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1   Polysaccharide food matrices for controlling the release, retention and

2   perception of flavours

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11

12 Abstract

13 Polysaccharides have many roles across both the food and pharmaceutics

14 industries. They are commonly used to enhance viscosity, stabilise emulsions

15 and to add bulk to food products. In the pharmaceutics industry, they are also

16 utilised for their mucoadhesive nature. Mucoadhesive polysaccharides can

17 facilitate retention of active ingredients at mucosal sites for a prolonged time

18 and formulations can be designed to control their release and bioavailability.

19 This study investigates how polysaccharides, with differing physicochemical

20 properties (e.g. functional groups and molecular weight), affect the release

21 and perception of flavour compounds from films. Polysaccharide films were

22 prepared using either high or low viscosity carboxymethyl cellulose, pullulan

23 or hydroxypropyl methylcellulose. Glucose, vanillin or a combination of both

24 was also added to the films to assess the effect of flavour release and

25 perception over time. The films were assessed for glucose release *in vitro*,

26 swelling and disintegration times, and mucoadhesive ability. Results show

27 that flavour release and perception depend on the polysaccharide matrix

28 properties; this includes how quickly the films dissolves, the rate of release of

29 tastant compounds, and the mucoadhesive strength of the polysaccharide. A

30 higher viscosity and slower disintegration time resulted in slower release of

31 glucose *in vitro* and flavour perception *in vivo*.

32

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

34

35 1. Introduction

36 Flavour perception requires the release of taste and aroma compounds from

37 the food matrix and the subsequent transport of those compounds to the

38 respective receptors. This process is dependent on many factors including the

39 properties of the compound, the components of the food matrix constituents,

40 food structure, how it is manipulated in the mouth and the physiological

41 conditions of the mouth, nose and throat during consumption of the food.

42 Furthermore, the onset and duration of flavour delivery is dependent on

43 factors such as partitioning, mass transport and diffusion. These factors play

44 varying roles and combined, result in a characteristic flavour profile for a food.

45

46 Typically, the polysaccharides, proteins and fats present in liquid food

47 systems determine the structure. The influence of these large molecules on

48 smaller molecules, such as aroma and tastant compounds, has been

49 investigated with various studies concluding that viscosity changes

50 (Hollowood, Linforth, & Taylor, 2002; Izutsu, Taneya, Kikuchi, & Sone, 1981;

51 Kokini, Bistany, Poole, & Stier, 1982; Secouard, Malhiac, Grisel, & Decroix,

52 2003; Stevenson & Mahmut, 2011) and physical entrapment of compounds

53 (Keršiene, Adams, Dubra, Kimpe, & Leskauskaite, 2008; Kora, Souchon,

Latrille, Martin, & Marin, 2004; Kuo & Lee, 2014) together explain perceptual

differences (S. L. Cook, Bull, Methven, Parker, & Khutoryanskiy, 2017). These

studies tend to focus on the matrix structure and the release characteristics

when contemplating changes in perception.

Chemical interactions between the flavour compounds and the food matrix is

also important (Heilig, Heimpel, Sonne, Schieberle, & Hinrichs, 2016;

Rodríguez-Bencomo et al., 2011; Scherf, Pflaum, Koehler, & Hofmann, 2015).

Factors such as charge of the flavour compound and other food constituents

will influence interactions between the two. For example, sodium is positively

charged and will therefore interact with negatively charged polysaccharides,

such as carboxymethyl cellulose, affecting the ions availability to elicit a salt

taste (Scherf et al., 2015). Retention of flavour compounds in the matrix will

obviously decrease their perception, as they will not reach the respective

receptors to be perceived and risk being swallowed in the food bolus before

triggering perception. However, if the matrix also adheres to the oral mucosa

then fewer tastant molecules may be swallowed allowing for release of the

flavour over time.

Many studies have investigated the impact on aroma release when reducing

fat in foods (Arancibia, Jublot, Costell, & Bayarri, 2011; Bayarri, Taylor, &

Hort, 2006). They have found, in general that aroma retention in the matrix of

a high fat food will increase as the P (partition coefficient of a molecule

between a lipophillic and an aqueous phase, usually octanol and water,

respectively) of the aroma compound increases. This means it will favour

79   being in the fatty matrix over partitioning into the aqueous saliva. Hydrophilic

80   compounds (log P equal to or less than zero) on the other hand tend to be

81   less dependent on changing fat levels (Arancibia, Castro, Jublot, Costell, &

82   Bayarri, 2015; Arancibia et al., 2011). In low fat systems, the release of

83   hydrophobic aromas will be faster leading to an unbalanced flavour profile.

84

85   More recently, interactions between food components and the oral and nasal

86   mucosa have been investigated. Specifically, interactions between flavour

87   molecules and the oral mucosa may explain persistence of aromas in certain

88   foods (Esteban-Fernández, Rocha-Alcubilla, Muñoz-González, Moreno-

89   Arribas, & Pozo-Bayón, 2016; Sánchez-López, Ziere, Martins, Zimmermann,

90   & Yeretzian, 2016). Furthermore, interactions between food matrices and the

91   oral mucosa have been of interest with regard to negative sensory

92   characteristics of dairy products (Bull et al., 2015; Hilal Y et al., 2015; Withers,

93   Cook, Methven, Godney, & Khutoryanskiy, 2013) and the impact of fat

94   reduction on perception of foods (De Hoog, Prinz, Huntjens, Dresselhuis, &

95   Van Aken, 2006; Dresselhuis, van Aken, de Hoog, & Martien, 2008).

96

97   Many polysaccharides are mucoadhesive, meaning they adhere to mucosal

98   surfaces in the body via intermolecular forces (hydrogen bonding, electrostatic

99   attraction, hydrophobic interactions and covalent bonds) and physical

100   penetration and entanglement of polymer chains (Andrews, Laverty, & Jones,

101   2009; Huang, Leobandung, Foss, & Peppas, 2000; Jabbari, Wisniewski, &

102   Peppas, 1993).   Though this phenomenon has been of interest and well

103   utilised in the pharmaceutics field for decades, the importance in the food

104   industry is beginning to gain interest (Bull et al., 2015; S. L. Cook, Bull, et al.,

105   2017; S. L. Cook, Woods, Methven, Parker, & Khutoryanskiy, 2018; Gibbins &

106   Carpenter, 2013; Hilal Y et al., 2015; Malone, Appelqvist, & Norton, 2003;

107   Withers et al., 2013).

108

109   Mucoadhesive polymers can retain and control the release of active

110   pharmaceutical ingredients (APIs) at mucosal surfaces including those in the

111   oral cavity (Andrews et al., 2009). The mechanisms of mucoadhesion have

112   been described in the literature numerous times (Peppas & Huang, 2004;

113   Shaikh, Singh, Garland, Woolfson, & Donnelly, 2011; Smart, 2005, 2014). The

114   physicochemical interactions depend on the polymeric substance (e.g ionic

115   groups, chain length), the state of hydration of the polymer, the mucosal

116   secretions (e.g. pH, thickness, mucin concentration) and the epithelial

117   structure and morphology (e.g. roughness and presence of micro cracks). The

118   fact that mucoadhesive polymers can retain small molecules at mucosal

119   surfaces and control their release will be important for the food industry to

120   consider as these frequently used polysaccharides may also retain tastant

121   and aroma molecules in a similar way (S. L. Cook, Woods, et al., 2018).

122

123   Many polysaccharides used in the food industry that are also mucoadhesive

124   include, but are not limited to; carboxymethyl cellulose (Yehia, El-Gazayerly,

125   & Basalious, 2008, 2009), sodium alginate (Juliano, Gavini, Cossu, Bonferoni,

126   & Giunchedi, 2004; Richardson, Dettmar, Hampson, & Melia, 2004) and

127   pectin (Kaur & Kaur, 2012; Thirawong, Nunthanid, Puttipipatkhachorn, &

128   Sriamornsak, 2007). Buccal films are a formulation type made by dissolving a

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

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

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

154 with more viscous, slower dissolving polysaccharides will reduce the intensity

155 but prolong the perception of flavours over time. Furthermore, the

156 mucoadhesive properties of the matrices were assessed and related to

157 flavour delivery. This study, therefore, provides a foundation of understanding

158 of the mechanisms by which mucoadhesive ingredients can alter the

159 perception of flavour over time, which may help in the development of

160 reformulated products.

161

162 2. Methods

163 *2.1. Materials*

164 Four different polysaccharides were chosen for this study due to their differing

165 chemical properties (Table 1). Pullulan (PUL) (Hayashibara nagase europa

166 group, Düsseldorf, Germany) was chosen as a non-ionic, low viscosity and

167 fast dissolving film former. Hydroxypropyl methyl cellulose (HPMC) (product

168 code METHOCEL K4M, Dow The Chemical Company, Staines, UK) was

169 chosen as a high viscosity, non-ionic film former. Two carboxymethyl cellulose

170 products were used, one low molecular weight (LCMC) (product code

171 AKUCELL AF 0305, AkzoNoble, Amsterdam, The Netherlands) and one high

172 molecular weight (HCMC) (product code WALOCEL 4500, Dow The Chemical

173 Company, Staines, UK). Carboxymethyl cellulose was chosen as it is well

174 known for its mucoadhesive properties due to its ionic nature and high

175 viscosity.

176

177

178

179 Table 1. Polysaccharide characteristics

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

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

181 * Sodium content determined by flame photometry

182

183 *2.2. Samples*

184 Films were prepared by dissolving polysaccharides in deionised water (2%

185 w/v) with glucose, vanillin (Sigma- Aldrich, St. Louis, Missouri, United States)

186 or glucose and vanillin (Table 2). The solution (30g) was weighed into circular

187 petri dishes (90 mm) and placed in an oven at 65°C for 20 hours. Once the

188 films were dry they were removed from the petri dish and cut into squares

189 (approx. 1cm^2). Glucose containing films weighed 100 mg and the aroma only

190 films 30 mg. This was to ensure that each sample contained the same amount

191 of polysaccharide. The water activity (a_w) of the films was measured after the

192 drying process using a HygroLab C1 Bench-Top Water Activity Monitor.

193

194

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

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

196

197   *2.3. Artificial saliva*

198   Artificial saliva (AS) was used for all *in vitro* experiments to emulate conditions

199   in the mouth. This was adapted from Madsen *et al.* (2013) and consisted of

200   0.21 g/L $NaHCO_3$, 0.43 g/L NaCl, 0.75 g/L KCl, 0.22 g/L $CaCl_2 \cdot 2H_2O$, 0.91 g/L

201     $NaH_2PO_4 \cdot 2H_2O$ dispersed in deionized water. For the mucoadhesion

202   experiment 2.5 g/L pig gastric mucin (PGM) type II (Sigma- Aldrich, St. Louis,

203   Missouri, United States) was also added. The pH of the AS was adjusted to

204   6.8 and kept at 37 °C during experiments and at 4 °C when not in use.

205

206   *2.4. Swelling and disintegration*

207   Swelling studies were carried out in an incubator set to 37 °C. Each film was

208   placed on to netting and fully submerged in a petri dish with 40 mL of AS. At

209   set time periods the sample was removed from the AS, excess water was

210   carefully absorbed with tissue paper and the film on the netting was weighed.

211   This process was repeated until the weight had returned to that of the netting

212   alone. Each type of film was tested 6 times with duplicate batch repeats. Film

213   thickness was measured before these experiments with a micrometer. The

214 maximum swelling ratio was determined by dividing the weight of the film at

215 set time points with the original weight of the film.

216

217 *2.5. Dissolution*

218 Each film containing glucose was placed onto netting and carefully

219 submerged into an individual beaker with 200 mL AS. The solution was stirred

220 by a magnetic stirrer bar at a constant rate throughout the experiment. At set

221 time points 1 mL aliquots of the AS medium were removed and put into

222 labelled Eppendorfs for analysis. The glucose in the samples was quantified

223 spectrophotometrically using an Amplex Red, glucose oxidase kit following

224 the advised protocol (Fisher Scientific, Loughborough, UK). Each sample was

225 tested 6 times with duplicate batch repeats. The time taken to release 50 and

226 100 % of the glucose was calculated from the results.

227

228 *2.6. In vitro* mucoadhesion

229 Adhesion experiments were carried out using a texture analyser (TA) with a

230 10mm cylindrical probe (on a TA-XT plus, Stable Micro Systems, UK). Porcine

231 tongues were collected from a local butcher (P D Jennings, Hurst, UK) less

232 than 24 hours after slaughter. They were stored on ice whilst the majority of

233 muscle and connective tissue was removed leaving a thin section of the

234 surface mucosa. These sections were stored at -20°C until required when

235 they were thawed in the fridge for 3 hours before use.

236

237 Each area of the tongue was cut into 1 cm^2 sections and secured on the

238 bottom platform of the TA. The film sample to be tested was stuck to the

239 probe with double-sided sticky tape. Before each experiment, the tongue

240 tissue section was conditioned with 100µL of AS and incubated at 37°C. The

241 contact time between the probe and the tissue was 60 seconds before pulling

242 apart with a removal speed of 1mm/s.

243

244 *2.7. In vivo* retention

245 The study was given a favourable opinion for conduct by the University of

246 Reading, School of Chemistry, Food and Pharmacy (study number 27/15).

247 Five volunteers (3 males and 2 females, age range 23-30) were asked to

248 place a film sample on their tongue and keep it between the tongue and roof

249 of their mouth for the duration of the experiment. They were instructed to treat

250 the film like a hard candy with some manipulation by the tongue. The

251 experiment was timed and volunteers were asked to note the time (s) when

252 the film began to adhere, when the adherence ceased and when the film

253 dissolved. They were also asked where in the mouth the film adhered to.

254 Adherence was noted as an inability to move the film with their tongue.

255

256 *2.8. Sensory perception*

257 Time intensity; profiling involves trained sensory panellists continuously

258 recording the intensity of one or two attributes over a specified time. This

259 enables perception to be captured during consumption and can be

260 summarised as parameters such as onset, persistence and duration. Over a

261 period of three weeks, 8 trained panellists from the University of Reading

262 Sensory Science Centre panel scored each of the film samples in duplicate.

263 There were 12 samples in total. For each polysaccharide, films were made

264 with either glucose alone, vanillin alone or glucose with vanillin. Each week

265 was used for one set of polysaccharide films. For example, in week 1 the

266 glucose only films were scored, in a balanced order, for sweetness over time.

267

268 Training took place before each scoring week to familiarise the panel to the

269 samples and the time intensity protocol. Each film was presented to the panel

270 and a discussion of the different flavour release behaviours for each of them

271 took place. During these sessions, the panel were given 3 standards for both

272 glucose and vanillin. Glucose standards were 8%, 4% and 2%, and aroma

273 samples were 0.02%, 0.01%, and 0.005%. The panellists decided where

274 these standards scored on the line scale with their strongest standard

275 representing 100 on a standard 100-point scale. These standards were given

276 to the panellists at the start of each scoring sessions to re-familiarise them

277 with the standard intensities.

278

279 Panellists were trained on single and dual attribute time intensity scoring

280 using Compusense@hand software (Ontario, Canada) and feedback was

281 given to those who were not showing good reproducibility. The time intensity

282 test lasted for 5 minutes, which was the agreed amount of time that the

283 panellists could concentrate for without fatigue or boredom. The attributes

284 scored were sweet for glucose only films, vanilla for aroma only films and both

285 sweet and vanilla for the combined films. Panellists were also trained on how

286 to manipulate the sample in the mouth. They were asked to gently rub the film

287 between the tongue and roof of the mouth to facilitate flavour release.

288 Panellists were instructed to treat each sample the same way to avoid biasing

289 release.

290

291 Each week the panellists were given a training session on the first day

292 followed by two days of scoring the samples. Four samples were served

293 monadically, in a petri dish, in a balanced order with individual blinding codes

294 each day with the duplicate being served on a consecutive scoring day.

295 Panellists were provided with isolated sensory booths, computers with

296 Compusense Software and warm water for palate cleansing. There was a 2-

297 minute delay between samples to allow for palate cleansing. Time intensity

298 curves were produced for each panellist and each sample in duplicate.

299

300 *2.9. Statistical analysis*

301 One way or two way repeated measures ANOVA (rmANOVA) was used for

302 the appropriate test. Bonferroni or Tukey's HSD corrections were used on

303 pairwise analysis to account for multiple comparisons, at a significance level

304 of $p \leq 0.05$.

305

306 3. Results & Discussion

307 *3.1. Film characteristics*

308 A range of standard methods were used to characterise the polymeric films

309 (Morales & McConville, 2011; Nair et al., 2013). Each film was measured for

310 thickness, water activity (a_w), glucose release, and swelling / disintegration

311 times (Table 3).

312

313 The thickness of the films varied between the different polysaccharides and

314 between the films with and without glucose. The order of film thickness was

315 HPMC>HCMC>LCMC>PUL. This is not surprising as HPMC and HCMC were

316 higher viscosity grades than LCMC and PUL and therefore will occupy more

317 space, retain more water and form thicker films. Glucose films were thicker

318 than those without glucose, which was expected, as the glucose was in

319 addition to the polysaccharides. The thickness of a film will impact the

320 dissolution rate as a thicker film will have a smaller surface area to volume

321 ratio and this can slow water uptake from the surrounding medium. This will

322 impact mucoadhesion as hydration of the dosage form is integral for polymer -

323 mucin interactions to occur.

324

325 PUL and LCMC films fully dissolved after a similar time; however, LCMC films

326 swelled more before beginning to disintegrate (Table 3 & Figure S1). This is

327 because LCMC is more viscous than PUL (table 1) and possesses ionic

328 groups, which interact strongly with water molecules due to the higher osmotic

329 pressure induced by the high entropy of the counter-ions. LCMC and HCMC

330 films swelled considerably more than the non-ionic, PUL and HPMC films with

331 relation to their disintegration time. The carboxymethyl cellulose films

332 absorbed more water, forming a swollen gel-like layer, before beginning to

333 degrade. HCMC samples took the longest time to dissolve and swelled the

334 most due to their high viscosity. All films without glucose had higher swelling

335 ratios than their glucose containing counterparts and took longer to dissolve.

336 This is because the small, highly hydrophillic glucose molecules contained

337 within the film matrix will quickly dissolve into the surrounding medium,

338 leaving pores for the water molecules to enter, effectively increasing the

339 surface area of the film.

340

341 The glucose release from the films followed a similar pattern to the dissolution

342 rates. PUL and LCMC released glucose fully after 7.0 and 7.8 min

343 respectively, followed by HPMC (186 min) and then HCMC (300 min). HPMC

344 quickly released 50% of the total glucose in the film over a mean of 14

345 minutes. This fast initial release is most likely due to crystallisation of the

346 glucose molecules on the outside of the film. This was visually observed, as

347 these films were cloudy with a fine powder covering them. Furthermore, the

348 HPMC samples took a long time to fully dissolve, most likely due to the high

349 viscosity network it forms which will slow permeation of water molecules. The

350 HCMC released the glucose at a constant rate. The HCMC films swelled

351 considerably so the swollen, surface of the film contained loosely associated

352 polymer chains, which would then allow the glucose molecules to diffuse out

353 and dissolve in the surrounding medium. The increased surface area caused

354 by the high swelling degree of the HCMC films may facilitate glucose release,

355 however, the thick gel layer covering the outer surface of the film may also

356 decrease diffusion by physical entrapment. Additionally, the thick gel layer

357 may prevent matrix disintegration and affect subsequent water uptake when

358 unperturbed (Rodriguez, Bruneau, Barra, Alfonso, & Doelker, 2000). HPMC

359 did not swell substantially but took a long time to dissolve, therefore the

360 glucose molecules would essentially be trapped in the film matrix until is

361 started to erode.

362

363   Table 3. Characteristics of films

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

364   Films are separated into those without glucose and those with glucose. Each

365   value is the mean of 6 replications for the measured parameters (2 batch

366   repeats). Mean values within a column and film group not sharing the same

367   letter were significantly different from each other at $p \leq 0.05$ using Tukey's

368   HSD correction.

369

370   It was expected that changes in flavour perception over time would be

371   influenced by the parameters measured (Table 3). For example, it was

372   hypothesised that PUL films would result in a high intensity flavour that

373   decreased in intensity quickly as they dissolved faster and released glucose

374   quickly. Conversely, it was expected that as the HCMC would slow the

375 release of glucose and aroma and therefore reduce the initial intensity of

376 flavour but prolong the sensation over time. The results gained from this study

377 are in concordance with the authors expectations.

378

379 *3.2. Mucoadhesion in vitro*

380 Two values were obtained from the TA experiments; the maximum force

381 required to separate the probe from the tongue (peak force of detachment)

382 and the area under the curve (total work of adhesion). The mean values for

383 peak force of attachment and total work of adhesion decreased in order of

384 LCMC, HCMC, PUL and HPMC for films without glucose and LCMC, PUL,

385 HCMC and HPMC for films with. In films both with and without glucose the

386 LCMC film was significantly more mucoadhesive than the HPMC film (Figure

387 1a & b). The films without glucose required a significantly higher force to

388 separate the film from the tissue suggesting a stronger adhesive joint (Figure

389 1a). This is not surprising as the glucose content was high and therefore the

390 relative amount of polymer in contact with the tissue was smaller. The HPMC

391 films with glucose exerted the lowest total work of adhesion and peak force of

392 detachment (Figure 1). This is probably due to the non-ionic nature of HPMC

393 along with the large molecule size and slow swelling (Table 3 & Figure S1).

394

395 Mucoadhesion of solid polymeric substances is dependent on the hydration of

396 the formulation, which will create a polymeric mesh enabling the interactions

397 between polymer and mucin chains. The mucin used in the artificial saliva

398 were PGM purchased from Sigma-Aldrich, which is dehydrated and potentially

399 denatured due to production processes (Kocevar-Nared, Kristl, & Smid-

17

400 Korbar, 1997). Therefore, the interactions that may occur with salivary mucin

401 may not be represented by this commercial mucin. Furthermore, an adhesive

402 joint is formed due to the viscous gel formed between the film and the moist

403 mucosal surface. However, over-hydration of the film will lead to a slippery

404 mucilage being formed and will result in an adhesive joint failure. The swelling

405 ability of a polymeric substance is important for establishing a mucoadhesive

406 bond as this enables polymer chains to be available to interact with the

407 mucosa.
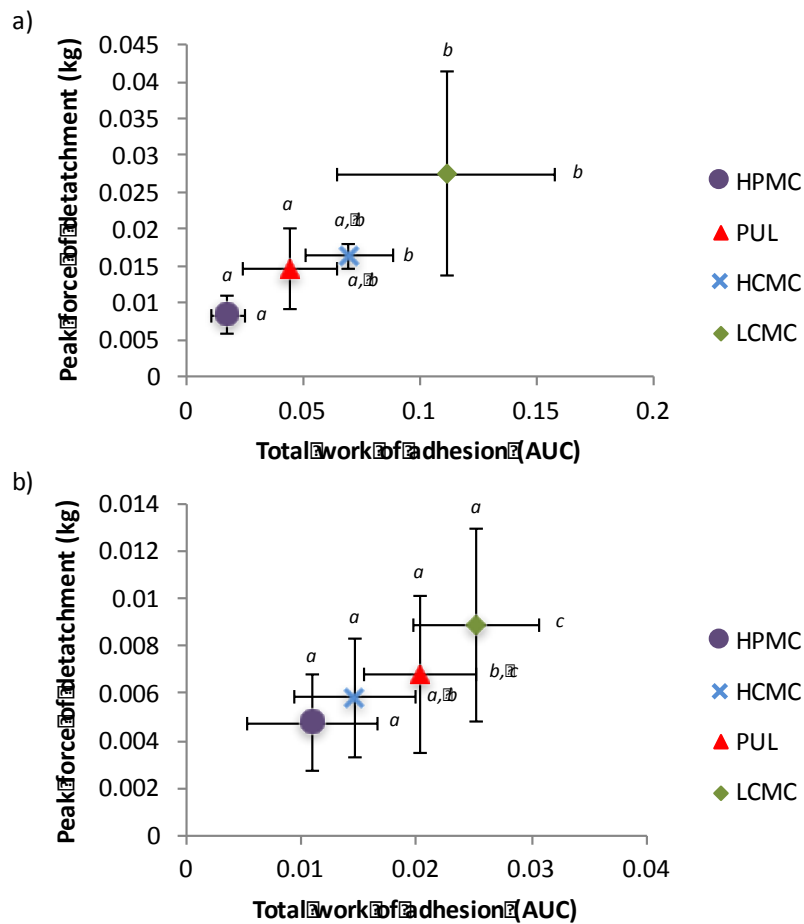
408

409 Figure 1. Total work of adhesion against the peak force of detachment for

410 films a) without glucose and b) with glucose. Results determined by texture

411 analysis. Data points are means of 6 measurements and error bars are SD.

412 Superscript letters represent statistically different groupings (p<0.05). Letters

413 on top of the data point refer to the y axis and those to the right hand side

414 refer to the x axis.

415

416 *3.3. Mucoadhesion in vivo*

417 *In vivo* mucoadhesion experiments were carried out with 5 panellists that were

418 asked to record the following: where the film stuck, for how long and when it

419 dissolved. All films, except for HPMC with glucose, were reported to adhere

420 for the duration of the time that the film was in the mouth (Figure 2a & b).

421 Adherence was mainly to the roof of the mouth but also the tongue. The time

422 that the films took to dissolve reflected the *in vitro* dissolution (Table 3) as

423 PUL and LCMC took the least amount of time to dissolve followed by HPMC

424 then HCMC. For films without glucose, HPMC and HCMC films did not differ

425 in time for dissolution *in vivo* (Figure 2a) despite the difference in the *in vitro*

426 test. This is probably due to the participants manipulating the film with their

427 tongue during these experiments, thereby exerting mechanical stress on the

428 film. Therefore, as the HCMC swells and takes up water to produce a gel–like

429 layer, the tongue pressure will remove it and therefore speed up the time of

430 erosion.

431

432

433
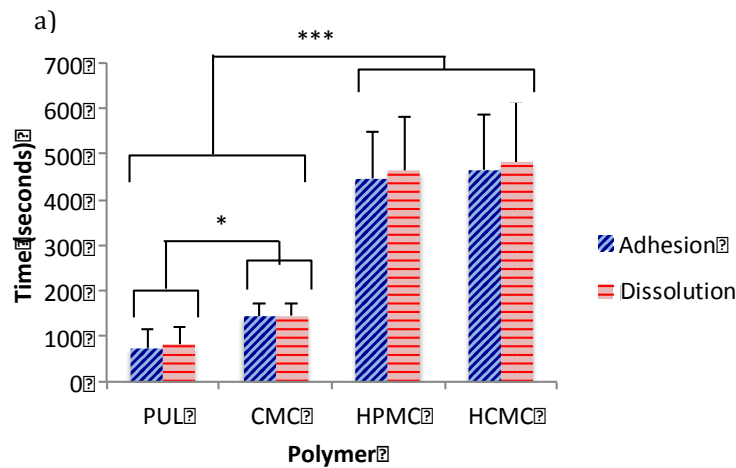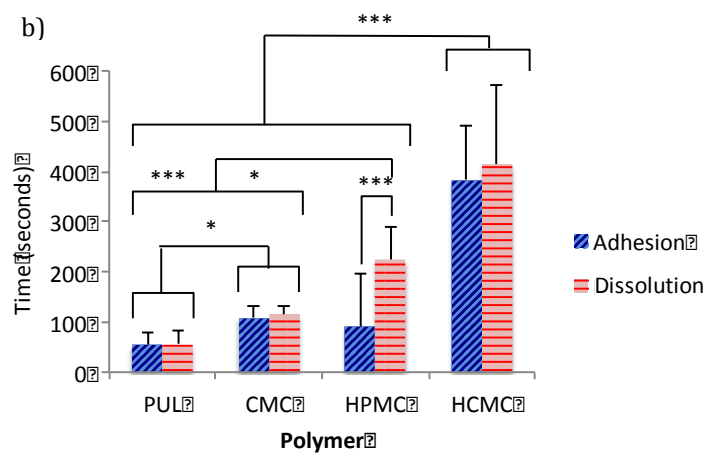
Figure 2. *In vivo* mucoadhesion of a) polymer films without glucose and b) polymer films with glucose. Each bar represents the mean of 10 separate data points, error bars represent standard deviation. N= 5 in duplicate. * = p<0.05, *** = p<0.001.

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

445 panellists reporting adherence after an initial delay. There are two

446 explanations to this. Firstly, AS was used in the *in vitro* experiments, which

447 contained Sigma-Aldrich PGM as opposed to human salivary mucin. This may

448 affect interactions between the polysaccharide matrix and the saliva due to

449 differences in denaturation states and response to pH. For example, mucin

450 chains must be flexible and uncoiled enough to allow interpenetration with

451 polymer chains. Secondly, the hydration of the oral cavity *in vivo* may be

452 different to that which was on the porcine tongue in the *in vitro* experiments.

453 This may have led to a stronger adhesion *in vivo*, as the film did not become

454 overhydrated.

455

456 The PUL film dissolving and adherence time was significantly quicker for

457 LCMC films in these experiments. The PUL films dissolved on average at 81

458 seconds compared to 145 seconds for the LCMC films during these

459 experiments. This is in contrast to the results obtained from the *in vitro*

460 dissolution tests (table 3) where they were not significantly different. This

461 difference was expected to have an impact on flavour release from LCMC

462 films compared to PUL. Film thickness is the most likely explanation for the

463 differences observed, LCMC films were thicker than PUL and therefore, when

464 in contact with the moist mucosal surface, will take longer to take up water. To

465 properly assess the impact of polysaccharide type on dissolution times, the

466 thickness of the films would need to be matched.

467

468 *3.4. Perception of tastant and aroma from films over time changes depending*

469 *on polysaccharide used*

470   Panellists produced time intensity curves for each sample and repeat. They

471   continuously scored either sweetness or vanilla, or both attributes at the same

472   time, over the course of 5 minutes using an unstructured line scale. Various

473   parameters were extrapolated from the curves including the area under the

474   curve (AUC), time to maximum intensity (T_{max}), maximum intensity (I_{max}),

475   duration of perception, and incline and decline angles (Figure S2). One-way

476   rmANOVA was used for each parameter

477

478   *3.4.1. Glucose only films*

479   Time intensity curves were averaged across all panellists and both replicates

480   (Figure 3). The mean sweetness AUC and I_{max} values for the films decreased

481   in order of PUL >LCMC >HPMC >HCMC with the reverse order for T_{max}

482   (Table 4) where PUL was significantly higher than HCMC and higher for all

483   other films for I_{max}. This suggests a fast onset of intensity for PUL and LCMC,

484   which is supported by their larger incline angles compared to HPMC and

485   HCMC. Furthermore, PUL and LCMC decline angles were also larger than the

486   other two film types suggesting a quicker rate of decline. These results were

487   expected as *in vitro* results (Table 3) show that PUL and LCMC films were

488   faster dissolving and release glucose quicker than HPMC and HCMC films

489   (table 3). Although the total duration of perception was not significantly

490   different between the films, there was a trend that HPMC and HCMC films

491   prolonged the flavour perception compared to PUL and LCMC (see "duration"

492   in table 4).

493

494   Regarding mucoadhesion, the HPMC films containing glucose were found to

495   have poor adhesive abilities (Figures 1 & 2). In the perception experiments

496   panellists were asked not to swallow these films and, therefore, the perception

497   may have been artificially prolonged due to consciously keeping the film in the

498   mouth. During normal consumption in a real food system the material would

499   be chewed into a bolus and, without mucoadhesive ability, it may well be

500   swallowed with the food bolus thereby negating any further release. On the

501   other hand, HCMC films showed strong adhesion (Figures 1 & 2) and

502   therefore would be more likely to adhere to the oral cavity for longer,

503   prolonging the release.

504

505   *3.4.2. Vanillin only films*

506   For films containing the polysaccharide and vanillin the mean scores for I_{max}

507   decreased in order of PUL>LCMC>HPMC>HCMC (Table 4, Figure 3b).

508   Where PUL was significantly higher than HCMC. T_{max} and AUC were not

509   dependent on polysaccharide type. The duration of perception was longest in

510   the HPMC samples followed by HCMC. This suggests that although the total

511   intensity of perception was the same for each film, the aroma was delivered at

512   a slightly lower intensity for longer in the HPMC and HCMC samples. This is

513   supported by the decline angles being larger for PUL and LCMC samples

514   suggesting the intensity decreased more quickly in these films.

515

516   To date, the only studies investigating aroma release and perception in food

517   thickened with polysaccharides are in liquid and semi-solid foods. These

518   studies have found confounding results with regard to interactions between

519   aroma molecules and the food matrix. Arancibia et al. (2011)   found that

520   thickener type affected total aroma release from dairy desserts with CMC

521   thickened samples reducing the cumulative release of hydrophobic aroma

522   (linalool) compared to starch. Furthermore, a follow up study by Arancibia,

523   Castro, Jublot, Costell, & Bayarri (2015)   found that thickener type affected

524   both hydrophilic aroma (cis-3-hexen-1-ol) and hydrophobic (linalool) aroma.

525   The CMC thickened dairy desserts reduced the release of both aromas,

526   though it had more of an impact on the hydrophilic compound. Cook, Linforth,

527   et al., (2003)   on the other hand found that in-nose measurements of

528   hydrophobic aroma release were not dependent on thickener type or on an

529   increase in viscosity. These studies exemplify the complex behaviour of

530   aroma release and its dependence on the food matrix.

531

532   In this current study, vanillin, a slightly hydrophobic molecule with a log P of

533   1.2, was used as the aroma. Perception results show that films made with

534   slow dissolving polysaccharides (HPMC and HCMC) reduced the I_{max} but

535   prolonged the duration of perception. Perception results for the aroma only

536   films were not as distinguishable as the films containing glucose. This may be

537   because the panel found scoring the aroma only films particularly difficult as

538   they contained no tastant along with the aroma, which does not normally

539   occur in food products.

540

541   *3.4.3. Glucose & vanillin films*

542   Dual attribute time intensity was used to simultaneously and continuously

543   monitor sweetness and vanilla attributes over 5 minutes. Results for the

544 sweetness attribute were similar for the dual attribute and single attribute tests

545 (Table 4, Figure 3c and d). The AUC and I_{max} were highest for PUL and

546 lowest for HCMC. HPMC and HCMC took longer to reach T_{max} compared to

547 PUL and LCMC.

548

549 The AUC for the vanilla attribute did not significantly differ with the different

550 polysaccharides (Table 4). HPMC and HCMC had reduced I_{max} and increased

551 T_{max} results compared to PUL and LCMC. The total duration of perception

552 was striking in these films with the HCMC averaging 53 seconds longer than

553 PUL. HPMC also increased the duration significantly compared to PUL and

554 LCMC. Although not statistically significant, LCMC followed the trend of

555 prolonging the perception compared to PUL. The incline angles for HPMC and

556 HCMC were, again, smaller than PUL and CMC suggesting a slower rate of

557 onset.

558

559 These results suggest that PUL films give a quick burst of flavour that

560 declines quickly. LCMC films are almost as quick to release as PUL but take

561 somewhat longer to reach I_{max}. HPMC has a slower onset to reach I_{max} and

562 the perception continues for longer than LCMC and PUL. Finally, HCMC films

563 have the slowest onset with a steady release over time. This is particularly

564 evident for the vanilla attribute, which prolongs the perception for longer than

565 the faster dissolving films.

566

567 Although from this perception data HPMC films appear to give a sustained,

568 medium level intensity of flavour, this formulation was not particularly

mucoadhesive and, therefore, it would most likely be swallowed along with the bolus in a real food system. Participants were instructed not to chew or swallow the film and many suggested that this would have been possible if they were eating normally. However, the other formulations were firmly adhered to the roof or tongue tissue and would not be easily swallowed.

Table 4. Parameters from time intensity results.

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

576   8 panellists scored each sample in duplicate therefore each result is the mean

577   of 16 separate results. Statistical analysis was done for each attribute

578   separately comparing the different polysaccharides. Different letters represent

579   significantly different groupings for each set of data.
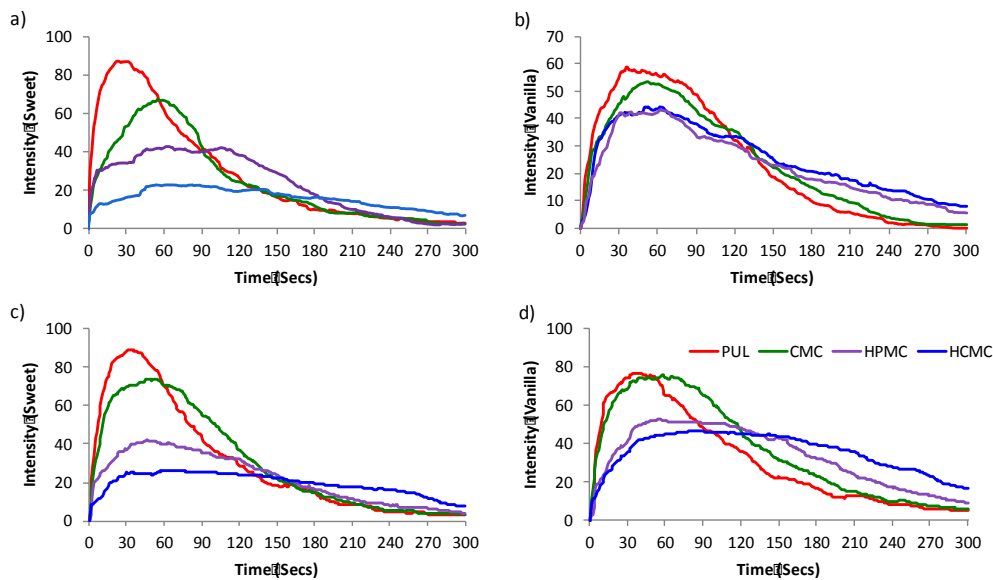
580

581   Figure 3. A panel of 8 trained panellists scored different polysaccharide films

582   in duplicate for either sweetness of vanilla perception over time. Time intensity

583   curves for a) glucose only films, b) vanillin only films were produced from

584   single attribute time intensity tests. Dual attribute time intensity tests produced

585   the curves for glucose and vanillin films in c) and d).

586

587   *3.5. Comparing perception results to in vivo dissolution*

588   During the in vivo experiments where participants were asked to record the

589   adhesion and dissolution of the films, PUL was reported to dissolve after an

590   average of 57 seconds. When comparing these timings to the perception data

591   it is clear that perception of flavour is continuing after the film has completely

592   dissolved (Table 4 & Figure 3). There are two explanations for this. Firstly, the

593   glucose and aroma molecules may still be present at the respective receptors,

594 thereby initiating a response. Secondly, as the intensity of sweetness was

595 very high, an adaptation type response could occur where the sweet signal is

596 switched on for a longer time even after the stimulus has gone.

597

598 The physiological differences between participants were not collected for the

599 in vivo mucoadhesion nor the sensory perception experiments. Factors such

600 as salivary flow and constituents varies between individuals (Fenoli-

601 Palomares et al., 2004) and will therefore impact the mucoadhesive strength

602 and rate of film dissolution. Despite not adding these covariates in analysis,

603 there were still significant results gained from the experiments.

604

605 *3.6. Comparisons between different film types*

606 Time intensity results were compared between 5 panellists who were

607 consistent for both experiments. The AUC for the vanilla attribute differed

608 between films with and without glucose (Figure 4). Significant increases in the

609 total perception intensity (AUC) of vanilla were observed for LCMC, HPMC

610 and HCMC films containing vanillin plus glucose compared to those without

611 glucose.

612

613 During single attribute time intensity, the attribute is scored horizontally but

614 during dual attribute, one must be scored vertically. The vanilla attribute was

615 scored vertically in the dual attribute tests, which may have affected the

616 results. Duizer, Bloom, & Findlay, (1995) investigated this issue and found

617 that scoring an attribute vertically lead to approximately 13% increase in

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

619   only factor.

620

621   A more likely explanation is that the presence of glucose in the films

622   enhanced the aroma through cross modality (D. J. Cook et al., 2003; Niimi,

623   Eddy, Overington, Heenan, et al., 2014; Niimi, Eddy, Overington, Silcock, et

624   al., 2014). T_{max} was also significantly ($p < 0.05$) increased for vanillin in the

625   HCMC films going from 26 to 89 seconds (Figure S3). This suggests that

626   when glucose was present the perception of aroma had a slower onset, which

627   lasted for longer and was sustained.
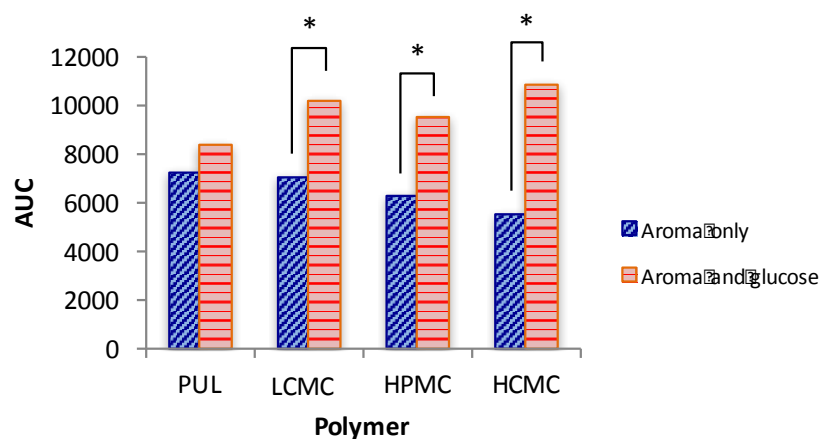
628

629   Figure 4. Comparisons of the area under the curve for the vanilla attribute of

630   films with and without glucose. * denotes significant differences $p = <0.05$

631   using Bonferroni correction.

632

633   *4. Conclusions*

634   This study has shown that polysaccharides affect the retention, release and

635   perception of flavour compounds, dependant on the physicochemical

636   properties of the polysaccharide matrix. The viscosity and swelling ability of

637 the polysaccharide influences the release of flavour molecules from the

638 matrix. This in turn has an impact on the flavour perception. Fast dissolving

639 polysaccharides resulted in a quick burst of flavour at high intensity that

640 tapered more quickly whereas slow dissolving films gave a slower onset and a

641 more consistent release over time. The mucoadhesive ability of the films will

642 influence how long the matrix stays in the mouth whilst releasing the flavour

643 compounds before being swallowed. Furthermore, in line with previous

644 literature, this study shows that aroma intensity is dependent on the

645 perception of a congruent tastant, giving more evidence for cross modal

646 interactions.

647

648 The mucoadhesive nature of some of the polysaccharides tested will have an

649 effect on flavour delivery over time as those that adhere to the oral cavity will

650 continue to release flavour whilst those that are not mucoadhesive will be

651 swallowed. This study investigated flavour release from very simple food

652 matrices, polysaccharide films; of course in a real food there will be many

653 other food components that could affect flavour release. However, this study

654 provides some fundamental understanding of how different polysaccharide

655 matrices affect flavour release. Results from this study can be used to inform

656 the food industry of the impact that the addition of these polysaccharides can

657 have on temporal flavour perception. Possible applications include topical

658 coatings, confectionary, low fat and low sugar foods. However, there is a need

659 for further research into this area to understand the full impact on the

660 organoleptic properties of foods.

661

662 Acknowledgements

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