within the LVR. A frequency sweep from 0.6 to 6.3 rad/s was carried out for each PS.

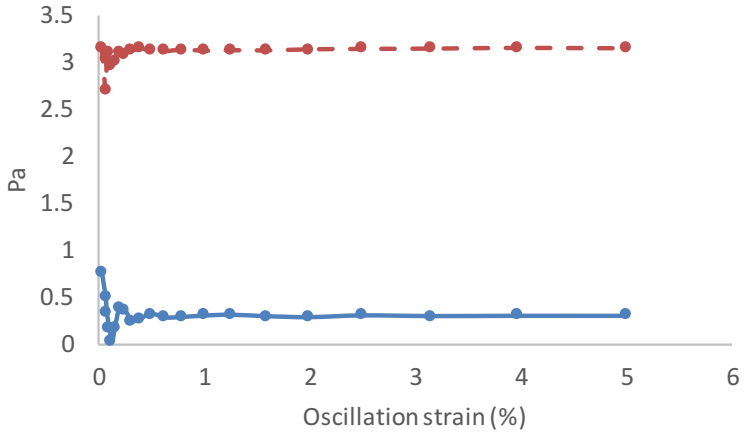


Figure 3.1. Strain sweep for 5.5% LCMC to determine linear viscoelastic region. Dashed line represents loss modulus and solid line represents storage modulus.

All the samples were shear thinning and differed from each other in their behaviour. Therefore, it is impossible to match the rheological behaviour of these samples over a range of shear. A somewhat arbitrary angular frequency of 1.3 rad/s was chosen at which the viscosities were matched. This low frequency was selected as during the retention experiments there is little shear force applied to the sample. Readings at a lower frequency were unreliable and had huge variations between repeats. It is important to control for viscosity as increases in viscosity enhance the mucoadhesive strength of solutions [23].

3.2.3. Ex vivo retention model

A method to assess mucoadhesion using fluorescent microscopy to visualise PS retention has been developed in our group [6,18]. The method, adapted from Cave *et al.* (2012) [24], is a dynamic procedure that enables indirect quantification of solid or liquid polymeric formulations retained on mucosal membranes after being washed with an artificial eluent. PSs are either labelled with a fluorophore or have unbound sodium fluorescein mixed into the solution. The labelling of PS was done

so that the retention of the PS chains could be visualised under the microscope, however, the labelling process changes the chemical properties of the PS (e.g. linking the fluorophore to the carboxylic acid group), therefore, unlabelled PS with free fluorophore were also tested and compared to the labelled samples to see if there were any differences observed. Each retention experiment was repeated 3 times for each PS type and concentration.

3.2.3.1. Fluorescent labelling of polymers

A method for labelling, LCMC, SA and LMEP was developed by the conjugation of the PS carboxylic acid groups with the amine group of fluorescein amine. Calculations were made so that 1 mol % of monomer units was labelled if the reaction was 100 % efficient. It was thought that this low labelling would limit the impact on mucoadhesion. Labelling was achieved by dissolving PSs for 3 hours at room temperature in phosphate buffered saline (PBS) at pH 6. After which, the addition of 1 equivalent (to the amount of fluorescein to be added) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide facilitated the reaction between carboxylic acid groups of the PS and the amino-groups of the fluorescein amine. To stabilise the reaction intermediate, 2 equivalents of N-hydroxysulfosuccinimide sodium salt was added to the solution. This was left for 15 minutes stirring at room temperature before adjusting to pH 7 and adding 1 equivalent of fluorescein amine. The mixture was left stirring, in the dark, at room temperature for 24 hours. To purify the PSs from any unreacted fluorescein amine, the solution was dialysed in 3L bottles for 2-3 days, changing the dialysis water 3-4 times a day, until the dialysis water did not contain any fluorescence. This was checked with UV spectrophotometry. Solutions were freeze-dried, sealed and stored in the dark until required.

Purity of labelled polymers was determined by thin layer chromatography (TLC) to confirm there wasn't any unbound fluorescein in samples. A drop of labelled polymer sample was placed onto the TLC paper next to a free fluorophore reference sample. The TLC paper was looked at under UV light to confirm that the fluorescence of the labelled PS stayed at the bottom of the TLC paper whilst the reference sample travelled upwards indicating that the fluorophore was indeed conjugated to the PS.

3.2.3.2. Tissue preparation

Porcine tongues were collected from P & D Jennings butchers (Hurst, UK) a maximum of 24 hours after slaughter. During transportation and preparation in the lab the excised tongues were kept in a bag on ice whilst the majority of muscle and connective tissue was removed with razor blades and medical scalpels. The remaining epithelia and connective tissue measured around 2- 3 mm thick. Tissue was taken from the front, rear and side portions of the tongue (Image 3.1.). Tissue sections were sealed in airtight bags and frozen at -20 ºC until required. Many studies have supported the claim that there is no difference between fresh or frozen tissue when testing for mucoadhesion [25,26]. When required, tissue was thawed in the fridge for 2 hours and cut into 1 cm^2 samples from three areas of the tongue; front, side and rear. Samples were kept moist with artificial saliva (AS) during thawing.

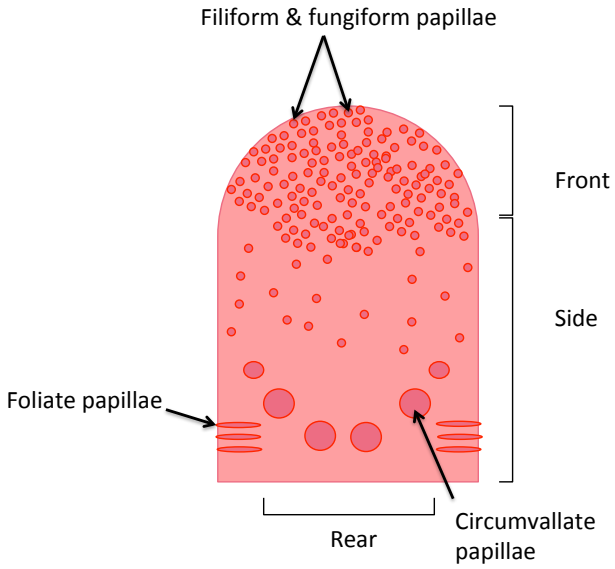


Image 3.1. Schematic drawing of the different areas of the tongue selected due to the differing morphologies and characteristics.

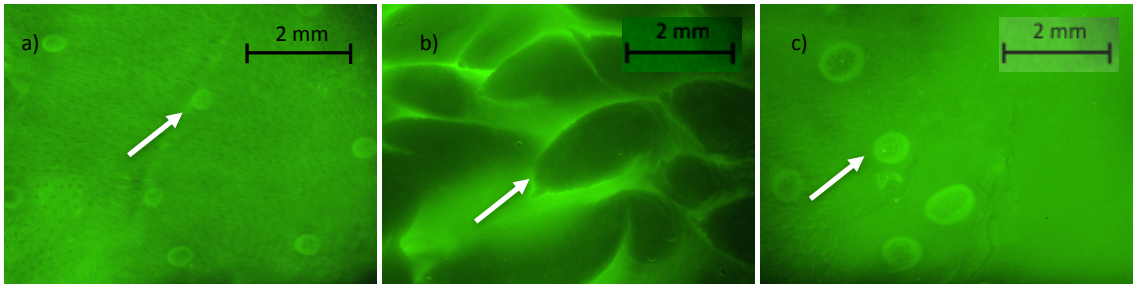


Image 3.2. Example fluorescent images of the a) front, b) rear and c) side of the porcine tongue tissue. Images (x8 magnification) were captured after 0.1% sodium fluorescein solution was added to the tissue. Arrows indicate papillae in a) and c), and finger-like protrusions in b).

The different areas of the tongue were selected due to their different morphologies and properties (Image 3.1. & 3.2.). The apex of the tongue is densely packed with filliform and fungiform papillae. Filliform are small, keratinized papillae that play a mechanical role in food manipulation due to the roughness they contribute. Their presence results in an increased surface area at the front of the tongue. Fungiform are mushroom shaped papillae interspersed within the filliform papillae

throughout the tongue, which contain many taste buds and are innervated by the submandibular ganglion. The side of the tongue has fewer papillae and is mostly smooth non-keratinised mucosa. The rear portion is populated by a few large circumvallate papillae and foliate papillae.

3.2.3.3. Artificial saliva

A buffer solution to simulate saliva was adapted from (Madsen *et al.,* 2013) and was used as the eluent to wash over the tissue. Madsen *et al* (2013) tested this artificial saliva (AS) in wash off experiments and found that the retention results were like those when they used human saliva. As this current study is using the same principle experiment, this AS was chosen to best simulate conditions in the mouth. The AS was comprised of 4 mM $CaCl_2$, 10 mM KCl, 2mM $NaHCO_3$, 7mM NaCl, 6.7mM KH_2PO_4 and 2.5% pig gastric mucin (PGM) (SigmaAldrich). The pH was adjusted to 7 after PGM addition. Table 3.1. shows the concentrations of solutes present in the AS compared to human saliva [27].

Table 3.1. Concentrations of constituents present in artificial saliva compared to those found in human saliva from Dawes & Dong (1995) [27] .

Constituent	**Artificial saliva (mmol/L)**	**Unstimulated human saliva (mmol/L)**
Calcium	1.44	1.32 ± 0.11
Potassium	7.1	19.42 ± 0.79
Sodium	3.27	4.66 ± 0.79
Chloride	11.63	15.10 ± 0.79
Phosphate	4.6	5.4 ± 0.48

3.2.3.4. Labelled vs unlabelled

Retention experiments took place in an incubator set to 37 °C (Image 3.3.). An automatic pump with a syringe filled with AS was used to wash over the tissue sample at a set flow rate of 6 mL/min. A movable plastic slide was made to hold the tissue section, which was mounted on a microscope slide, so that when the AS ran over the tissue the AS flowed into a waste beaker. Images of the fluorescent sample on the tissue sections were taken on a Leica MZ10F fluorescent microscope before washing and then after set amounts of washing.

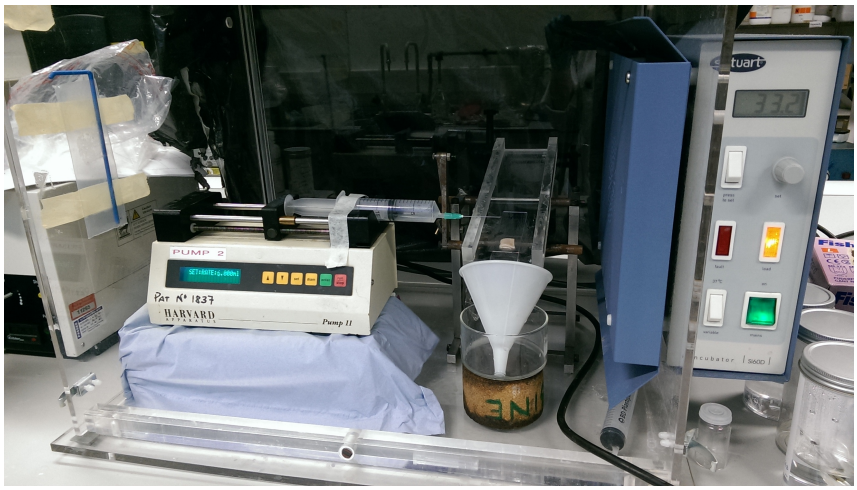


Image 3.3. Retention experiment set up.

PS that were either labelled with fluorophore or mixed with unbound fluorophore were dissolved at concentrations necessary for all to be similar viscosity at low shear in DW (Table 3.2.). The viscosity chosen was based mainly on the labelled PS needing to be concentrated enough to visualise the fluorescence.

The AS in the syringe and the tissue was brought up to 37 °C before each experiment. Tissue sections were conditioned with 1 mL AS (pump speed 6 mL/min) to ensure that all tissue started with the same amount of hydration and AS coverage. The pump speed was selected after trying various speeds and

determining this speed allowed for continuous drops of AS onto the tissue. Before the PS solution was applied with a syringe, a photograph was taken of the tissue to adjust for background fluorescence. This was necessary for the labelled PS experiments as the exposure needed to be high to visualise the fluorescence. After depositing a 30 µL drop of fluorescent PS, another photo was taken that was later normalised to be 100%. The tissue section was then washed with AS using the automatic pump and photographs were taken after 1, 2, 3, 5, 10, 15 and 20 mL washes. The photographs were analysed using ImageJ software to quantify the mean fluorescence intensity of the area where the PS was applied. Using this information, the retention of the sample can be quantified in terms of fluorescence intensity.

Table 3.2. Labelled polysaccharide concentrations used and η* at 1.3 rad/s shear rate. Each sample was tested 3 times with standard deviation (SD) shown.

Polysaccharide	**Labelled**	**Concentration (w/w %)**	**η* (Pa.s)**
LCMC	✓	5	0.49 ± 0.05
LCMC	-	5	0.57 ± 0.03
SA	✓	1.5	0.41 ± 0.02
SA	-	1.25	0.49 ± 0.11
LMEP	✓	2.5	0.44 ± 0.09
LMEP	-	5	0.47 ± 0.18

Control experiments were carried out to ensure that the relationship between fluorescence intensity and PS concentration was linear. These control experiments were done in a 96 well plate. A series of dilutions were made of a fluorescent PS sample and images were taken. To ensure that the fluorescence was linearly

correlated to the volume of the liquid, different volumes of a PS sample was put into each well and images were taken.

Sodium fluorescein in water (0.01%) was used as a negative control to ensure that there was no retention of the fluorophore without PS. Furthermore; unlabelled starch at matched viscosity was used as a non-ionic PS that does not form a viscous interconnected polymer chain network.

3.2.3.5 Unlabelled polymers at different concentrations

PS were mixed with sodium fluorescein instead of being chemically labelled to determine if this is necessary or if the same results are obtained. A concern for using unbound fluorophore in these experiments is that the fluorophore may wash off without the polymer or conversely, penetrate the tissue, making it difficult to determine what is being measured. However, drawbacks of labelling the polymers include alterations that the labelling procedure may have on the chemical properties of the polymer and hydrolysis of polymer chains during the long dialysis. Furthermore, the fluorophore is attached to the carboxylic groups of the monomer units, which will decrease the overall availability of these groups to interact with the mucosa.

In addition to comparing between labelled and unlabelled, three different viscosities of unlabelled PS were tested on the front of *ex vivo* porcine tongues to determine the impact of the viscosity of solutions on the mucoadhesive strength. The concentrations and viscosity of solutions at 1.3 rad/s shear are shown in Table 3.3. The same experiment was carried out as explained in section 3.2.3.4.

As a negative control, 0.01% fluorophore in deionsed water was used as well as a starch sample matched at the same viscosity at 1.3 rad/s (Figure 3.8.). Starch was chosen as the PS negative control as it increases viscosity but is not necessarily noted for its mucoadhesive abilities. This is most likely because starch does not fully hydrate in water, rather it forms swollen granules, and therefore does not form a viscous polymer network where mucin chains can interpenetrate. Furthermore, starch is non-ionic and does not contain any strong hydrogen bonding groups unlike the other PS. Therefore, starch was to control for the viscosity as a factor in mucoadhesion and the water negative control was to ensure that the free fluorophore was not penetrating the tissue. Table 3.3. shows the concentrations of the PS used and their viscosities.

3.2.4. Statistical analysis

For the rheology experiments two-way ANOVA was used for the different PS at various concentrations with PS type and viscosity as treatment effects. Two-way ANOVA with 3 factors was used for retention experiments to assess differences between PS and area over time. Two-way ANOVA was used for determining differences of WO_{50} values with WO_{50} and tongue area or PS as factors. A $p<0.05$ was used to determine significance.

3.3. Results and Discussion

3.3.1. Rheology of labelled and unlabelled polysaccharides

Figure 3.2. shows the viscosities of the different concentrations of each labelled PS solutions. The labelled PS were tested at the following concentrations: for SA 1.5, 2.5, 3 and 3.25 % were tested; for LMEP, 2.5, 3, 4 and 5 % were tested; for LCMC, 2, 5, 3 and 5.5%. These measurements were taken in order to find the concentrations

where the viscosities were matched at low shear rate. Figure 3.3. shows the viscosities of unlabelled PS samples at different concentrations. The unlabelled PS were tested at the following concentrations: for SA 0.45, 1, 1.25 and 1.5%; for LMEP 1.25, 4.25, 4.5, 5 and 6 %; for LCMC 1.4, 2.5, 5 and 5.5 %. The viscosities chosen for retention experiments are detailed in tables 3.2. & 3.3.

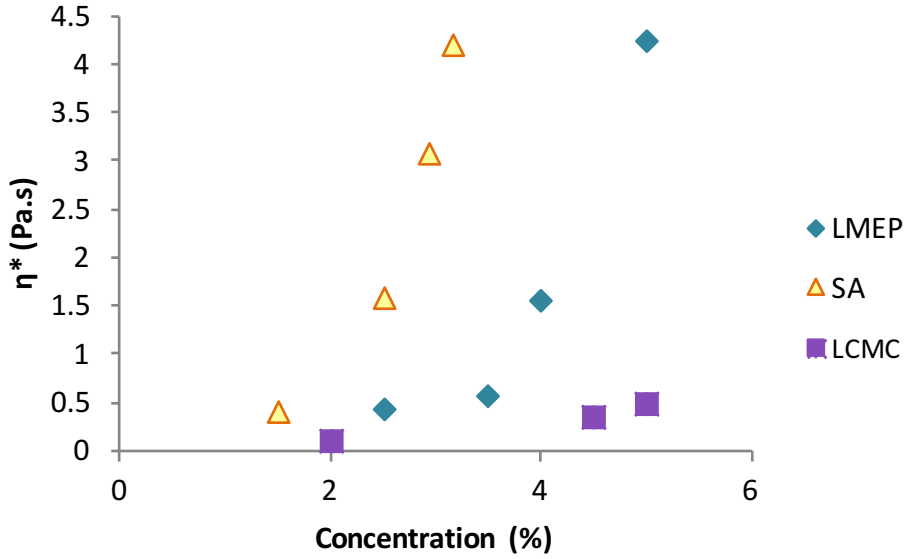


Figure 3.2. Complex viscosity (η*) of labelled polysaccharide solutions at 1.3 rad/s against concentration (w/v %). Error bars are too small to be seen here.

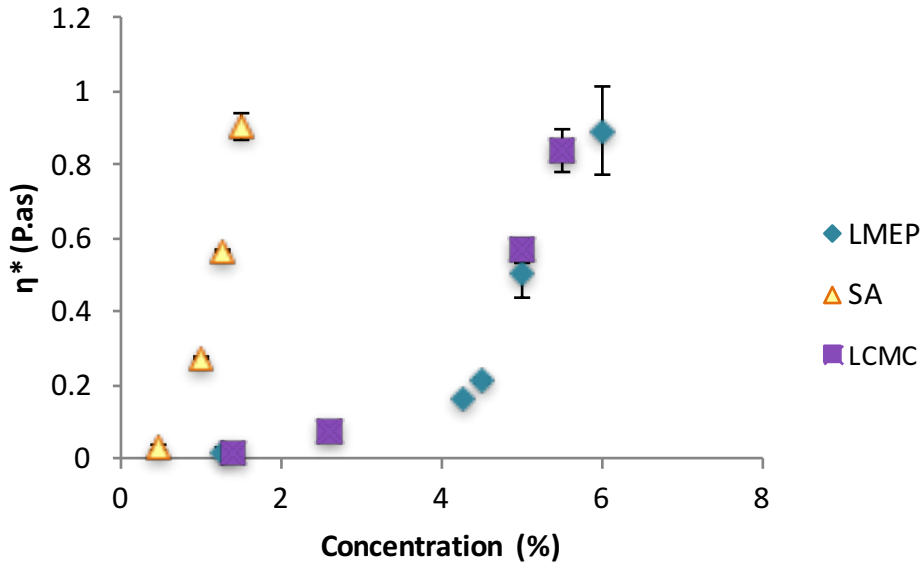


Figure 3.3. Complex viscosity (η*) of unlabelled polysaccharide solutions at 1.3 rad/s against concentration (w/v %). Error bars shown are standard deviation.

All PS samples increased in viscosity exponentially as the concentration increased. The concentration of labelled LMEP required to match the other PS viscosities was lower than the unlabelled LMEP of the same viscosity (Table 3.2.). This is most likely due to the pH of the solutions. The labelled LMEP was adjusted to pH 7 during the labelling process, however, when dissolved in DW to the desired concentration it was found to have a pH of only 6. The unlabelled LMEP, on the other hand, was adjusted to pH 7 after sodium fluorescein addition. As the pH is decreased for LMEP solutions, the viscosity also increases [28].

3.3.2. Labelled polymers retention

Figures 3.4. – 3.6. show the wash off profiles of labelled PS samples on the different areas of *ex vivo* porcine tongue. Overall, the PS adhered most to the front of the tongue ($p<0.05$) (Figure 3.4.). This is most likely due to the increased surface area due to the many small, protruding papillae present on this surface. According to the mechanical theory of mucoadhesion, an increased surface area, produced by micro-cracks and rough surfaces, increases the mucoadhesive strength. Therefore, a smooth surface, such as the side of the tongue, is not as susceptible to mucoadhesion. This is reflected in the results, the retention on the side of the tongue was significantly ($p>0.05$) less than the front and rear (Figure 3.4.). The rear of the tongue also shows good mucoadhesion (Figure 3.4. b) and this has fewer, larger papillae and finger-like protrusions.

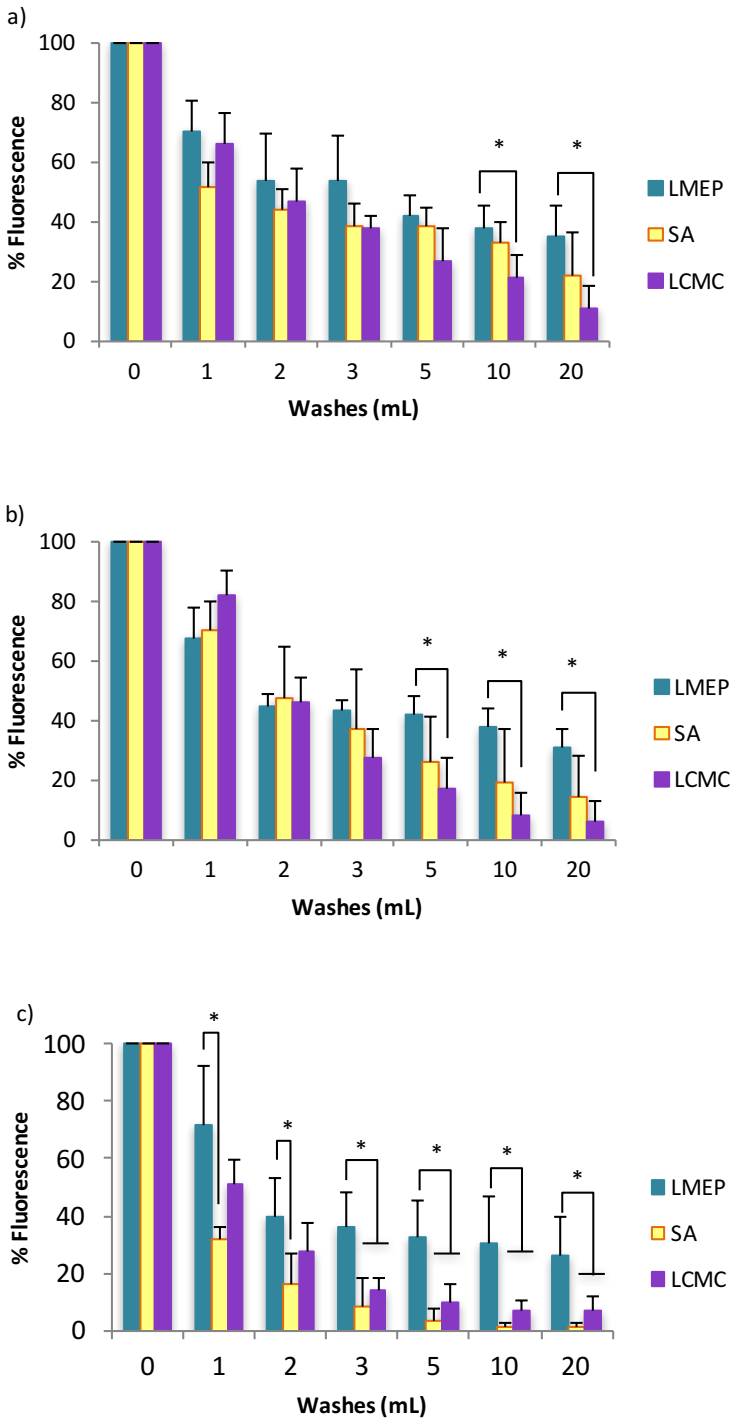


Figure 3.4. Labelled polysaccharide retention on the front a), rear b) and side c) of porcine tongue. Error bars represent standard deviation. * Signifies $p<0.05$.

The labelled PS used here are polyelectrolytes due to the many charged carboxylic acid groups they possess. LMEP and SA are known to gel in the presence of calcium ions [29,30]. The AS used contained calcium chloride so although the viscosities of

the solutions were matched before application to the tissue, once the AS was washed over samples, the Ca^{+} ions were free to interact with the PS chains to form a gel on the outer layer of the droplet. This may make it more difficult to remove by washing over the tissue with little force. However, this does not seem to be the case with the SA samples as they were not significantly ($p<0.05$) more retentive than LCMC samples, which do not gel.

An explanation for why LMEP was more retentive compared to SA might be the higher concentration used. LMEP was at 2.5% (w/v) and SA was at 1.5% (w/v). Therefore, the total amount of polymer chains available to form a gel in the presence of calcium and to interact with the mucin and tissue topology was reduced in the SA.

3.3.3. Comparison of labelled and unlabelled polymers retention

The viscosities and shear thinning behaviour of the labelled and unlabelled polymers were not significantly ($p<0.05$) different (Figure 3.5. a, 3.6. a, 3.7. a), however, the concentrations needed to be at that same viscosity was different for SA and LMEP (Table 3.2). The retention profiles of the labelled and unlabelled polymers did not differ significantly from one another on the front and rear of the tongue for all PS, which suggests that free fluorophore is a good way to measure retention of the PS. Table 3.2. shows the concentrations of the labelled and unmlabelled PS and their viscosities.

Table 3.2. Labelled polysaccharide concentrations used and η* at 1.3 rad/s shear rate. Each sample was tested 3 times with standard deviation (SD) shown.

Polysaccharide	**Labelled**	**Concentration (w/w %)**	**η* (Pa.s)**
LCMC	✓	5	0.49 ± 0.05
LCMC	-	5	0.57 ± 0.03
SA	✓	1.5	0.41 ± 0.02
SA	-	1.25	0.49 ± 0.11
LMEP	✓	2.5	0.44 ± 0.09
LMEP	-	5	0.47 ± 0.18

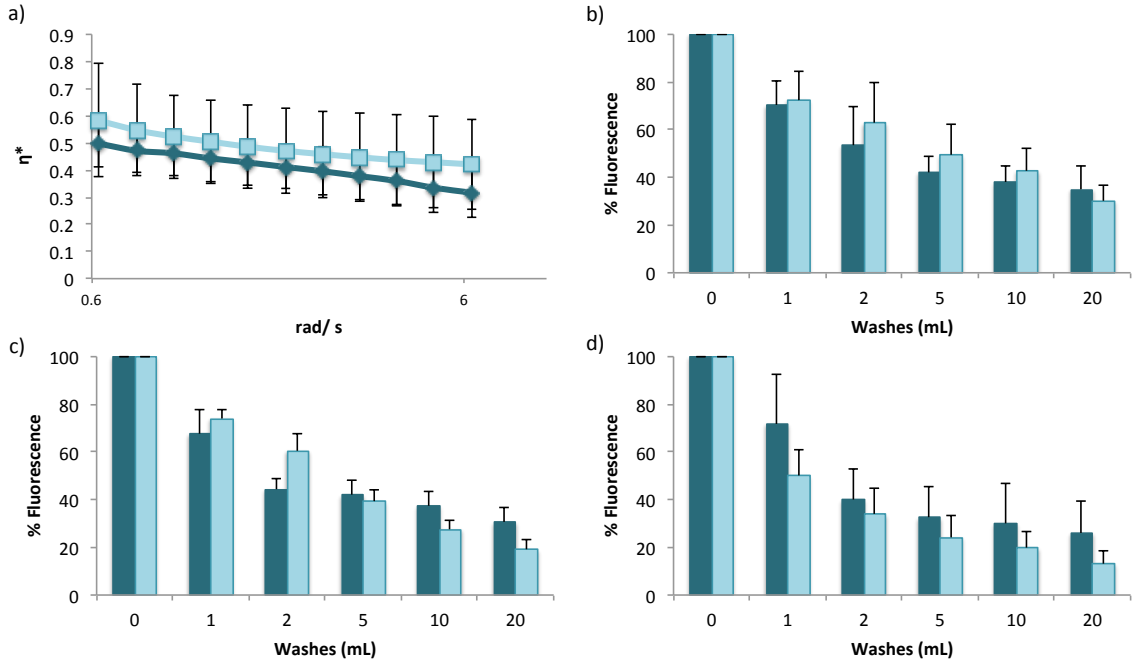


Figure 3.5. Complex viscosity (η*) of LMEP samples a) and retention profiles on the front b), rear c) and side d) of porcine tongue. Labelled polysaccharide (dark) and unlabelled PS (light). N = 3 for retention experiments. Error bars show standard deviation, * denotes significant difference p <0.05.

The retention profiles for labelled and unlabelled, LMEP and LCMC did not differ accross all three areas of the tongue (Figures 3.5. & 3.6.). The retention profiles for

SA did not significantly differ between the labelled and unlabelled PS on the front and rear of the tongue (Figure 3.7. b & c). However, there were significant ($p<0.05$) differences found on the side of the tongue (Figure 3.7. d). The unlabelled SA concentration was 1.25% for a viscosity of 0.49 Pa.s (+/-0.11) at 1.3 rad/s compared to 1.5% concentration required for the labelled SA for a similar viscosity of 0.41 Pa.s (+/-0.02) at the same frequency (figure 3.7. a). The rheology of unlabelled SA at a concentration of 1.5% is almost doubled to 0.88 Pa.s (+/- 0.28) compared to 1.25 % unlabelled. This could suggest that during the labelling process, the SA underwent some hydrolysis to reduce its viscosity. However, as there is only a significant difference on the side of the tongue and not the front and rear it may be an artefact of the tissue.

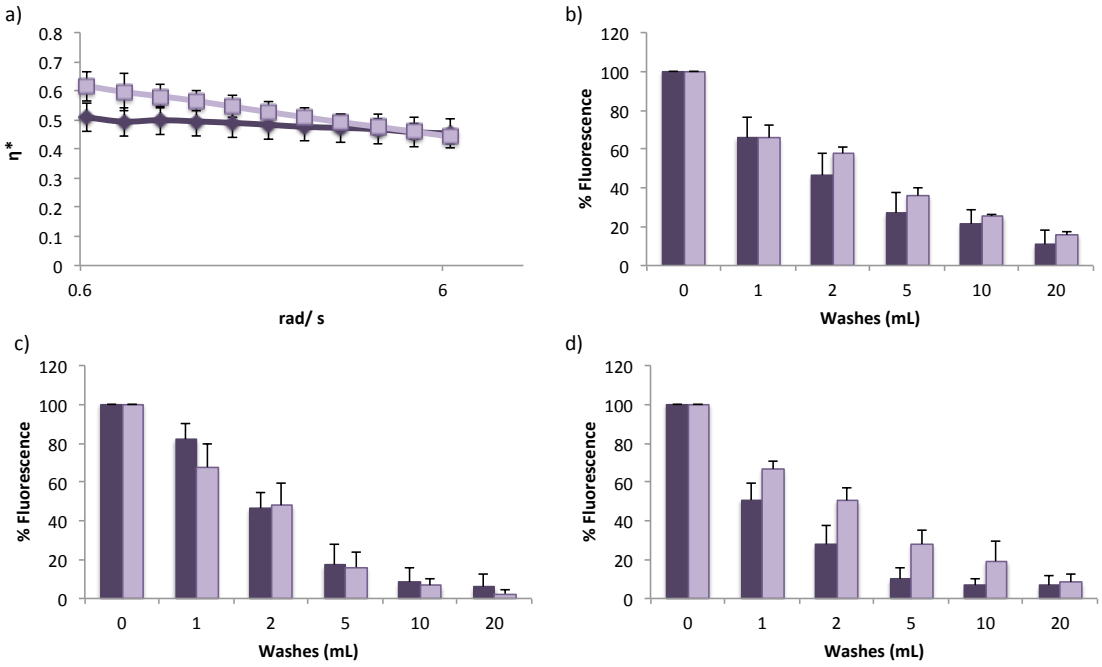


Figure 3.6. Complex viscosity (η*) of LCMC samples a) and retention profiles on the front b), rear c) and side d) of porcine tongue. Labelled polysaccharide (dark) and unlabelled PS (light). N = 3 for retention experiments. Error bars show standard deviation, * denotes significant difference $p < 0.05$.

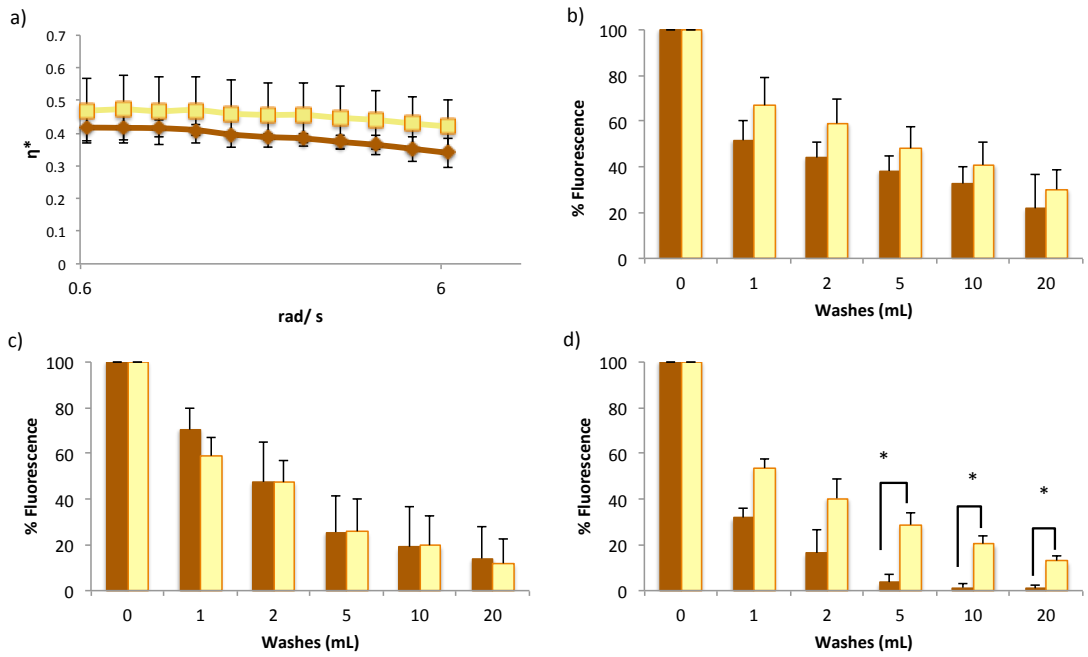


Figure 3.7. Complex viscosity (η*) of SA samples a) and retention profiles on the front b), rear c) and side d) of porcine tongue. Labelled polysaccharide (dark) and unlabelled PS (light). N = 3 for retention experiments. Error bars show standard deviation, * denotes significant difference p <0.05.

From the wash off graphs, for labelled and unlabelled PS samples, a trend line was fitted and the amount of AS needed to wash off 50% of the sample (WO_{50}) was calculated for each repeat and each area of the tongue (Table 3.4.). WO_{50} values are used to give more information about the retention of the sample, especially if the experiment ended before 50% was washed off [21]. The WO_{50} values are higher for unlabelled LMEP on the front and rear of the tongue than the other samples. Labelled LMEP was found to be the most retentive ($p<0.05$) compared to SA and LCMC, however, the difference in retention was found to be after 50% had been removed (Figures 3.5., 3.6. & 3.7.).

Table 3.4. WO_{50} (mL) values for labelled and unlabelled polysaccharides (PS) on the different areas of pig tongue. Different letters in the same column represent significant ($p<0.05$) differences between samples.

Labelled	PS	Front WO_{50} (±SD)	Rear WO_{50} (±SD)	Side WO_{50} (±SD)
✓	LCMC	2.35 (1.43) [a]	1.81 (0.10) [a]	1.25 (0.24) [a]
-	LCMC	2.48 (1.69) [a]	2.11 (0.45) [a]	2.12 (0.38) [b]
✓	SA	1.40 (0.40) [a]	2.25 (1.12) [a]	0.76 (0.22) [a]
-	SA	3.44 (1.71) [a, b]	1.89 (0.79) [a]	1.60 (0.40) [a]
✓	LMEP	2.54 (1.43) [a]	1.93 (0.31) [a]	1.37 (0.51) [a]
-	LMEP	7.09 (4.51) [b]	3.64 (1.47) [b]	1.37 (0.30) [a]

A limiting factor when comparing labelled and unlabelled samples is the intensity of the fluorescence. The labelled PS had very low labelling efficiency equivalent to a concentration of below 0.001 % fluorescein amine, whilst the unlabelled PS had 0.01% sodium fluorescein final concentration. This meant that the fluorescent microscope had to be set to a higher exposure time to get a bright enough image to measure intensity. Due to the higher exposure time, the background fluorescence from the tissue became a factor. This was not an issue with the unlabelled PS as the flourophore was bright enough to be visualised with a very low exposure time. Further to this, the fact that so few of the monomers were labelled for each polymer means that it is possible that not every PS chain was labelled and the degree of labelling may vary from one polymer chain to another. Therefore, it may be difficult to draw a conclusion that once the fluorescence detected had disappeared that meant that all of the polymer chains had been washed off.

3.3.4. Unlabelled polymers with negative controls

Table 3.3. shows the concentrations of the PS used and their viscosities. Figure 3.8.

shows the rheology profile of PS compared to the starch. Starch is a lot more shear thinning than the other PS which will have an effect when the PS is masticated.

Table 3.3. Unlabelled polysaccharide concentrations used and η* at 1.3 rad/s shear rate. 9 measurements were taken for each polymer sample ± standard deviation (SD). Viscosity ranking for each PS refers to high (H), medium (M) and low (L) viscosity.

Polysaccharides	**Viscosity Rank**	**Concentration (w/w %)**	**η* (Pa.s)**	
LMEP	H	6	0.89	± 0.12
LMEP	M	5	0.47	± 0.18
LMEP	L	1.25	0.011	± 0.002
LCMC	H	5.5	0.98	± 0.08
LCMC	M	5	0.57	± 0.03
LCMC	L	1.4	0.015	± 0.002
SA	H	1.5	0.88	± 0.28
SA	M	1.25	0.49	± 0.11
SA	L	0.45	0.024	± 0.004

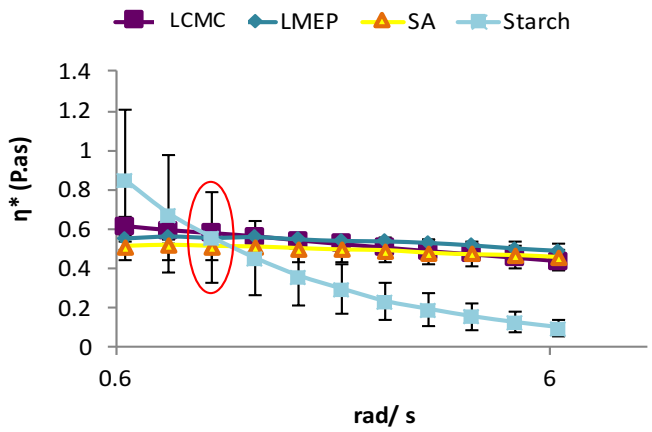


Figure 3.8. Complex viscosity (η*) of unlabelled PS and starch over increasing frequency. Error bars represent SD. Red circle is where viscosities were matched.

Figure 3.9. shows the retention of the samples on the different areas of the tongue. The negative water control shows that the unbound fluorophore does not penetrate into the tissue as the majority of it washes off after the first wash. The starch sample is somewhat retentive but is still significantly ($p>0.05$) less retentive compared to the other PS.

3.3.5. Viscosity dependant retention of unlabelled polymers

The concentration and thus viscosity of a polymer solution is an important consideration for mucoadhesion. The concentration of polymer is thought to reach an optimum when the strength of mucoadhesion is at maximum. Past this point, the polymer chains may become impervious to hydration and therefore, mucin chains are unable to penetrate and interact [31,32]. Generally, an increase in viscosity will lead to an increased mucoashesive bond between the polymer substance and the mucosa and the formulation becomes more resistant to applied stress.

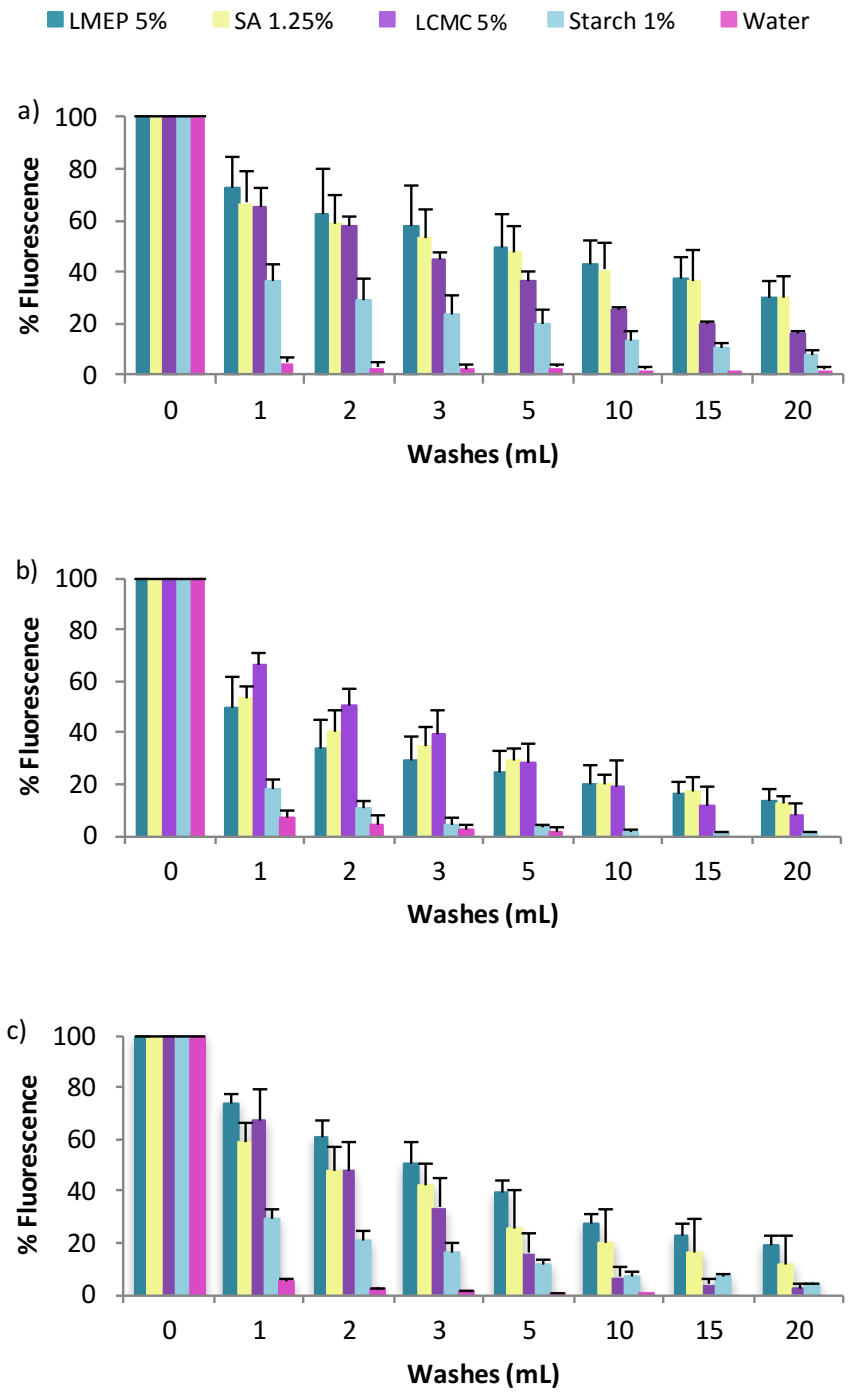
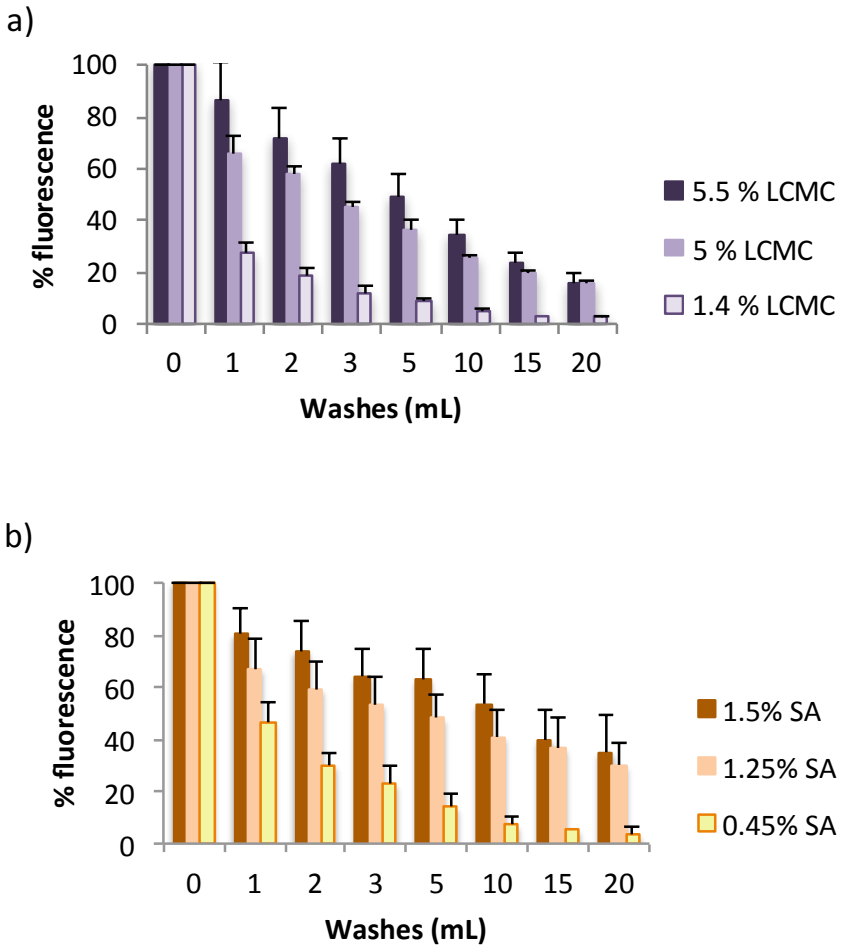


Figure 3.9. Retention profiles of polysaccharide samples and sodium fluorescein in water on the front (a), rear (b) and side (c) of *ex vivo* porcine tongue. Error bars are SD.

Three viscosities of each PS were tested in the retention experiment to measure the effect of viscosity on mucoadhesive strength. For each PS, viscosities were

chosen to be low, medium and high viscosity (Table 3.3.). The results show that for all PS the lowest viscosity solutions were significantly ($p>0.001$) different to the medium and high concentrations (Figure 3.10.). This suggests that higher viscosity increases the mucoadhesive strength. More concentrations would be required to investigate this further for this experimental method.



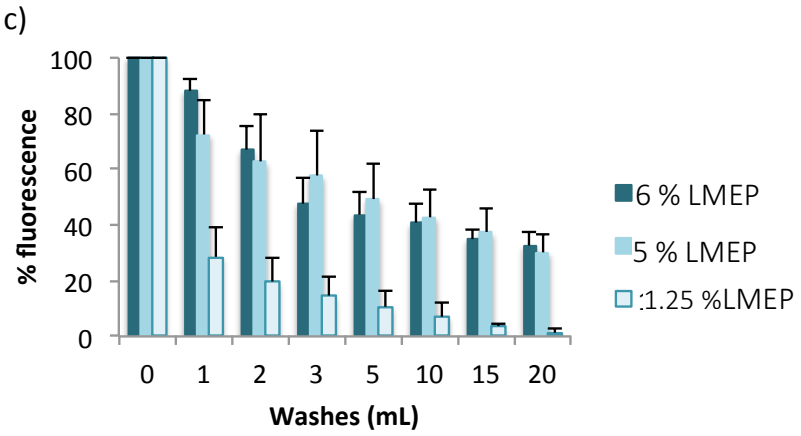


Figure 3.10. Retention profiles of different concentrations of a) LCMC, b) SA & c) LMEP. All lowest concentration for each polysaccharide are significantly different ($p<0.05$) than medium and high concentrations.

There are no significant differences between the medium and high viscosity solutions for all polymers on the front of the tongue (Figure 3.10.). This could suggest that an optimum concentration has been reached for these polymers. Another possibility is that the concentration required to make a significant difference increases exponentially as viscosity increases. Futhermore, the gap between concentrations used was not equal between low, medium and high as the relationship between concentration and viscosity is not linear (Figures 3.2. & 3.3.).

The lowest viscosity samples for each PS retained significantly ($p<0.001$) less at after each wash (Figure 3.10.). The high and low viscosity samples had significantly ($p<0.05$) different WO_{50} values for all three PS (Figure 3.11.). Furthermore, Figure 3.11. shows the linear relationship between η^* and WO_{50} for LCMC ($r^2 = 0.98$) and SA ($r^2 = 0.91$) samples. The medium viscosity LMEP sample does not follow the trend and is due to one repeat having a much higher WO_{50} value than the others.

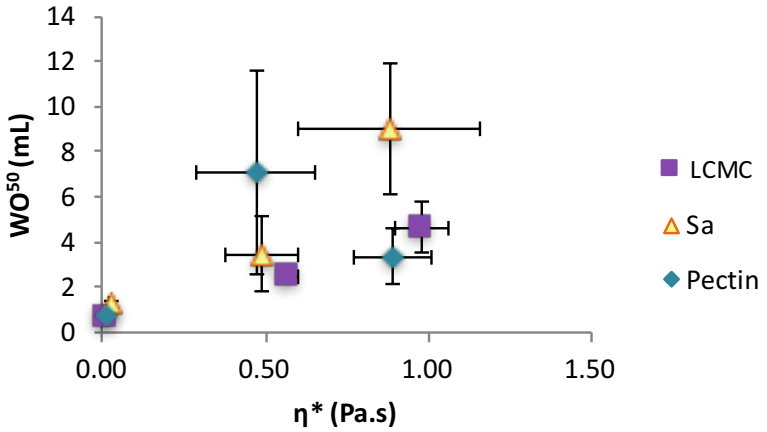


Figure 3.11. The relationship between the WO^{50} values and the complex viscosity (η*) of samples. Eorror bars represent ± standard deviation.

3.4. Conclusions

Food grade polymers were tested for their mucoadhesive strength on the tongue, a tissue rarely investigated in these types of tests. All three mucoadhesives were found to exhibit good mucoadhesion properties in liquid formulations, on different areas of the tongue compared to starch and water as negative controls. The limited differences between the labelled and unlabelled polymers suggest that it may not be necessary to label the polymers for this experiment. However, it will depend on the polymer and what is being measured. A good approach may be to use labelled polymers and free fluorophore of different excitation wavelengths in the same experiment to compare the difference. The different concentrations used confirm the sensitivity of this experiment, where a low viscosity is washed off relatively quickly compared to higher viscosity solutions.

3.5. References

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Chapter 4: Time intensity of tastants in mucoadhesive polysaccharide matrices

4. 1. Introduction

The mucoadhesive nature of LMEP, LCMC and SA was established in chapter 3 using an *in vitro* method to assess retention of liquid formulations. The ability of these PSs to retain tastants in the mouth for longer has not yet been investigated. This chapter is the preliminary work to elucidate whether mucoadhesive PSs retain the tastants, sodium and glucose, for longer compared to water. Furthermore, the impact on tastant perception was measured by time intensity profiling with a trained sensory panel.

4.1.1. Taste perception

The perceived flavour experience of a given food is influenced by many factors. These include physicochemical interactions between the flavour compounds and the food matrix, physiochemical interactions with the oral anatomy, cross modal interactions between the senses, and psychological factors such as expectation, emotion and familiarity. Therefore, flavour perception can be influenced at any of these levels.

Chemosensory flavour perception can be broken up into two distinct parts; aroma and taste perception. Taste is experienced due to the tastants present in the foodstuff. Five basic taste perceptions are confirmed to exist, these are salty, sweet, bitter, sour and umami. These perceptions are elicited by tastants. Tastants are the molecules that bind to the receptors on taste cells which, following transduction, result in the perception of one of the five tastes. For example, sucrose is a sweet tastant and monosodium glutamate is an umami tastant. At a purely physiological

level, for perception of any of these five tastes to occur, the tastant must reach the appropriate receptor or ion channel. For this to occur, the tastant must be released from the food matrix, diluted in the saliva and diffuse into the taste bud pores of the papillae, where many taste receptor cells are located. Fungiform and foliate papillae house many taste buds. Each taste bud contains between 50 and 100 taste cells with receptors and ion channels capable of eliciting a taste response [1].

Salt taste is elicited by cations such as sodium, passing through ion-gated channels, such as the epithelial sodium channel (ENaC), located on taste cells [2]. This influx of positively charged ions leads to depolarisation of the taste cell membrane, which in turn causes an influx of Ca^{+}. This leads to the release of neurotransmitters that activate the neurons associated with the taste cell, leading to an action potential. Glucose, on the other hand, is perceived as sweet when the glucose molecules bind to Type I G-coupled protein receptors (T1R2 and T1R3) on the outside of the cell, which leads to a signal cascade inside the cell. This too ends in a depolarisation of the cell membrane causing an action potential to be fired [3].

4.1.2. Salt reduction in industry

Over the past few decades, the food industry has reduced the salt content of processed food products. The pressure for the industry to reduce sodium is due to the relationship between high sodium diets and hypertension, leading to cardiovascular disease [4]. Various methods have been employed in order to reduce salt whilst maintaining an acceptable taste and flavour profile. One of the most successful approaches taken was an industry wide gradual reduction of salt contents, thereby easing the change on the consumers. This gradual adaptation has been reflected in the literature [5] where a gradual decrease of salt added to white

bread over several weeks maintained consumer acceptance. Similarly, repeated exposure studies have shown that consumers learn to like the lower salt alternatives [6].

Another option for salt reduction is the replacement with other salts such as potassium chloride, although the perception of saltiness is weaker and potassium has also been shown to be perceived as bitter [7]. Aroma has also been used to enhance the saltiness of food with a combination of salt replacers and congruent aromas [8]. Other methods to further reduce salt content whilst maintaining salty perception include reducing salt particle size for topically applied flavourings such as those used on crisps [9,10]. Heterogeneous distribution of salt and congruent aromas have also been found to enhance saltiness in a variety of snacks [11–13]. Air fillers have been investigated as a means to enhance the saltiness perception within hydrogels and have been found to successfully reduce salt content by 80% without a loss of salt perception [14].

4.1.3. Perception alterations due to polysaccharides

Flavour and textural alterations caused by the addition of PSs to food have been of interest for a long time. PSs are highly water-soluble, thickening and gelling agents that impart desired textural characteristics to a food product. Most PSs used in the food industry are virtually tasteless, however, the impact they have on flavour perception of the foods to which they are added can be drastic. Where PSs are used to increase viscosity, this will have a subsequent effect on aroma release from the fluid matrix and may reduce mobility of tastants and hence reduce their ability to bind to taste receptors.

Much of the literature investigating the impact of PSs on flavour perception has used simple systems such as aqueous solutions [15–17], emulsions [18,19] or model dairy drinks and desserts [20–23]. The type of PSs used has been found to show variability in flavour perception independent of viscosity factors [24]. Specifically, PSs that exhibit random coil structure in solution have a larger impact on taste perception than granular PSs such as starch [24]. Furthermore, chain length of the PS is important to consider as longer chains will overlap more creating a mesh of polymer chains that can physically trap flavour molecules within [25].

Although it is generally accepted that as viscosity increases, the perception of taste and flavour decreases and there are various theories that account for these phenomena. Baines & Morris (1988 & 1987) [26] [27] were the first to show that flavour perception decreased when the PS concentration reached a critical point such that the polymer chains overlapped with one another, deemed the coil overlap concentration (c*). At c* an abrupt increase in viscosity is observed along with a decrease in flavour perception. However, they did not offer an explanation as to why both tastant and aroma were affected when the mass transfer of these molecules is very different.

Later, Cook et al. [15,16] added to this theory by observing perception changes in solutions thickened with hydroxypropylmethyl cellulose (HPMC). One study investigated the impact on basic tastes and found that above c*, perception was decreased for sweet and salty attributes but not for bitter and sour [16]. The authors suggest psychological factors may play a role with tactile tri-geminal stimulus being altered by enhanced viscosity. Increases in viscosity are associated

more with sweet foods rather than bitter or sour, which tend to be thinner; so an enhanced viscosity may be associated with a stronger sweet taste which the PSs will not provide [16]. A follow-on study investigated the delivery of aromas to the nasal cavity as well as perceptual changes occurring above c* in HPMC thickened solutions. The authors found that although perception of aromas decreased for solutions above c*, the amount delivered was not influenced by PS concentration. Instead the authors conclude that a reduction in congruent tastant delivery and thus perception, has a cross modal impact to reduce the associated aroma [15,28].

The studies investigating changes in sensory perception of foods thickened with PSs are somewhat limited in their experimental design. The large majority ask panellists to score the sample intensity of any given attribute at a static time point [17,27,29,30]. Whilst some measure in-nose or headspace aroma release over time, the dynamic flavour perception is not usually investigated. Usually scoring takes place whilst the sample is in the mouth and a reduction in intensity is recorded with PS thickeners. The aim of this work is to explore the change in flavour delivery over time, which may be altered by the addition of mucoadhesive PSs as food additives.

How PS thickeners influence the organoleptic properties has been the topic of many research papers. However, due to the variety of PSs and infinite combinations of PS, food types and flavour compounds, the food industry would benefit from further research into the underlying mechanisms governing flavour perception. The fact that many PSs are also mucoadhesive is seldom taken into consideration. The aim of this chapter is to elucidate the impact of mucoadhesive PSs in thickened solutions on salt and sweet perception. Furthermore, the

concentration of tastant present in the mouth over time was investigated to determine if mucoadhesive PSs retain tastant molecules within their matrix in the oral cavity.

4.2. Methods

4.2.1. Materials

LMEP, SA, and LCMC were used as detailed in Chapter 2. All other chemicals and reagents were purchased from Sigma Aldrich. The viscosity of the PS solutions were matched at 1.3 rad/s for a viscosity of 17 mPa.s (± 6) (Chapter 3). Concentrations required for this viscosity are in Table 4.1.

4.2.2. Sample preparation

PS were dispersed in DW and stirred for 3 hours before either sodium chloride or glucose was added. LMEP was adjusted to pH 7 with sodium bicarbonate, in order to be the same pH as the other PS. For the salt perception experiment the amount of sodium in each sample was controlled. The PS inherently contains varying amounts of sodium so an Economical Flame Photometer (230 VAC, 50/60 Hz) was used to determine sodium content of each PS solution.

Standards were made ranging from 2 mg/L to 10 mg/L sodium with sodium chloride (Sigma Alridch, Poole, UK) in DW. DW was used as a blank to set the flame photometer to 0 and the highest standard was set to 100. A calibration curve was produced with a R^2 of 0.99 (Appendix 1). PS containing samples were diluted 1000 times with DW and read on the flame photometer. The appropriate amount of NaCl was added to each sample so that the final concentration of sodium was 0.3% (Table 4.1).

Table 4.1 Sample formulation. NaCl added to achieve total sodium equivalence (0.3%w/v) in all samples, considering the sodium inherent in each PS.

Sample	Matrix	Concentration (% w/v in water)	mg/100mL NaCl added	g/100mL glucose added
1	SA	0.45%	181	0
2	LMEP	1.25%	111	0
3	LCMC	1.40%	121	0
4	Water	-	300	0
5	SA	0.45%	0	8
6	LMEP	1.25%	0	8
7	LCMC	1.40%	0	8
8	Water	-	0	8

4.2.3. Perception of tastants in polysaccharide matrices

Sensory experiments were designed to elucidate whether viscous, mucoadhesive PSs prolonged the perception of sodium and glucose over time compared to water. Eleven trained panellists from the University of Reading Sensory Science Centre were used to assess the samples. Panellists were trained to assess samples using a single attribute, either sweetness for the glucose containing samples or saltiness for the NaCl containing samples. Time intensity (TI) sensory testing was used to measure the intensity of salt and sweet perception over defined time periods. TI testing allows the panellists to record the dynamic changes in perception over time for the attribute. Prior to experiments, panellists were trained on the Compusense software used for TI testing.

Each sample was tested in duplicate and labelled with 3 digit random codes. Panellists were seated in isolated sensory booths with a computer, filtered portable water (at room temperature) and crackers (Carr's Water Biscuits, United

Biscuits, UK) for pallet cleansing. Panellists were presented the samples monadically in a balanced design to gather data for 3 time points; 5, 30 and 120 s. Panellists tested either the salt model or the sweet model first and the order was balanced throughout all panellists. Panellists held the sample in their mouth for 10 s, with little manipulation and were then prompted to swallow and start scoring instantly. The type of PS in the samples was fixed each session so that individual panellists had the same polymer for the salt test and the sweet test but the PS would differ between panellists each session. For example, a panellist could have been given a sample of LCMC with glucose and asked to score sweetness for 5 s then another of the same sample for 30 s and finally 120 s. After this session the panellist would test LCMC with NaCl at all three time points.

4.2.4. Saliva collection

Before the start of each session, panellists' saliva was collected to determine their baseline sodium concentration. After swallowing each sample and scoring for the predetermined time interval, panellists immediately scraped their tongue with their teeth and expectorated their whole saliva into labelled tubes, for analysis of tastant concentration. TI data and saliva was collected during the same experiment so that real time perception of taste was recorded along with quantification of tastant remaining in the mouth.

Whole saliva samples were diluted with DW; 40 ml DW for salt samples, 10 ml DW for glucose samples. All saliva samples were centrifuged at 3000rpm for 30 minutes to remove cells. The supernatant was stored in eppendorf tubes at -20°C until analysis.

4.2.5. Flame photometry

Saliva samples were analysed by flame photometry for sodium concentration like that described in 4.2.2. however, most samples were dilute enough to be within the calibration so no further dilution was needed. Those that has readings higher than 100 were diluted 2-fold and were then within the calibration range.

4.2.6. Capillary electrophoresis

The capillary electrophoresis (CE) method used was adapted from Soga (2000). Briefly, a HP3D CE with diode-array detector and Agilent ChemStation software (Santa Clara, CA, USA) was used. Electrophoretic separation was performed at a constant pressure of 50 mbar, with a 6 second injection of sample, followed by a four second injection of buffer. A fused silica capillary (Agilent, Stockport, UK) was used which measured 50 μm i.d., 112.5 cm in length, with an effective length of 104 cm to the detector, maintained at 15 °C. An anionic buffer (pH 12.1) purchased from Agilent (Palo Alto, CA) was used for sample separation and the column was preconditioned for 10 minutes with buffer before each run. A constant voltage of 30 kV was applied with a negative polarity. Detection was at 350/20 nm for 40 min. External standards were used for the quantification of the analytes of interest. These standards were 0, 0.5, 1, 3 and 4 mg/mL glucose in DW.

4.2.5. Statistical analysis

Time intensity parameters (Figure 4.1.) were extrapolated from the raw data produced in the experiments. Imax, Tmax, AUC, decline angle, incline angle, plateau and duration were analysed using one-way, repeated measures ANOVA (rmANOVA) with PS as a treatment effect and panellists as random effect. The

saliva concentrations of tastants were analysed using two-way, rmANOVA with PS and time as treatment effects. Data were analysed in SPSS version 21 (IBM, UK) and a value of $p<0.05$ was used to determine significance.

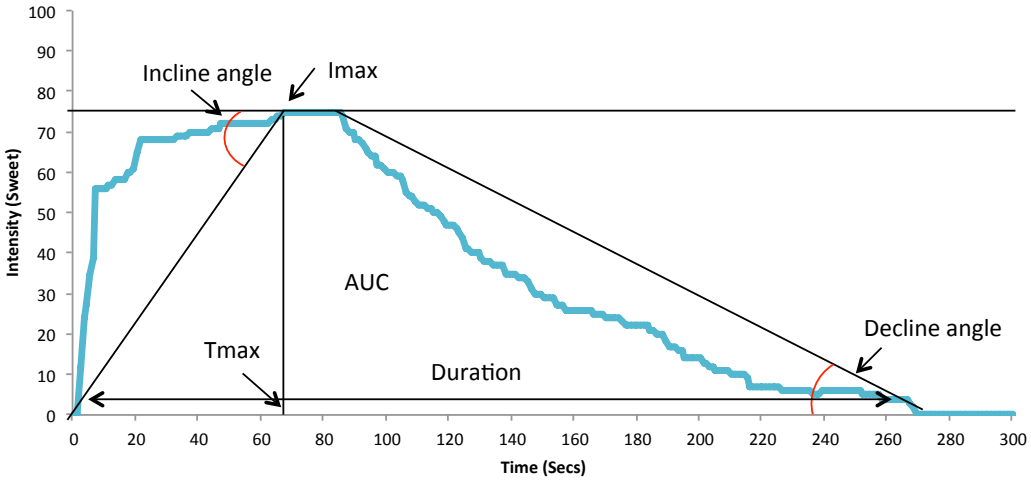

Figure 4.1. Example time intensity curve with extracted parameters indicated. Imax is the maximum intensity reached, Tmax is the time it took to reach the maximum intensity, the area under the curve (AUC) is the "total" perception value, duration is how long the attribute was experienced for, incline angle is the angle created between the start time and the maximum intensity time and value, and decline angle is the angle from the peak intensity value to the end of perception.

4.3. Results and Discussion

4.3.1. Salt perception of samples

Eleven trained panellists scored 3 different PS matrices and a water control containing sodium on the saltiness of the sample over time post swallowing. Table 4.2. shows the extrapolated parameters from time intensity curves produced by each panellist for the 120 second experiment scorings. There was no significant ($p<0.05$) difference between the samples for the duration, time to maximum intensity (Tmax) and rate of increase of salty taste (incline angle). The maximum

intensity (Imax) scored for the LCMC and water only samples were higher than for the SA samples. Furthermore, the area under the curve (AUC) for LCMC was significantly ($p>0.05$) higher than for the LMEP and SA matrices. This suggests that the LCMC and water samples did not differ from each other in the perception of saltiness over time, but the SA sample was significantly ($p<0.05$) reduced in the perception of saltiness.

This reduction in saltiness may be explained by the slightly higher viscosity of the SA sample. Samples were matched at a high shear rate of 50 rad/s as this is quoted as the shear rate of the mouth [32,33]. However, below this shear rate, SA had a higher viscosity than the other two PS samples. The relationship between the shear rate and flavour perception in viscous solutions is not completely understood and the shear rates in the mouth are thought to vary drastically [34,35]. Therefore, the viscosity of these samples above and below 50 rad/s may also be important.

Table 4.2. Time intensity parameters from salt perception experiments from 11 panellists scoring each sample in duplicate. Different letters vertically represent statistically different groupings.

Matrix	Tmax (s)	Imax	Plateau (s)	Incline (°)	Decline (°)	AUC	Duration (s)
LMEP	19.58 [a]	37.13 [a, b]	10.8 [a]	40.31 [a]	19.54 [a, b]	2490.77 [a]	101.89 [a]
SA	19.58 [a]	32.96 [a]	19.15 [a]	34.71 [a]	13.44 [a]	2315.63 [a]	109.78 [a]
LCMC	19.67 [a]	44.96 [b]	11.15 [a]	45.4 [a]	16.6 [a, b]	3314.52 [b]	104.61 [a]
Water	14.71 [a]	44.91 [b]	9.95 [a]	42.01 [a]	17.5 [b]	3089.48 [a, b]	115.94 [a]

4.3.2. Sodium retention in the mouth

The panellists' saliva was collected 5, 30 and 120 s after the sample was swallowed. For each time point a new sample was given as the panellists had removed all their saliva by scraping for each time point. As saliva contains sodium ions, baseline concentrations were established and subtracted from the results for each panellist (mean 1.1mg/ whole saliva ± 0.3). The amount of sodium in panellists' saliva after swallowing decreased with time (5, 30 and 120 s) (Figure 4.2.). In addition, at 5s and 30 s, the sodium content of the saliva was higher when LCMC was used, compared to the other three matrices. At 30 s, the sodium content of the saliva was higher when LMEP was used compared to SA and water. There was no difference between SA containing samples and the water only samples at any time.

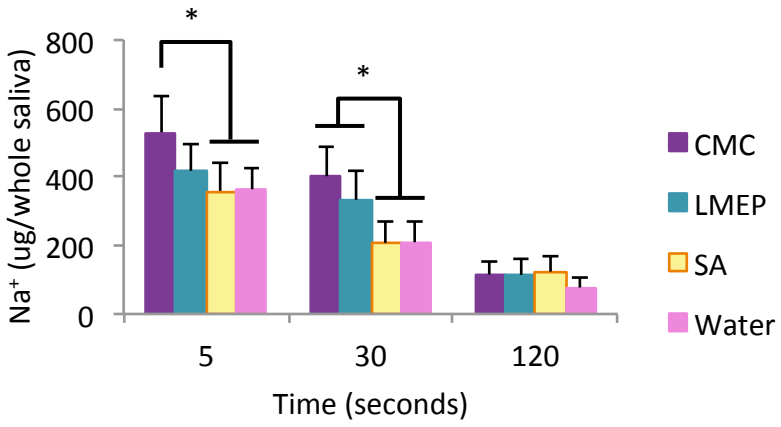

Figure 4.2. Sodium concentration in panellists' saliva at 3 time points after swallowing. Error bars represent standard error of the mean. N= 11 in duplicate and * signifies $p < 0.05$.

As all PS containing samples were matched for sodium content, the amounts present in the panellists' saliva is representative of the amount left in the mouth after swallowing minus the individuals' baseline sodium concentration. The results

suggest that mucoadhesive PSs (LCMC and LMEP) retain sodium ions better than water samples. However, the results also show that samples with a SA matrix do not retain sodium ions better than water alone. A possible explanation for this is that the SA containing samples were less mucoadhesive, however, the *in vitro* retention studies conducted in Chapter 3 would suggest otherwise. Another consideration is the concentration of the PS in the samples. LCMC and LMEP contained 1.4 and 1.25 %, respectively whereas SA was used at only 0.45 %, in order to achieve equivalent sample viscosity. This difference in concentration means there are less PS chains to interact with Na^+ in solution or physically entrap the Na^+ so if the PS is being retained on the oral surfaces, the Na^+ associated with it will be less if the concentration of the PS is lower.

When comparing the *in vivo* retention and salt perception data it is interesting that although both LMEP and LCMC appeared to retain sodium after swallowing (Figure 4.2.) they were perceived differently. This perceptual difference suggests that the amount of sodium in the panellists' saliva does not necessarily represent how much is reaching the taste receptor cells necessary to elicit a salt perception. The LMEP sample may retain the sodium but if it is not in contact with the receptor cells then it will not result in a salty perception. Interestingly, the LCMC containing samples did not differ in the perception of saltiness compared to the water samples, but retained the sodium for longer in the panellists' mouths. This may be because although LCMC reduced mass transfer of sodium ions to the receptors, more sodium ions will be swallowed from the water only samples.

4.3.3. Sweet perception of samples

All the parameters extrapolated from the time intensity curves for the sweet,

except decline angle, were not significantly different between samples (Table 4.3.). The decline angle for the water only matrix was significantly ($p<0.05$) higher than that for the LMEP matrix. This indicates that the perception decreased quicker after the water only sample compared to the LMEP containing sample.

Apart from the decline angle of water and LMEP matrices the sweetness scoring over time for the different matrices containing glucose did not differ. This suggests that the perception was not greatly altered by the presence of the PSs at this viscosity. The minimal differences in perception may be due to the PS concentration being low enough not to interfere with mass transfer of the glucose molecules to the taste buds. Hollowood *et al.* (2002) [17] found that as the concentration of PS in solution increased above a critical point (c*), the perception of salt and sweet was stunted. This decrease in intensity was more pronounced in the salt samples with the sweet samples needing a higher viscosity to reduce the perception significantly. This could explain the results from this chapter.

Table 4.3. Time intensity parameters from sweet perception experiments from 11 panellists scoring each sample in duplicate. Different letters vertically represent statistically different groupings.

Matrix	**Tmax (s)**	**Imax**	**Plateau (s)**	**Incline (°)**	**Decline (°)**	**AUC**	**Duration (s)**
LMEP	24.83 [a]	42.91 [a]	11.38 [a]	42.86 [a]	15.93 [a]	2959.27 [a]	117.88 [a]
SA	24.75 [a]	39.21 [a]	19.5 [a]	37.96 [a]	19.22 [a, b]	2807.35 [a]	117 [a]
LCMC	15.96 [a]	43 [a]	17.25 [a]	53.77 [a]	19.46 [a, b]	3212.31 [a]	116.31 [a]
Water	19.75 [a]	44.33 [a]	24.25 [a]	42.32 [a]	21.58 [b]	3347.60 [a]	118.31 [a]

4.3.4. Glucose retention in the mouth

A complete set of results were collected for concentrations of glucose in saliva only at the 30 s after swallowing time point, therefore, statistical analysis was performed only on this data (Data incomplete at remaining time points due to instrumental break down). At 30 s after swallowing, the glucose concentration in panellists' saliva was higher after the LCMC containing samples compared to the LMEP matrix (Figure 4.3.).

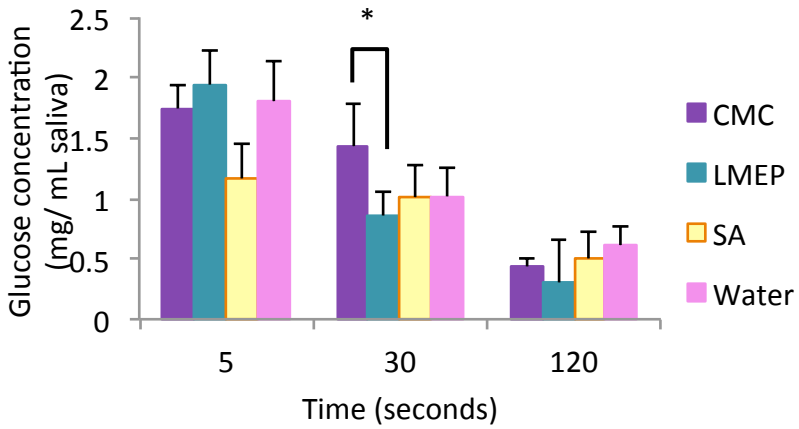

Figure 4.3. Glucose concentration in panellists' saliva at 3 time points after swallowing. Error bars represent standard error of the mean. N= 11 in duplicate and * signifies $p < 0.05$.

The data are inconclusive due to the lack of time points other than 30 s post swallow. It is therefore difficult to draw any firm conclusions relating the perception data to the *in vivo* retention data. However, for the 30 second time point the LCMC containing samples were significantly ($p<0.05$) higher than the LMEP samples with a similar trend for other samples. Although it appears that there may have been more glucose retained after the LCMC samples, the perception data did not differ from the other PS or water samples. This could be because the amount that was retained was not high enough to produce a measurable difference in

perception or it could be that the LCMC created a barrier between the glucose and its receptor, essentially inhibiting diffusion.

As the saliva samples are no longer available from this experiment the missing data can not be recovered. However, an experiment could be set up where saliva is collected from a smaller number of participants after swallowing a sample with PS and glucose. A glucose oxidase assay could be used to analyse the glucose concentration instead of CE, which would be time efficient and be less likely to encounter technical failures.

4.4. Conclusions

Aqueous PS matrices alter the temporal perception and retention of sodium. Increasing the viscosity of samples with LMEP and SA resulted in reduced intensity and overall perception of sodium compared to LCMC-containing samples and water alone. The retention of sodium in the mouth was found to be higher in two of the mucoadhesive samples, LCMC and LMEP; but the SA sample was not different to the water sample. This evidence suggested a complicated relationship between the concentration of tastant present in the mouth and the perception of that tastant. This disconnect between actual amount of tastant present and the perception of the tastant may be due to physical effects such as a reduction in mass transfer of the tastant or adaptation effects. There was not a difference in perception of sweetness in samples thickened with PSs. The data is inconclusive for the retention of glucose in the mouth, however, the trend was like the salt experiments where LCMC tended to retain more tastant.

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Chapter 5: Mucoadhesive polysaccharides modulate sodium retention, release and taste perception

5.1. Introduction

Results from chapter 4 suggested that samples with a LCMC matrix retained more sodium in the mouth after swallowing. Therefore, in this chapter samples with LCMC as the matrix was compared to a relatively non-mucoadhesive matrix and water only. Amylase resistant starch (detailed in Chapter 2) was used as a relatively non-mucoadhesive control. LCMC is a linear polysaccharide made of β 1 -4 linked glucose units with some of the hydroxyl groups substituted with carboxymethyl groups to render it soluble in water. Starch, on the other hand, is a branched polysaccharide consisting of many glucose units joined by α 1 - 4 glycosidic bonds in the form of amylose (helical) or amylopectin (linear). Unlike LCMC, starch swells within granules, unless gelatinised, limiting the formation of interconnecting chains.

Flavour balance is a challenge presented in low fat food formulations as the reduction of the hydrophobic matrix of a food results in the increased release of hydrophobic aroma compounds from food matrices[1–3]. This results in an aroma release that peaks and rapidly falls compared to higher fat counterparts where the release is more uniform over time [4]. Some studies have found that although perception of aroma from higher viscosity solutions can be lower, in-nose aroma concentration stays the same and that the determining factor for perception is the congruent tastant perception [5,6]. Therefore, if mucoadhesives can deliver tastants at a lower rate over time, then aroma perception may be adjusted

accordingly, resulting in a product with a flavour profile like that of a high fat product.

Lian et al. (2004) and Malone and Appelqvist (2003) attempted to prolong aroma delivery using gelled emulsion particles of calcium alginate. The results suggest that aroma release can be controlled by particle size. Emulsions and encapsulation of aromas have been widely researched, however, utilising mucoadhesion to prolong flavour delivery is a relatively novel concept. For the past few decades mucoadhesion has been researched in relation to pharmaceutical applications, however, more recently the potential for their use in food products to prolong flavour delivery has been considered [8–10]. The work in this chapter investigates the temporal retention, release and subsequent perception of sodium, a tastant, in a model aqueous food prepared with either starch, LCMC or just water.

The work in this chapter is the first to show that food grade mucoadhesives are retained on the tongue *ex vivo*, alter the temporal perception of saltiness over time compared to non-mucoadhesives, and prolong sodium retention in the mouth despite a reduction in perception. Perception data was collected after consuming samples using a sensory method called progressive profiling to understand changes in perception over time. Furthermore, an *in vivo* retention experiment was developed to ascertain the differences in sodium levels retained by the mucoadhesive sample compared to non-mucoadhesive samples. Chapter 4 was used as preliminary evidence that LCMC can retain sodium ions for longer in the mouth and the work in this chapter builds on that evidence. The hypothesis for this work was that a mucoadhesive PS would retain sodium for longer in the

mouth delaying clearance and prolonging flavour perception compared to a non mucoadhesive control and water alone.

5.2. Methods

5.2.1. Materials

Three aqueous matrices containing the same amount of sodium were prepared for all parts of this experiment; the samples were made with DW alone, or DW plus LCMC as the mucoadhesive polysaccharide, or an amylase resistant starch (detailed in Chapter 2). The aqueous samples were freshly prepared on the day they were used as the starch containing samples needed to be assessed within 2 hours of hydration. Both LCMC and starch were dispersed in DW to obtain a final concentration of 2.6% (w/w). This concentration was chosen as the samples resembled drink consistency that one might encounter. LCMC samples were prepared on the morning before experiments and left in the fridge for at least 3 hours to remove air bubbles. Starch and water samples were prepared no longer than 30 min before commencing experiments to prevent the starch from thinning.

All samples contained the same concentration of sodium (final concentration 0.18% Na^+ or 786 μM) either from NaCl salt added or Na^+ inherently present in the polysaccharide. The LCMC contains a high amount of Na^+ to make it soluble in water. Flame photometry (Economical Flame Photometer; 230 VAC, 50/60 Hz) was used to determine the amount of Na^+ in LCMC (51.5 mg/g) and therefore, the amount of NaCl added to these samples was adjusted to account for this inherent sodium concentration (method detailed in Chapter 4.2.2.). This ensured that the dosage of sodium in each sample was the same, but the amount of accompanying chloride was different.

5.2.2. Viscosity

The viscosities of the LCMC and the starch sample were determined using a TA AR2000 rheometer with 40mm parallel plate geometry (TA Instruments, Herts, UK). LCMC samples were removed from the fridge at least an hour before the test to bring it up to room temperature. The starch sample was measured no more than half an hour after it was made. Solutions were stirred before 600 µL was taken up with a plastic syringe and placed onto the bottom parallel plate of the rheometer. The rheometer was set to equilibrate the sample to temperature (37°C) before beginning sweeps. A 40 mm parallel plate was used with a 400 µm gap between the plates. Each sample was tested 9 times, 3 batch repeats and 3 analytical replicas.

After the initial amplitude sweep to determine the linear viscoelastic regions of the samples, the amplitude was set to 1% strain and frequency sweeps were carried out to determine the complex viscosity (η*) over increasing frequency (Figure S1a & b). Various concentrations of LCMC were measured to match the 2.6% (w/w) starch viscosity (55 mPa.s) at a shear rate of 50 rad/s (Figure S3) as this is typically quoted as the shear rate of the mouth [11–13].

5.2.3. Ex vivo retention experiments

The experiment detailed in Chapter 3 (3.2.3.) was used for the work in this Chapter also. The retention experiment allows indirect quantification of the amount of sample retained on a mucosal surface after being repeatedly washed with an artificial eluent. Sodium fluorescein was added to the aqueous samples to visualise retention of the matrix. *Ex vivo* porcine tongue was used as the mucosal substrate and an AS formulation was used adapted from Madsen *et al* (2013), as

the eluent (Chapter 3.2.3.3.). Each experiment was repeated 3 times on three different tongues.

5.2.4. Progressive profile

The University of Reading screened and trained sensory panel of 11 people were trained to assess three attributes in the samples using a progressive profile method. A progressive profile is a temporal method that allows panellists to score the intensity of attributes after swallowing with scoring carried out at set time points. This is opposed to using a quantitative descriptive analysis (QDA) which is a static assessment made by the panellist immediately after consumption. A QDA is not able to capture information about the development of attributes over time. Each sample was tested in duplicate on separate days.

The panel were given the samples in training so they could decide which attributes they would use to differentiate samples. They chose saltiness, adhesion and mouthcoating to best describe the samples. Panellists were trained on the saltiness attribute with a range standard samples that varied in concentration. They were given 0.4% NaCl in water as their extreme anchor. Two more standards 0.2% and 0.1% were given that were approximately 50% and 25% of the line scale. These were given to the panellists during training sessions and for reference before experiments. Adhesion was defined as the stickiness of the sample to the roof of the mouth and mouthcoating was defined as the feeling of something present on the mouth lining.

Progressive profiling produces a time-dependent descriptive profile showing the intensity of attributes over specific time during or after consumption. The test was

made in Compusense using standard unstructured line scales. In this experiment, the progressive profile scoring took place after the sample was swallowed to gain insights into the influence of adhesion on salt perception. Panellists were given 5 mL of each sample in opaque shot glasses and asked to score the attributes immediately after swallowing. They were then instructed to sit quietly and swallow a consistent number of times (dependant on the panellists individual defined times in 1 min), predetermined during training, for 20 s until the next scoring session. Panellists took an average of 10 s to score the samples and therefore the time interval between scores was, on average, 30 s. Compusense collected data and the raw data was exported and analysed in SPSS.

5.2.5. In vivo sodium retention

An *in vivo* retention study was designed to determine the actual amounts of sodium retained in the mouth after consumption. It is well known that mucoadhesives retain small compounds at mucosal sites, hence, it was hypothesised that this would be the case with sodium ions. Five participants were recruited, 1 female and 4 males, between the ages of 22 and 30. Ethical approval was sought and granted by the University of Reading's School of Chemistry, Food and Pharmacy ethics committee prior to experiments (project code 27/15). Participants were asked to brush their teeth and rinse their mouth thoroughly with filtered water 15 min before they started each session. Each sample was tested in triplicate so each data point reported was a mean of 15 individual saliva collections.

5.2.5.1. Saliva collection

For each session, the participants were given one of the three matrices containing sodium. Compusense software was used for timing each experiment and the breaks between each sample. For each sample, the participant was presented with 5 mL and asked to hold the sample in the mouth for 10 s before spitting out the sample into a disposable spittoon. To avoid excessive consumption of sodium chloride participants spat out the sample instead of swallowing. This first expectoration was not measured as this was in place of the participants swallowing. After this initial spitting, a timer started and once it had finished, the participant was prompted to scrape their tongue with their teeth and rid their whole mouth of saliva into a pre-weighed, appropriately labelled tubes that would later be weighed and analysed. The timer counted down from either 5, 30, 60, 120, 180, 240 or 300 s to gather measurements of sodium retained at each of these time points. For every time point, a new sample was presented to the participant to accurately measure how much would be retained at each time point over the total 5 min period. There was at least a two-minute break between each sample in the series. Timings were randomised and swallowing was controlled during each experiment so that each individual participant was swallowing the same amount of times for each sample and all time points. Due to individual variances of saliva production the number of swallows per person was different.

5.2.5.2. Analysis of sodium in saliva

The tubes were weighed before and after collection to determine the amount of saliva collected. The saliva samples were diluted with 40 mL DW and agitated so the sodium concentration could be determined by flame photometry set for sodium detection. Sodium chloride standards were used for a calibration curve

ranging from 0 mg/ L to 10 mg/ L Na^+ which was in the linear range. A blank saliva sample was taken each day before experiments started to measure the sodium present in resting saliva. These blanks were averaged over the 9 sessions to give a value for baseline sodium content of each participant's saliva. The average blank was then subtracted from the results obtained from the experiments.

5.2.6. In vivo retention

Five volunteers were used for testing the *in vivo* retention of samples. Data was collected by asking participants to consume the sample and then at set time points to expectorate their whole saliva into pre-weighed tubes, which were later analysed using flame photometry. Each time point was carried out in triplicate for each matrix type. Saliva was collected to measure how much sodium was retained after the bulk of the sample had been swallowed. It was hypothesised that the presence of the mucoadhesive polysaccharide, LCMC, would enhance the retention of sodium ions in the oral cavity.

5.2.7. Statistical analysis

For all experiments two way rmANOVA was used. Time and sample were set as treatment effects. Bonferroni adjustments were made for multiple comparisons of time points. Fisher's Least Significant Difference was used when comparing between the three matrices. Data were analysed in SPSS version 21 (IBM, UK) and a value of $p<0.05$ was used to determine significance.

5.3. Results & Discussion

5.3.1. In vitro retention of solutions

Rheology results found that LCMC is relatively non-shear thinning whereas starch was very shear thinning (Figure 5.1.). Although viscosity can be quoted at a single shear rate, the shear behaviour of the sample will be an important factor when considering the impact on mucoadhesion and retention of molecules.

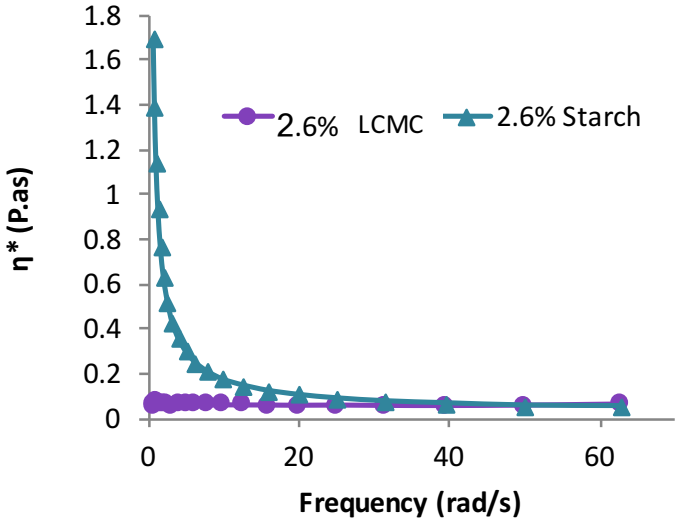

Figure 5.1. Complex viscosity (η*) of LCMC and Starch over increasing frequency.

Figure 5.2. shows the retention profiles of the LCMC, starch (matched viscosities) and water samples on different areas of *ex vivo* pig tongue. The different areas of the tongue have different retention profiles with the front of the tongue retaining the polysaccharide matrices longer than the rear and side of the pig tongue. This is in accordance with previous results investigating milk protein retention on different tongue areas [15]. This difference is probably due to the morphology of the front surface of the tongue, as it possesses a high density of fungiform and filiform papillae, increasing the surface area and surface roughness, facilitating mucoadhesion. The rear of the tongue has larger protrusions and the side is mostly smooth, non-keratinised tissue with few papillae present.

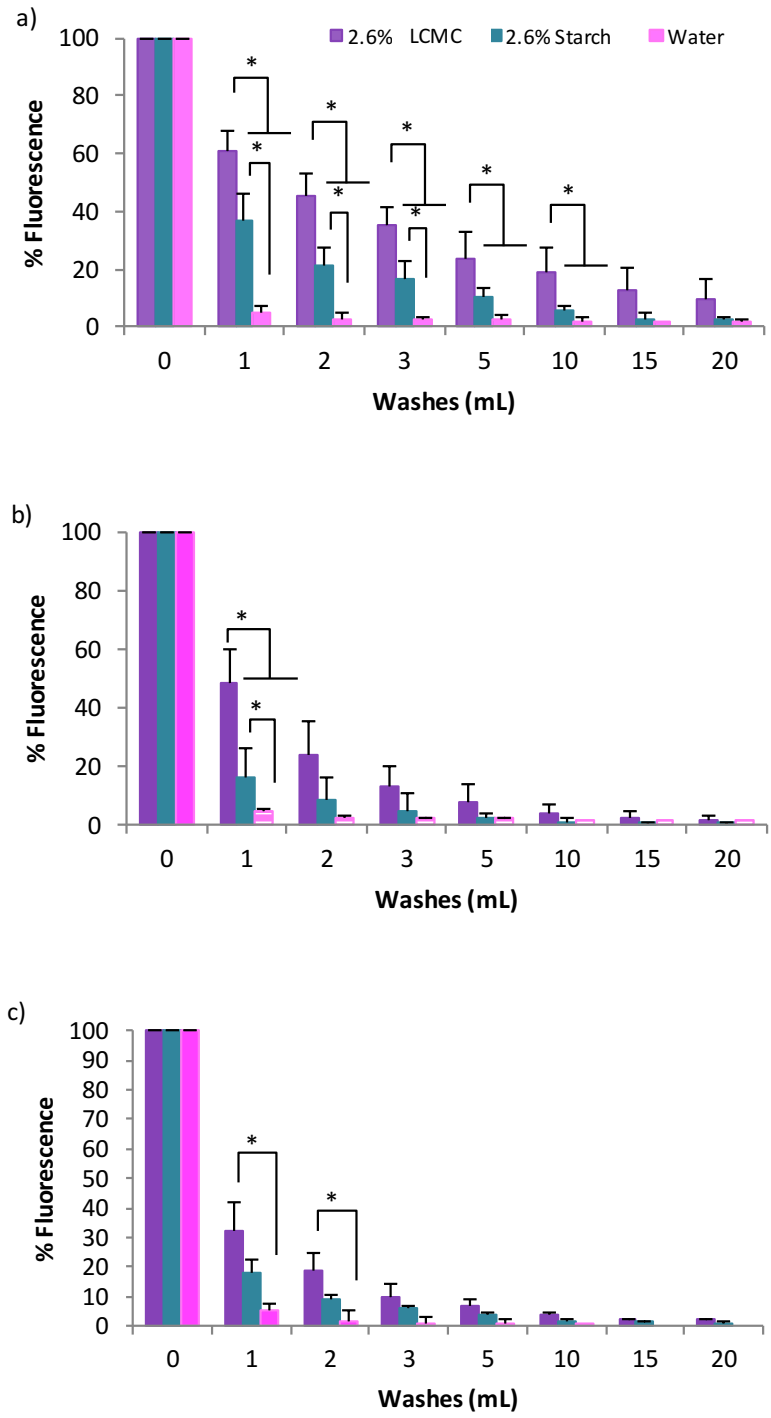

Figure 5.2. Retention profiles of matrices on the front (a), rear (b) and side (c) of *ex vivo* pig tongue. Statistically significant differences ($p < 0.05$) represented by *. Error bars are ± standard deviation.

As a control, SF in water was applied to the tissue and washed off. Figure 5.2. shows that this solution was not retained on any of the areas of the tongue after

the first wash with 1 mL AS. This shows that the dye is not being retained on the tissue without the presence of the PS matrix. The starch sample was retained on the tongue longer than the water sample, which is most likely due to viscosity factors. On the front of the tongue most of the sample containing starch was washed off after 5 mLs of AS, whereas for the rear and side of the tongue 3 and 2 mL was sufficient, respectively. LCMC on the other hand was still visible after 20 mL of AS washing on the front of the tongue.

During these experiments the shear force that the sample is put under is that from the droplet encountering the tissue. The shear rate that the sample viscosities were matched at was relatively high to emulate the reported shear conditions in the mouth. Therefore, at lower shear rates there is a large discrepancy between viscosities, with starch having a much higher viscosity than LCMC (Figure 5.1.). Despite this higher viscosity at lower shear, LCMC was retained for longer than starch on the front of the tongue with a similar trend in the other areas. This suggests that viscosity is not the only driving factor for mucoadhesion, though an increase in viscosity does result in enhanced mucoadhesion (Chapter 3) [16]. The solubility of a polymeric substance in the mucosal secretion will also play an important role in the mucoadhesion observed. In this study both PS are hydrophilic and will, therefore, be soluble in saliva, which has a neutral pH.

There are many possible reasons why LCMC is more retentive on the tongue mucosa than starch. Starch is a shear thinning PS used for its thickening properties in a range of liquid and semi solid food applications. Starch was chosen as a negative control for mucoadhesion in this experiment as it thickens solutions whilst being relatively non- adherent to the mucosal surface of the mouth, as

illustrated by the *in vitro* retention (Figure 5.2.). Starch has a granular structure in solution as the polymer chains swell and form colloidal hydrated particles that exhibit limited chain entanglement [17]. Nutilis is a modified form of starch to make it amylase resistant, however, it still exhibits a granular, swollen texture rather than a continuous network of polymer chains [18]. This granular structure will affect the ability of the polymer chains to interpenetrate within the mucus layer to form physical entanglements with mucin, promoting adhesion. Conversely, LCMC polymer chains can settle into the micro cracks (papillae) that are present on the surface of the tongue leading to an increased polymer – surface interface. Furthermore, LCMC is an anionic polysaccharide due to the presence of COO^- groups. This will contribute to mucoadhesion through hydrogen bonds and van der Waals forces with the mucin oligosaccharide side chains.

5.3.2. Sensory perception: Saltiness

The saltiness intensity of samples was scored on unstructured line scales several times over 6 minutes. The results for this attribute show that all three samples decreased in the intensity of saltiness over time (Figure 5.3.). Saltiness perception was significantly ($p<0.001$) higher in the water samples compare to starch over time ($p <0.05$), however, after 2 min the difference between them became non-significant. The saltiness of the LCMC sample was reduced compared to starch ($p <0.01$) and water ($p <0.001$) initially, and this difference persisted over time (Figure 5.3.). Saltiness intensity was significantly ($p<0.001$) higher for water samples compared to samples with LCMC at all time points. The starch samples were significantly ($p<0.001$) higher than LCMC until 480 s after which the scores were not significantly different.

There are various factors to consider with salt taste perception such as viscosity, matrix-tastant interactions and adaptation. An increase in viscosity is known to reduce the diffusion of tastant molecules as predicted by the Stokes-Einstein and Wilke- Chang equations [19] and subsequently decrease taste perception in foods[20–23]. The Stokes-Einstein equation predicts that diffusion is dependent on the square root of the viscosity. Furthermore, interactions between ionic thickeners can slow the diffusion of charged molecules and recent research suggests that sodium ion availability from food matrices is the most important factor to consider for salt taste [24]. The interactions are often due to adsorption, entrapment in microregions, complexation, encapsulation, and hydrogen bonding [25]. Therefore, if the tastant is being chemically or physically prevented from diffusing out of the food matrix to reach taste bud receptors, then perception will be stunted.

How well the matrix mixes with saliva has also been proposed as an explanation to why starch does not impede perception like random coil polysaccharides [17]. Another possibility is that adaptation effects are artificially turning down the saltiness signal [26], however, apdaptation would be more likely with stronger tasting solutions than weaker more prolonged taste.

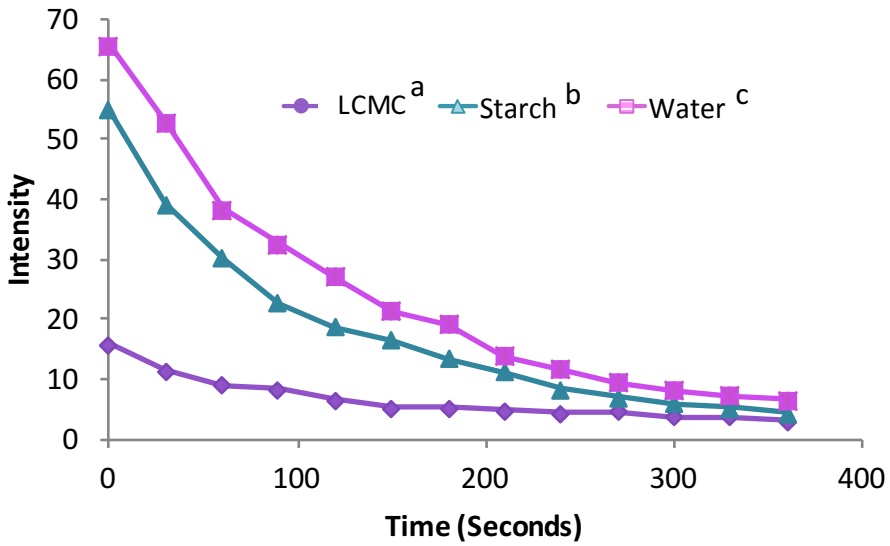

Figure 5.3. Progressive profiling data for *Saltiness*. Each data point represents the mean for the 11 panellists and their duplicate tests. Error bars are not included in this graph as there is large individual variation in scores over time. The letters next to the sample key represent statistically significant groupings ($p < 0.05$).

The most likely explanation for the results found in this study, however, is the anion effect restricting the perception of sodium [27]. Although sodium ions themselves are responsible for activating taste cells for a salt response, the anion associated with it serves an important purpose. To be perceived, sodium ions must diffuse from the food matrix into the saliva where they then diffuse into the papillae where the taste bud receptor cells are located. The anion associated with the sodium cation has great implications on the amount of saltiness perceived from a given concentration of sodium. The anion effect explains why smaller anions such as chloride facilitate a salty perception and larger anions do not [27–30]. Briefly, as the sodium ions diffuse paracellularly to permeate the basolateral cells of a taste bud pore, anions larger than chloride do not diffuse as readily. This leads to the development of a transepithelial potential and hyperpolarisation of

the taste cell preventing an action potential. In the experiments in this study, the sodium levels were matched regardless of the counter ion so it makes sense that with LCMC being the anion in this circumstance, the sodium ions will not produce a salty perception. [17].

Due to these complications, it is not clear whether the presence of a mucoadhesive would prolong the taste perception of saltiness as the salt perception was already lower with LCMC at the start of the profile due to the large anion effect. The amount of added NaCl to the LCMC samples was 25% of that added to the other samples. The average intensity (0-100) recorded by participants at the first scoring point was 16 for LCMC, 55 for starch and 66 for water. This means that the LCMC scores were 29% of the score for starch and 24% of the score for the water samples. It could therefore be argued if the amount of NaCl added was the same for all the samples then the LCMC samples may not have had such a reduction in intensity.

5.3.3. Sensory perception: Adhesion & Mouthcoating

Panellists scored the attributes adhesion and mouthcoating at the same time as scoring the saltiness attribute. As these attributes are closely linked and have a similar response from the panellists, they will be discussed together. The scores for adhesion (Figure 5.4.a) and mouthcoating (Figure 5.4.b) were significantly ($p<0.05$) higher for LCMC containing samples compared to starch and water only samples. During training the panel described the LCMC samples as sticky and gummy whereas the starch was described as globular and gritty in texture.

Immediately after swallowing and 30 s later the starch samples were perceived as more adhesive than water, which is unsurprising considering the increased viscosity and bulk it imparts to the sample. LCMC on the other hand, scored significantly ($p<0.05$) higher for adhesion up to 210 s for water ($p >0.05$) and 480 s for starch ($p >0.05$) (Figure 5.4.a). Adhesion scores were paralleled by mouthcoating scores (Figure 5.4.b), though starch scored higher for this attribute overall, presumably because it spreads throughout the oral cavity well but is extremely shear thinning (Figure 5.1.) so not particularly sticky when manipulated with the tongue. Mouthcoating scores for starch were initially higher than the water samples but dropped quickly, whereas LCMC was significantly ($p<0.05$) higher than the other samples for over 2 minutes after swallowing (Figure 5.4.b).

Although panellists perceived that starch coated their mouth somewhat after swallowing, it was not adhesive in the same way as LCMC. These results are in line with the *in vitro* retention experiments (Figure 5.2.), where LCMC retained for longer on the tongue than starch. This prolonged adherence of the liquid formulation could be beneficial when delivering flavour molecules in liquid and semi solid food products.

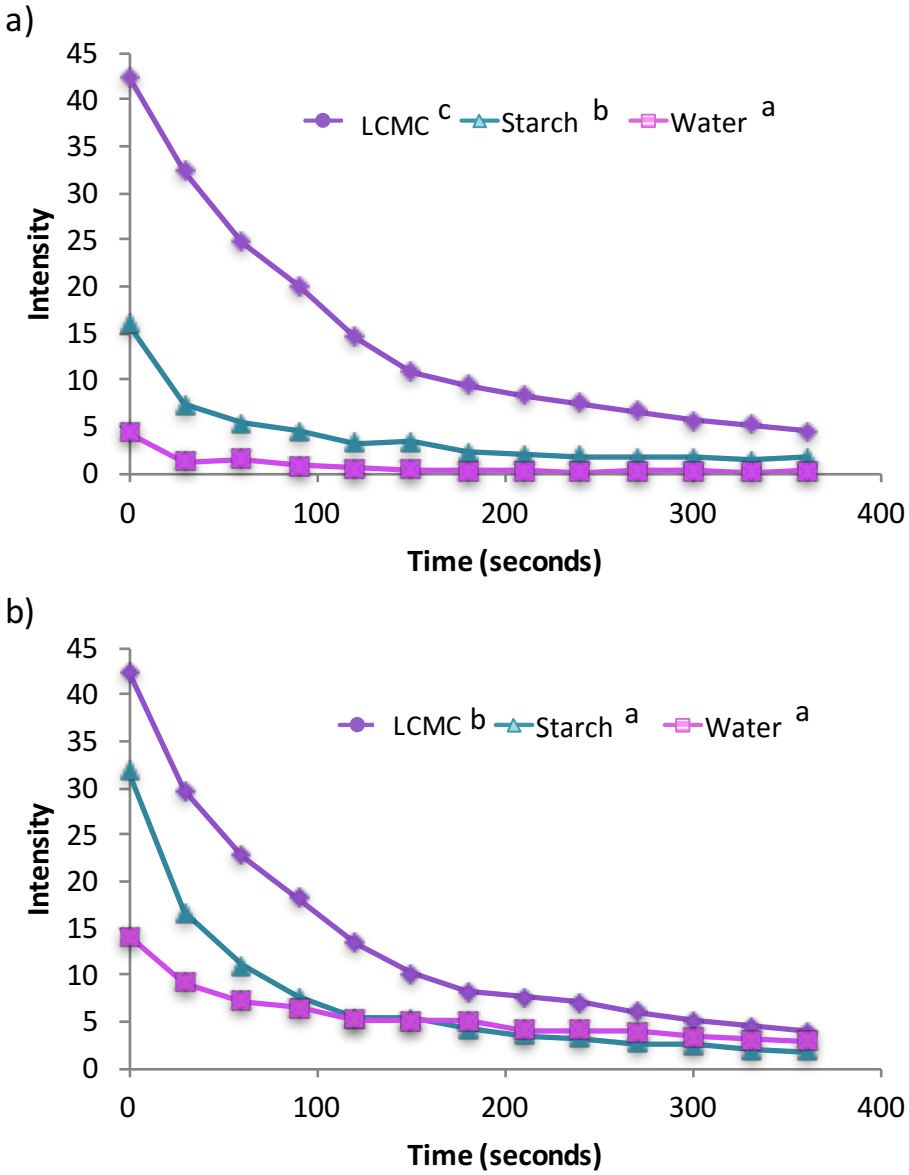

Figure 5.4. Progressive profiling data for a) *adhesion* and b) *mouthcoating*. Each data point represents the mean for the 11 panellists and their duplicate scoring. Error bars are not included in this graph as there is large individual variation in scores over time. The letters next to the sample key represent statistically significant groupings. Different letters represent a significant difference of $p < 0.05$.

5.3.4. In vivo salt retention

Figure 5.5. shows the total amount of sodium present in the participants' expectorated saliva at each time point. The total sodium amounts in the panellists'

saliva after consuming the samples containing LCMC were higher than the starch ($p < 0.05$) and water ($p < 0.05$) samples (Figure 5.5.). This suggests that the LCMC samples were better at retaining the sodium ions due to enhanced adhesion of the matrix, which retains the ions associated with the PS network in the mouth for a prolonged period. This is supported by the results from the *in vitro* retention experiments (Figure 5.2.) and the sensory perception scores for adhesion and mouthcoating (Figures 5.4. a & b). Although the perception of sodium was stunted due to the anion effect, the actual amounts of sodium were higher and retained for longer.

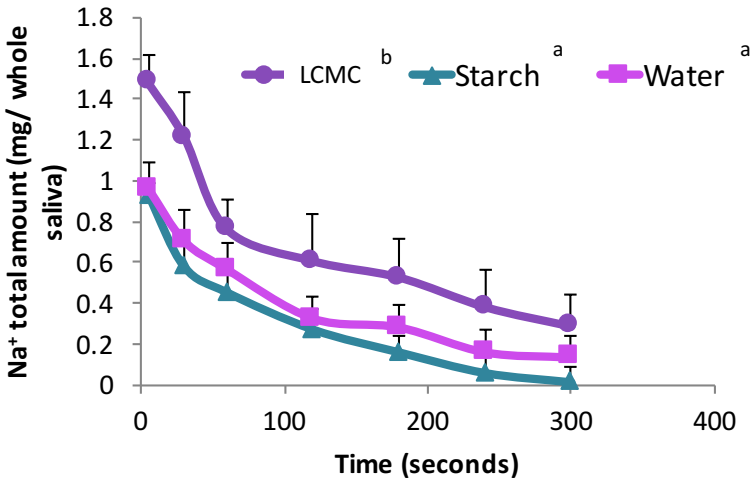

Figure 5.5. Amount of sodium present in participants' whole saliva over a 5-minute period. Each data point represents the mean of 15 (5 participants, 3 repeats) saliva collections analysis. Error bars represent ± standard error mean. The letters next to the sample key represent statistically significant groupings of $p < 0.05$ using Bonferroni correction.

LCMC is an ionic PS and this ionic nature lends itself to mucoadhesion due to ionic and hydrogen bond formation and Van der Waals interactions with the oral

mucosa. However, the drawback of this ionic PS, from a nutritional perspective, is that LCMC inherently has sodium associated with the negatively charged carboxylic acid groups. This is the case for many ionic PSs and therefore adding these types of PSs to foods will increase the sodium content without necessarily adding to the salty taste. In this work, the sodium contents of the samples were matched in order to ascertain whether the inherent sodium in LCMC would elicit a salt response and prolong this perception over time. However, the amount of sodium inherently in the LCMC samples meant that the amount of NaCl added to the LCMC samples was a quarter of that which was added to the other samples. If there were equal amounts of NaCl added then the anion effect would be minimized and perhaps there would be a prolonged perception of saltiness. Of course, this would then mean that there was much more sodium in those samples making it less ideal from an application point of view.

As mucoadhesion is correlated with viscosity (Chapter 3), a non-ionic polysaccharide could be used to overcome the excess sodium issue. The mucoadhesive strength of polymers does not solely rely on viscosity; however, in liquid and semi solid formulations this may be an overriding factor. The rheological behaviour is also an important consideration as LCMC is relatively less shear thinning compared to starch, which may explain the retention further. The force required to remove the LCMC samples may need to be higher than for the starch for example. Therefore, similar cellulose derivatives that are non-ionic such as hydroxypropyl methylcellulose may be retentive due to the rheological behaviour but will not have the associated sodium with them. Liquid mucoadhesion is heavily influenced by viscosity, the more viscous a sample is the more resistant it is to force. It is, therefore, difficult to control for viscosity in such

experiments as most polysaccharides that can form viscous solutions are also going to exert some mucoadhesive strength. Furthermore, polymer chain flexibility that facilitates chain entanglement is inherently related to mucoadhesion, so this further complicates the endeavour to find a polymer that exhibits flexibility in solution and is not mucoadhesive. Therefore, starch was chosen as one of the few polymeric substances that thicken solutions without forming an interconnecting polymer chain network.

Although water was not statistically different to starch at retaining sodium ions there was a general trend that more sodium was retained in the water samples over the different time points (Figure 5.5.). This retention could be explained due to the viscosity of starch; some of the sodium ions would reside in the starch matrix and be swallowed in the bolus as it is not mucoadhesive, thus reducing the amount left in the mouth. As there is no bolus formation in the water samples and water poses no physical barrier to the mucosa, the sodium ions are free to diffuse into the taste bud pores to be perceived and remain in the mouth.

5.4. Conclusions

The results from this work show that a matrix containing mucoadhesive LCMC prolongs the adherence of the matrix to the mucosa *in vitro* and *in vivo* studies show that it also retains the model tastant, sodium, within it for longer than starch and water matrices. However, this work found that, due to the large anion effect, the perception of the retained sodium was diminished. This work suggests that mucoadhesive matrices could be used to control the release of flavour compounds after consumption when the anion effect is not an issue.

5.5. References

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Chapter 6: Polysaccharide matrices control the release, retention and perception of flavours

6.1. Introduction

In previous chapters the mucoadhesive strength of various PS was measured and the effect of mucoadhesive PSs on the perception and retention of tastants was investigated. Evidence from Chapters 5 suggests that although LCMC retained sodium longer than water and starch samples, the perception was stunted due to the anion effect. Therefore, the work on this chapter investigated the effect of different PS matrices on sweet based flavourings to avoid issues due to the anion effect. One of the main aims for this body of work was to investigate the use of mucoadhesives in solid food systems. The previous chapters have all focused on liquid formulations, therefore, the work in this chapter investigated solid PS matrices and the effect of flavour release and perception over time.

6.1.1. Flavour interactions

Flavour perception occurs via the release of flavour compounds from the food matrix and the subsequent transport of those compounds to the respective receptors in the nose and mouth. This whole process is dependent on various factors including the chemical properties of the flavour compounds (e.g. polarity), the nature of the food matrix, the physiological conditions of the mouth, nose and throat during consumption of the food, and psychological factors such as memory and emotion. These play varying roles depending on the food that is being consumed and will affect the amount and rate of flavour compounds reaching the receptors. These factors result in a characteristic flavour profile for a particular

food. The rate and onset of flavour delivery is dependent on factors such as partitioning, mass transport and diffusion.

In liquid food systems PSs, proteins and fat influence the structure of the food. The impact they have on smaller molecules, such as aroma and tastant compounds, has been investigated mainly in relation to viscosity increases [1–5] and physical entrapment of such molecules [6–8]. The impact of these structural changes on perception is well documented, however, perception does not always reflect flavour release. More recently, the interactions between food components and the oral and nasal mucosa are being investigated. Specifically, interactions between flavour molecules and the oral and nasal mucosa may explain persistence of aromas in certain foods [9,10].

To date, the literature on taste – texture and aroma- texture interactions has mainly focused on liquid and semi solid foods with, often, contradictory results reported. The mixed findings are most likely due to the chemical and physical composition of the food matrix rather than purely an increased viscosity. Furthermore, studies often focus on changes of fat levels and/ or the addition of various PSs at various concentrations and combinations with flavour molecules. Although mixed results have been obtained, in general, an increase in viscosity by the addition of PSs results in a decreased perception of sweet and salty tastants [11–14]. For example, Cook et al., (2002) found that HPMC samples above c* reduced the sweetness intensities of aspartame, sucrose, fructose and neohesperidin dihydrochalcone and the saltiness of sodium chloride. Physicochemical explanations have been proposed for the reduction in taste perception due to tastant interaction with the food matrix and physical

entrapment of the tastant molecules within the food matrix prohibiting the diffusion to the taste buds in order to be perceived [8,15].

Aroma – texture interactions are equally as troublesome to characterise due to the countless combinations of food ingredients and aroma compounds. Clearly there is not just one mechanism to describe all these possible combinations. Some studies, using real time *in vivo* measurements, found that although perceptual changes occurred with foods thickened with PSs the aroma release and amount delivered was unchanged [6,16]. Aroma – tastant – cognition interactions are thought to play a role in aroma perception as a decrease in the perception of a tastant caused by an increase in viscosity has been found to lead to a decrease in the congruent aroma despite aroma delivery concentration remaining the same [16,17].

Flavour molecules can be volatile (aromas) or non-volatile (tastants) and food matrices range tremendously. While the physicochemical properties of the flavour compounds are important in determining their interaction with the matrix, the state of the matrix is equally important. For example, polysaccharides can be present in viscous liquids (e.g. mayonnaise), rubbery solids (e.g. bread dough) or glass (e.g. a low moisture snack product). Therefore, it is impossible to generalize the interaction of a particular flavour molecule with a particular polysaccharide in all its forms.

6.1.2. Flavour release

In the mouth, flavour retention in the food matrix largely depends on the composition of food and how well it mixes with saliva. Retention of flavour compounds in the matrix will obviously decrease the perception of those

compounds as they will not reach the respective receptors to be perceived and risk being swallowed. Many studies that have investigated the impact on aroma release when reducing fat in foods [18,19] have found that, in general, aroma retention in the matrix of a high fat food will increase as the logP of the aroma increases meaning the more hydrophobic aroma compounds will favour being in the fatty matrix rather than partitioning into the aqueous saliva. For hydrophilic compounds (log P equal to or less than zero) the reverse happens as an increase in fat levels may increase the rate of release from the matrix. It makes sense then that the release of hydrophobic aromas will be faster in lower fat systems, which can lead to an unbalanced flavour profile when attempting to develop low fat alternatives.

Prolonging the retention of aromas in the food matrix, without a high fat content, may combat this issue. The encapsulation of aroma compounds with hydrocolloids has been a popular research area in previous years for liquid foods, especially emulsions [20–22]. Volatile aroma compounds can be encapsulated using various hydrocolloids to physically entrap them and control their release over time. Gelled emulsions, using cross-linked Ca-alginate, have worked reasonably well for controlling the release of hydrophobic aroma compounds to mimic higher fat emulsions [23,24].

6.1.3. Mucoadhesive films

Buccal films are commonly used to deliver drugs either locally or systemically due to the ease of application, avoidance of first-pass metabolism and high blood flow [25]. The use of mucoadhesive PSs in these formulations means that the film will adhere to the buccal tissue and will not be swallowed. Various films have been

designed to release drugs systemically, such as propranolol hydrochloride for high blood pressure [26], and locally acting metronidazole for periodontal disease [27]. Polymers such as carbopol, CMC, SA, HPMC, chitosan and modified starches have been used in these formulations [28]. The polymers chosen for the formulations are based on their chemical properties depending on what drugs are being incorporated, the desired rate of release and residence time.

As mentioned in previous chapters, PSs are employed in a variety of industries and there is a huge overlap between the food and pharmaceutics industries. Listerine Pocketpaks® are an example of where the food and pharmaceutical applications overlap in terms of the use of PSs. Pocketpaks® are thin PS films containing essential oils to kill bacteria whilst simultaneously freshening breath with a variety of flavourings. These fast dissolving films are made with pullulan (PUL) as the film forming excipient. PUL (detailed in Chapter 2) has a very low viscosity and excellent film forming properties. It adheres to wet surfaces, most likely due to its rapid water uptake and disintegration. Pocketpaks® are designed to dissolve rapidly when in contact with a moist surface and this results in a quick burst of flavour.

The clear majority of studies investigating the impact of PSs on flavour relate to liquid and semi solid food products as this is where they are most frequently employed. The work in this chapter is concerned with how PSs effect flavour perception in a solid system. Various food grade PSs that differ in their chemical and physical properties were cast into films and the effect on the release, retention and perception of glucose and vanillin were assessed.

PSs were cast into films containing glucose and/ or vanillin. The release and perception of flavour attributes over time is dependent on many factors including mass transfer and flavour-matrix interactions. Whilst this study takes those factors into consideration, a further interaction between the food matrix and the oral anatomy, mucoadhesion, is investigated. The hypothesis for this work was that films made with viscous, slow dissolving polysaccharides that adhere to oral surfaces for a prolonged time will reduce the intensity but prolong the perception of flavours over time. This work, therefore, provides fundamental data relating to how food ingredients influence the perception of flavours over time and how new products may be developed.

6.2. Methods

6.2.1. Materials

Four PSs were chosen for this study due to their differing chemical properties (Chapter 2). PUL was chosen as a non- ionic, low viscosity and fast dissolving film former, HPMC was chosen as a high viscosity, non-ionic film former and two CMC viscosity grades were used, one low viscosity (LCMC) and one high viscosity (HCMC). Vanillin was purchased from Sigma- Aldrich (Missouri, US) and glucose powder was purchased from a local supermarket.

6.2.2. Samples

Films were prepared by dissolving PS in DW (3 % w/v) with either glucose, vanillin or glucose and vanillin (Table 6.2.). PS solution (30 g) was weighed into circular petri dishes and placed in an oven at 65°C for 20 hours. After this time, the films were dry and could be removed from the petri dish. They were cut into squares and weighed. Glucose containing films weighed 100 mg and the aroma

only films weighed 30 mg. This weight difference was to ensure that each sample contained the same amount of PS.

Table 6.2. Final concentrations of ingredients in each type of film.

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

6.2.3. Artificial saliva

AS was used for all *in vitro* experiments to emulate conditions in the mouth. This formulation was adapted from Madsen *et al.* (2013) and consisted of 0.21 g/L $NaHCO_3$, 0.43 g/L $NaCl_2$, 0.75 g/L KCl, 0.22 g/L $CaCl_2{\cdot}2H_2O$, 0.91 g/L $NaH_2PO4{\cdot}2H_2O$ and 2.5 g/L pig gastric mucin type II dispersed in DW. The pH was adjusted to 6.8 and kept at 37 °C during experiments and stored at 4 °C when not in use.

6.2.4. Swelling profiles of films

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 and netting was removed from the AS and weighed. Excess water was carefully absorbed with tissue paper so this did not add to the weight. The process was repeated until the weight had returned to that of the netting alone. Each sample was tested 6 times with duplicate batch repeats. Film thickness was measured before these experiments with a micrometer.

6.2.5. Dissolution of glucose from films

Films containing glucose were placed onto netting and carefully submerged into a beaker with 200 mL AS. The solution was kept stirring at a constant rate throughout the experiment. At set time points, 1 mL aliquots of the AS medium were removed and put into labelled epindorfs for analysis. The glucose concentrations in the samples were quantified spectrophotometrically using an Amplex Red, glucose oxidase kit (Fisher Scientific, Loughborough, UK). Each sample was tested 6 times with duplicate batch repeats.

6.2.6. Vanillin headspace analysis from films

The vanillin in the films was analyzed by SPME/GC-MS. Each film was placed in a 20 ml SPME vial with 5 mL DW the volatiles were extracted using a DVB/Carboxen/PDMS Stableflex fibre (SupelCo, Poole, U.K.). The samples were equilibrated at 60 °C for 60 min with intermittent stirring prior to exposure to the fibre for 10 min at 40 °C. The fibre was desorbed in the injection port for 20 min and the volatile compounds analysed using an Agilent 7890A gas chromatograph equipped with a Zebron ZB-5MSi column (30 m x 0.25 mm i.d. x 1 um film thickness) coupled to an Agilent 5975C MSD. Helium was the carrier gas (1.2 ml/min). After desorption, the oven was maintained at 40 °C for 10 min, then raised to 250 °C at 4°C/min. Mass spectra were recorded in electron impact mode at an ionization voltage of 70 eV and source temperature of 230 °C. A scan range of m/z 29-400 with a scan time of 0.69 s was employed and the data was collected by ChemStation. Vanillin was identified at 152 m/z.

External calibration curves were prepared for each PS with vanillin standards at 18, 50, 75 and 120 mg/L. Standards were prepared by dissolving 30 mg PS in DW water and spiking with the appropriate amount of vanillin to give the desired

concentrations. Each standard was made up at least 3 hours before testing. Each standard was tested in triplicate and for each film type, 6 different samples were tested, two batch repeats and three repeats within each batch of different areas of the film.

6.2.7. In vitro mucoadhesion

Adhesion experiments were carried out on a TA-XT plus texture analyser (TA) with a 10mm cylindrical probe. Porcine tongues were collected from P.D. Jennings butchers (Hurst) less than 24 hours after slaughter. They were stored on ice whilst most of the 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 film was tested on three pieces of tissue from 3 different tongues.

The dorsal of the tongue was cut into 1 cm^2 sections and each section was secured on the bottom platform of the TA with a metal plate. The film sample to be tested was secured 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 set for 60 s with a removal speed of 1mm/s.

6.2.8. In vivo retention

Ethical approval was sought from the University of Reading, School of Chemistry, Food and Pharmacy (Project 27/15). Five volunteers (3 males, 2 females. Age range, 23-30) were asked to place each film sample on their tongue and keep it between the tongue and roof of the mouth for the duration of the experiment. They were instructed to treat the film like a boiled sweet with some manipulation

with the tongue. During each experiment, they were asked to note if the film stuck and where to, when the film began to adhere, when the adhesion ceased and when the film dissolved. Adhesion was noted as an inability to move the film with their tongue. This experiment was done in triplicate.

6.2.9. Time intensity

A three-part experimental design was used for these experiments. Over three weeks 8 trained panellists from the University of Reading's Sensory Science Centre panel scored each of the film samples in duplicate. There are 12 samples in total, 4 with glucose alone, 4 with aroma alone and 4 with glucose and aroma. Each week was used for each set of films. For example, week 1 was the glucose only films. Sensory experiments were carried out in duplicate.

Training took place before each week to familiarise the panel with the samples and the time intensity test. 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 vanilla and sweet attributes varying in intensity. The panellists decided where these standards came on the line scale with their strongest standard representing 100 on a standard 100-point scale. These standards were given to the panellists during all scoring sessions to familiarise themselves with the intensities.

Panellists were trained on single and dual attribute time intensity scoring using Compusense at hand software 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 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 presented in a balanced order each day with the duplicate being served on a consecutive scoring day. Panellists were provided with an isolated sensory booth, a computer with Compusense Software and warm water for palate cleansing. Panellists were instructed to place each sample on their tongue and start the time intensity timer immediately. Panellists scored the appropriate attribute that corresponded to the film they tasted. For example, for the glucose only films they were just scoring sweetness but for the films with both glucose and aroma they scored both vanilla and sweetness at the same time. Panellists were asked to treat the film like a boiled sweet by keeping it between their tongue and roof of their mouth with gentle manipulation with the tongue. Time intensity curves were produced for each panellist and each sample in duplicate.

6.2.10. Statistical analysis

Time intensity parameters (Figure 4.1.) were extrapolated from the raw data produced in the experiments. Imax, Tmax, AUC, decline angle, incline angle, plateau and duration were analysed using one-way, repeated measures ANOVA (rmANOVA) with PS as a treatment effect and panellists as random effect.

Bonferroni correction was applied to multiple pairwise analysis for two-way rmANOVAs to account for multiple comparisons where time and PS were factors. Data were analysed in SPSS version 21 (IBM, UK) and a value of $p<0.05$ was used to determine significance.

6.3. Results & Discussion

6.3.1. Film characteristics

A range of standard methods was used to characterise the polymeric films [30,31]. Each film was measured for thickness, water activity (a_w), glucose release, and swelling and disintegration times (Table 6.3.).

The thickness of the films varied between the different PSs and between films with and without glucose. Film thickness was dependant on viscosity and glucose content. HCMC and HPMC were thicker than LCMC and PUL, and glucose films were thicker than those without glucose. The thickness of a film will influence the dissolution rate as a thicker film will have a smaller surface area to volume 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.

Table 6.3. Characteristics of films.

Matrix	**Glucose content (%)**	**a_w (mean)**	**Thickness (mm)**	**Dissolve time (min)**	**Max swelling ratio**	**50% glucose release**	**100% glucose release**

								(min)		**(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*

Films are separated into those with polymer alone and those with polymer and glucose. Each value is the mean of 6 replications for the measured parameters (2 batch repeats). *a, b, c* groupings signify significant ($p<0.05$) differences between the mean values obtained using pairwise comparison with Tukey HSD correction. These comparisons are made within each group (i.e. polymer films without glucose and polymer films with glucose).

PUL and LCMC films fully dissolved after a similar time; however, LCMC films swelled more before beginning to disintegrate (Figure 6.1. & 6.2.). This is because LCMC is more viscous than PUL and possesses ionic groups, which interact with water molecules. LCMC and HCMC films swelled considerably more than the non-ionic, PUL and HPMC films with relation to their disintegration time. The CMC films absorb 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 the high viscosity. All films without glucose had higher swelling ratios than their glucose containing counterparts and took longer to dissolve. This is mostly likely 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. As a film swells it takes up water from the surrounding area. If this is a moist mucosal surface then this will facilitate mucoadhesion as an adhesive joint will be

initiated due to high viscosity of the sample. Furthermore, the swelling of polymer chains leaves them free for interaction with the mucin molecules, strengthening mucoadhesion.

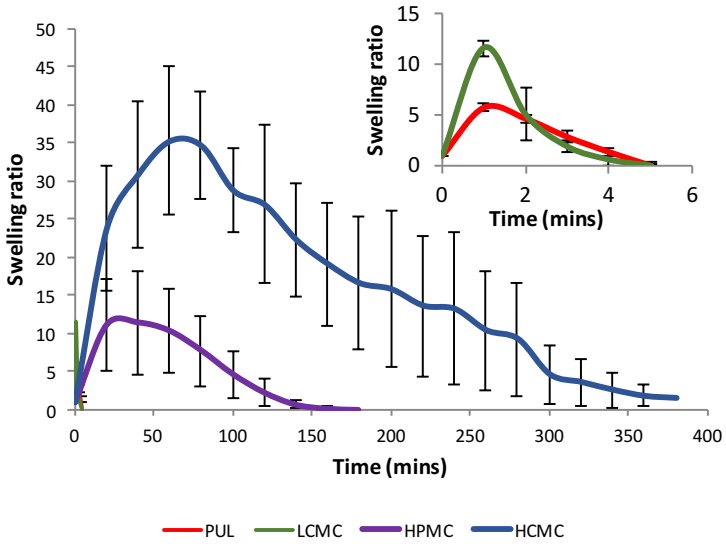

Figure 6.1. Polysaccharide films without glucose swelling and disintegration. Error bars represent SD.

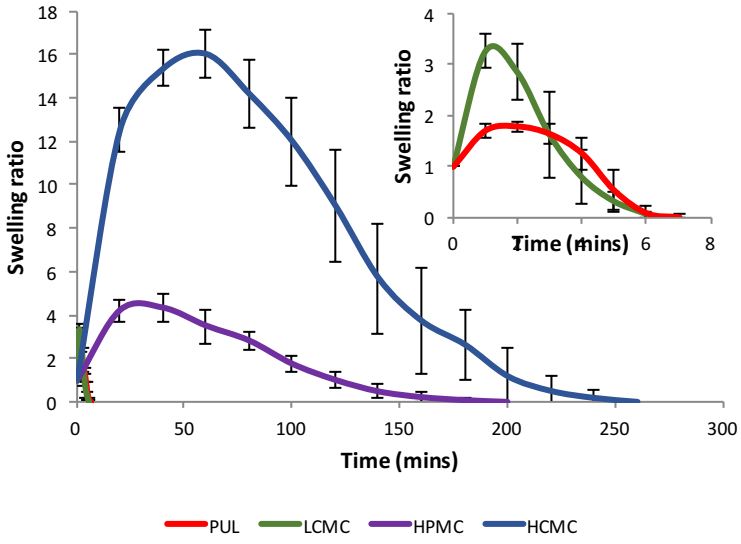

Figure 6.2. Polysaccharide films with glucose swelling and disintegration. Error bars represent SD.

Glucose release from the films followed a similar pattern to the dissolving rates (Figure 6.3.). 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 (Table 6.3. & Figure 6.3.). This 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 gel it forms which will slow permeation of water molecules.

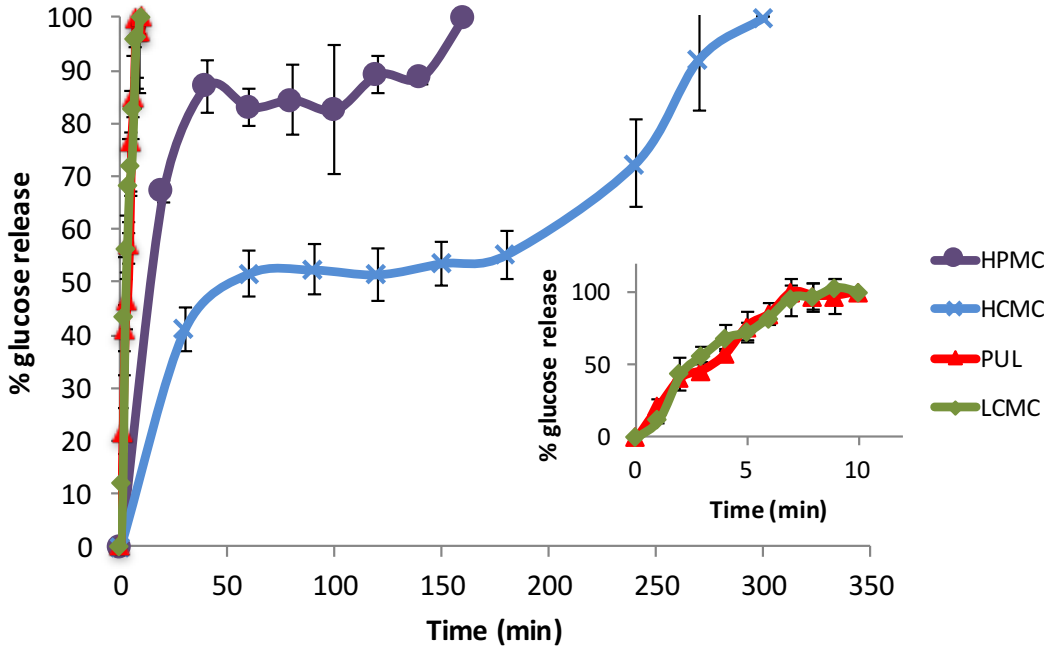

Figure 6.3. Glucose dissolution from films over time. Inset graph is a magnified view of PUL and LCMC release curves. Error bars represent SD.

HCMC films released the glucose quickly in the first 50 min then more slowly thereafter. The HCMC films swelled considerably so the swelled, gel-like surface of the film contained loosely associated polymer chains, which will 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 or viscous resistance. Additionally, the thick gel layer may prevent matrix disintegration and affect subsequent water uptake when unperturbed [32]. 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.

It was expected that changes in flavour perception over time would be influenced by the parameters measured (Table 6.3.). 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 delivery over time.

6.3.2. Headspace concentrations of vanillin from films

Standard curves were produced by GC-MS to determine the amount of vanillin in each PS film (Figure 6.4.). For each PS set (except HCMC) the peak value obtained from the GC- MS analysis decreased linearly as the concentration of vanillin decreased. The peak area for HCMC did not change significantly for increasing concentrations. These results show that the release of vanillin is dependent on the PS matrix present. HCMC was by far the most viscous PS used for these experiments and the huge variability of vanillin release could be due to physical entrapment of the aroma or chemical interactions (e.g. hydrogen bonds) occurring between the vanillin hydroxyl groups and the carboxyl groups of the HCMC.

At the highest concentration (120 mg/L) there were significant ($p<0.05$) differences between PS type. LCMC released less vanillin than PUL and HPMC suggesting a retention of the compound in the PS matrix. HPMC is higher viscosity than LCMC so it is possible that the retention was caused by interactions between the CMC and vanillin preventing the partitioning into the headspace.

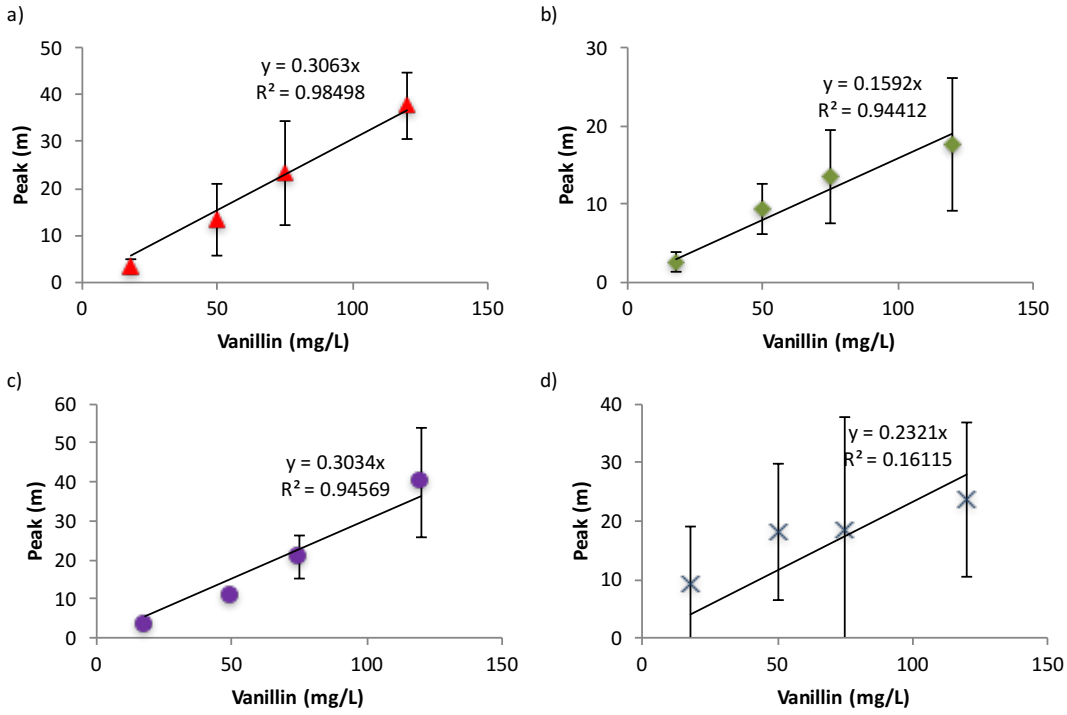

Figure 6.4. Standard curves of vanillin release from a) PUL, b) LCMC, c) HPMC and d) HCMC matrices. Error bars represent SD. Each value is a mean of 3 repeats.

The concentration of vanillin in the films used for sensory analysis were not significantly ($p>0.1$) different from each other (Table 6.4.). The variation of the films peak concentration was less than the standard solutions. A possible explanation for this is that the films were left overnight to dissolve in the 5 mL DW and therefore may have reached an equilibrium unlike some of the standards, some of which were left to equilibrate for just 3 hours prior to testing.

Table 6.4. Vanillin concentration in polysaccharide films with and without glucose. Concentrations determined by standard curves (Figure 6.4.). Letters represent significantly ($p<0.05$) different groupings.

Polymer	**With glucose**	**Vanillin concentration (mg/L) (± SD)**
PUL	Y	110 (24)[a]
PUL	N	125 (42)[a]
CMC	Y	105 (23)[a]
CMC	N	155 (59)[a]
HPMC	Y	122 (20)[a]
HPMC	N	163 (34)[a]
HCMC	Y	161 (68)[a]
HCMC	N	142 (76)[a]

6.3.2. Mucoadhesion in vitro

Two methods were used to assess mucoadhesion of the flavour containing films. Firstly, *in vitro* tensile experiments were carried out based on previous literature [31,33]. Two values were obtained from the TA experiments; the maximum forced required to separate the probe from the tongue (peak force of detachment) and the area under the curve (total work of adhesion). The results from these tensile experiments found the order of mucoadhesion to be LCMC> HCMC> PUL > HPMC for the films without glucose and LCMC> PUL> HCMC> HPMC for those with glucose (Figure 6.5. a & b). The films without glucose required a significantly ($p<0.05$) higher force to separate the film from the tissue suggesting a stronger adhesive joint (Figure 6.5. a). 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 work of adhesion with a similar trend for peak force of detachment (Figure 6.5.). This is probably due to the non-ionic nature of HPMC along with the large molecule size and slow swelling (Table 6.3. & Figure 6.2.).

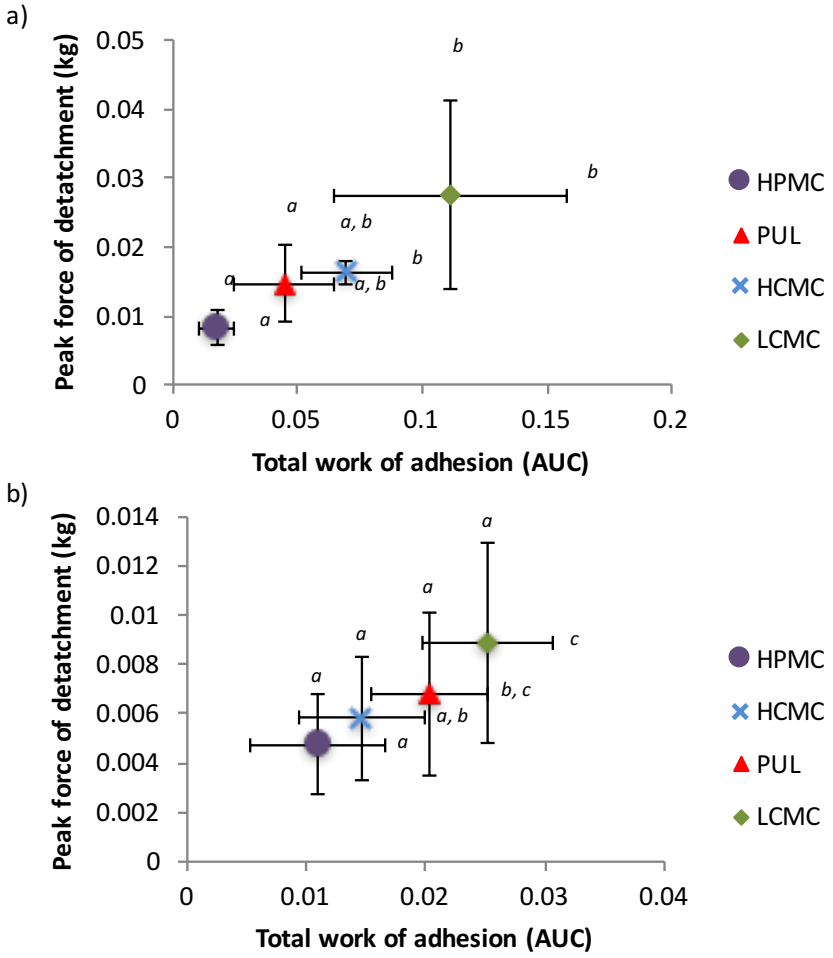

Figure 6.5. Total work of adhesion against the peak force of detachment for films a) without glucose and b) with glucose. Results determined by texture analysis. Data points are means of 6 measurements and error bars are SD. Superscript letters represent statistically different groupings ($p<0.05$). Letters on top of the data point refer to the y axis and those to the right-hand side refer to the x axis.

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. 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 freed up to interact with the mucosa.

6.3.3. Mucoadhesion in vivo

In vivo mucoadhesion experiments were carried out with 5 panellists that were asked to record the following: where the film adhered, the length of time it adhered and when it dissolved. All films, except for HPMC with glucose, were reported to adhere for the duration of the time the film was in the mouth (Figure 6.6. a & b). Adhesion 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 6.3.) 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 6.6) despite the difference in the *in vitro* test. This difference 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.

a)

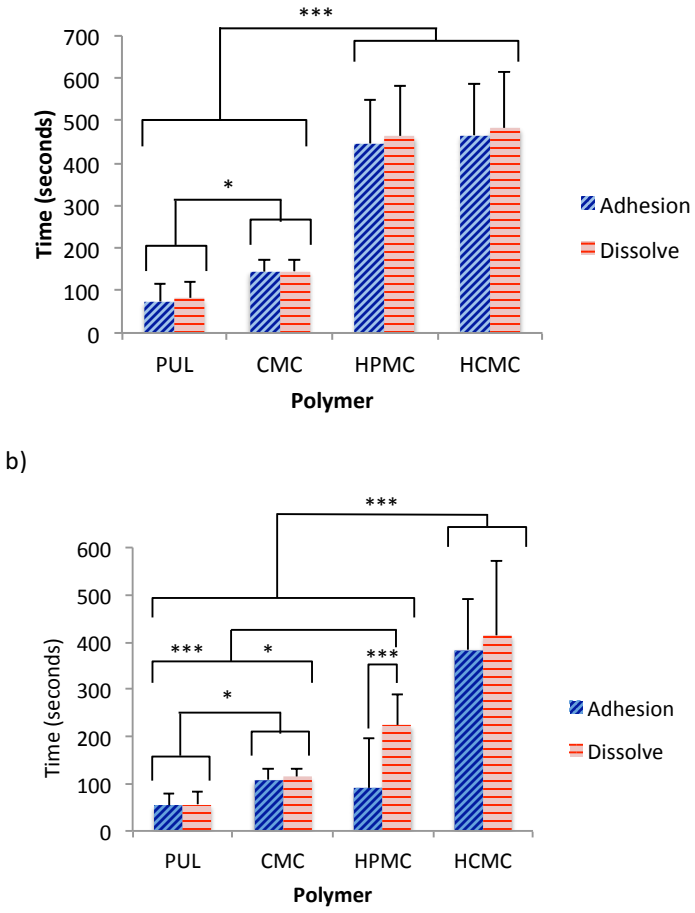

Figure 6.6. *In vivo* adhesion and dissolution times for a) polymer films without glucose and b) polymer films with glucose. Each bars represents the mean of 10 separate data points, error bars represent standard deviation. N= 5 in duplicate. * = $p<0.05$, *** = $p<0.001$.

The HPMC films with glucose were reported to adhere for a significantly ($p<0.05$) 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 6.6. b). These results reflect the *in vitro* tensile experiments that found HPMC to be significantly ($p<0.05$) 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 adhesion after an initial delay. There are two explanations to this

discrepancy. Firstly, AS was used in the *in vitro* experiments, which contained pig gastric mucin as opposed to human salivary mucin. This may affect interactions between the PS matrix and the saliva. 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 difference in hydration may have led to a stronger adhesion *in vivo*, as the film did not become overhydrated.

The PUL film dissolving and adhesion time was significantly ($p<0.05$) quicker than LCMC films in these experiments. The PUL films dissolved on average at 81 s compared to 145 s for the LCMC films. These dissolution times contrast with the results obtained from the *in vitro* dissolution tests (table 6.3.) where they were not significantly different. The differences found in the characterisation of films was expected to have an impact on flavour release from LCMC films compared to PUL.

6.3.4. Perception

Panellists produced time intensity curves for each sample and repeat. They 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 (Tmax), maximum intensity (Imax), duration of perception, and incline and decline angles (Chapter 4, Figure 4.1.).

6.3.4.1. Glucose only films

Time intensity curves were averaged for all panellists and their repeats for the sweetness attribute (Figure 6.7. a). The AUC and Imax of sweetness for the films was PUL >LCMC >HPMC >HCMC with the reverse order for Tmax (Table 6.5.).

These parameters suggest 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 6.3.) show that PUL and LCMC films were faster dissolving and release glucose quicker than HPMC and HCMC films. Although the total duration of perception was not significantly ($p>0.05$) different between the films, there is a trend that HPMC and HCMC films prolong the flavour perception compared to PUL and LCMC (Table 6.5.).

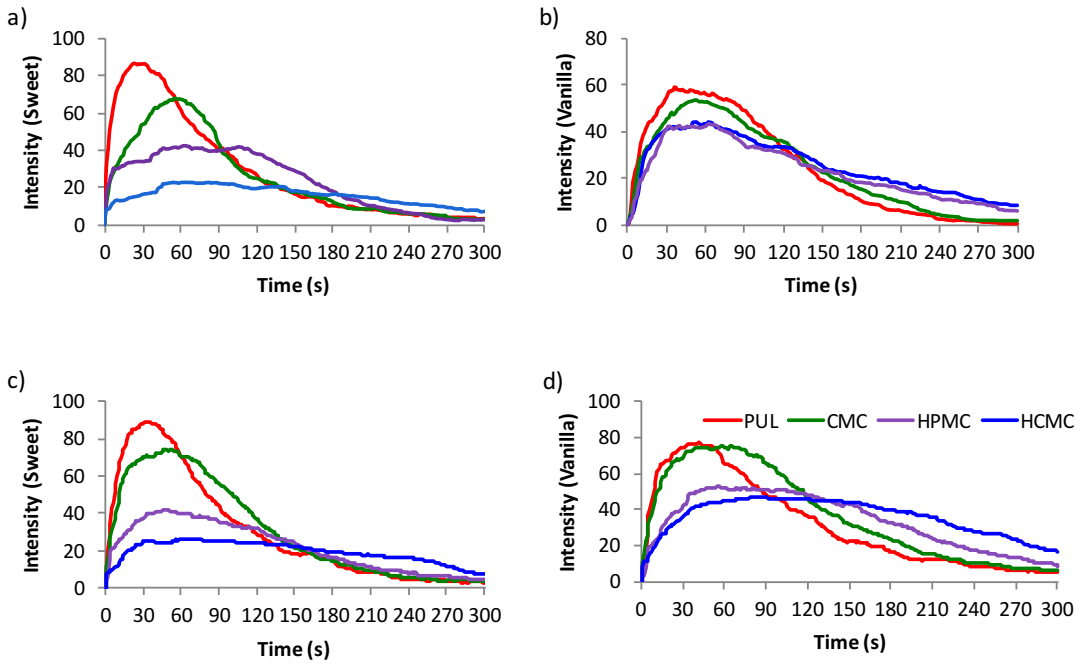

Figure 6.7. Time intensity curves of sweetness and vanilla perception over for a) glucose only films, b) vanillin only films and c) and d) for glucose and vanillin films scored by dual attribute time intensity.

Table 6.5. Parameters from time intensity results.

Matrix	Attribute	Polymer	AUC	Imax	Tmax (s)	Duration (s)	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.

Regarding mucoadhesion, the HPMC films containing glucose were found to have poor adhesive abilities (Figure 6.5.). In the perception experiments panellists were asked not to swallow these films and therefore the perception may have been artificially prolonged do 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 be swallowed with the food bolus thereby negating any release afterwards. On the other hand, HCMC films showed strong adhesion (Figure 6.5.) and therefore would be more likely to adhere to the oral cavity for longer, prolonging the release.

6.3.4.2. Vanillin only films

For films containing the PS and vanillin the Imax intensity ordering was PUL>LCMC>HPMC>HCMC (Table 6.5.). Tmax and AUC were not dependent on PS 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 flavour was delivered at a slightly lower intensity for longer in the HPMC and HCMC samples. This assumption is supported by the decline angles being larger for PUL and LCMC samples suggesting the intensity decreased quicker in these films.

To date, the only studies investigating aroma release and perception in food thickened with PSs are liquid and semi solid foods. These studies have found mixed 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., (2003b) on the other hand found that in-nose measurements of hydrophobic aroma release were not dependent on thickener type or 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 relatively hydrophilic molecule with a log P of 1.2, was used as the aroma. Perception results show that films made with slow dissolving polysaccharides (HPMC & HCMC) reduced the Imax but prolonged the duration of perception. Perception results for the aroma only films were not as distinguishing as the films containing glucose. This indiscrimination 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.

6.3.4.3. Glucose & vanillin films

Dual attribute time intensity was used to simultaneously monitor sweetness and vanilla attributes over 5 minutes. Results for the sweetness attribute were similar for the dual attribute and single attribute tests (Table 6.5.). The AUC and Imax were highest for PUL and lowest for HCMC. HPMC and HCMC took longer to reach Tmax compared to PUL and LCMC.

The AUC for the vanilla attribute did not significantly differ with the different PSs (Table 6.5.). HPMC and HCMC had reduced Imax and increased Tmax results compared to PUL and LCMC. The total duration of perception was striking in these films with the HCMC averaging 53 s longer than PUL. HPMC also increased the duration significantly ($p<0.05$) 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 LCMC 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 Imax. HPMC has a slower on set to reach Imax and the perception continues for longer than LCMC and PUL. Finally, HCMC films have the slowest onset with a steady release over time. This delay 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 mucoadhesive (Figure 6.6.) 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 swallowing 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.

6.3.5. Comparing perception results to in vivo dissolution

During the *in vivo* experiments where participants were asked to record the adhesion and dissolving time of the films, PUL was reported to dissolve after an average of 57 s. When comparing these timings to the perception data it is clear that perception of flavour is continuing after the film has completely dissolved (Table 6.5. & Figure 6.6.). 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 may have occured where the sweet signal is switched on for a longer time even after the stimulus at the taste bud has gone.

6.3.6. Comparisons between different film types

Time intensity results were compared between 5 panellists who were consistent for all experiments. The AUC for the vanilla attribute differed between films with and without glucose (Figure 6.8.). Significant ($p<0.05$) increases 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 that this is the only factor.

A more likely explanation is that the presence of glucose in the films enhanced the aroma through cross modality [16,36,37]. Tmax was also significantly ($p<0.05$) increased for vanillin in the HCMC films incresasing from 26 to 89 s (Figure 6.7.). This suggests that when glucose was present the perception had a longer onset of aroma perception, which lasted for longer and was sustained.

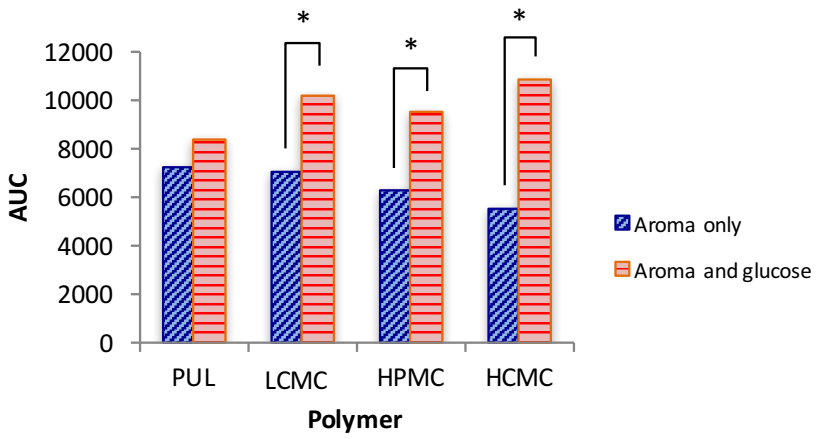

Figure 6.8. Comparisons of the area under the curve for vanilla attribute of films with and without glucose. * denotes significant differences $p < 0.05$.

6.4. Conclusions

The work in this chapter has shown that PSs affect the retention, release and perception of flavour compounds, dependant on the physicochemical properties of the PSs matrix. The viscosity and swelling ability of the PS influences the release of flavour molecules from the matrix. This in turn has an impact on the flavour perception accordingly. Fast dissolving PS films gave a quick burst of flavour at high intensity that tapered 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. This is the first investigation highlighting the importance of characterising the mucoadhesion properties of PSs in food systems regarding their influence on flavour perception. Results from this study can be used to inform the food industry of the impact which addition of such PSs can have on temporal flavour perception.

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Chapter 7: Application of mucoadhesive polysaccharides

7.1. Introduction

Commercial snack seasonings are developed by blending base flavourings, fillers and top notes to produce well-balanced flavour profiles on a final product. Tastants such as salt and sugar make up the majority of the base flavourings and fillers such as whey permeate powder and maltodextrin are used to add bulk and aid in the blending of ingredients [1]. Sugar and salt also provide the crystalline material which provides a suitable structure on which to add the liquid flavourings and enables good flow properties. Flavour enhancers such as monosodium glutamate (MSG) and yeast extract are also often used to enhance savoury seasonings [2]. The characteristic notes of a flavouring are usually due to the top notes that are in the seasoning such as a smokey note in a paprika flavouring. These tend to be more expensive ingredients, often highly volatile and oil-based. Some ingredients will give mouthfeel or textural characteristics such as cheese flavouring to give a fatty mouthfeel [3].

There are various ways to apply the seasoning to a dry snack food and this is dependent on 4 main factors; the type of snack, the method of cooking it, how much fat is required in the final product, and flow properties of the seasoning. For example, a slurry with oil and the seasoning can be sprayed directly onto extruded snacks and the oil is used as the adherent for the seasoning [3]. However, in snacks that are baked to reduce the fat content, a small amount of oil is typically used to sparingly coat the snack and the seasoning applied topically. This redcued fat content obviously results in more seasoning drop off than the slurry method and various attempts have been made to combat this issue including the use of

adhesive PSs [4]. Furthermore, PSs have been used to reduce oil absorption in fried snacks by forming an impenetrable barrier between the hot oil and the food matrix [5].

Popcorn can be seasoned in a variety of ways depending on the desired nutritional outcome. For example, a sugar-based glaze can be applied to the popped corn containing the seasoning and further seasoning can then be added topically if required. The glazing step can be bypassed in order to create popcorn varieties with lower fat and sugar contents. The steam and moisture produced from popping the corn, along with a limited amount of oil can be used to adhere dry seasonings to the popcorn, however, this too would lead to a large amount of seasoning drop off, reducing the overall flavour impact and increasing waste.

The results from chapter 6 indicate that PSs can have an impact on the delivery and subsequent perception of flavours over time. This change in flavour delivery could be utilised in solid snack food products to augment the delivery of flavourings over time [6–8]. The work in this chapter aimed to investigate if PSs could be used as mucoadhesives within a snack seasoning to prolong flavour delivery in a real food application. Popcorn was chosen as the base food due to its ease of application, absence of inherent tastants and oil, and relevance to the sponsor of this PhD thesis, McCormick (UK) Ltd.

7.2. Methods

7.2.1. Seasoning recipe, snack base and coating method

Recipes for vanilla and Eton mess seasonings for popcorn were formulated using the ingredients listed in tables 7.1. A vanilla flavouring and a strawberry

flavouring was obtained from Create (North Somerset, UK) to give the characteristic flavours to the popcorn, respectively. Seasonings contained maltodextrin as a base filler, where the control formulation contained 43-56 % maltodextrin Table 7.1.). The modified formulations replaced the majority of this maltodextrin (20% of by weight of the seasoning) with the mucoadhesive PS (HCMC)

Table 7.1. Vanilla and Eton mess popcorn seasoning ingredients and their proportions. Seasonings were made up in 300g batched.

Ingredients	**Proportion (%w/w) in vanilla seasoning**	**Proportion (%w/w) in Eton mess seasoning**
NaCl	6	6
Sucrose	15	15
Rapeseed oil	0.8	0.75
Maltodextrin	35.8	22.9
Whey permeate powder	20	20
Vanilla flavouring	4	0
Butter sweet	1	0
Citric acid	0.4	0.4
Silicon dioxide	0.4	0.4
Strawberry powder	0	3.3
Beetroot powder	0	1
Strawberry flavouring	0	2
Creamy mouthfeel	0	1.3
Remaining bulk (maltodextrin in control; HCMC in modified formulation)	20	20
Total	**100**	**100**

Popcorn (EastEnd, Tesco, UK) was popped in a microwavable popcorn bowl for 2 min 30 s. The popped corn (100g) was transferred into a large metal bowl and set aside. All the ingredients for the glaze (Table 7.2.) were heated on an electric hob

(medium heat) in small saucepan until the mixture started to bubble. Once boiling, a timer was started and the mixture simmered for 5 minutes, after which the hot glaze was poured over the popcorn. The popcorn was stirred for 2 minutes to evenly coat the popcorn pieces with the glaze. The coated popcorn was transferred into a preheated oven at 100°C. At 15 minute intervals, the popcorn was stirred to make sure the drying process was uniform. After 1 hour, the bowl was removed from the oven, the popcorn was spread out onto greased proof paper and left to cool for an hour.

Table 7.2. Glaze ingredients and their proportions. The glaze was made up in 300g batches for covering of one batch of popcorn.

Ingredient	**Proportion (%w/w) in glaze**
Water	14
Glucose syrup	20.7
Extra fine caster sugar	51.7
Rapeseed oil	9.3
Salt	0.3
Vanilla or Eton mess seasoning	4
Total	**100**

After cooling, the popcorn was transferred into a large metal spherical seasoning drum on an automatic rotator. The drum turning speed was set to 60 rpm and was heated externally with a heat gun during the seasoning process. This heating was to warm the glaze to help the seasoning to adhere. The 12g of seasoning to be topically applied was put into a seasoning shaker and shaken over the popcorn whilst it was rotating. The seasoning contained either 20% HCMC or solely maltodextrin filler as the standard.

7.2.2. Addition of polysaccharides

Various combinations and ways to incorporate the polysaccharides and varying amounts of PS substitution were trialled (Appendix 2). The PS (HCMC) was always added to the dry seasoning mix, replacing some of the maltodextrin used as a bulking agent. The various % (w/w) substitutions were made and tested (Appendix 2), 20% substitution was taken forward for further testing as the preliminary test showed difference between the products (Table 7.3.). There were three options to incorporate the PS into the final product. These were: addition of PS containing seasoning half in the glaze and half topically, standard seasoning addition to the glaze and PS containing seasoning topically or PS containing seasoning in glaze and standard seasoning applied topically. The latter option was not explored in this chapter. The first option, addition of PS containing seasoning in the glaze and topically, was attempted, however, due to the drastically enhanced viscosity of the glaze it was decided this option was not viable from a food manufacturing perspective. Instead the second option, where seasoning without the PS was added to the glaze and seasoning with PS was applied topically was employed.

7.2.3. Quantitative descriptive analysis of vanilla popcorn

For all sensory experiments in this chapter the following was used. A trained sensory panel at McCormick (UK) Ltd consisting of 10 women and 2 men with an average age of 45 ± 17 years. Experiments were done in duplicate with 15 minute intervals between each sample. Panellists were in isolated sensory booths at 20°C under artificial daylight lighting with fresh water as a pallet cleanser. Samples were presented monadically with a balanced order between panellists and sessions. Panellists were instructed to taste 3 individual pieces of popcorn before

scoring the flavour attributes. Intensity scoring was captured with Compusense software on a structured line scale (Figure 7.1.).

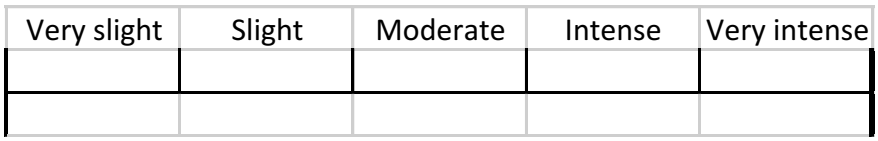

Figure 7.1. The structured line scale that panellists used to score each attribute. Numerical values were assigned to where the panellists clicked ranging from 0-15.

Panellists were trained for 4 hours over two days. The two samples were presented with 3 digit random number blinding codes. Panellists first tasted and described the product individually and then refined their attributes into a consensus vocabulary with the help of the panel leader and reference standards. Vanilla sugar (Taylor & Colledge) and vanilla frosting (Tesco) were used as reference. A consensus vocabulary was established incorporating 4 aroma, 8 flavour and 5 aftertaste attributes (for definitions see Appendix 3). After taste was scored 30 seconds after panellists swallowed the sample.

7.2.4. Progressive profiling of vanilla popcorn

Progressive profiling is a temporal sensory method that allows panellists to score up to 5 attributes at set time points during the eating process. During training the panellists and the sensory panel leader decided on three key attributes; toffee, vanilla and salt to be used for the profiling. These were chosen after preliminary tests on paper to see how the attributes changed over time. Training in the booths on the Compusense software was done on these attributes, concentrating on one attribute at a time at first then all three together so the panellists became familiar with the experiment set up.

Panellists were instructed to place 3 individual pieces of popcorn in their mouths and chew whilst scoring the flavour attributes then after 20 seconds to swallow the popcorn. For this experiment time intervals of 10 seconds were chosen to capture changes in the intensity of the attributes during mastication and after swallowing. A total of 9 scores were taken over a 90 second period. Each popcorn type was tested in duplicate.

7.2.5. Difference testing with untrained consumers

A triangle test was carried out at the University of Reading with 25 untrained consumers (age range 21- 44). The vanilla flavour popcorn and the Eton mess flavoured popcorn were tested. The test was explained to the consumers and they were asked to rinse their mouths thoroughly with water between each sample. Participants were sat in isolated sensory booths at 20°C under artificial daylight lighting using Compusense software to record the data. Samples were presented in a balanced order between panellists. The participants were asked to taste each sample, rinse thoroughly between samples and then decide which they thought was the odd one out (the different sample). This was a preliminary test to see if further differentiation testing with the appropriate number of assessors (at least 70 people) was required.

7.2.6. Statistical analysis

One-way rmANOVA was used for QDA results with sample type as the factor. Two-way rmANOVA were used for the progressive profiling results with time and sample type as factors. XLSTAT version 2017.1 was used for data analysis. Tukey HSD was used as a correction for multiple comparisons and a significance level of $p \leq 0.05$ was accepted.

7.3. Results & discussion

The preliminary testing of popcorn samples with the addition of mucoadhesive PS showed differences with the modified popcorn being less crunchy, sweet and having a slimy mouthfeel (Table 7.3.). These were with 5 assessors of the flavour team at McCormick (UK) Ltd.

Table 7.3. Preliminary taste screening test of standard and modified vanilla popcorn with 5 assessors.

Product	**Summary of Comments (5 assessors)**	**Mean Liking Score (1-5)**
Standard	Good TF, crunchy, sweet, creamy, nice, sticky, less slimy.	4.7
Modified (20% HCMC)	Less crunchy, longer AT, more toffee, sweet, caramel, soft crunch, softer, milky, TF down, slimy MF.	3.9

Key: Total flavour (TF), After taste (AT), Mouthfeel (MF).

7.3.1. Sensory QDA profiling of the vanilla popcorn samples

Of 17 attributes rated, 5 were significantly ($p<0.05$) different between samples (Figure 7.2.). These attributes were overall aroma, flavour impact, coconut oil, toffee and umami flavour where the test sample scored lower than the standard. There was also a similar trend with the floral/ perfume aroma and aftertaste. However, the aftertaste attributes did not differ significantly between the two samples.

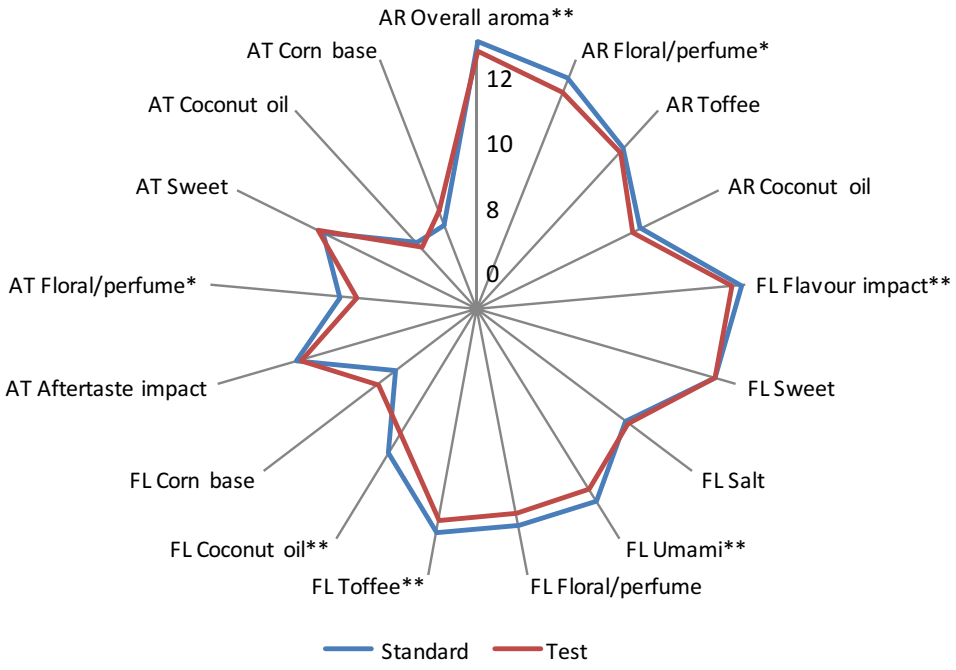

Figure 7.2. Quantitative descriptive analysis results of the test sample compared to the standard. Significance values of p <0.1 for * and p <0.05 for **. Scored in duplicated n= 12.

The reduction of flavour impact for the test sample was not surprising as results from Chapter 6 demonstrated that, in a model system, HCMC reduced the intensity of flavour but prolonged it over time. The mucoadhesive PS was applied to the popcorn as a dry topical seasoning. Once the PS encountered the moist oral cavity, it is expected that the PS chains will hydrate and form a viscous network in the mouth. This network may then trap the tastant and aroma molecules and prevent them from activating the appropriate receptors. However, due to the mucoadhesive nature of the PS it was hypothesised that although the intensity would be reduced, the flavour would be released slowly after swallowing the sample as the PS will remain in the mouth with some of the seasoning. The results from this QDA do not support this hypothesis; however, the test was not time

constrained so panellists would be scoring the aftertaste at different times to each other.

There may have been some misunderstanding between panellists about the floral aroma attribute which described the vanilla notes. Some of the panellists were scoring the vanilla attribute with what they described as a deep and dark vanilla flavour in mind whilst others were using the references to score the vanilla, thinking of lighter, fruity flavour and putting any "dark" attribute into the toffee score. These attributes were difficult to separate due to the complex nature of the vanilla flavouring along with the glaze.

The panellists did not describe any mouthfeel attributes within the QDA vocabulary. This was surprising as during the preliminary screening (Table 7.4. & Appendix 2) several assessors noted a slimy mouthfeel for the test sample. There are several possible explanations for this omission, firstly, the panel are used to concentrating on the flavour and aroma of products as the texture of the products they frequently assess are very similar. However, they do assess mouthfeel characteristics and during training a few of the assessors mentioned a mouth-watering characteristic of the test sample but not everyone was in consensus. This may mean that there is individual variability in perception of any altered mouthfeel. This could be due to individuals saliva production, the amount of mucin present and the way they manipulate their food [9].

7.3.2. Progressive profiling of the Vanilla Popcorn Samples

Results from the progressive profiling show that the intensity of attributes changed significantly ($p<0.001$) over time, but there were no significant

differences between samples (Figures 7.3. - 7.5.). For all 3 attributes the peak intensity was before swallowing and the intensity dropped after swallowing. Although there was a tendency of the test sample to be rated higher for toffee and salt perception over time compared to the standard, this was not significant (p=0.503 for toffee and p=0.518 for salt)

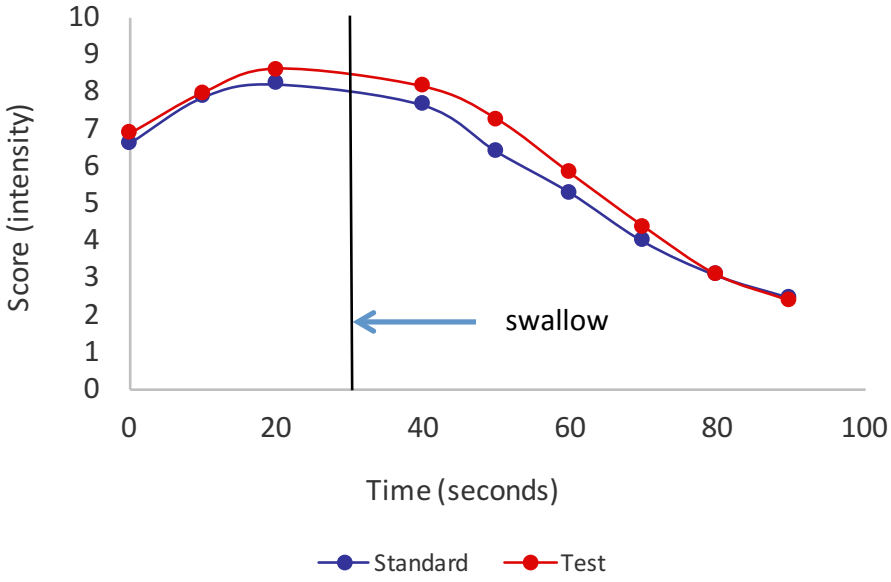

Figure 7.3. Progressive profiling results from trained panel for *toffee* attribute. Intensity scale was 0-15. There were no significant differences between the samples over time (p=0.503 for sample, p=>0.000 for time and p=0.778 for sample*time).

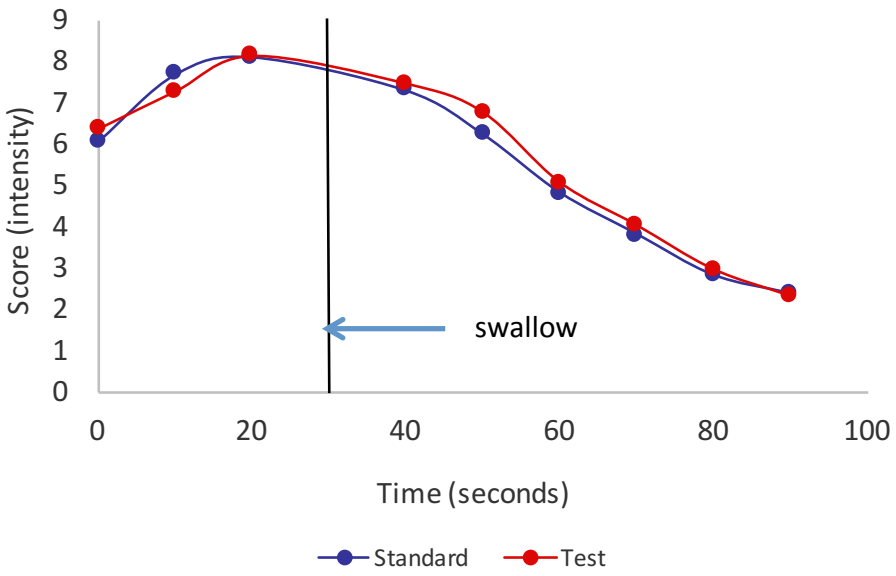

Figure 7.4. Progressive profiling results from trained panel for *vanilla* attribute. Intensity scale was 0-15. There were no significant differences between the samples over time (p=0.976 for sample, p=>0.000 for time and p=0.643 for sample*time).

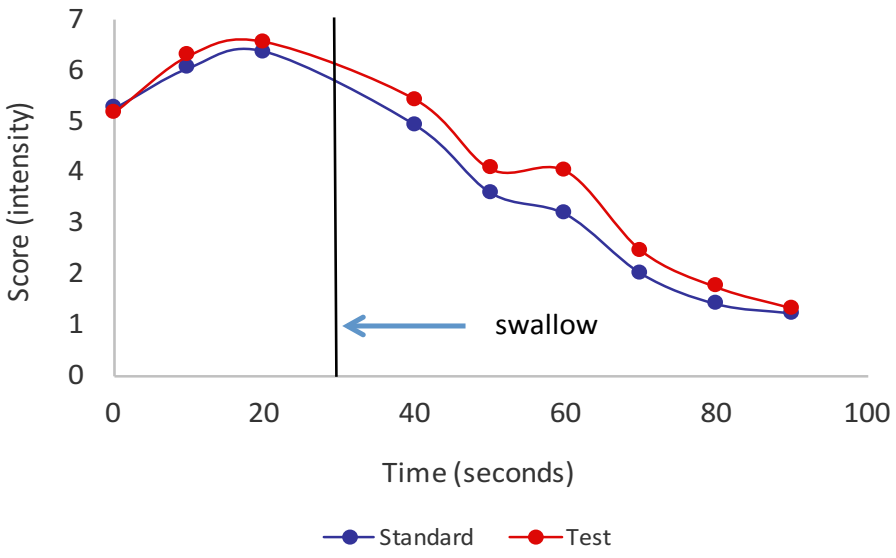

Figure 7.5. Progressive profiling results from trained panel for *salt* attribute. Intensity scale was 0-15. There were no significant differences between the samples over time (p=0.518 for sample, p=>0.000 for time and p=0.376 for sample*time).

The results from the training for progressive profiling showed promising trends in the data (Appendix 4). During training, the panellists ran through the Compusense test scoring all three attributes for both samples, however, all panellists received the same sample at the same time. These preliminary results show significant ($p<0.05$) differences between the test and the standard popcorn with the former scoring higher in toffee and lower in vanilla over time. The differences were not reflected in final rating where there were no significant differences between the samples, although the trend for toffee was the same. It was concluded that the panellists learnt to recognise the samples as, unfortunately, the same blinding codes were used during the training session in the discussion room as and the training test in the booths but different ones were used during scoring sessions. Therefore, these results cannot be used to support the hypothesis.

Although the panellists said that they perceived differences between the samples during training, neither the QDA nor the progressive profiling tests could identify what those differences were. A possibility is that the panel were detecting differences in mouthfeel characteristics that they could not explain. Another possibility is that there are differences between the products but they are not obvious enough to be significant when in a blind test. Furthermore, popcorn as a base is difficult to control due to; 1. the coverage of glaze and 2. the coverage of seasoning. Therefore, the heterogeneous nature and piece to piece variability make it difficult to tell subtle differences between different seasoning blends.

7.3.3. Difference test with consumers

Two sets of samples were investigated by difference test; a vanilla set and an Eton mess set. The Eton mess flavour was introduced as it was thought that the vanilla flavouring may be too complex to differentiate the different samples. Results from the triangle test show that the assessors were not able to distinguish the different samples (Table 7.4.). From these results, it was decided that further testing would not take place as they did not provide promising evidence that a difference would be found.

Table 7.4. Results from triangle test of untrained assessors.

Popcorn flavour	**Correctly identified the different sample**	**Did not correctly identify the different sample**	**Significance (p value)**
Vanilla	7	18	0.778
Eton mess	11	14	0.178

More participants correctly identified the different sample for the Eton mess flavour which could mean there is variability in the effect of PS on different flavourings. The impact of PSs in the seasonings was clearly minimal despite differences being perceived by individuals that had some information about the project (Table 7.4.). Further differentiation tests with these samples would require 140 participants to have enough power for statistical testing. Comments from the triangle test did not concur with each other with some saying the test sample higher in sweetness and some saying the standard was higher. There was not a trend with what the participants thought the difference was. In addition to the possible explanations set out in section 7.3.3. a further factor that may have played an essential role here is that the consumers may not have thoroughly rinsed

between samples and therefore, sample cross over would be detrimental to determining any differences between samples.

7.4. Conclusions

The results from the application work suggest that the addition of PS in this way to popcorn may not be a worthwhile pursuit. Although preliminary testing with a small number of people suggested there were differences between the products, in properly executed experiments the differences were minimal. Furthermore, those that could detect a difference tended to like the test sample less than the standard (Table 7.4; 4 out of 5 assessors rated the test sample lower in liking than the standard). At least 100 people should be used for a hedonic test, however, during the preliminary trials the test samples consistently scored lower than the standard. These results may suggest that in larger studies this may also be the case. However, in the preliminary tests, the assessors are experts as finding differences in the products and some had prior knowledge of the project to know which attributes to pay most attention to. When the assessors are unware of what attributes might be changing, no effect was found. The results suggest that there may be subtle differences between the products such as those found in the QDA but individual variability may mean that the differences are not always detectable.

7.5. References

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Chapter 8: General conclusions & future work

The literature review in Chapter 1.2 exhibited the limited research into the area of mucoadhesive PS and food, and highlighted opportunities to improve the apparent gap in knowledge. Whilst the adhesion of native food ingredients such as fat [1,2] and aroma compounds [3] have been investigated, the role of mucoadhesive PSs requires more attention. The research described in chapters 3 – 7 for this PhD thesis was undertaken to elucidate the impact and potential benefits that mucoadhesive PSs have on flavour retention and perception in liquid and solid foods.

8.1. Food grade polysaccharides are mucoadhesive

In agreement with various studies in the literature [4–6], the work in chapter 3 found that LCMC, LMEP and SA are mucoadhesive on porcine tongue tissue. The mucoadhesive strength was concentration and PS type dependant. The results showing mucoadhesive strength increases as concentration increases (along with viscosity) is in line with current literature [7,8]. The results in chapter 3 found starch to be a relatively weak mucoadhesive compared to the other PSs used. This is most likely due to the granular structure of starch in solution [9] which does not lend itself to mucoadhesion.

To validate the retention method in chapter 3, PSs were either labelled or mixed with sodium fluorescein and the retention profiles compared. The results showed minimal differences between labelled and unlabelled PS solutions. Due to the time and difficulty of labelling PS, it was concluded that it was not necessary to label the PSs for the experiments in this work. Using unlabelled PS solutions also meant that the amount of fluorescence could be controlled between samples whereas the

labelled PSs differed in their fluorescence intensities. The method was used in subsequent chapters for unlabelled PSs including the starch samples.

The mucoadhesive nature of many PSs may be an important property that can affect the organoleptic properties of a food product that contains them. The strong adhesion to the tongue may influence the perception of flavour compounds. The viscous solution could either prevent flavour compounds reaching the respective receptors indefinitely or could facilitate a time dependant delivery of flavour compounds as they are held near the taste buds rather than being swallowed in a food bolus.

8.2. Mucoadhesives alter flavour delivery and perception

The results from chapter 4 show that sodium and potentially glucose molecules are retained in the mouth longer after swallowing samples with a LCMC matrix compared to other PSs matrices and water. The LCMC containing solutions did not significantly change the intensity or duration of sweetness or saltiness perception over time compared to water only samples. The results from this work suggest that the in-mouth concentration of tastants does not necessarily reflect the intensity of the perception of them. This disconnect may be because the PSs reduce the mass transfer of tastants out of the matrix and therefore never reach the receptor to elicit a sensory response [10,11].

Chapter 3 results from the retention studies found that LCMC washed off more easily than SA LMEP at higher viscosities. However, at the low viscosity used for experiments in chapter 4, there was minimal difference between retention profiles of all PSs used. A possible explanation as to why LCMC retained more tastant is

that the concentration used was higher than the other two PS. The impact on tastant perception differed between the two tastants and the different matrices used. For the salty solutions, there were some slight differences in perception between the different PSs. For example, the total perception of saltiness over a two-minute period was reduced in SA and LMEP containing samples compared to LCMC containing samples. However, they were not significantly reduced compared to water samples. If the effect was purely to do with viscosity then one would expect the water sample to have the highest intensity. Furthermore, for the sweet samples the parameters extrapolated from the time intensity curves found very few differences between the samples. This could suggest that the PS containing samples did not have much impact on tastant delivery at such low PS concentrations.

Chapter 5 expanded on the preliminary results in chapter 4 by adapting the methods used to capture sensory data and weighing saliva to allow for more accurate quantification of saliva sodium levels. In chapter 5, LCMC was tested against a starch containing sample matched at the same viscosity (50 rad/s) and a water sample. Results in this chapter found that LCMC substantially reduced the perception of saltiness over time compared to the water and starch containing samples but the mouthcoating and adhesion perception was scored higher for LCMC. It was concluded that the reduction in salt perception was due to the anion effect for the LCMC samples. However, the *in vivo* sodium concentrations over time were higher for the LCMC containing samples than starch and water. This suggests that the mucoadhesive LCMC can retain sodium for longer in the mouth, however, the perception of saltiness is stunted due to the large anion effect.

Chapter 6 explored the impact of different PS matrices on the release and perception of flavour in solid films based on pharmaceutical formulations. Films were chosen as a model system to move towards the potential use of mucoadhesive PSs in solid foods. The results show that the release and subsequent perception of flavourings can be controlled by the composition of the food matrix. The swelling, dissolution and mucoadhesion properties of the PS matrices were important determining factors of how the flavourings were perceived over time.

Results from chapters 3- 6 in this work found that mucoadhesive PSs have differing effects on flavour retention and delivery depending on the type of mucoadhesive and the viscosity grade. In liquid systems, the saltiness of sodium was reduced due to viscosity and anion effects, and in solid systems sweetness and vanilla aroma can be controlled by using PSs with different mucoadhesive strength, dissolving and dissolution speeds.

8.3. Influences on the food industry

Chapter 7 details the work carried out at McCormick (UK) Ltd, Haddenham as part of the fulfilment of the BBSRC CASE studentship that funded this PhD. HCMC was selected as the mucoadhesive to use for the studies in this chapter. Experiments were based on the results collected in chapter 6 where HCMC augmented the delivery of flavour over time. Two different options were assessed to incorporate the mucoadhesive PS into a popcorn seasoning. The heterogeneity of the popcorn pieces was thought to be the main contributing factor to why significant differences were not perceived for mucoadhesive PS containing snacks. The impact of having mucoadhesive PSs in snack foods may be better elucidated using another type of snack such as crackers or cereal bars.

Although the application for mucoadhesive PSs attempted in chapter 7 was not successful, there are many other avenues of applications. The understanding of mucoadhesion in food substances could have impacts on the food industry, whether mucoadhesives are added as a functional ingredient, or whether native mucoadhesives in the food are manipulated to control sensory properties. By understanding the properties of mucoadhesive food components, a higher level of control could be achieved in the texture and flavour of a food product.

8.4. Future work

This work contained in this thesis can be used as a foundation for the investigation of other mucoadhesive PSs and their use in a range of food systems. Further work could focus on the impact of the mucoadhesive properties of PSs (e.g. xanthan gum, carrageenan, pectin) already employed in the food industry for thickening and stabilising liquid and semi solid food products or focus on the potential for using mucoadhesive PSs in new product design to control texture and the delivery of flavourings over time. Fields where new product design could benefit includes confectionary, sports products and health foods.

Chapter 6 investigated the impact on the release of vanillin, a relatively hydrophilic aroma compound. Vanillin was purposely chosen for its solubility in aqueous solutions so that it would not partition out of the PS matrix when they were dehydrated to form a film. The way this experiment was designed meant that the impact of different PS matrices could not be assessed for more hydrophobic compounds such as those mentioned above. However, this does not mean that they would not have an effect in a real food product that will be a more complex

matrix. There is potential that mucoadhesive PS could control the delivery of hydrophobic aroma compounds. Although some studies suggest that release of hydrophobic aromas are not effected by PSs [12,13] others have found that PSs do reduce the release [14]. This highlights the complex relationship between PSs and the impact on aroma compound retention and release but also the potential for their use to control aroma release. Future work could focus on elucidating the impacts of mucoadhesive PSs on hydrophobic aromas over time.

Another potential application is the use of high viscosity mucoadhesive PSs (e.g. CMC) for confectionary where a persistent flavour would be desirable. For example, a prolonged sweet flavour may reduce the amount of food consumed and therefore aid with weight loss. Another possibility is the use in breath fresheners such as mints. Quick dissolving films made of pullulan have been used for this purpose (Listerine Pocketpacks®), however, slower dissolving films could be made so that the flavour lasts longer. This effect could be extended to many different pleasant flavours.

The potential for using PSs as a way to adhere topical seasonings to snacks has been explored by Armstrong (2013) [15]. In their work, they apply a thin film of PS to a cracker and this is used to adhere the seasoning to the cracker. The results are promising with regard to reducing the amount of seasoning drop off, however, the impact of these hydrocolloid coatings on the sensory properties of the crackers was not investigated. It would be interesting to see if there were any changes to the flavour delivery and any changes to the textural characteristics of the crackers.

Finally, investigating the potential for mouthfeel and textural changes by mucoadhesive PS could be useful. This has already been explored for liquid and semi solid foods such as dairy products and desserts [13,16,17] but the impact of mucoadhesion on the texture of these product still requires some attention. Tribology techniques can be employed to understand the lubricative properties of PSs with saliva in the mouth and how this may translate to an oily/ slippery mouthfeel when reducing fat.

8.5. References

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Appendix 1: Flame photometry standard curve

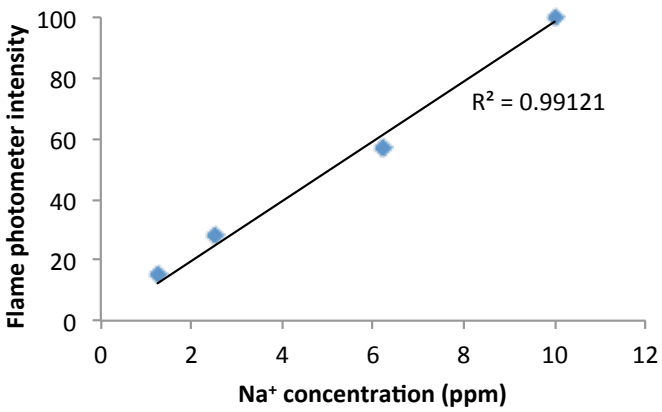

Figure A1.1. Sodium standard curve produced by flame photometry.

Appendix 2: Screening results for popcorn with different formulations

Table A2.1. Preliminary screening for the various popcorn formulations.

Seasoning	Product	Summary of Comments	Mean Liking Score (1-5)
24g in glaze (n=5)	Standard	Sweet, less chewy, vanilla OK, crispy, butter, nice crunch, caramelised sugar, good AT.	4.5
	Modified (13.3 % HCMC)	Less crispy, less vanilla, more sweet, longer AT, TF down, chewy, nice AT.	3.9
12g (standard) in glaze and 12g topical (n=4)	Standard	Nice creamy vanilla, buttery, good overall impact.	4.25
	Modified (13.3 % HCMC) topical	Vanilla down, burnt up, caramel, TF down, slimy MF, greasy MF, buttery, base up.	3.25
12g in glaze and 12g topical (n=4)	Standard	Very sweet, creamy vanilla, good body, clean.	4.25
	Modified (13.3 % HCMC) 12g topical	Woody, spicey, cooling, strange MF, slimy MF, buttery, less vanilla, sweet, creamy.	3
	Modified (16.6 % HCMC) 12g topical	Toffee, caramel, vanilla ice cream, good MF, long AT, creamy, slimy MF, low vanilla, burnt, TF down, slimy MF, jelly like.	3.13
	Modified (13.3 % HCMC) in 12g glaze and 12g topical	Good MF, low TF, eggy, low vanilla, low sweet.	2
12g	Standard	Sweet, low vanilla, crispy,	4

(standard)		chewy, good TF.	
in glaze and 12g topical (n=3)	Modified (16.6 % HCMC) 12g topical	Slimy MF, good TF, crunchy, less crunchy, buttery, low vanilla.	2
	Modified (16.6 % LCMC) 12g topical	Good texture, bttery, low vanilla, crispy, TF down, light, sweeter.	3.5
	Modified (16.6 % HPMC) 12g topical	Chewy, slimy MF, vanilla, sweet, good TF, less vanilla, less sweet.	2.83
12g (standard)	Standard	Good TF, vanilla, sweet, sticky, nice crunch, caramel.	4.7
in glaze and 12g topical (n=5)	Modified (20 % HCMC) 12g topical	Less crunchy, longer AT, toffee, caramel, more toffee, TF down, slimy MF.	3.9

Key: Total flavour (TF), After taste (AT), Mouthfeel (MF). 1= dislike strongly, 2= dislike moderately, 3= neither like nor dislike, 4= like moderately, 5= like strongly

Appendix 3: Vanilla popcorn attributes for QDA

Attribute	Definition
AR Aroma Impact	Overall aroma impact, rated from weak to intense
AR Floral perfumed	Aroma associated with vanilla, perfumed notes rated from weak to intense Vanilla flavoured frosting (Tesco), vanilla bean dusting sugar (Taylor & Colledge)
AR Toffee	Aroma associated with toffee, caramel, butterscotch, rated from weak to intense
AR Coconut oil	Aroma associated with coconut butter, coconut oil, rated from weak to intense
FL Flavour Impact	Overall flavour impact, rated from weak to intense
FL Sweet	Basic flavour of sweet, from weak to intense
FL Salt	Basic flavour of salt, rated from weak to intense
FL Umami	Basic flavour of umami, rated from weak to intense
FL Floral perfumed	Flavour associated with vanilla, perfumed notes, fruity notes, rated from weak to intense Vanilla flavoured frosting (Tesco), vanilla bean dusting sugar (Taylor & Colledge)
FL Toffee	Flavour associated with toffee, caramel, butterscotch, rated from weak to intense
FL Coconut oil	Flavour associated with coconut butter, coconut oil, rated from weak to intense
FL Corn base	Flavour associated with the base, rated from weak to intense
AT Overall aftertaste	Overall aftertaste, rated from weak to intense
AT Floral perfumed	Aroma associated with vanilla, perfumed notes rated from weak to intense

	Vanilla flavoured frosting (Tesco), vanilla bean dusting sugar (Taylor & Colledge)
AT Sweet	Overall sweetness, basic of sweetness, rated from weak to intense
AT Coconut oil	Aroma associated with coconut butter, coconut oil, rated from weak to intense
AT Corn base	Aroma associated with the base, rated from weak to intense

Key: Aroma (AR), Flavour (FL), Aftertaste (AT).

Appendix 4: Training for progressive profiling

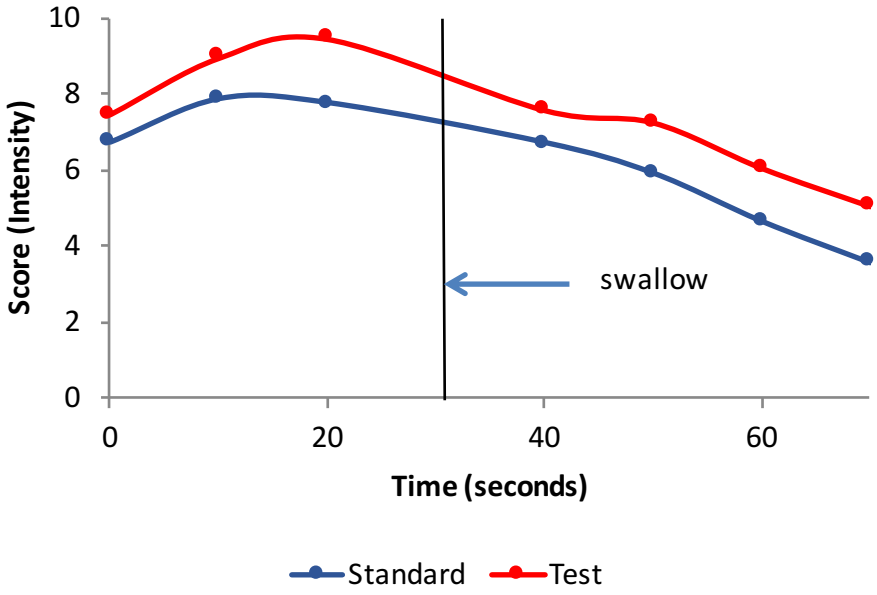

Figure A4.1. Training results for *toffee* attribute over 100 seconds. N= 11, p >0.000 for time and p 0.003 for sample. Sample x time interaction is p>0.000.

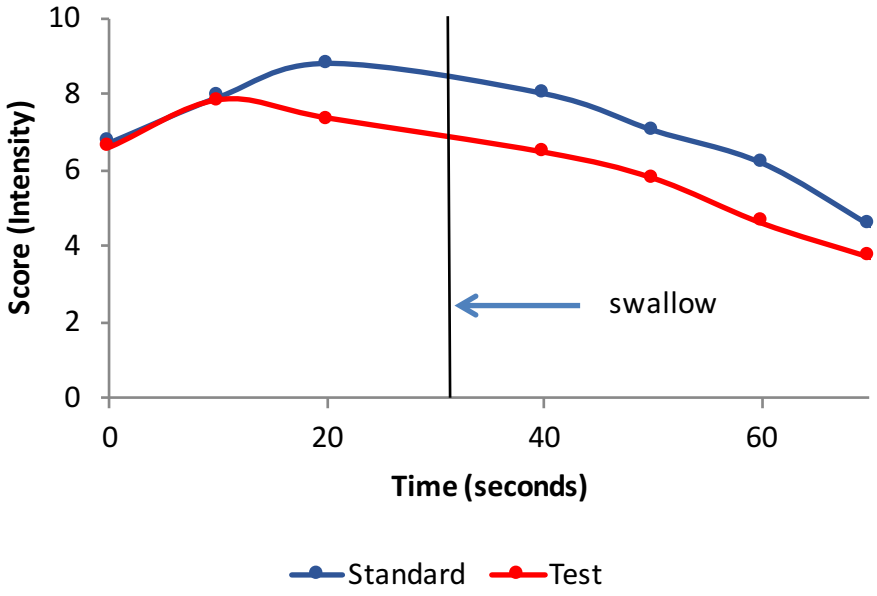

Figure A4.2. Training results for *vanilla* attribute over 100 seconds. N= 11, p >0.000 for time and p 0.011 for sample. Sample x time interaction is p>0.000.

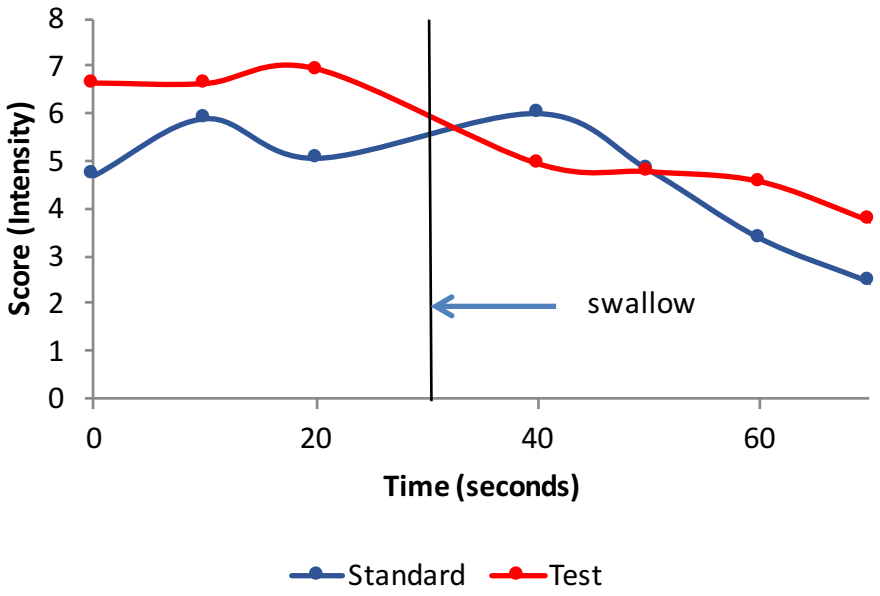

Figure A4.3. Training results for *salt* attribute over 100 seconds. N= 11, p >0.000 for time and p 0.003 for sample. Sample x time interaction is p>0.000.