Food & Function

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2019, **10**, 4242

Non-chemically modified waxy rice starch stabilised wow emulsions for salt reduction

Miroslaw Kasprzak, †[a] Peter Wilde, [b] Sandra E. Hill, [a] Stephen E. Harding, [a] Rebecca Ford [a] and Bettina Wolf *‡[a]

Water-in-oil-in-water emulsions containing an internalised salt solution were stabilised with non-chemically modified waxy rice starch (WRS), and octinyl succinic anhydride (OSA) as reference, to release salt during oral processing due to amylase-induced destabilisation. Salt levels were 1.5 g salt and 0.47 g salt per 100 g external and internal aqueous phases, respectively. Variables were the starch content (2, 3, 4 g per 100 g emulsion; 20 g oil per 100 g emulsion), level of polyglycerol polyricinoleate (PGPR) as a lipophilic emulsifier (0.29, 0.57 g per 100 g emulsion) and ambient-pressure processing temperature for WRS gelatinisation, the non-chemical modification process, (75 ± 3, 88 ± 5 °C). OSA starch was used under previously applied conditions (2, 3, 4 g starch, 0.57 g PGPR per 100 g emulsion, 25 ± 5 °C). Emulsions were stable for three months, except OSA and lower level PGPR low temperature processed WRS emulsions lost salt into the external emulsion phase. One day after processing, encapsulation efficiency (EE) was as predicted from the composition for OSA emulsions, while at the same PGPR content an external aqueous phase was incorporated into the oil droplets of the WRS emulsion increasing EE. Salt release was assessed *in vitro* and through sensory evaluation using paired comparison testing. The results revealed that the efficacy of this salt reduction approach was enhanced for gelatinised WRS compared to OSA starch stabilised emulsions. Consumer tests on a tomato soup, to validate this salt reduction approach for a real food, revealed a possible 25% salt reduction, compared to current UK products.

Received 4th October 2018,
Accepted 10th June 2019

DOI: 10.1039/c8fo01938j

rsc.li/food-function

Introduction

The average global consumption of salt remains above the recommended level of 5 g day^{-1}.[1] This is despite the well-established fact that the overconsumption of salt promotes the development of hypertension or cardiovascular disease.[2] In an attempt to reduce the consumption of salt through processed foods, a number of salt reduction strategies have been demonstrated and successfully applied by the industry. These include reduction by stealth,[3] modulation of the salt crystal size, and compartmentalisation in solid foods such as crisps and bread.[4–6] The latter two approaches are challenging to apply in high moisture foods simply because salt dissolves in water. The compartmentalisation approach, the basis of the research reported here, is based on the observation that consumers notice a change in tastant concentration delivered to the taste buds as the tastant is released in "bursts" during the oral processing of the food, with salt probably being the most frequently investigated tastant in the context of this approach.[7–10] However, this approach has recently been demonstrated to show promise in liquid emulsion-based foods through encapsulation of a concentrated salt solution inside the oil droplets of a water-in-oil-in-water (wow) emulsion.[11] The emulsion droplets were stabilised by an emulsifier designed to destabilise during oral processing allowing the encapsulated concentrated salt solution to interact with the taste buds, thereby imparting a change in tastant concentration and increased saltiness perception. The obvious choice of an emulsifier was a starch-based emulsifier, hydrolysing in contact with the salivary amylase. In the cited literature, chemically modified octinyl succinic anhydride (OSA) starch was used, which was later optimised,[12] and all sensory testing to draw conclusions about showing potential to allow a plus 20% reduction of salt in emulsion-based foods was based on assessing emulsions alone.

The OSA starch optimisation consisted of lowering the degree of substitution of hydroxyl groups per glucose unit with OSA, to a level where emulsions were still stable, thereby retaining a larger number of reaction sites for the amylase mediated emulsion destabilisation increasing the *in vitro*

[a]*School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK. E-mail: b.wolf@bham.ac.uk*
[b]*Quadram Institute Bioscience, Norwich Research Park, Norwich, Norfolk, NR4 7UA, UK*
† Present address: Devro Limited, Chryston G69 0JE, UK.
‡ Present address: School of Chemical Engineering, University of Birmingham, Birmingham B15 2TT, UK. E-mail: b.wolf@bham.ac.uk

assessed salt release. Here it was hypothesised that replacing the OSA starch with non-chemically modified starch will increase the efficacy of this salt reduction approach. *In situ* gelatinised waxy rice starch (WRS), recently identified to successfully stabilise oil-in-water emulsions,[13] was selected as the non-chemically modified starch.

Amylase mediated salt release was assessed *in vitro* with a conductivity-based assay, variations of which are frequently applied for this purpose.[14] Appropriately calibrated to alleviate the impact of ions present in the digestive juice containing the amylase to simulate salivary conditions as closely as possible, the absence of ions in the product formulation rendered conductivity easy to assess physico-chemical properties to assess changes in salt concentration. The comparative release efficiency of selected formulations was validated by sensory evaluation. As an extension of our previous work,[13] the process parameters of the frictional heat based ambient pressure starch modification process were controlled more tightly in terms of temperature, applying two narrow temperature windows. One was selected to be around the endset temperature of the WRS gelatinisation domain, previously determined for the same batch of WRS by differential scanning calorimetry.[13] The second temperature was higher, but still below boiling to enable processing at ambient pressure, and attained by lengthening the processing time at the same speed of the high shear overhead process. It was hypothesised that the additional shear energy would increase the level of starch materials available for interfacial stabilisation. Therefore, smaller emulsion droplets would be stabilised providing an overall larger interfacial area to release encapsulated salt following enzymatic droplet destabilisation. As a consequence, saltiness perception would be heightened. Another parameter taken into consideration in this study was the concentration of polyglycerol polyricinoleate (PGPR) in the oil phase, present to stabilise the internal water-in-oil emulsion. PGPR is still the most successfully applied surfactant for this purpose.[15] However, it is generally regarded as not label friendly and, as per the food additive directive 95/2/EC of the European Parliament, limited in application too, for example, 4 g per 1 kg dressing, a relevant product area for this salt reduction strategy. The same amount of PGPR as in the previously cited studies based on OSA starch,[11,12] and half of this amount were applied here. It was hypothesised that the breakdown of the internal emulsion would be more efficient at the lower level of PGPR since the water-in-oil emulsion would be less stable and therefore more readily release the salt solution for taste perception. The relevant physical–chemical properties of the various emulsions are reported followed by *in vitro* salt release data and validation by sensory assessment for four sample pairs based on six emulsion samples.

Finally, the design and outcomes of a brief study applying a WRS stabilised wow emulsion to salt reduction in a tomato soup are reported in the Appendix, validating this salt reduction approach not only for a model food, *i.e.* an emulsion,[11,12] but for a real food.

Materials and methods

Materials

The two starches used in this study to stabilise the external oil/water interface were clean label native WRS (Synergie Nutrylon, Urlick and Short Ltd, Pontefract, UK) and OSA-starch (NC46 Creamer, Univar, Bradford, UK). As previously reported,[13] the onset, peak and endset temperature of gelatinisation of this WRS was (60.1 ± 0.3) °C, (67.5 ± 0.1) °C and (74.6 ± 0.3) °C respectively and gelatinisation enthalpy was 12.5 ± 0.8 J g^{-1}. The OSA starch was included as the reference emulsifier previously applied in the oral processing of wow emulsion-based salt reduction strategy. Water used throughout was Milli-Q water (conductivity 0.5 μS cm^{-3}). The oil phase comprised sunflower oil purchased from a local supermarket and PGPR (PGPR 90, DuPont, Kettering, UK). Both aqueous emulsion phases contained sodium chloride (S/3160/60 (99.7% pure), Fisher Scientific, Loughborough, UK). Sodium azide (101671965, Sigma Aldrich, Gillingham, UK) was added as an antimicrobial agent at a level of 0.02 g per 100 g to emulsion water phases that were not destined for sensory analysis. Porcine amylase with an activity of 10 U mg^{-1} (1002227364, Sigma Aldrich, Gillingham, UK), sodium dihydrogen orthophosphate (EC231-449-2, Sigma Aldrich, Gillingham, UK) and disodium hydrogen orthophosphate (S/4520/53, Fisher Scientific, Loughborough, UK) were acquired for the *in vitro* starch digestion assay. All materials were used as received.

Emulsion preparation

The wow emulsions were prepared following a two-step process. The internal water-in-oil (w_1/o) emulsion was prepared first and then incorporated into the external water phase (w_2). w_1 and w_2 contained 1.5 g salt per 100 g and 0.47 g salt per 100 g, respectively. w_1 was stabilised at two levels of PGPR in the oil phase; 1.43 g per 100 g and 2.86 g per 100 g. The oil phase was stabilised with varying amounts of gelatinised WRS contained in w_2: 2.8 g per 100 g, 4.2 g per 100 g and 5.6 g per 100 g, corresponding to 2 g per 100 g, 3 g per 100 g and 4 g per 100 g based on emulsion. OSA starch was applied as a commercial starch based reference emulsifier, but on only at the higher level of PGPR in the oil phase, corresponding to the level of our previous study.[13]

The final emulsions contained a total of 0.47 g salt per 100 g, which is comparable to current commercial soups low in salt. 0.13 g salt per 100 g emulsion were contained in the internal aqueous phase. The oil content (including PGPR) in the final wow emulsions was 20 g per 100 g. The content of PGPR in the final emulsions was either 0.29 g per 100 g or 0.57 g per 100 g.

Emulsions were prepared in batches of 100 g using a high shear overhead mixer (L5M, emulsor screen, Silverson, Chesham, UK) as follows. Initially, the desired amount of PGPR (1 or 2 g) was mixed with sunflower oil (68 or 69 g) during processing for 2 min at 8000 rpm. w_1 (1.5 g salt per 100 g; 30 g) was then added and emulsified for 2 min at 8000 rpm. Next, w_2 was prepared. In the case of OSA starch, the

appropriate amount of starch (2, 3 or 4 g) was added into salt solution (0.5 g salt per 100 g; 67.4 with water (2, 1 or 0 g) added to obtain 71.4 g of the aqueous phase for all three starch levels) and processed for 5 min at 8000 rpm followed by emulsifying in w_1/o (28.6 g) for 5 min at 8000 rpm. The glass beaker utilised for processing the OSA starch stabilised emulsion was immersed in an ice bath to process at 25 ± 5 °C.

In the case of WRS, following the addition of starch (2, 3 or 4 g) and water (2, 1 or 0 g to make up 71.4 g of w_2) into the salt solution (0.5 g salt per 100 g; 67.4 g), w_2 was processed for 5 min at 8000 rpm. This increased the dispersion temperature to around 60 °C. w_1/o (28.6 g) was then added and the mixture was processed for 4 ± 1 min or 6.5 ± 1.5 min at 8000 rpm to reach a maximum processing temperature of 75 ± 3 °C or 88 ± 5 °C, respectively. All emulsions were prepared and analysed in duplicate.

The emulsions destined for sensory analysis did not contain sodium azide and were prepared in a food preparation area.

Microstructure

The microstructure of the processed wow emulsions was assessed by bright field microscopy (EVOS fl, Life Technologies, Paisley, UK). To prepare the glass slides, 1 mL of emulsion was mixed with 10 mL of water in a small glass beaker using a spatula. Around 0.5 mL of the diluted emulsion was then dropped onto the glass slide followed by carefully sliding over a glass cover slip before mounting the slide onto the microscope. *In vitro* and *in vivo* digested emulsions were imaged following the same protocol.

Droplet size

Droplet size distributions of the wow emulsions were acquired using a low angle diffraction particle analyser (LS 13 320, Beckman Coulter, High Wycombe, UK) fitted with a liquid dispersion cell (Universal Liquid Module) containing water. After background measurement, an appropriate amount of emulsion as indicated by the equipment software was added into water and three diffraction patterns were acquired. Their average was analysed by using the equipment software based on the refractive index of 1.33 for water as the continuous dispersion phase and 1.54 for the material adsorbed at the surface of the oil droplets following a published method.[16] As some of the droplet size distributions were multimodal, characteristic distribution values reported are $x_{10,2}$, $x_{50,2}$ and $x_{90,2}$ as the droplet diameter below which 10, 50 and 90% respectively of the distribution lay, based on the surface area. The replicate emulsions were analysed in duplicate and reported data are based on the average.

Encapsulation efficiency

A conductivity-based method was applied to assess the level of salt in the external emulsion phase after emulsion preparation and during storage. The data were used to calculate the encapsulation efficiency (EE) as defined by eqn (1).[17]

$$\mathrm{EE}(\%) = \frac{\text{g salt per 100 g emulsion} - \text{g salt in } w_2 \text{ per 100 g emulsion}}{\text{g salt per 100 g emulsion}} \times 100\% \quad (1)$$

Initially, calibration curves to convert conductivity into salt concentration were generated as follows. A number of solutions (9) containing between 0 and 70 mg of sodium chloride were mixed with 12 g of water in a 50 mL glass tube while gently stirring (500 rpm) at 20 °C on a magnetic stirrer. 12 g of the wow emulsion prepared as described above, omitting the addition of salt to w_1 and w_2, were then added while recording conductivity using a SevenExcellence conductivity meter (Mettler Toledo, Leicester, UK) connected with a 4-pole platinum conductivity cell with a chemical resistant glass body (inLab 710, 0.01–500 mS cm^{-1}, Mettler Toledo, Leicester, UK) for 1 min. Calibration curves were acquired for two emulsions to ensure that formulation would not affect the reading. These emulsions contained 2.8 g or 5.6 g of WRS per 100 g w_2 and were processed at a higher temperature. The calibration curves overlapped. The mixing step of this conductivity-based assay affected the dynamic values recorded; therefore conductivity after equilibrium was reached is reported. The data point recorded at 30 s was chosen as by then the conductivity reading varied by less than 5% within 5 seconds across all samples.

Considering the composition of the emulsions, the EE as defined by eqn (1) would be 28% unless emulsion processing or storage has led to transfer of salt from one aqueous phase into the other. A value lower than 28% would indicate the loss of w_1 into w_2 during the second emulsification step or diffusion of salt out of the oil droplets during sample storage. A higher value on the other hand would correspond to incorporation of w_2 into the oil droplets during processing or diffusion of salt from w_2 into w_1.

Viscosity

The steady shear viscosity behaviour of the wow emulsions subjected to sensory analysis was analysed at 37 °C to facilitate the discussion of the sensory data. A rotational rheometer (MCR301, Anton Paar, Graz, Austria) fitted with a concentric cylinder geometry (C27, Anton Paar, Graz, Austria) was used. The shear rate was stepwise increased from 0.1 to 500 s^{-1} and then decreased to 0.1 s^{-1}. Due to pour data reproducibility for some samples at shear rates of less than around 1 s^{-1}, only data between 10 and 500 s^{-1} were considered for data fitting. In this range, the samples showed power law model behaviour, see eqn (2). Some of the emulsions showed hysteresis so the model was only applied to the decreasing shear rate ramp. The results are reported as the power law parameters.

$$\eta(\dot{\gamma}) = k \cdot \dot{\gamma}^{n-1} \quad (2)$$

where η = viscosity (Pa s), $\dot{\gamma}$ = shear rate(s^{-1}), k = flow consistency index (Pa s^n) and n = flow behaviour index. In addition to

the model parameters, the results of the viscosity assessment are reported as average viscosity at 50 s^{-1} on the decreasing shear rate ramp. The choice of 50 s^{-1} is justified where the results are presented.

Methods of additional characterisation of gelatinised waxy rice starch

Water solubility index. The water solubility index (WSI) of the heat treated WRS was examined in view to understanding differences in emulsion stabilisation and digestion properties of emulsions processed at the two temperatures applied in this study (75 ± 3 °C and 88 ± 5 °C). A previously published procedure was followed in principle but adjusted in terms of sample preparation[18] as follows. An aqueous dispersion containing 4 g WRS per 100 g was processed like the emulsion at 75 °C and 88 °C, respectively. 7.5 g of starch dispersion were then mixed with 10 g of water in 50 mL test tubes and centrifuged (Rotina 380R, Andreas Hettich, Tuttlingen, Germany) at 1750 g and 20 °C for 10 min. The supernatant was transferred to an aluminium tray and dried overnight at 105 °C until constant weight. The WSI was calculated as the weight of the dry solids in the supernatant expressed as a percentage of the original weight of the sample. The original weight of the sample was corrected for the moisture content based on mass loss during drying overnight at 105 °C.

Viscosity. The steady shear viscosity behaviour of the WRS based external emulsion phases was analysed at the two temperatures at which the emulsions were processed (75 °C and 88 °C) to provide information about the level of granule disruption depending on temperature. The samples were prepared with the same high shear overhead mixer as the emulsions and then immediately transferred to the pre-heated rotational rheometer. The rheometer set up, measurement protocol and data analysis were the same as for the emulsions, except for the measurement temperature and the power law model was applied to either shear rate ramp as these samples showed no hysteresis.

Salt release methods

***In vitro* assessment.** The same set-up as for measuring encapsulation efficiency was used for the *in vitro* assessment of the amylase mediated salt release. Instead of mixing 12 g of emulsion with 12 g of water, however, for both, the acquisition of calibration curves (utilising the same two emulsion formulations as in the acquisition of the calibrating curve to assess the EE) and measurement of salt release, 12 g of the emulsion were mixed with 12 mL of porcine amylase in phosphate buffer solution (100 U per 1 ml, 0.01 M). The level of amylase activity was close to the average of 92.5 U per 1 mL reported for human salivary amylase,[19] resulting in a final enzyme activity of 50 U mL^{-1} mixture. Mixing temperature was 37 °C which was the optimal temperature for amylase activity. As for the EE assay, the two calibration curves overlapped and *in vitro* salt release data reported are based on the plateau value at 30 s. Measurements were conducted in triplicate one or two days after replicate emulsion preparation and data were averaged. The results are presented as % salt in the external emulsion phase with an indication of the amount of salt present in this phase prior to *in vitro* digestion as computed from the encapsulation efficiency. 100% salt in the external emulsion phase would signify total emulsion breakdown (all salts initially added to w_1 present in w_2). Thus, % salt in the external emulsion phase provides immediate information about the efficiency of this salt reduction strategy.

Sensory evaluation. For the sensory evaluation of the emulsion samples, ethical approval was obtained from the University of Nottingham Medical Research Ethics Committee. The evaluation was conducted in compliance with relevant laws and informed consent was obtained from the subjects.

Perceived saltiness was assessed with the method of paired comparison (2-Alternate Forced Choice tests, BS ISO 5495:2007). Emulsions were selected for testing based on the results of the *in vitro* assay, and are detailed in Table 4. 104 naive assessors (64 female, 40 male) between 19 and 70 years of age (mean age 30) were recruited from students and staff of the University of Nottingham and asked to assess sample pairs for saltiness indicating the sample they perceived as most salty. 10 mL of the emulsion were presented in containers labelled with a random three-digit code and the sample pairs were presented in a randomised, balanced order. After each sample and each sample pair, volunteers were instructed to cleanse their palate with unsalted crackers (Matzo Crackers, Rakusen's, UK) and mineral water (Evian, Danone, France). After each sample pair, a break of 3 minutes was enforced. Four sample pairs were presented in one sensory session.

Statistical analysis

Sensory data were analysed by the two-sided binomial test for difference, $\alpha < 0.05$. If no significant difference was found, similarity was calculated with parameters set to $\alpha = 0.2$, $\beta = 0.05$ and pd = 30%. Statistical analysis of the characteristic droplet size distribution parameters ($x_{10,2}$, $x_{50,2}$, $x_{90,2}$), encapsulation efficiency and salt release data was based on the one-way ANOVAs, using GenStat (15 Edition, VSN International, Hemel Hempstead, UK). All data are expressed as least squares means with differences considered statistically significant at $p < 0.05$.

Results and discussion

Emulsion properties

Emulsions were assessed for the microstructure and encapsulation efficiency. All of the OSA starch and WRS stabilised emulsions prepared during this study were duplex emulsions as evidenced in Fig. 1 by the dark appearing emulsion droplets typically observed for this type of microstructure.[11,20–23] The micrographs were acquired 1 day after sample preparation and at the same magnification to illustrate differences in the droplet size. One day after emulsion preparation, the OSA starch stabilised emulsion had the smallest w_1/o droplets while the WRS emulsions containing a higher level of PGPR

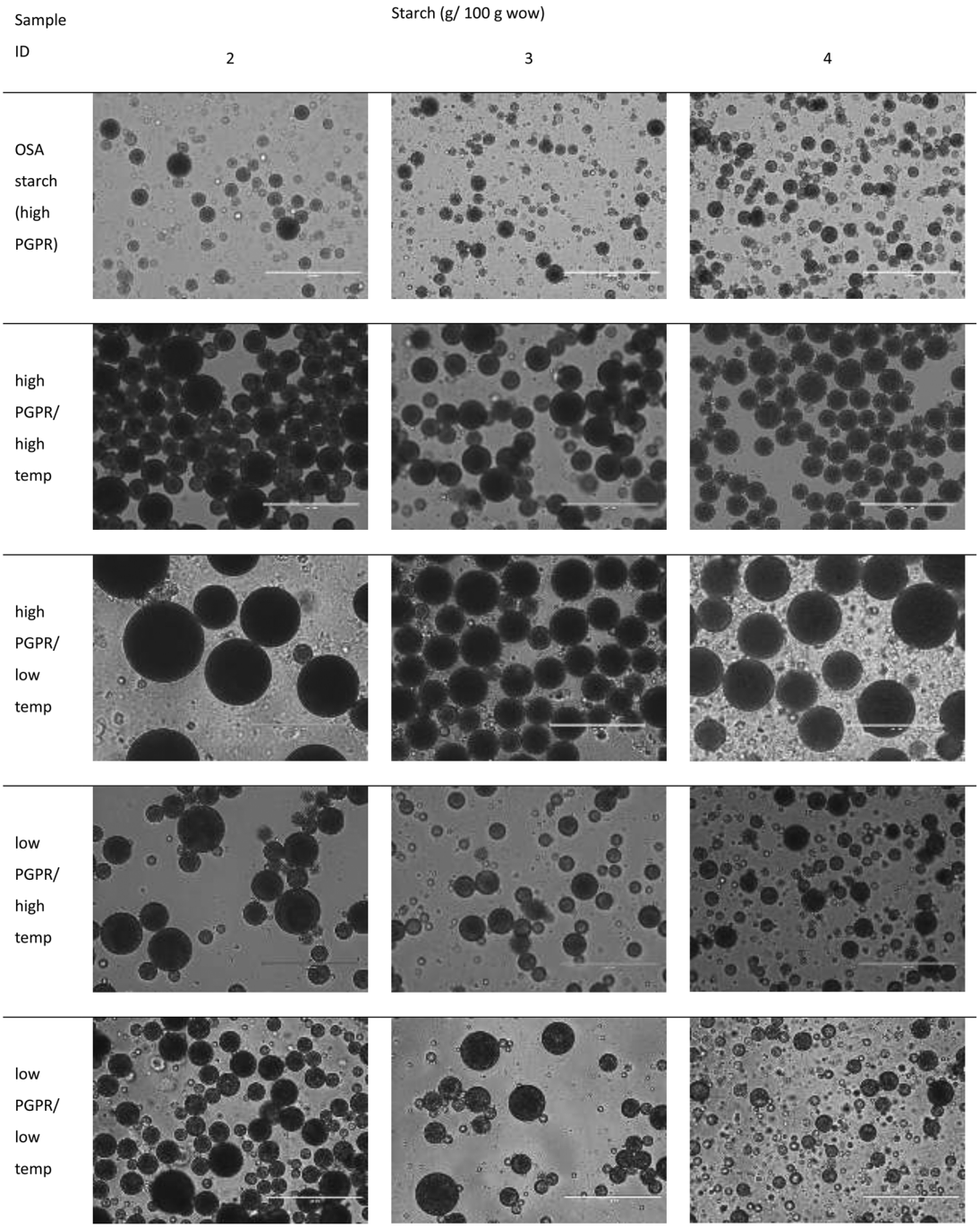

Fig. 1 Bright field micrographs of emulsions taken 1 day after processing. All images were taken at the same magnification with the scale bar corresponding to 100 μm.

processed at a lower temperature featured the largest w_1/o droplets. These observations were confirmed by the droplet size data presented in Fig. 2 as the characteristic droplet diameters $x_{10,2}$, $x_{50,2}$ and $x_{90,2}$. These descriptors for the droplet size distributions were chosen since the surface area-based density distributions showed a variety of characteristic shapes.

Some were monomodal, some bimodal with two distinct peaks and some polymodal. Data for the emulsion stabilised with 3 g WRS per 100 g containing a higher level of PGPR and processed at the lower temperature were excluded from Fig. 2 since these data showed much poorer reproducibly than any of the other data. This was probably a coincidence since the emulsions stabilised with 2 g and 4 g WRS per 100 g gave consistent data under these conditions (higher level of PGPR and processed at the lower temperature).

The discussion of the characteristic droplet size parameters of the fresh emulsions follows on from the discussion of their evolution during storage. Along the way the results of the prop-

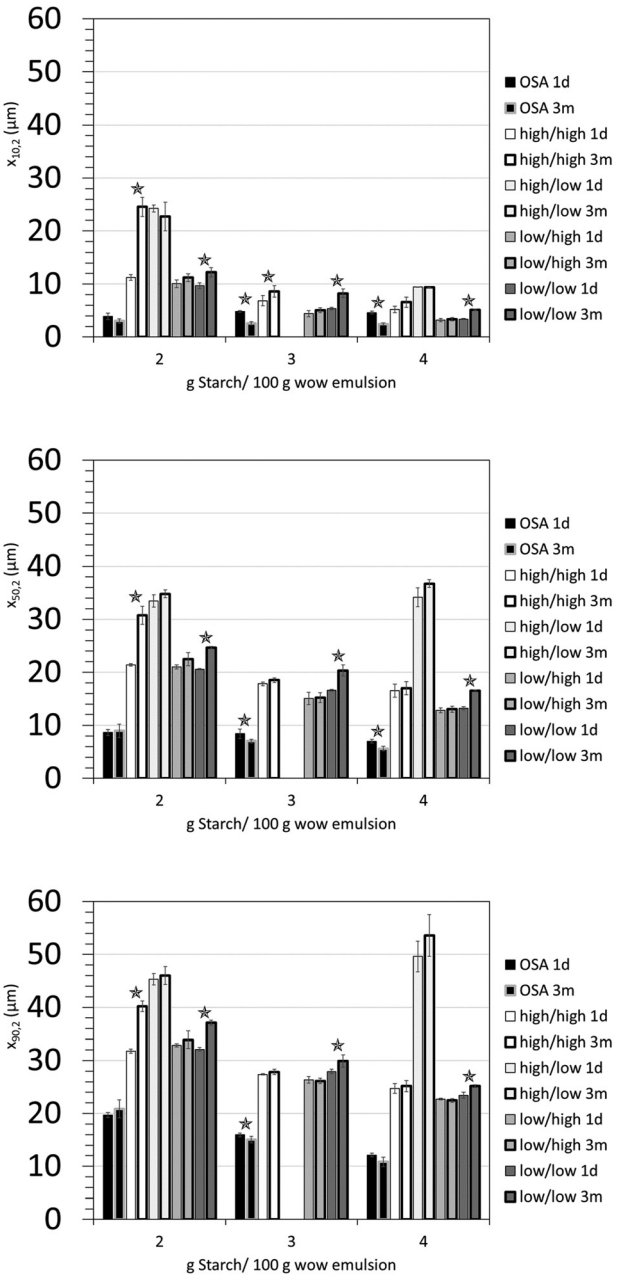

Fig. 2 Characteristic droplet size distribution parameters $x_{10,2}$ (top), $x_{50,2}$ (middle) and $x_{90,2}$ (bottom) of all emulsions as calculated from small angle laser diffraction analysis carried out 1 day (1 d) and 3 months (3 m) after emulsion preparation and storage at 20 °C. Low and high before and after "/" indicate the level of PGPR and processing temperature (75 ± 3 °C, 88 ± 5 °C) respectively. OSA starch emulsions were processed with high PGPR and 25 ± 5 °C. Note that no data are included for 3 g per 100 g high/low 1 d and 3 m as the data showed poor reproducibility for reasons that were not obvious. A start symbol above a 1 d and 3 m data pair indicates the significant difference of the droplet size parameter over storage.

erty analyses affecting the initial droplet size (encapsulation efficiency, starch solubility index and viscosity) are introduced. All three WRS stabilised emulsions prepared with a lower amount of PGPR and a lower temperature coarsened over the 3 months storage period as evidenced by a significant increase in the value of all three characteristic droplet size parameters. The emulsion based on 2 g WRS per 100 g, the higher amount of PGPR and processing at the higher temperature also coarsened. In the case of the 3 g WRS per 100 g emulsion, a significant increase in the characteristic droplet size value was only found for $x_{10,2}$. The OSA starch stabilised emulsions on the other hand showed some shrinkage. Coarsening is most likely the result of insufficient surface coverage of the droplets with WRS following processing, leading to coalescence over storage. It appears that processing the starch at the lower temperature is less beneficial with regard to the emulsion stabilising properties of the gelatinised starch compared to processing at the higher temperature. Microscopic inspection of the processed starch revealed the presence of what is generally known as starch ghosts following processing at the lower temperature. These were no longer present following processing at the higher temperature indicative of a higher proportion of granule disruption and thus soluble starch polymer present in the external emulsion phase. The WSI of the WRS was 91.7 ± 0.3 g per 100 g and 97.9 ± 0.2 g per 100 g for processing at 75 °C and 88 °C, respectively.

Viscosity was also measured to obtain additional evidence of structural differences between the starch-rich phases of the emulsions processed at the two different temperatures. The viscosity behaviour followed power law behaviour, see Table 1 for the power law parameters. The flow behaviour index decreased with the increase in starch concentration which agrees with previously reported results for gelatinised starches.[26] At the higher of the two temperatures, the flow behaviour index was lower for each concentration, indicative of a higher degree of structure in the sample, *i.e.*, of a higher degree of granule disruption at the higher processing temperature, in line with the results of the WSI assay. The higher processing temperature was reached by processing for longer, for which a decrease of the flow behaviour index has previously been reported.[24] Accordingly, the flow consistency index increased with increasing processing temperature.

The encapsulation efficiency is reported in Fig. 3. None of the WRS stabilised emulsions showed a theoretical value of 28% for encapsulation efficiency 1 day after processing, but all three OSA starch stabilised emulsions did. The OSA starch

Table 1 Power law indices for processed aqueous starch dispersions. For consistency, samples are identified with their starch concentration based on the wow emulsion. Means in the same column with different superscripts differ significantly (one-way ANOVA, $p < 0.05$)

Sample (g starch per 100 g wow)	Temperature (°C)	k (mPa s^n)	n
2	75	2.8 ± 0.1^{d}	0.99 ± 0.01^{a}
3		5.9 ± 0.4^{c}	0.97 ± 0.01^{ab}
4		13.6 ± 1.2^{b}	0.92 ± 0.02^{c}
2	88	4.4 ± 0.3^{cd}	0.95 ± 0.01^{b}
3		14.4 ± 0.8^{b}	0.87 ± 0.01^{d}
4		23.3 ± 1.3^{a}	0.86 ± 0.01^{d}

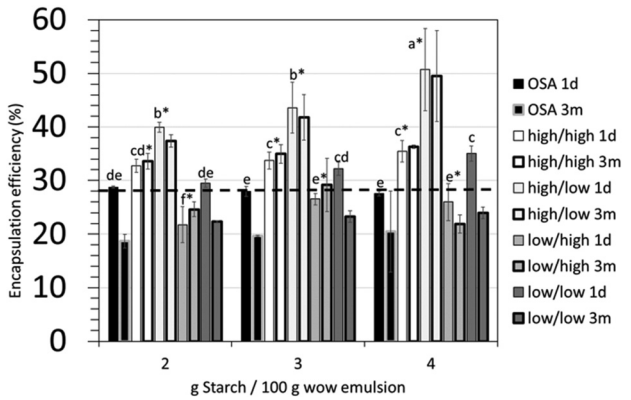

Fig. 3 Encapsulation efficiency (eqn (2)) of all emulsions determined 1 day (1 d) and 3 months (3 m) after emulsion preparation and storage at 20 °C. Low and high before and after "/" indicate the level of PGPR and processing temperature (75 ± 3 °C, 88 ± 5 °C) respectively. OSA starch emulsions were processed at a high level of PGPR and 25 ± 5 °C. The dashed line indicates the theoretical value of 28%, as based on the composition. Different letters indicate statistically significant differences between the day 1 data (p = 0.05). An asterisk (*) above day 1 data indicates that encapsulation efficiency at 3 m was not statistically significant different (p = 0.05).

applied here was more interfacially active than the gelatinised WRS.[13] This suggests that the newly created interface during droplet break-up was stabilised by OSA starch molecules before either external aqueous emulsion phase could be emulsified into the oil phase, thereby increasing the EE, or internal aqueous phase combined with an external aqueous phase, leading to a value of less than 28% for EE. The properties of the WRS stabilised emulsions as imparted by processing were affected by two more variables than for the OSA starch stabilised emulsions. These were the level of PGPR and the processing temperature, which were constant at a high level of PGPR and 25 ± 5 °C, respectively, for the OSA starch stabilised emulsions. Either factor could have contributed to the deviation of the value for the encapsulation efficiency from the theoretical value of 28% as well as to the differences in characteristic droplet size parameters as measured 1 day after processing. With the exception of the three "low/low" emulsions, and in contrast to the OSA starch stabilised emulsions, the encapsulation efficiency of the WRS stabilised emulsions was stable over the 3 month storage period. The encapsulation efficiency of the "low/low" emulsions decreased and, with the knowledge that the droplets in these emulsions coarsened over storage, it can only be assumed that a proportion of w_1 was lost into w_2 during oil droplet coalescence. The other emulsion showing coalescence through an increase of all three characteristic droplet size parameters over storage, 2 g WRS per 100 g emulsion "high/high", was stable with regard to encapsulation efficiency. This suggests a higher stability of w_1 in this emulsion due to the higher concentration of PGPR. The level of PGPR in the oil phase also appeared to have played a role in the process induced droplet size together with the starch concentration and the processing temperature, see data for 1 d in Fig. 2. The interplay is complex and probably most straightforward for the emulsions processed at the higher temperature. As not unexpected, the droplet size decreased with increasing starch concentration for both levels of PGPR in the oil phase. However, at 3 and 4 g WRS per 100 g emulsion, the droplet size was smaller at the lower level of PGPR. The corresponding data for encapsulation efficiency, see Fig. 3, suggest that, at the higher level of PGPR, a proportion of w_2 was incorporated into the oil droplets during processing. The encapsulation efficiency was greater than the theoretical value of 28% for all three starch concentrations, whereas it was lower than 28% at the lower level of PGPR present in the oil phase. This in turn suggests that some of w_1 was lost into w_2 during processing. While at each of the three starch concentrations, processed at the higher temperature, the differences in encapsulation efficiency between the two levels of PGPR were significant, see Fig. 3, in terms of the droplet size, this was only the case for the two higher starch concentrations. It has already been discussed that the emulsion coarsened over storage when only 2 g WRS per 100 g emulsion was present, so coalescence during processing due to the lower degree of gelatinisation and hence lower viscosity was most likely the reason for this observation. Processing the emulsion at the lower temperature at the same level of starch led to emulsions with a larger droplet size at the higher level of PGPR in the system. The weaker emulsion stability of this system has already been discussed. It appears that the effect of PGPR incorporating w_2 into the oil droplets was more pronounced (at the lower processing temperature), possibly due to the lower viscosity of the continuous emulsion phase, see Table 1, enabling incorporation of this phase followed by stabilisation by a larger amount of not already interfacially adsorbed PGPR present in the oil phase. At the lower level of PGPR (at the lower processing temperature), the data for encapsulation efficiency still suggest incorporation of w_2 into the oil droplets during processing. This trend was increasing with increasing starch concentration although the droplet size, see $x_{50,2}$ and $x_{90,2}$ Fig. 2, decreased in the same direction. This is contradictory but would be the result of dynamic microstructure development during emulsion processing including concurrent droplet coalescence and break-up.

Salt release

Salt release was assessed *in vitro*, utilising a conductivity-based assay, and *in vivo* with a sensory panel. Both assays were applied between 1 and 2 days after emulsion preparation. The amylase present in the assay led to the partial destabilisation of the emulsion microstructure as illustrated in Fig. 4 and 5 following *in vitro* digestion and expectoration after chewing (not following a chewing protocol), respectively. Both types of digested emulsions showed a range of but similar microstructures. While some of the microstructures of the original w/o/w emulsion were retained (Fig. 4 & 5 left), there were clearly also coalescence and loss of the internal droplet phase (Fig. 4 & 5 middle) and the development of a bi-continuous oil–water microstructure (Fig. 4 & 5 right), with the oil phase clearly featuring included droplets.

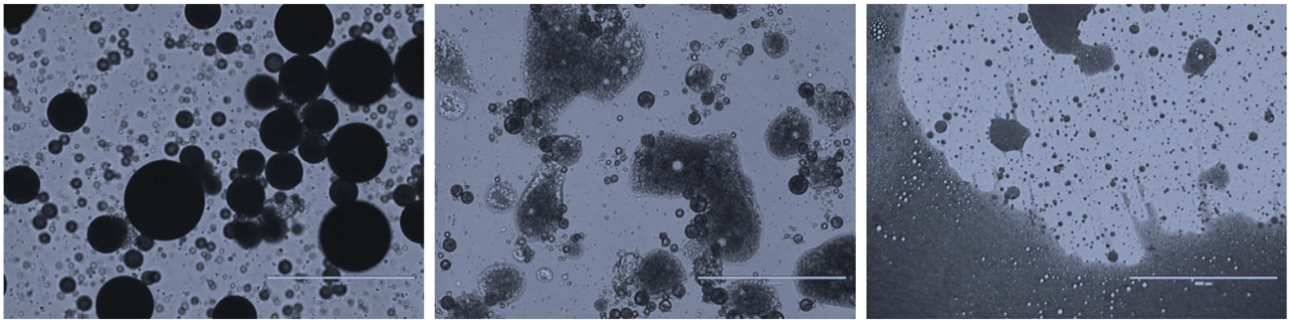

Fig. 4 Micrographs of emulsions after 30 s of *in vitro* digestion assay showcasing the different microstructures seen across all of the emulsions. Left and middle: 2 g WRS per 100 g emulsion, low PGPR, low temperature processing. Scale bar = 200 μm. Right: 4 g WRS per 100 g emulsion, low PGPR, low temperature. Scale bar = 400 μm.

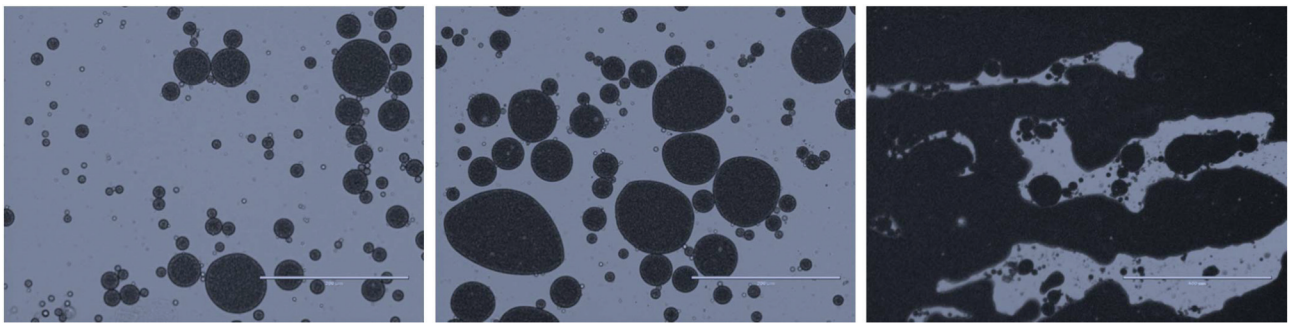

Fig. 5 Micrographs of emulsions after 30 s of oral processing showcasing the different microstructures seen across all of the emulsions. Left and middle: 2 g WRS per 100 g emulsion, high PGPR, high temperature processing. Scale bar = 200 μm. Right: 4 g WRS per 100 g emulsion, high PGPR, high temperature. Scale bar = 400 μm.

In vitro evaluation

The quantitative salt release data based on the *in vitro* assay are shown in Fig. 6. The concentration of salt in the external emulsion phase following *in vitro* digestion varied from just under 70% to around 90% of the total salt contained in the emulsion. Processing at the lower level of PGPR and at the higher temperature resulted in the highest values and could therefore be regarded as the most successful formulation. However, the difference between before and after *in vitro* digestion was the lowest for the 2% WRS emulsion of this group, see Table 2. These differences are critical to the success of this salt reduction approach since it builds on generating variation in tastant concentration during oral processing. So, this emulsion might perform poorer in a sensory test compared to the other two emulsions of this group. The benefit of the higher processing temperature lies in the higher degree of granule disruption, as reflected in the results for viscosity and WSI, providing more reaction sides for hydrolysis by amylase.[25] Analysing the increase in the content of salt in the external emulsion phase following *in vitro* digestion revealed that processing at the higher level of PGPR, still at the higher temperature, brought about a higher increase of 21%, at 4 g starch per 100 g emulsion, compared to 17% as the maximum at the lower level of PGPR, at 3 g starch per 100 g emulsion. The largest difference between before and after *in vitro* digestion was recorded for an emulsion processed at the lower temperature, specifically the emulsion containing both the highest level of starch and PGPR. This result is explained by the fact that this emulsion had the highest encapsulation efficiency. The result that in this group of emulsions, containing the higher level of PGPR and processed at the lower temperature, the lowest final content of salt in the external emulsion phase was found for the emulsion containing the intermediate amount of starch should not be over interpreted. The standard deviation is relatively large and the error bar overlaps with that of the emulsion containing the lowest level of starch and it reaches the bottom end of the emulsion containing the higher level of starch. In fact, the error bars overlapped in all five groups of emulsions. The statistical analysis indicated in Fig. 6 was based on all 15 emulsions to aid selection of emulsion samples for sensory analysis. As mentioned in the methods section, 6 emulsions were selected for sensory analysis and combined into 4 sample pairs, see Table 4. While the processing temperature was not a selection criterion, by coincidence, all of the selected WRS stabilised emulsions were processed at the higher temperature. One of the selection criteria for the sample pairs included emulsion viscosity as a well-recognised material property impacting tastant perception.[28,29] The power law parameters fitted to the viscosity data acquired between

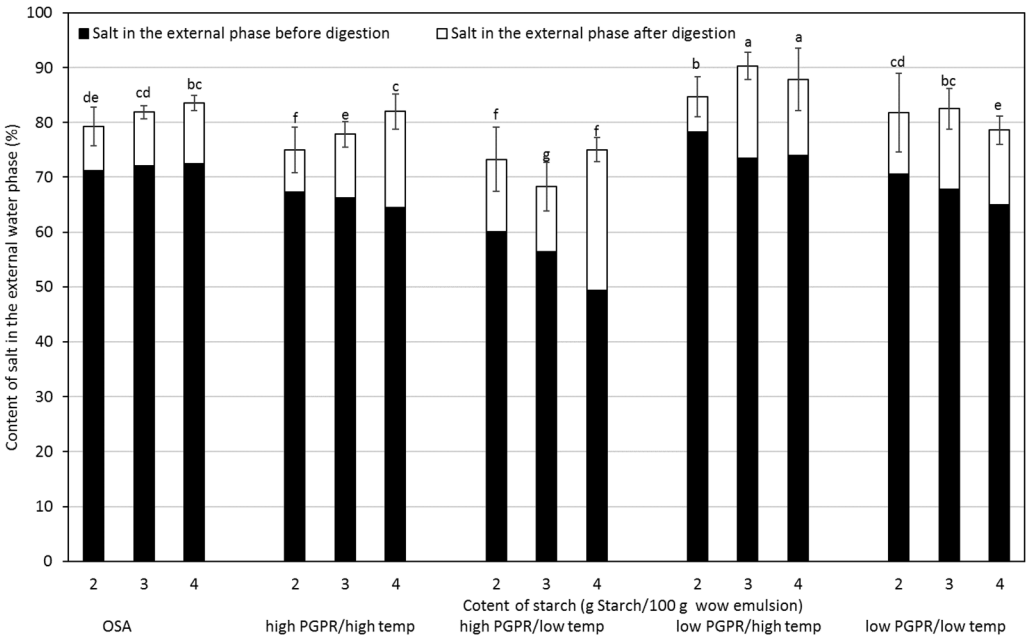

Fig. 6 Content of salt in the external emulsion phase as measured by conductivity before and after application of the *in vitro* digestion assay. Different letters indicate statistically significant differences in the salt content in the external emulsion phase after *in vitro* digestion (p = 0.05).

Table 2 Difference in the salt content in the external emulsion phase between before and after *in vitro* digestion

Sample				
Starch				
Type	g per 100 g wow	Level of PGPR	Processing temperature	Difference (%)
OSA	2	High	n/a	10
OSA	3			12
OSA	4			13
Waxy rice	2	High	High	10
Waxy rice	3			15
Waxy rice	4			21
Waxy rice	2	High	Low	18
Waxy rice	3			17
Waxy rice	4			34
Waxy rice	2	Low	High	8
Waxy rice	3			17
Waxy rice	4			16
Waxy rice	2	Low	Low	14
Waxy rice	3			18
Waxy rice	4			17

Table 3 Power law parameters for emulsions included in the sensory assessment

Sample	k (mPa s^n)	n
3 g OSA per 100 g wow	108 ± 1^{e}	0.81 ± 0.00^{a}
4 g OSA per 100 g wow	187 ± 23^{d}	0.79 ± 0.02^{b}
2 g WRS per 100 g wow, low PGPR/high temp	99 ± 9^{e}	0.80 ± 0.00^{ab}
3 g WRS per 100 g wow, low PGPR/high temp	257 ± 2^{c}	0.76 ± 0.00^{c}
3 g WRS per 100 g wow, high PGPR/high temp	523 ± 46^{a}	0.72 ± 0.00 ^{d}
4 g WRS per 100 g wow, low PGPR/high temp	446 ± 12^{b}	0.75 ± 0.01^{c}

10 s^{-1} and 500 s^{-1} on the decreasing shear rate ramp are reported in Table 3. Additionally, the values at 50 s^{-1} are included in Table 4 to facilitate comparison between the samples. This shear rate was chosen as it is widely accepted as the representative shear rate for oral processing of liquid foods and most often considered in attempts to correlate sensory data to viscosity data.[26] The power law parameters in Table 4 follow an expected trend for increasing starch content in the emulsions under otherwise constant formulation conditions, *i.e.*, level of PGPR (low) and processing temperature (high). The flow consistency index increased as starch aggregates that were not interfacially adsorbed thickened the external aqueous emulsion phase. Also, the droplets were smaller, see Fig. 2, increasing internal surface area in the emulsion and thus friction leading to the viscosity increase. The flow behaviour index decreased with increasing concentration of starch, although the increase between 3 and 4 g starch per 100 g wow was not significant. The WRS emulsion with a higher PGPR content was the most viscous but also the most shear thinning of the WRS emulsion included in the sensory assessment. The encapsulation efficiency measured for this emulsion was significantly higher, see Fig. 3. It was argued that this was due to a fraction of w_2 being incorporated into the oil droplets during the 2nd emulsification step. Hence, the dispersed phase volume of this emulsion was increased and, in line with established theory,[27] viscosity increased.

The first sample pair (PC1) was selected to verify whether saltiness perception would follow the trend of the *in vitro*

Table 4 Sample pairs, viscosity, encapsulation efficiency EE, characteristic droplet size $x_{50,2}$, number of panellists assessing each pair and results of the sensory assessment, compared to the results of the *in vitro* assay. All WRS emulsions in the sample pairs were processed at the higher temperature. The values for EE and $x_{50,2}$ correspond to those reported in Fig. 2 and 3 and were included here to facilitate discussion

	Sample							Results		
	Starch							Sensory*		
Pair	Type	g per 100 g wow	Level of PGPR	Viscosity (mPa s) at 50 s^{-1} and 37 °C	EE (%)	$x_{50,2}$ (μm)	No. assessors selecting sample as most salty	Difference test	Similarity test	*In vitro*
PC1	Waxy rice	3	Low	101 ± 1	26.5 ± 1.0	15.1 ± 1.1	65	Saltier ($p < 0.01$)	n/a	12% more salt externally
	Waxy rice	3	High	184 ± 9	33.7 ± 1.6	17.8 ± 0.3	39			
PC2	Waxy rice	4	Low	169 ± 3	26.0 ± 3.4	12.8 ± 0.5	52	Not significantly different ($p > 0.05$)	Similar ($\beta < 0.05$)	Not significantly different
	OSA	4	High	82 ± 5	27.5 ± 0.5	7.0 ± 0.3	52			
PC3	Waxy rice	2	Low	47 ± 4	21.8 ± 3.4	21.0 ± 0.4	53	Not significantly different ($p > 0.05$)	Similar ($\beta < 0.05$)	3% more salt externally
	Waxy rice	4	Low	169 ± 3	26.0 ± 3.4	12.8 ± 0.5	51			
PC4	Waxy rice	3	Low	101 ± 01	26.5 ± 1.0	15.1 ± 1.4	59	Not significantly different ($p > 0.05$)	Not sufficiently similar ($\beta > 0.05$)	8% more salt externally
	OSA	3	High	51 ± 0	27.9 ± 0.9	8.5 ± 0.9	45			

*The difference was perceived significantly saltier at p value $\alpha < 0.05$. The similarity test was set at $\alpha = 0.2$, $\beta = 0.05$ and pd = 30% as outlined in BS ISO 5495:2007.

data – increased concentration of salt in the external emulsion phase at the conclusion of the digestion process at the reduced content of PGPR as the only variable except for viscosity. The difference in the content of salt before and at the conclusion of the *in vitro* assay was the same, see Table 2. The viscosity of the emulsion, measured at 50 s^{-1} and 37 °C, with the higher final content of salt in the external emulsion phase was significantly lower, see Table 4. While this viscosity difference would not affect the *in vitro* result as equilibrium data were reported, taste perception is a dynamic process and known to be affected by viscosity as aforementioned. The second sample pair (PC2) was composed of two emulsions prepared with the two different types of starch applied in this study leading to the same final salt content available for taste perception, although the OSA stabilised emulsion showed a higher encapsulation efficiency, see Fig. 3, and therefore a larger difference in the content of salt before and at the conclusion of the *in vitro* assay, see Table 4. So, the tastant difference through oral processing would be higher and this emulsion might potentially be identified as the saltier emulsion of this pair. Concurrently, the viscosity of this emulsion was higher, which could be expected to counteract enhanced saltiness perception. The WRS based emulsion of PC2 (4 g starch per 100 g emulsion and low level of PGPR) was also paired with a WRS sample (2 g starch per 100 g emulsion and low level of PGPR) showing similar differences to the OSA starch sample, *i.e.*, lower viscosity and larger difference in the content of salt before and at the conclusion of the *in vitro* assay. The higher viscosity sample showed a slightly but significantly higher content of salt at the conclusion of the *in vitro* assay. This pair (PC3) was included in the sensory assessment to verify whether the difference in the salt content would outweigh the expected negative impact on salt perception of the 3-fold higher viscosity. Finally, the fourth sample pair (PC4) included the emulsion showing the highest content of salt in the external emulsion phase at the conclusion of the *in vitro* assay when stabilised with WRS and OSA starch, respectively. In the case of the OSA starch, the result for the emulsion containing 3 g starch per 100 g was not significantly different to the result at 4 g starch per 100 g emulsion. Therefore, the emulsion with a lower content of starch was selected so the concentration of starch was the same in this sample pair, despite the therefore larger difference in viscosity. The encapsulation efficiency of the WRS stabilised emulsion was slightly lower but the final content of salt in the external emulsion phase following *in vitro* digestion was significantly higher. At the same time this emulsion was more viscous than the OSA starch stabilised emulsion.

Saltiness perception. The results of the sensory assessment are shown in Table 4. While not considered in the selection of the samples for sensory assessment, encapsulation efficiency and characteristic droplet size, represented by $x_{50,2}$, data were included to facilitate the discussion of the results. No significant difference ($p > 0.05$) in perceived saltiness was found for pairs 2, 3 and 4. The similarity test identified that the emulsions presented in PC2 and PC3 were perceived to be

sufficiently similar in saltiness. In the case of PC4, the similarity test revealed that the samples were neither significantly different nor similar.

The sensory assessment validated the results of the *in vitro* assay for PC1 and PC2. The two samples of PC1 were found to be significantly different in both types of assessment. While a similar difference in the salt content in the external emulsion phase between before and after *in vitro* digestion, see Table 2, as well as the comparable droplet size, see Table 4, was presented by both samples of PC1, the saltier perceived sample was both less viscous and of a higher salt content in the external emulsion phase preventing the possibility of a conclusive statement for the driver of increased saltiness perception. It is only possible to conclude that for PC1 the *in vivo* assessment validated the result of the *in vitro* assessment.

In the case of PC2, the two emulsions were not significantly different and the sensory results for similarity confirmed that the two emulsions were perceivably similar for saltiness. Again, the sensory assessment confirmed the *in vitro* result for this sample pair. Both samples of this pair contained 4 g starch per 100 g emulsion, one containing WRS and the other one OSA starch, and encapsulation efficiency and the final salt content in the external emulsion phase following *in vitro* digestion were comparable. However, the OSA starch emulsion had smaller droplets and was a lot less viscous than the WRS emulsion. Both observations would suggest that this emulsion should release more salt, because of the larger surface area for release, and viscosity is largely inversely correlated with perceived taste intensity.[28,29] The droplet size argument assumes that droplet break-up in the two emulsions of this sample pair, both through the mechanical action of the *in vitro* assay and oral processing, was comparable. The validation of this argument is not straightforward due to the interfacial kinetics involved in the digestive process and experimental data to this effect are yet to be acquired. Additionally, the stress field during oral processing is largely unknown and a number of assumptions would need to be made to this effect. It might be worth noting though that, generally, smaller droplets and interfaces with a higher interfacial tension require larger interfacial stresses for break-up. Here, while the droplets of the OSA starch emulsion were smaller, the OSA starch used in this study reduced the interfacial tensions further than the non-chemically modified WRS as we reported previously.[13]

With regard to PC3, the *in vitro* results indicated a significantly higher content of salt in the external emulsion phase for one of the two emulsions of this sample pair at the conclusion of the *in vitro* assay while the assessors rated these two emulsions as similar in saltiness. The emulsion showing the higher final salt content in the external emulsion phase contained 4 g WRS per 100 g emulsion and its viscosity at 50 s^{-1} and in-mouth temperature were roughly three times higher compared to those of the other emulsion of this sample pair, containing 2 g WRS per 100 g emulsion. The droplet size was smaller and the encapsulation efficiency was higher creating a larger surface area and a difference in the salt content during consumption to be noticed as taste enhancement, and thus saltier by consumers, appeared to have counterbalanced the negative impact of the much larger viscosity on saltiness perception. So, the results of this PC provide some insight into how to design these emulsions at different viscosity, or thickness as it would be rated by consumers, without compromise in saltiness perception.

The sample pair assessed in PC4 was similar to the sample pair of PC2 in as far that both emulsions contained the same amount of starch while one was stabilised with WRS and the other one with OSA starch. They were also comparable in terms of the differences and similarities in material properties, see Table 4. The encapsulation efficiency across the 4 samples of these two sample pairs was similar whereas the samples of PC4 were less viscous due to the lower amount of starch in their formulation. Based on the material property data available for these emulsion systems, this lower viscosity level offers the only explanation for the indifferent results obtained for these two sample pairs in *in vitro* assay (not significantly different for PC2 *versus* significantly different for PC4) and the sensory assessment for saltiness (not significantly different but similar for PC2 *versus* not significantly different but not sufficiently similar for PC4).

Conclusions

In situ gelatinised WRS was successfully applied to stabilise the external interface in wow emulsions containing salt in either aqueous phase. A higher degree of WRS granule disruption due to gelatinisation at higher temperature, 90 °C as opposed to around 75 °C, resulted in emulsions that were microstructurally stable for at least 3 months. Stabilising the internal aqueous phase with 0.57 g PGPR per 100 g emulsion compared to 0.29 g per 100 g led to incorporation of the external aqueous phase into the oil droplets during the second step of the two-step wow emulsification process applied here. The same observation did not apply to the OSA starch stabilised reference emulsions, also containing 0.57 g PGPR per 100 g emulsion, which was hypothesised to be due to the higher interfacial activity of the OSA starch compared to the non-chemically modified WRS.

Considering the difference in the salt content in the external aqueous phase before and after *in vitro* digestion as a success factor, since this salt reduction approach is built on taste enhancement by delivering change of tastant to the taste buds, a lower concentration of the lipophilic emulsifier and a higher level of starch conversion for the stabilisation of the oil droplet interface were beneficial. The sensory assessment of four sample pairs, composed of six emulsions including four WRS emulsions processed 90 °C and two OSA starch stabilised emulsions, revealed that WRS stabilised emulsions could be of less favourable viscosity (higher) and droplet size (larger) characteristics compared to OSA starch stabilised emulsions delivering the same level of saltiness. We ascribed this result to the non-chemically modified nature of the WRS starch emulsifier, enhancing amylase-based hydrolysis and thus

emulsion destabilisation is required as a pre-requisite for this salt reduction approach to be successful.

Conflicts of interest

There are no conflicts of interest to declare.

Appendix: Real food validation

As mentioned at the end of the introduction of the main article, we conducted a short application study of a WRS stabilised wow emulsion to salt reduction in tomato soup and report this at the end of this paper.

Preparation of tomato soup samples

Tomato soup samples were prepared by mixing a tomato soup base (containing no oil or salt) with either a salt-free wow emulsion and salt, or a salt containing wow emulsion. All ingredients for the soup base were purchased from a local supermarket (Sainsbury's) and processed in a food processor (Thermomix TM31, Vorwerk, Sunninghill, UK) as follows. Peeled potatoes (100 g) and carrots (50 g) were chopped for 10 s at 10 200 rpm. Afterwards, tomatoes (400 g), tap water (100 g), onions (150 g) and garlic (5 g) were added and mixed for 10 min at 1100 rpm. Temperature was set to 100 °C, which was reached after approximately 8 min. Dried basil (1 g) was then added and mixed for 5 min at 1100 rpm, at 100 °C. The soup base was stored at 4 °C.

The wow emulsions were prepared as described previously, based on 5 g WRS per 100 g w_2, 1.43 g PGPR per 100 g oil and the higher processing temperature of 88 ± 5 °C. Three emulsions were prepared with either no salt, 1.2 g or 1.41 g per 100 g emulsion of which 0.13 g per 100 g emulsion were contained inside the oil droplets. Finally, three soup samples were prepared by processing in the food processor for 30 s at 2000 rpm, by adding 40 g of the wow emulsion to the salt and soup base. The salt content was 0.4 g per 100 g of soup with no salt inside the oil droplets, termed reference sample as this level of salt corresponds to the level in a number of commercial tomato soups currently available in the UK, 0.3 g and 0.35 g (some of which was inside the oil droplets) per 100 g respectively.

Sensory evaluation of tomato soup samples

The sensory evaluation of the tomato soup samples comprised consumer testing of the soups utilising a paired comparison test and a liking test. The ethical approval obtained for the sensory evaluation of the emulsion samples was extended to cover the tomato soup samples and their testing was approved by the same committee. Again, the evaluation was conducted in compliance with relevant laws and informed consent was obtained from the subjects. 116 naive subjects (62 female, 54 male) between 18 and 70 years of age (mean age 31) were recruited from a convenience sample of students, staff and visitors to The University of Nottingham.

Perceived saltiness was assessed by paired comparison tests (2-Alternate Forced Choice tests, BS ISO 5495:2007) and subjective liking of the soups was measured using a 9-point hedonic scale.[30] Additional questions regarding the soups' saltiness and texture were also included using just-about right (JAR) scales to establish whether the soups with a lower salt content would be acceptable.

The 0.4 g salt per 100 g and the 0.35 g salt per 100 g soups were compared for perceived saltiness using the paired comparison test. The two samples (15 g) were presented in a randomised order in containers labelled with a random three-digit code. Volunteers were asked to taste the samples, cleansing their palate with water and crackers in-between, and select the sample they perceived to be the saltiest. After the test, the volunteers were instructed to cleanse their palate for 1 min with mineral water (Evian, Danone, France) before being invited to take part in the liking test. The liking test included the 0.3 g salt per 100 g and the 0.35 g salt per 100 g soups. In sequence, the consumers were presented with the sample (30 g) labelled with different random three-digit codes in a randomised, balanced order. They were asked how much they liked the soup using a 9-point hedonic scale, what they thought of the level of saltiness using a 5-point just-about-right (JAR) scale (5 – far too much salt, 3 – just-about-right, 1 – not salty enough) and what they thought of the texture (5 – far too thick, 3 – just-about-right, 1 – far too thin). The two JAR scales for saltiness and texture were presented in a randomised order. The volunteers were asked to cleanse their palate for 1 min in between the two acceptability tests. Paired comparison data were analysed by the two-sided binomial test for difference, $\alpha < 0.05$. If no significant difference was found, similarity was calculated with parameters set to $\alpha = 0.2$, $\beta = 0.05$ and pd = 30%. Liking data were analysed by comparing the mean liking scores using a paired *t*-test. JAR data were collapsed to top two box (too much) and bottom two box (too little) and percentage frequencies were reported. If the percentage of consumers in the 'too little' or 'too much' categories was greater than 20%, the decrease in acceptability (mean drop in liking) was calculated. A net penalty (mean drop × proportion of respondants scoring 'too much' or 'too little') less than 0.25 is considered to have a low impact on liking.

Findings

53 out of the 116 consumers selected the test soup (0.35 g salt per 100 g) as saltier, and 63 selected the reference soup (0.4 g salt per 100 g). This means that these two soups were not perceived to be significantly different ($\alpha = 0.4$) for saltiness. They were in fact found to be significantly similar with a 95% confidence interval (pd = 30%). Therefore, these data suggest that a

reduction in salt by 12.5% compared to the level of similar products currently on the market (0.4 g salt per 100 g) can be achieved without compromise in saltiness perception. This validates the presented salt reduction approach for this type of product.

In order to assess whether a further reduction in salt would be possible, the consumers were asked about their liking of the lower (0.35 g salt per 100 g) and an even lower (0.3 g salt per 100 g) salt content soup. The mean liking score for the 0.35 g and 0.3 g salt per 100 g soup was 6.37 ± 1.44 and 6.31 ± 1.58, respectively. A paired *t*-test revealed no significant difference in the liking scores between these two samples (α = 0.7), indicating that a further reduction in salt could be possible without compromising consumer liking. For the JAR data, the saltiness of the low salt content soup (0.35 g per 100 g) was perceived as just-about-right by 60%, not salty enough by 18% of consumers and too salty by 22% of consumers. The mean drop in liking due to not being salty enough was 0.58 (on a 9-point hedonic scale), with a net penalty of 0.11 revealing a low impact on acceptability. The even lower salt content soup (0.3 g salt per 100 g) was perceived as just-about-right by 53%, not salty enough by 27% and too salty by 21% of consumers. The mean drop in liking due to not being salty enough was 0.46 (on a 9-point hedonic scale) with a net penalty of 0.12 revealing a low impact on acceptability.

Texture was perceived as just-about-right by 73% (too thin by 12% and too thick by 15%) of consumers for the lower salt content soup, and JAR by 72% (too thin by 10% and too thick by 17%) of consumers for the even lower salt content soup. As the percentage of consumers in the 'too thin' or 'too thick' categories was not greater than 20%, mean drops were not calculated. It can be concluded that the non-chemically modified starch-based wow emulsion approach presented here could enable a 25% salt reduction in a tomato soup compared to levels offered on UK supermarket shelves (before 12.5% lower in salt soups were introduced).

Acknowledgements

This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/M027139/1]. The authors thank Qian Yang and Liling Zhang for support with sensory, the consumers for testing the samples and Elizabeth Starr for formulating the tomato soup.

References

1 WHO, *Guideline: Sodium intake for adults and children*, World Health Organization, 2012.
2 B. McKenzie, J. A. Santos, K. Trieu, S. R. Thout, C. Johnson, J. Arcand, J. Webster and R. McLean, The Science of Salt: A focused review on salt-related knowledge, attitudes and behaviors, and gender differences, *J. Clin. Hypertens.*, 2018, **20**, 850–866.
3 A. Regan, M. P. Kent, M. M. Raats, A. McConnon, P. Wall and L. Dubois, Applying a Consumer Behavior Lens to Salt Reduction Initiatives, *Nutrients*, 2017, **9**, 9.
4 R. Rama, N. Chiu, M. Carvalho Da Silva, L. Hewson, J. Hort and I. D. Fisk, Impact of Salt Crystal Size on in-Mouth Delivery of Sodium and Saltiness Perception from Snack Foods, *J. Texture Stud.*, 2013, **44**, 338–345.
5 M. Noort, J. Bult, M. Stieger and R. Hamer, Saltiness enhancement in bread by inhomogeneous spatial distribution of sodium chloride, *J. Cereal Sci.*, 2010, **52**, 378–386.
6 X. Tian and I. D. Fisk, Salt release from potato crisps, *Food Funct.*, 2012, **3**, 376–380.
7 C. Morris, A. L. Koliandris, B. Wolf, J. Hort and A. Taylor, Effect of Pulsed or Continuous Delivery of Salt on Sensory Perception Over Short Time Intervals, *Chemosens. Percept.*, 2009, **2**, 1–8.
8 J. Busch, C. Tournier, J. E. Knoop, G. Kooyman and G. Smit, Temporal Contrast of Salt Delivery in Mouth Increases Salt Perception, *Chem. Senses*, 2009, **34**, 341–348.
9 C. Morris, C. Labarre, A. L. Koliandris, L. Hewson, B. Wolf, A. J. Taylor and J. Hort, Effect of pulsed delivery and bouillon base on saltiness and bitterness perceptions of salt delivery profiles partially substituted with KCl, *Food Qual. Prefer.*, 2010, **21**, 489–494.
10 J. B. Thomazo, A. Burbidge and B. Le Reverend, Frequency-Amplitude Cross Interaction during Pulsatile Taste Delivery Using Gustometers, *Front. Neurosci.*, 2016, **10**, 5.
11 N. Chiu, L. Hewson, I. Fisk and B. Wolf, Programmed emulsions for sodium reduction in emulsion based foods, *Food Funct.*, 2015, **6**, 1428–1434.
12 N. Chiu, A. Tarrega, C. Parmenter, L. Hewson, B. Wolf and I. D. Fisk, Optimisation of octinyl succinic anhydride starch stablised w(1)/o/w(2) emulsions for oral destablisation of encapsulated salt and enhanced saltiness, *Food Hydrocolloids*, 2017, **69**, 450–458.
13 M. M. Kasprzak, W. Macnaughtan, S. Harding, P. Wilde and B. Wolf, Stabilisation of oil-in-water emulsions with non-chemical modified gelatinised starch, *Food Hydrocolloids*, 2018, **81**, 409–418.
14 C. de Loubens, M. Panouillé, A. Saint-Eve, I. Déléris, I. C. Tréléa and I. Souchon, Mechanistic model of in vitro salt release from model dairy gels based on standardized breakdown test simulating mastication, *J. Food Eng.*, 2011, **105**, 161–168.
15 G. Muschiolik and E. Dickinson, Double emulsions relevant in food systems: Preparation, stability, and applications, *Compr. Rev. Food Sci. Food Saf.*, 2017, **16**, 532–555.
16 M. Sjoo, S. C. Emek, T. Hall, M. Rayner and M. Wahlgren, Barrier properties of heat treated starch Pickering emulsions, *J. Colloid Interface Sci.*, 2015, **450**, 182–188.
17 S. G. M. Ong, L. C. Ming, K. S. Lee and K. H. Yuen, Influence of the Encapsulation Efficiency and Size of Liposome on the Oral Bioavailability of Griseofulvin-Loaded Liposomes, *Pharmaceutics*, 2016, **8**, 25.

18 R. A. Anderson, H. F. Conway, V. F. Pfeifer and E. L. Griffin, Roll and extrusion-cooking of grain sorghum grits, *Cereal Sci. Today*, 1969, **14**, 372–376.
19 A. L. Mandel, C. P. des Gachons, K. L. Plank, S. Alarcon and P. A. S. Breslin, Individual Differences in AMY1 Gene Copy Number, Salivary alpha-Amylase Levels, and the Perception of Oral Starch, *PLoS One*, 2010, **5**, 9.
20 A. Pawlik, P. W. Cox and I. T. Norton, Food grade duplex emulsions designed and stabilised with different osmotic pressures, *J. Colloid Interface Sci.*, 2010, **352**, 59–67.
21 N. Garti, A. Aserin and Y. Cohen, Mechanistic considerations on the release of electrolytes from multiple emulsions stablized by BSA and nonionic surfactants, *J. Controlled Release*, 1994, **29**, 41–51.
22 E. C. Rojas, J. A. Staton, V. T. John and K. D. Papadopoulos, Temperature-induced protein release from water-in-oil-in-water double emulsions, *Langmuir*, 2008, **24**, 7154–7160.
23 M. Lad, L. Hewson and B. Wolf, Enhancing saltiness in emulsion based foods, *Flavour*, 2012, **1**, 13.
24 M. S. Rohaya, M. Y. Maskat and A. G. Ma'aruf, Enhancing saltiness in emulsion based foods, *Sains Malays.*, 2013, **42**, 1707–1714.
25 J. Singh, A. Dartois and L. Kaur, Starch digestibility in food matrix: a review, *Trends Food Sci. Technol.*, 2010, **21**, 168–180.
26 N. Selway and J. R. Stokes, Starch digestibility in food matrix: a review, in *Annual Review of Food Science and Technology*, ed. M. P. Doyle and T. R. Klaenhammer, Annual Reviews, Palo Alto, 2014, vol. 5, pp. 373–393.
27 I. M. Krieger and T. J. Dougherty, A mechamism for non-Newtonian flow in suspensions of rigid spheres, *Trans. Soc. Rheol.*, 1959, **3**, 137–152.
28 C. M. Christensen, Effects of solution viscosity on perceived saltiness and sweetness, *Percept. Psychophys.*, 1980, **28**, 347–353.
29 D. J. Cook, T. A. Hollowood, R. S. T. Linforth and A. J. Taylor, Perception of taste intensity in solutions of random-coil polysaccharides above and below c*, *Food Qual. Prefer.*, 2002, **13**, 473–480.
30 S. Wichchukit and M. O'Mahony, The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives, *J. Sci. Food Agric.*, 2015, **95**, 2167–2178.