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In vitro oral processing of raw tomato: Novel insights into the role of endogenous fruit enzymes

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Abstract

During consumption of fruits, the breakdown of the fruit tissue due to oral processing (chewing, mixing with saliva) may activate or increase the rate of endogenous enzyme activities via the disruption of the cell wall, cellular de-compartmentalization and particle size reduction allowing the enzymes to reach their substrates. The aim of this study was to investigate the activity of one such endogenous fruit enzyme (pectin methylesterase (E.C. 3.1.1.11) during in vitro oral processing of raw tomatoes and associated changes in viscosity and microstructure. Oral processing of tomatoes purees was examined in the presence of artificial saliva at 37 °C. In vitro oral processing was followed using immunofluorescence microscopy, apparent viscosity measurements, spectrophotometric and titrimetric techniques. Results demonstrated that pectin methylesterase had slight but significant activity in the tomato fruit during in vitro oral processing generating methanol as a function of oral processing time, which was further evidenced using immunolabelling techniques to detect methylated pectin epitopes. A significant shear-thinning behaviour of the tomato puree was observed due to dilution and/or endogenous fruit enzyme activity. These results suggest that activity of other fruit enzymes, such as polygalacturonase, which catalysed the depolymerisation of unmethylated pectin chains might have resulted in a decrease in viscosity, which compensated for the increased potential for gel formation (if any) caused by PME. These interesting insights on role of endogenous fruit enzymes might pave the way to the understanding of fruit viscosity modification occurring in the mouth and help in rational design of new fruit based products.

Key words: Oral processing; Pectin methylesterase; Viscosity; Tomato; Artificial Saliva

Abbreviations: pectin methylesterase (PME), artificial saliva (AS).

Practical Applications

This work provides novel insights on role of endogenous fruit enzymes during in vitro oral processing in altering the viscosity of fruits. The in vitro oral processing of tomatoes at 37 °C was followed in presence of artificial saliva containing mucin by employing a range of complimentary physical and microstructural techniques. This study demonstrated for the first time that pectin methylesterase (PME) had a slight activity during oral processing of tomato generating methanol. Interestingly, tomato puree showed a shear-thinning behaviour during in vitro oral processing. This pseudo-plastic behaviour might be attributed to the dilution effects by artificial saliva as well as polygalacturonase activity, latter might have compensated for the increase in viscosity (if any) caused by PME. Thus, these results might pave the way to gain understanding of fruit viscosity modification occurring in the mouth and relevant for designing new fruit based products with tailored oral textural properties.

1. Introduction

Fruits are one of the main components of a healthy diet as they represent an important source of vitamins, minerals, sugars, fibres and other bioactive compounds, such as, carotenoids and polyphenols (Lai et al., 2015). Fruits become edible after the complex physiological process of ripening, which is associated with enzyme activity that leads to cell wall disassembly and tissue softening (Wang et al., 2018). Several enzymes have been found to modify polysaccharides of cell wall and middle lamella during ripening, including pectin methyl esterase, polygalacturonase and pectin lyase (Wang et al, 2018). They have different activities involving various substrates, and are active at different ripening and development stages. Some of them possess very low or undetectable activity during fruit storage but become active if the product is disrupted, for example during handling or food processing (Fischer and Bennett, 1991; Brummell and Harpster, 2001).

During consumption of fruits, the fruit tissue breaks down due to oral processing with associated physical and biochemical modifications, such as cell separation and particle size reduction (Chu et al., 2017), pH change, interactions with proteins and enzymes of saliva, eventually resulting in the bolus formation (Chen, 2009; Sarkar et al., 2009; Sarkar and Singh, 2012; Sarkar et al., 2017). These physical and biochemical changes as well as the length of oral residence time determine the sensorial and/or textural perception of the fruits in the mouth (Szczesniak, 2002; Laguna et al., 2016a; Laguna et al., 2016b; Laguna and Sarkar, 2016). Interestingly, the degree of such oral processing, such as interaction of saliva, oral processing time may increase or decrease the activity of some endogenous fruit enzymes and affect oral viscosity.

Previous literature has focussed on how food processing, such as thermal treatments and high pressure treatments (Ludikhuyze et al., 2003; Chakraborty et al., 2014) can be used to inactivate fruit endogenous enzymes that are involved in the alteration of pectin network and thus produce low viscous fruit-based products. However, whether the fruits' endogenous enzymes are active during oral processing of fruits and how such activity may affect oral viscosity and sensory perception of raw fruits and fruit-based products remains largely unexplored. Most fruits are naturally acidic,

79 containing organic acids that influence taste and astringency, but the impact of pH on saliva
80 properties, such as viscosity and enzyme activities (e.g. amylase) is often not considered. While the
81 activity of salivary amylases in relation to rheology has been previously explored (Evans et al., 1986),
82 the activity of endogenous food enzymes has not been previously investigated. The hypothesis is that
83 mixing fruit with saliva will lower pH and affect the activity of endogenous fruit enzymes. These
84 enzymes may be active during the oral phase and may impact on the rheological properties of food-
85 saliva mixtures, and eventually impact on sensory perception

86 In this study, we focused on pectin methylesterase (PME), since it is a key enzyme in tomato
87 ripening and its activity impacts on other fruit enzymes such as endo-polygalacturonase (Fischer and
88 Bennett, 1991; Brummell and Harpster, 2001; Paniagua et al., 2014). The PME catalyses the
89 demethylation of galacturonic acid units in pectin polymers resulting in the release of methanol and
90 formation of negatively charged carboxylate groups on the galacturonic acid moieties. Demethylation
91 increases the electrostatic interactions between carboxylate groups and with Ca^{2+} ion leading to the
92 formation of calcium cross-linkages (Almeida and Huber, 1999; Paniagua et al., 2014). The formation
93 of these interactions between pectin chains can be hypothesized to cause increase of oral viscosity
94 during oral processing. However, PME activity leads to increased substrate availability for endo-
95 polygalacturonase, which would in turn depolymerise pectin and result in a decrease in viscosity.

96 Hence, the aim of this study was to understand whether PME is active and influence the oral
97 processing of fruits. We hypothesize that fruit endogenous enzymes will be active during oral
98 processing and will alter the oral viscosity of fruits. Salad tomatoes (Lycopersicum esculentum) were
99 selected as the fruit of choice due to its wide spectrum of domestic and industrial applications. The
100 oral processing was evaluated using artificial saliva at 37 °C and characterized using a range of
101 complimentary physicochemical and immunolabelling techniques. To our knowledge, this is the first
102 study that investigates the activity and effects of endogenous fruit enzymes during oral processing of
103 tomatoes.

2. Materials and methods

2.1 Materials

Acetic acid, ammonium acetate, ammonium nitrate, dibasic sodium phosphate, Calcofluor white, formaldehyde, lactic acid sodium salt, monobasic sodium phosphate, porcine gastric mucin Type II, phosphate buffer saline, potassium citrate, potassium chloride, potassium phosphate, sodium chloride, urea, uric acid sodium salt, alcohol oxidase from Pichia pastoris (14 units/mg) were purchased from Sigma Chemical Co., St. Louis, MO, USA.. Acetylacetone at analytical grade was purchased from SLS, Nottingham, UK.

Salad tomatoes (Lycopersicum esculentum) at red light ripening stage were purchased from local supermarket (Tesco, UK).

2.2 In vitro oral processing

Artificial saliva (AS) was prepared using composition used in previous literature (Leung and Darvell, 1997; Sarkar et al., 2009; Laguna et al., 2017a). Porcine gastric mucin was used in quantity of 3 g/L to simulate the rheological properties of saliva, the pH was adjusted to 6.8 with NaOH 0.1 mol/L. Raw tomatoes were diced (diameter = 0.5 cm) and puree (diameter ≈ 2-5 mm) was obtained by homogenizing using a laboratory scale blender (Kenwood, UK). Then, the puree was mixed with AS at 37 °C in a water bath in different sample: puree ratios and shaken for 2 minutes.

2.3 pH analysis

The pH value was measured in oral processed samples with a pH-meter (Model 3520, Jenway, Stone, UK). Mean and standard deviation was calculated using five measurements carried out for each puree: AS ratio. Titratable acidity of the puree-AS mixture was measured using an automatic pH-stat device (Model TIM 854, Hach, Loveland, USA) as a function of oral processing time using 0.05 mol/L

NaOH solution with end-point at pH 6.8. Titratable acidity (mol%) was calculated using Equation (1) (Laguna et al., 2017b):

$$mol\% = \frac{mL\ of\ NaOH \cdot 0.05 mol/L}{g\ of\ sample} \times 100 \quad (1)$$

Titratable acidity was fitted to logistic function (equation (2)) which was optimised using Excel solver to minimise residual fit with the data. The logistic function has an asymptotic maximum with growth rate k (μmol min^{-1}). The model fit against data was conducted with Pearson correlation. Logistic growth factor k and t_0 ($t_{1/2}$ in case of mid-point in min) values are reported.

$$\text{Acidity}(\%) = \frac{\text{Max}(\%)}{\left(1+\exp\left(-k(t-t_0)\right)\right)}$$

2.4 Apparent oral viscosity

Apparent viscosity was measured with a viscometer (Model DV-2T, Brookfield, Middleboro, USA) with the RV02 spindle set (plate). The apparent viscosity of the oral processed samples was measured at 37 ± 0.2°C and at 25 ± 0.2°C for comparison. Combinations of spindle and rotational speed with a torque value between 10 and 100% were used to collect the data. Apparent viscosity was measured as function of time, rotational speed and temperature, means and standard deviations were collected for triplicate samples.

2.5 Pectin methylesterase (PME) activity

PME activity was evaluated by measuring the quantity of methanol released in the sample as end product of the reaction catalysed by the enzyme. The released methanol was measured using a spectrophotometric method (Klavons and Bennett, 1986). After 1 min and 1 h of in vitro oral processing, 1.5 mL of supernatant was collected in an Eppendorf tube (1.5 mL) and centrifuged for 2

minutes at 10,000 rpm (Eppendorf mini spin centrifuge). The supernatant was collected and centrifuged again at 10,000 rpm. Then, 50 µL of alcohol oxidase (0.03 units) in sodium phosphate buffer (pH = 7.5) was added to 50 µL of supernatant in a UV plate (GRE96ft UV-Star, Greiner, Stonehouse, UK) and shaken for 15 minutes at room temperature. Then, 100 µL of reagent solution was added (reagent made fresh by mixing 28 µL acetyl acetone, 28 µL glacial acetic acid, 1.54 g of ammonium acetate made up to 10 mL with water) . The plate was incubated at 60 °C for 10 minutes. Absorbance was recorded at 412 nm with a plate reader (Model Spark, Tecan, Switzerland), and compared to a calibration curve ($R^2 = 0.9927$) obtained using different methanol concentrations.

2.6 Immunolabeling and microscopy

Tomato puree samples before or after in vitro oral processing were fixed with 4% formaldehyde in PEM buffer (Leyton-Puig et al., 2016). Formaldehyde-fixed tomato-puree samples were washed 3 times for 10 minutes each with phosphate-buffered saline (PBS) before immuno-labelling. The cells were incubated in skimmed milk-PBS solution (10 mg/mL) (M-PBS) for 30 minutes at room temperature. Then, the cells were incubated in M-PBS solution containing 100 µL/mL of monoclonal anti-pectin antibodies i.e. JIM7, where the antibody recognises the homogalacturonan domain of pectic polysaccharides, recognises partially the methyl-esterified epitopes of homogalacturonan but does not bind to un-esterified homogalacturonan. (Clausen et al., 2003) or LM19, where it binds strongly to unesterified homogalacturonan. (Christiaens et al., 2011), for 1.5 h at room temperature. The primary antibody was washed from cells with M-PBS and then incubated for 1.5 h at room temperature in the dark in M-PBS solution containing secondary antibody anti-rat IgG (whole molecule) linked to fluorescent isothiocyanate (FITC). At the end of the incubation, cells were washed 3 times with PBS. Cells were also stained in Calcofluor white solution (2.5 mg/mL) for five minutes in darkness. Cells were examined with a light microscope equipped with epifluorescence irradiation; images were acquired with a digital camera.

2.7 Statistical analysis

The statistical analyses were carried out using Microsoft Excel 2016 and differences were considered significant (*) when $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$, were obtained. Titratable acidity was fitted to logistic function which was optimised using Excel solver to minimise residual fit with the data. The correlation with the model fit against data was conducted with Pearson correlation. Logistic growth factor k and $t_{1/2}$ values are reported.

3. Results and discussion

3.1 Acidity

The pH of oral processed samples in presence of different ratios with AS was measured. Results reported in Table 1 show that tomato puree-saliva mixtures at any of the ratios tested had significantly lower pH as compared to saliva alone ($p < 0.05$). The lower pH in the presence of tomato is outside the optimum for salivary α-amylase as the optimum pH conditions for ptyalin, the isoform present in human saliva is pH 5.6–6.9 (Valls et al., 2012). This suggests that the textural property of tomato puree perceived in the mouth will be most likely independent of the activity of starch hydrolysis (if any) by the amylase present. Tomato PME has been reported to have an alkaline pH optimum (around pH 8) but has good activity in acidic environments as measured in the saliva mixtures (Duvetter et al., 2006). The pH would favour the activity of polygalacturonase in particular (Verlent et al., 2005). Acidity may impact textural perception through effect of acid on protein aggregation and carbohydrate-protein interactions, which may result in increased viscosity of the food-saliva mixture.

[Table 1 here]

The titratable acidity (Figure 1) showed that acids were released faster in the 2:1 w/w ratio samples than the in 1:1 w/w samples, (AS: tomato puree), with k being -0.52 and -0.41 respectively. Correspondingly, the time needed to release the acids and reach plateau was longer than the time

needed for oral processing, with t_0 being 4.39 and 5.66 min, respectively, suggesting that not all acids were released (6.45 and 4.11 %) during the oral phase of digestion.

[Figure 1 here]

3.2 PME activity

The methanol concentration was calculated as mg of methanol per L of supernatant in the oral processed samples. The release of methanol from tomato fruits during in vitro oral processing was clearly evident even after 1 minute of oral processing time (Table 2). The quantity of methanol released after 1 hour was significantly higher than the quantity released after 1 minute ($p < 0.05$). This provides the first preliminary indication of activity of PME during oral processing, however, the oral residence time may play a key role in such reaction. Demethylated pectin is the ideal substrate for endo-polygalacturonase, which is also present in ripe tomato. While activity of endo-polygalacturonase was not measured in this study, it can be hypothesised that endo-polygalacturonase may also be active in the oral phase, and thus, explain decrease in viscosity of the food-saliva mixture. It might be further noted that the methanol concentration measured in the supernatant obtained after oral processing simulation is much lower than the safest dose (2 g) and toxic dose (8 g) for methanol (Paine and Davan, 2001). A potential role for dietary methanol as a signalling molecule in metabolism has been recently suggested (Dorokhov et al., 2012).

[Table 2 here]

3.3 Immunofluorescence microscopy

In non-oral processed tomato cells (Figure 2A), JIM7, which binds preferentially to highly methylated homogalacturonans labelled distinct regions of the cell surface were associated with cell corners identified by darker calcofluor staining (Ordaz-Ortiz et al., 2009). In contrast, in oral processed cells (Figure 2B), the JIM7 labelling of cell corners was lost indicating decrease in methylation or solubilisation of methylated pectin. The JIM7 epitope appeared to be easily solubilised from tomato parenchyma cell walls (Cornuault et al., 2018).

[Figure 2 here]

In non-oral processed tomato cells (Figure 3A), LM19, which binds preferentially to un-esterified homogaclacturonan was localised in distinct punctate areas of the cell wall, and the labelling was again lost after oral processing. There was a low background labelling by both JIM7 and LM19 before and after oral processing indicating that the oral processing does not completely destroy or solubilise all pectin. There was no apparent effect of oral processing on cellulose staining with calcofluor white, which indicates absence of cellulase activity. Also of significance is that intact cells were abundantly observed in the tomato puree. These large cells can be over 500 μm in size and likely could have a large impact on the oral flow properties and sensory perception. The presence of cells has also been detected in other fruit products and have been shown to resist gastrointestinal digestion (Chu et al., 2017). It is possible that oral processing caused the solubilisation of the water-soluble pectin fraction into artificial saliva. To understand this further, rheological measurements of oral processed samples were investigated.

[Figure 3 here]

3.4 Apparent viscosity

254 The orally processed tomatoes had three-orders of magnitude higher viscosity than artificial saliva
255 alone at oral processing conditions (Figure 4). This could be due to the presence of tomato cells, the
256 cross-linking of de-methylated pectin and the effect on low pH on molecular aggregation. Dilution
257 with AS had a significant shear thinning effect on the puree. However, the thinning effect might be
258 also attributed to the alteration of pH to near optimal pH for the endogenous fruit enzymes (e.g.
259 polygalactouronase, pectin lyase) to act and depolymerise pectin via hydrolytic and trans-elimination
260 cleavage (Niture et al., 2008).

261 [Figure 4 here]

262

263 This suggests that although PME activity was evident (Figures 2 and 3), the possible presence of
264 depolymerising activity might have dominated such effects and no PME-mediated increase in
265 viscosity was observed. It is recognized that cohesive gels can be formed by enzymatic de-
266 esterification of high-methoxy pectin by PME in the presence of calcium ions (O'Brien et al., 2009).
267 However, it is worth recognizing that the artificial saliva formulation used in this study did not contain
268 any Ca^{2+} ions to contribute to the gelation, which might not be the case in real human saliva (Schipper
269 et al., 2007). Thus, the exact interactions mediated by PME occurring in whole unstimulated human
270 saliva containing inherent calcium ions remains to be further explored.

271 As expected, oral processed tomatoes were non-Newtonian with pseudo-plastic behaviour (Figure
272 5). This was probably due to gradual break-up of the network-like tomato-puree aggregates into
273 smaller particles in the direction of flow. Also, the dependence between viscosity and time has been
274 evaluated and no relation was detected (Figure 5), even when the structural factors that contributes
275 to thixotropy (decrease of viscosity as function of time) were similar to those that determined the
276 pseudo-plastic behaviour, as well as a high solid content and the presence of pectin and fibres (Ramos
277 and Ibarz, 1998). Nevertheless, the rotational speeds used during the analysis might have destroyed
278 the structure of the tomato puree almost immediately. Therefore, it was not possible to exclude that
279 oral processed tomatoes may have thixotropic behaviour. Interestingly, the viscosity of oral

processed tomatoes was temperature-dependent, and heating the samples from 23°C to oral processing temperature (37 °C) caused a significant decrease of viscosity (Figure 5). Such decrease in viscosity might be also attributed to effect of PME in combination with other cell wall enzymes.

[Figure 5 here]

4. Conclusions

The results obtained in this study from immunofluorescence microscopy and from the measurements of the methanol concentration in the samples have demonstrated for the first time that PME is active during the oral processing of tomatoes, even at short oral residence times. However, in our experimental conditions, no increase in viscosity was observed during simulated oral processing. The tomato puree showed a shear thinning behaviour, which might be attributed to dilution by artificial saliva and/ or activity of other fruit enzymes, such as polygalacturonase or pectin lyase, which catalyse the depolymerisation of pectin chains into polyuronides with lower molecular weight. Such depolymerising activity might have caused a decrease of viscosity, which compensated for the increase (if any) caused by PME. Also, the real human saliva contains calcium ions inherently, which might contribute to formation of gel during oral processing, which is under future investigation. Thus, these results obtained might pave the way to gain understanding of fruit viscosity modification occurring in the mouth due to the endogenous fruit enzymes. Since viscosity and pH changes are known to affect fruit sensory characteristics, such as, flavour release and/or textural perception, a deeper knowledge of oral activity of fruits' endogenous enzymes is important to enable optimization (e.g. temperature, pressure) of fruit processing. In addition, knowledge of activity of fruit endogenous enzymes and their effects on oral viscosity can also provide a strategic route towards the design of tailored food products catering to disadvantaged populations (e.g. elderly, dysphagia patients).

Ethical Statements

Conflict of Interest: The authors declare that they do not have any conflict of interest.

Ethical Review: This study does not involve any human or animal testing.

Informed Consent: This study does not involve any human or animal testing.

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References

ALMEIDA, D.P.F., and HUBER, D.J. 1999. Apoplastic pH and inorganic ion levels in tomato fruit: A potential means for regulation of cell wall metabolism during ripening. Physiologia Plantarum 105, 506-512.

BRUMMELL, D.A., and HARPSTER, M.H. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Molecular Biology 47, 311–339.

CHAKRABORTY, S., KAUSHIK, N., RAO, P.S., and MISHRA, H.N. 2014. High-pressure inactivation of enzymes: A review on its recent applications on fruit purees and juices. Comprehensive Reviews in Food Science and Food Safety 13, 578-596.

CHEN, J. 2009. Food oral processing—A review. Food Hydrocolloids 23, 1-25.

CHRISTIAENS, S., VAN BUGGENHOUT, S., NGOUÉMAZONG, E.D., VANDEVENNE, E., FRAEYE, I., DUVETTER, T., VAN LOEY, A.M., and HENDRICKX, M.E. 2011. Anti-homogalacturonan antibodies: A way to explore the effect of processing on pectin in fruits and vegetables? Food Research International 44, 225-234.

CHU, J., IGBETAR, B.D., and ORFILA, C. 2017. Fibrous cellular structures are found in a commercial fruit smoothie and remain intact during simulated digestion. Journal of Nutrition & Food Sciences 7.

CLAUSEN, M.H., WILLATS, W.G.T., and KNOX, J.P. 2003. Synthetic methyl hexagalacturonate hapten inhibitors of anti-homogalacturonan monoclonal antibodies LM7, JIM5 and JIM7. Carbohydrate Research 338, 1797-1800.

CORNUAULT, V., POSE, S., and KNOX, J.P. 2018. Extraction, texture analysis and polysaccharide epitope mapping data of sequential extracts of strawberry, apple, tomato and aubergine fruit parenchyma. Data in Brief 17, 314-320.

337 DOROKHOV, Y.L., KOMAROVA, T.V., PETRUNIA, I.V., KOSORUKOV, V.S., ZINOVKIN,
338 R.A., SHINDYAPINA, A.V., FROLOVA, O.Y., and GLEBA, Y.Y. 2012. Methanol May
339 Function as a Cross-Kingdom Signal. PLoS One 7, 15.
340 DUVETTER, T., FRAEYE, I., SILA, D.N., VERLENT, I., SMOUT, C., HENDRICKX, M., and
341 VAN LOEY, A. 2006. Mode of de-esterification of alkaline and acidic pectin methyl
342 esterases at different pH conditions. J. Agric. Food Chem. 54, 7825-7831.
343 EVANS, I.D., HAISMAN, D.R., .ELSON, E.L., PASTERNAK, C., and MCCONNAUGHEY,
344 W.B. 1986. The effect of salivary amylase on the viscosity behaviour of gelatinised starch
345 suspensions and the mechanical properties of gelatinised starch granules. Journal of the
346 Science of Food and Agriculture 37, 573-590.
347 FISCHER, R.L., and BENNETT, A.B. 1991. Role of Cell Wall Hydrolases in Fruit Ripening.
348 Annual review of plant physiology and plant molecular biology 42, 675-703.
349 KLAVONS, J.A., and BENNETT, R.D. 1986. Determination of methanol using alcohol oxidase
350 and its application to methyl ester content of pectins. Journal of Agricultural and Food
351 Chemistry 34, 597-599.
352 LAGUNA, L., BARROWCLOUGH, R.A., CHEN, J., and SARKAR, A. 2016a. New approach to
353 food difficulty perception: Food structure, food oral processing and individual's physical
354 strength. Journal of Texture Studies 47, 413-422.
355 LAGUNA, L., FARRELL, G., BRYANT, M., MORINA, A., and SARKAR, A. 2017a. Relating
356 rheology and tribology of commercial dairy colloids to sensory perception. Food & Function
357 8, 563-573.
358 LAGUNA, L., HETHERINGTON, M.M., CHEN, J., ARTIGAS, G., and SARKAR, A. 2016b.
359 Measuring eating capability, liking and difficulty perception of older adults: A textural
360 consideration. Food Quality and Preference 53, 47-56.
361 LAGUNA, L., PICOUET, P., GUÀRDIA, M.D., RENARD, C.M.G.C., and SARKAR, A. 2017b.
362 In vitro gastrointestinal digestion of pea protein isolate as a function of pH, food matrices,

363 autoclaving, high-pressure and re-heat treatments. LWT - Food Science and Technology 84,
364 511-519.
365 LAGUNA, L., and SARKAR, A. 2016. Influence of mixed gel structuring with different degrees of
366 matrix inhomogeneity on oral residence time. Food Hydrocolloids 61, 286-299.
367 LAI, H.T.M., THREAPLETON, D.E., DAY, A.J., WILLIAMSON, G., CADE, J.E., and BURLEY,
368 V.J. 2015. Fruit intake and cardiovascular disease mortality in the UK Women's Cohort
369 Study. Eur. J. Epidemiol. 30, 1035-1048.
370 LEUNG, V.W.H., and DARVELL, B.W. 1997. Artificial salivas for in vitro studies of dental
371 materials. Journal of Dentistry 25, 475-484.
372 LEYTON-PUIG, D., KEDZIORA, K.M., ISOGAI, T., VAN DEN BROEK, B., JALINK, K., and
373 INNOCENTI, M. 2016. PFA fixation enables artifact-free super-resolution imaging of the
374 actin cytoskeleton and associated proteins. Biology Open 5, 1001-1009.
375 LUDIKHUYZE, L., VAN LOEY, A., INDRAWATI, SMOUT, C., and HENDRICKX, M. 2003.
376 Effects of combined pressure and temperature on enzymes related to quality of fruits and
377 vegetables: From kinetic information to process engineering aspects. Critical Reviews in
378 Food Science and Nutrition 43, 527-586.
379 NITURE, S.K., KUMAR, A.R., PARAB, P.B., and PANT, A. 2008. Inactivation of
380 polygalacturonase and pectate lyase produced by pH tolerant fungus Fusarium moniliforme
381 NCIM 1276 in a liquid medium and in the host tissue. Microbiological Research 163, 51-62.
382 O'BRIEN, A.B., PHILP, K., and MORRIS, E.R. 2009. Gelation of high-methoxy pectin by
383 enzymic de-esterification in the presence of calcium ions: a preliminary evaluation.
384 Carbohydrate Research 344, 1818-1823.
385 ORDAZ-ORTIZ, J.J., MARCUS, S.E., and KNOX, J.P. 2009. Cell wall microstructure analysis
386 implicates hemicellulose polysaccharides in cell adhesion in tomato fruit pericarp
387 parenchyma. Molecular plant 2, 910-921.

388 PAINE, A., and DAVAN, A.D. 2001. Defining a tolerable concentration of methanol in alcoholic
389 drinks. Human & experimental toxicology 20, 563-568.
390 PANIAGUA, C., POSÉ, S., MORRIS, V.J., KIRBY, A.R., QUESADA, M.A., and MERCADO,
391 J.A. 2014. Fruit softening and pectin disassembly: an overview of nanostructural pectin
392 modifications assessed by atomic force microscopy. Annals of Botany 114, 1375-1383.
393 RAMOS, A.M., and IBARZ, A. 1998. Thixotropy of orange concentrate and quince puree. Journal
394 of Texture Studies 29, 313-324.
395 SARKAR, A., GOH, K.K.T., and SINGH, H. 2009. Colloidal stability and interactions of milk-
396 protein-stabilized emulsions in an artificial saliva. Food Hydrocolloids 23, 1270-1278.
397 SARKAR, A., and SINGH, H. 2012. Oral behaviour of food emulsions. In Food Oral Processing.
398 Wiley-Blackwell, pp. 111-137.
399 SARKAR, A., YE, A., and SINGH, H. 2017. Oral processing of emulsion systems from a colloidal
400 perspective. Food & Function.
401 SCHIPPER, R.G., SILLETTI, E., and VINGERHOEDS, M.H. 2007. Saliva as research material:
402 Biochemical, physicochemical and practical aspects. Archives of Oral Biology 52, 1114-
403 1135.
404 SZCZESNIAK, A.S. 2002. Texture is a sensory property. Food Quality and Preference 13, 215-
405 225.
406 VALLS, C., ROJAS, C., PUJADAS, G., GARCIA-VALLVE, S., and MULERO, M. 2012.
407 Characterization of the activity and stability of amylase from saliva and detergent:
408 Laboratory practicals for studying the activity and stability of amylase from saliva and
409 various commercial detergents. Biochemistry and Molecular Biology Education 40, 254-
410 265.
411 VERLENT, I., SMOUT, C., DUVETTER, T., HENDRICKX, M.E., and VAN LOEY, A. 2005.
412 Effect of temperature and pressure on the activity of purified tomato polygalacturonase in

413 the presence of pectins with different patterns of methyl esterification. Innov. Food Sci.
414 Emerg. Technol. 6, 293-303.

415