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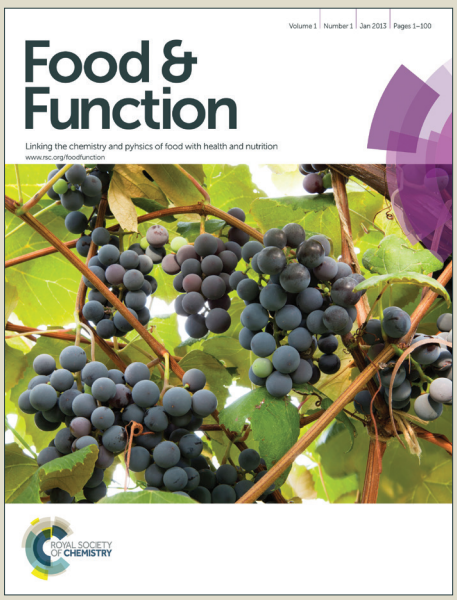

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Programmed emulsions for sodium reduction in emulsion based foods

Natalie Chiu, Louise Hewson, Ian Fisk and Bettina Wolf

Division of Food Sciences, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD

Key words: Food emulsion, wow emulsion, amylase, OSA-starch, salt perception, sodium reduction.

Abstract

In this research a microstructure approach to reduce sodium levels in emulsion based foods is presented. If successful, this strategy will enable reduction of sodium without affecting consumer satisfaction with regard to salty taste. The microstructure approach comprised of entrapment of sodium in the internal aqueous phase of water-in-oil-in-water emulsions. These were designed to destabilise during oral processing when in contact with the salivary enzyme amylase in combination with the mechanical manipulation of the emulsion between the tongue and palate. Oral destabilisation was achieved through breakdown of the emulsion that was stabilised with a commercially modified octenyl succinic anhydride (OSA)-starch. Microstructure breakdown and salt release was evaluated utilising *in vitro*, *in vivo* and sensory methods. For control emulsions, stabilised with orally inert proteins, no loss of structure and no release of sodium from the internal aqueous phase was found. The OSA-starch microstructure breakdown took the initial form of oil droplet coalescence. It is hypothesised that during this coalescence process sodium from the internalised aqueous phase is partially released and is therefore available for perception. Indeed, programmed emulsions showed an enhancement in saltiness perception; a 23.7 % reduction in sodium could be achieved without compromise in salty taste ($p<0.05$; 120 consumers). This study shows a promising new approach for sodium reduction in liquid and semi-liquid emulsion based foods.

Introduction

The need to lower sodium in our diet is recognised by both the food industry and consumers, but due to the complexity of the role of sodium in food, challenges still remain in achieving processed foods with sodium levels below governmental targets. High sodium intake has been widely reported to cause adverse health, in particular the development of hypertension. This subsequently increases the risk of developing cardiovascular and renal diseases [1-3]. Salt is one of the most common sources of sodium and the consumption in developed countries range between 8.75 and 14.01 g/ day [4], significantly exceeding the daily salt intake levels of 5 g/ d recommended by the WHO [5]. In Western diets, excessive salt intake is reported to mainly originate from processed foods which contribute approximately 75 – 80 % of total salt intake [6]. Therefore reducing salt across this category will significantly contribute to an overall dietary decrease although this can only be successful provided there is no compromise in acceptability by the consumer. The role of sodium in food not only includes delivery of salty taste, but also flavour enhancement, texture formation and as a processing aid. These complex multifaceted functions need to be overcome together to achieve true sodium reduction and viable healthier alternatives for consumers.

For foods such as bread and crisps successful strategies to reduce sodium have been demonstrated and healthier product alternatives have been commercialised. Successful strategies include the stepwise reduction to adjust consumer expectation, however this approach is only viable for foods consumed on a regular and/ or frequent basis [7, 8]; maximising the delivery efficiency of tastants [9, 10]; the use of inhomogeneous sodium concentration distributions [11] and replacement of sodium with non-sodium salts [12]. One way of maximising tastant delivery efficiency can be

achieved by concentrating sodium within small regions of the dry food thereby offering bursts of sodium release during oral processing and thus enhancing saltiness. This rapid delivery of a stimulus to the receptor reduces adaptation and consequently increases the resulting taste perception [13] and was successfully applied to bread as a sodium reduction strategy [11]. Adaptation is observed when receptors are repeatedly or extensively stimulated [14], resulting in a decrease in signal transduction or perception of that stimulus. In conclusion, the use of varying levels of stimulus delivered across an eating event is a promising route to enable the reduction of the total concentration of a stimulus whilst maintaining perception, this is proposed to be used to reduce sodium without compromising acceptability.

Emulsion based foods belong to the category of liquid and semi-liquid foods. This adds to the complexity, as sodium is water soluble hence localising sodium within different parts of the food requires some form of encapsulation. Gradual reduction combined with recipe reformulation is one of the most successful approach. Unfortunately, the complex taste interactions between sodium, other tastants and aromas limit what can be achieved, although a 23.7 % sodium content reduction in wet soups has previously been reported [15]. Studies conducted to reduce adaptation through pulsed delivery have shown mixed results for the enhancement of saltiness perception [16, 17]The success of this approach appears to very much depend on the timing of short and intense stimulus delivery and the overall length of experimental protocol. One group of researchers chose 15 s delivery profiles of salty water and concluded that saltiness perception was proportional to the overall amount of salt delivered within these 15 s [16] whilst the 30 s profiles chosen by another group showed greater promise for this approach[17].

The delivery of short intense bursts of sodium to the taste receptors are proposed to be achieved through entrapment of salt in the internal water phase of water-in-oil-in-water (wow) emulsions. It is well known that wow emulsions can be used for targeted release of water-soluble or oil-soluble actives during digestion [18] [19]. In the present case, the complex emulsion system was designed to destabilise during oral processing to release internalised sodium through formulation with emulsifying OSA-starch. Figure 1 shows the anticipated pathway of oral destabilisation of a starch stabilised wow emulsion. The interfacially adsorbed starch (starch shell, Figure 1) is hypothesised to be weakened through the action of salivary amylase and two scenarios of emulsion breakdown are proposed. The interface will destabilise and droplets coalesce (Figure 1A) releasing the high sodium entrapped water phase into the oral cavity. In this process, surface active salivary proteins may adsorb at the droplet interface. Furthermore, intensive manipulation between tongue and palate during oral processing in combination with the emulsifying action of salivary proteins may lead to phase inversion (Figure 1B). This hypothesis is based on the knowledge that fat continuous spreads and chocolate "phase invert" during oral processing into an oil-in-water emulsion, the microstructure of which directly impacts mouthfeel and flavour release [20, 21].

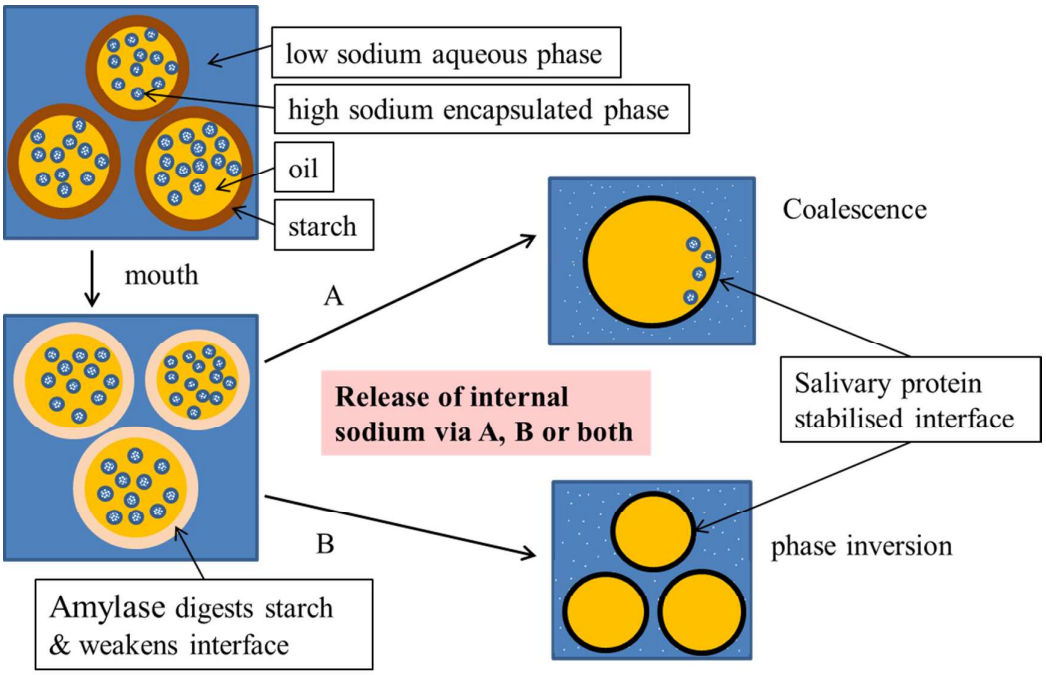

Figure 1 Schematic of the anticipated pathway of oral destabilisation of a starch stabilised wow emulsion.

Quinoa starch granules chemically modified with octinyl succinic anhydride (OSA) have been used to successfully encapsulate 1.6 % salt in the internal water phase of a wow emulsion with encapsulation efficiency, over 90 % remaining after 21 days [22]. The internal interface was stabilised with polyglycerol polyricinoleate (PGPR) added to the oil phase prior to emulsification. The commercially available OSA-starch was used to stabilise the external interface. Although not previously demonstrated for OSA-starches, or indeed interfacially adsorbed starches, starch digestion through salivary amylase has been shown to be relevant to the time scale of oral processing [23, 24]. Ferry and co-workers [23] explained sensory scores for thickness for starch thickened savoury liquids with the panellists' amylase activity linking higher enzyme activities to lower thickness scores.

In this research, wow emulsions formulated to orally destabilise by salivary amylase have been compared to orally inert stable emulsions formulated with protein. The

104 enzyme mediated destabilisation mechanism was evaluated for its ability to release
105 internalised sodium to enhance saltiness perception. This delivery rate of sodium was
106 assessed using *in vitro* methods and sensory evaluation was used to assess saltiness
107 perception.

Materials and methods

Materials

All materials used to prepare the emulsions were food grade and used without modification. Sunflower oil and table salt was obtained from a local supermarket, polyglycerol polyricinoleate (PGPR 90) to stabilise the internal water phase (w_1) was donated by Danisco (Beaminster, Dorset, UK) and the OSA-waxy maize starch, N-creamer 46 (NC46), used to stabilise the external phase of the wow emulsion was provided by Univar (Widnes, UK). Alternatively, orally inert pea protein isolate (PPI) obtained from Myprotein (Manchester, UK) was used. For sample analysis sodium chloride/ salt (NaCl) (99 %), porcine salivary α-amylase, hydrochloric acid (HCl), calcium chloride ($CaCl_2$), 4-Morpholinepropanesulfonic acid sodium salt (MOPS sodium salt), dimethyl sulfoxide (DMSO), ethanol and sodium azide were obtained from Sigma-Aldrich (Gillingham, UK). Sodium azide was used as an antimicrobial agent for samples that were not destined for sensory analysis. Sodium hydroxide (NaOH) was obtained from VWR International Ltd. (Lutterworth, UK). Glacial acetic acid was obtained from Fisher Scientific (Loughborough, UK). Thermostable α-amylase, amyloglucosidase, D-glucose and standardised regular maize starch were provided as part of the Megazyme total starch assay kit (Megazyme, Co., Wicklow, Ireland). Deionised water (15Mohm/cm) was used throughout.

Emulsion preparation and analysis

A stepwise approach was used to formulate wow emulsions. A water-in-oil emulsion (w_1/o) was initially formulated and it was then incorporated into the external water phase (w_2) to create a wow emulsion. A high shear overhead mixer (Silverson L5M

with an emulsor screen, (Chesham, UK) was used for all steps of emulsion processing. The internal water phase (w_1) consisted of 30 g aqueous NaCl solution (0 to 0.171 mol/ L NaCl) and the oil (o) phase (70 g) contained 2.8 % w/w PGPR 90 (premixed at 4000 rpm for 1 min). The aqueous phase was added to the oil phase and mixed for 2 min at 4000 rpm.

To produce the wow emulsion, w_1/o emulsions were mixed at a ratio of 1:1 with w_2. The external water phase contained 4 % w/w emulsifier (NC46 or PPI) with varying levels of NaCl (0 to 0.171 mol/ L NaCl) and mixed at 4000 rpm for 2 min.

The composition of the emulsions is shown in Table 1 prior to *in vitro* and *in vivo* testing, excluding those used for sensory analysis. The composition of emulsions for sensory analysis is included in Table 2.

Table 1 Composition of the wow emulsions submitted to *in vitro* and *in vivo* testing (excluding those used for sensory testing).

Sample code	External emulsifier	NaCl concentration (mol/ L)	
		w_1	w_2
A_1	NC46	0.171	0.171
A_2	NC46	0.171	0
A_3	NC46	0	0.171
B_1	PPI	0.171	0.171
B_2	PPI	0.171	0
B_3	PPI	0	0.171

Droplet size distributions of w_1 and w_1/o were acquired using image analysis captured 1 day after processing. For image acquisition, a digital inverted transmission light microscope (EVOS fl, Life Technologies Ltd., Paisley, UK) fitted with a 20x bright field, long working distance objective (AMEP4624, Life Technologies Ltd., Paisley, UK) was used. The images were processed with public domain image analysis software (ImageJ, NIH, Bethesda, USA). Six hundred droplets in three samples of each formulation were analysed and the Sauter mean diameter ($d_{3,2}$) was calculated using Microsoft Excel. Mean and standard deviation for each formulation were reported as an indication of emulsion droplet size.

In vitro analysis of sodium release $_2$

In vitro analysis of sodium release was measured from the formulated wow emulsions using a method adapted from literature [25]. 10 mL of emulsion was mixed on a magnetic stirrer at 37 °C with 5 mL of aqueous solution containing carbonate buffer at pH7. Porcine salivary α-amylase was added under continuous stirring. The final solution had an enzyme level of 50 units/ mL, human salivary α-amylase activity has been previously reported to range between 50 and 400 units/ mL [26, 27].

Immediately after enzyme addition a sodium ion specific electrode (Jenway, Stone, UK) was placed into the solution and conductivity recorded for 20 s to monitor the release of sodium from w_1 to w_2. After 20 s 1 mL of 2 M HCl was added to the sample to inactivate the enzyme and 0.02 % sodium azide mixed into the sample to prevent microbial spoilage. Total starch was then quantified as described below.

Oral breakdown of emulsions and saltiness perception

The "product" of oral processing of the wow emulsions was examined on the basis of 6 recruited volunteers from students and staff of the University of Nottingham (3 male and 3 female aged 19-30) and signed informed consent was obtained from participants. The oral processing protocol was as follows: the volunteers were provided with 10 mL of emulsion sample presented in a cup and asked to place all of the sample volume into their mouth, followed by pressing the tongue against the palate three times and at 20 s the sample was expectorated. Following expectoration 1 mL of 2 M HCl and 0.02% sodium azide was added and a total starch assay was conducted as in the case of the *in vitro* protocol.

Saltiness perception was evaluated using the method of paired comparison tests (2-Alternate Forced Choice tests, BS ISO 5495:2007). 120 assessors (78 women, 42 men, aged 19-57) were recruited from students and staff of the University of Nottingham and signed informed consent was obtained from each panellist before the study commenced. The description of the sample sets included in the paired comparison tests to determine overall perceived saltiness between two wow emulsions, varying in level of salt in one of the two aqueous phases or in the external emulsifier system (PPI or NC46), is included in Table 2. 10 mL of sample was presented to the panellists in randomised, balanced order across the panel in containers labelled with a random three-digit code. Sensory evaluation was conducted 1 day after sample preparation. Following the oral processing protocol used to collect the expectorated samples, assessors were instructed to taste the samples in the order presented and identify the sample they perceived to be saltier. Panellists were also instructed to cleanse their palate before and between samples with green apples (Granny Smith variety), unsalted crackers (99 % Fat Free, Rakusen's Leeds, UK) and

194 mineral water (Evian, Danone, France). The test was used in forced-choice mode, so
195 panellists were required to give an answer even if the perceived difference was
196 negligible and panellists were given the opportunity to comment on the samples.
197 Results were compared to tables A.2 and A.3 in BS EN ISO 5495:2007 to determine
198 difference and similarity respectively [28].
199

200 **Total starch assay**

201 Following the standard published protocol, total starch was analysed prior and after *in*
202 *vitro* and *in vivo* digestion (AOAC Method 996.11, Megazyme International Ireland
203 Ltd.).
204 Prior to conducting the analysis MOPS buffer and sodium acetate buffer was
205 prepared. MOPS buffer was prepared by dissolving 11.55 g of MOPS sodium salt in
206 900 mL of water and adjusted to pH 7.0 by the addition of 1 M HCl. Calcium chloride
207 (0.74 g) and 0.2 g of sodium azide was dissolved in the solution and adjusted to 1 L.
208 The sodium acetate buffer was prepared with 11.6 mL of glacial acetic acid to 900 mL
209 water adjusted to pH 4.5 by 1 M sodium hydroxide solution, 0.2 g sodium azide was
210 dissolved and the volume was adjusted to 1 L. Samples were washed in 5 mL of
211 aqueous ethanol (80 % v/v), and incubated at 80-85 °C for 5 min. An additional 5 mL
212 of 80 % v/v aqueous ethanol was added and the sample was then centrifuged for 10
213 min at 1,800 g and the supernatant was discarded. The pellet was re-suspended in 10
214 mL of 80 % v/v aqueous ethanol, stirred on a vortex mixer, centrifuged as previously
215 described. The supernatant was poured off and immediately 2 mL of DMSO was
216 added and stirred in vortex mixer. The content was placed in boiling water bath for 5
217 min. Thermostable α-amylase (3 mL) prepared as 1 part of α-amylase to 30 parts
218 sodium acetate buffer and 50 mM MOPS buffer was added and heated in boiling

water bath for 6 min. Sodium acetate buffer (4 mL and 0.1 mL amyloglucosidase (20 U) was added to the samples followed by mixing and incubation at 50 °C for 30 min. The entire content was transferred to a 100 mL volumetric flask and the container rinsed with distilled water. The volume was adjusted to 100 mL using distilled water. An aliquot of the solution was centrifuged at 1,800 g for 10 min. The concentration of glucose in the clear filtrate was then measured using a glucose analyser (Analox GM9 Analyser, London, UK).

Results and discussion

Emulsion microstructures

Distribution of the salt and choice of stabiliser had no impact on the Sauter diameter of the included w_1 phase droplets or the w_1/o droplets, as shown in Figure 2. The Sauter mean diameter ($d_{3,2}$) of the w_1/o droplets in all of the 6 wow emulsions ranged between 14.7 and 16.5 μm and there were no statistically significant differences (p>0.05). The Sauter mean diameter of the internalised water droplets was between 3.2 and 4.7 μm and again, across the sample set there was no statistically significant differences (p>0.05). Hence, it is valid to assume that droplet size does not represent a factor in these wow emulsions that would impact on sodium release and saltiness perception. Microscopic evidence is shown in Figure 3; droplet-in-droplet microstructure and dark appearance of the oil droplets typical observed for this microstructure are clearly recognisable[29-32].

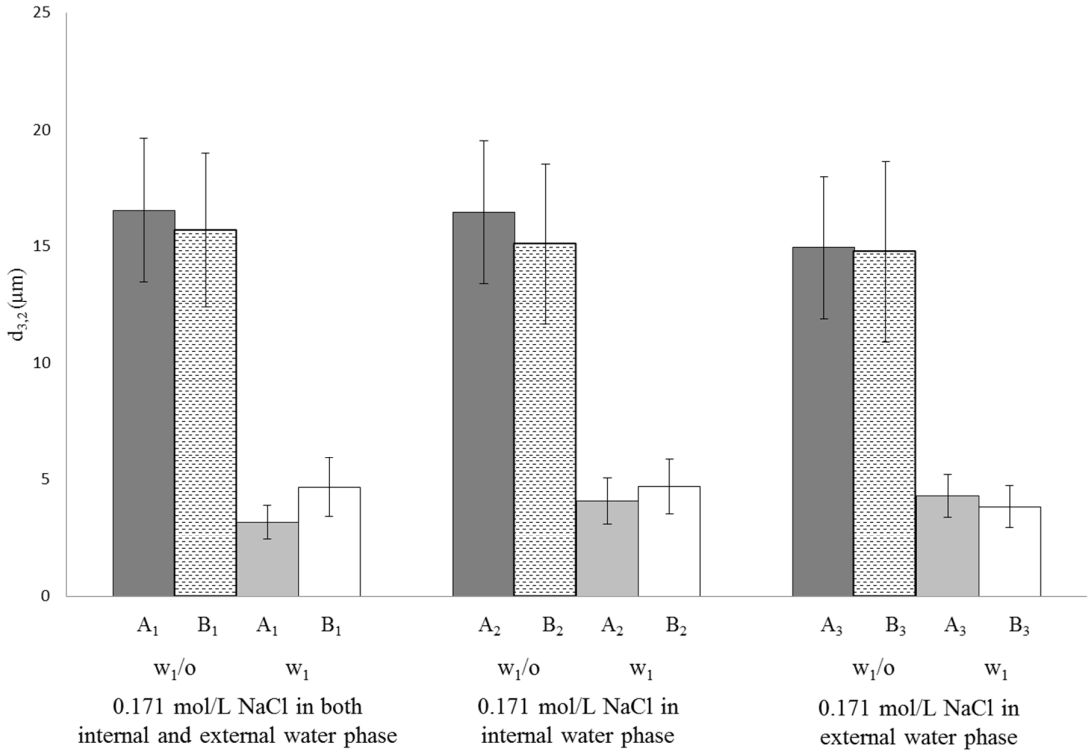

Figure 2 Sauter mean diameters ($d_{3,2}$) acquired by image analysis after 1 day of storage at 20°C. ■ w_1/o droplets stabilised with NC46; ▤ w_1/o droplets stabilised with PPI, ▢ w_1 droplets in NC46 stabilised emulsion, □ w_1 droplets in PPI stabilised emulsion.

Effect of *in vitro* and *in vivo* digestion on emulsion microstructure

Both PPI and NC46 stabilised wow emulsions were challenge tested for amylase mediated destabilisation using *in vitro* and *in vivo* digestion over 20 seconds. The changes in microstructure as a result of this challenge are shown in Figure 3.

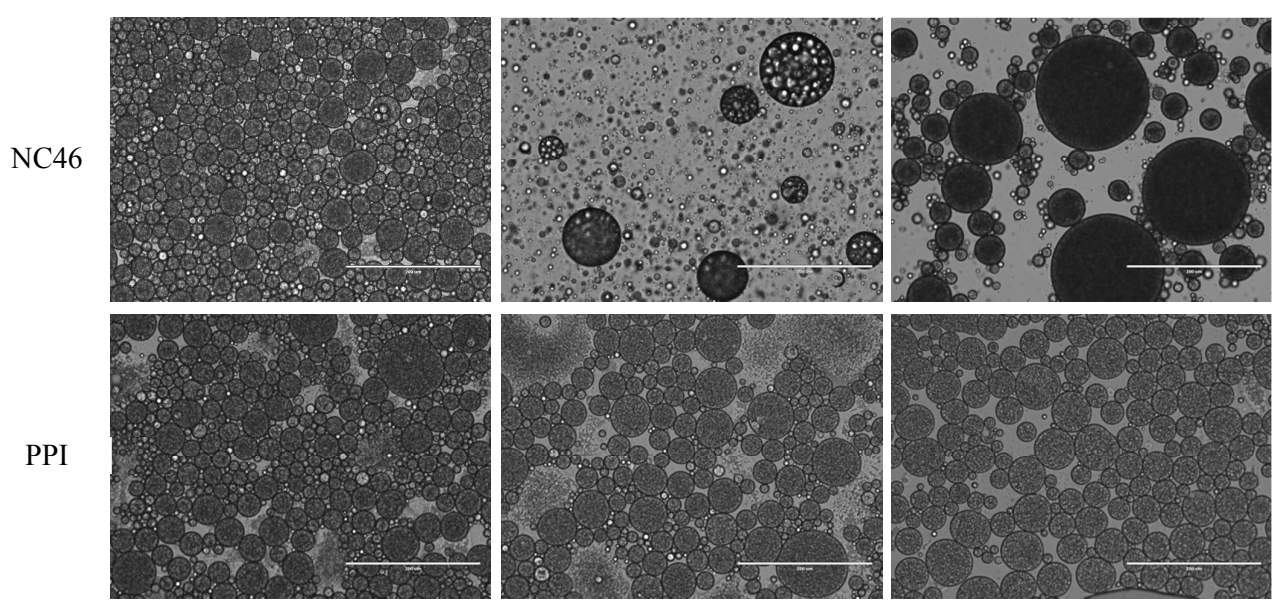

Figure 3 Micrographs before and after *in vitro* and *in vivo* digestion of wow emulsions stabilised with 2 % NC46 and PPI. The internal and external aqueous phase of both types of emulsion contains salt at 0.171 mol/L. The scale bar in each image corresponds to 200 μm.

For the NC46 stabilised emulsion there are substantial microstructure changes after *in vitro* and *in vivo* digestion whereas changes in the PPI stabilised emulsion are much more subtle. In the case of the NC46 stabilised emulsion, digestion has led to destabilisation of the oil droplet interface causing the oil droplets to coalesce as much larger droplets are found in the digested samples compared to before digestion. The larger internalised droplets recognisable in the *in vitro* digested sample suggest partial coalescence of the w_1 droplets whereas there is no such evidence for the sample imaged after *in vivo* digestion. The coalescence processes have led to the release of the internalised aqueous phase as indicated by the presence of void oil droplets seen in the digested samples. This implies that oral shear combined with salivary digestive enzymes is effective at imparting partial release of the internal water phase of starch stabilised complex emulsions. In contrast, the PPI stabilised emulsion showed no clear evidence of this type of instability process occurring during *in vitro* and *in vivo* digestion; the original emulsion microstructure is largely retained.

Starch degradation through the action of the porcine amylase or oral amylase was analysed using a total starch assay. *In vivo* digestion resulted in significant (p<0.05) reduction of total starch (2.14 g total starch/ 100 g was reduced to 1.69 g total starch/ 100 g) whereas a smaller but still significant (p<0.05) reduction was found after *in vitro* digestion (to 1.9 g total starch/ 100 g). It should be noted that the reduction was lower during *in vitro* digestion indicating that enzymes present orally may be more effective at digesting the OSA-starch [33], the more intense mechanical action during oral processing compared to the *in vitro* protocol may have contributed to the enhanced degradation of total starch or that the subject's enzyme activity may be higher than that presented in the *in vitro* assay.

The OSA treatment involves esterification of OSA at select free hydroxyl groups at the surface of the starch granules. The esterification process has been previously shown to be spatially heterogeneous on the surface of the granule as well as across the granule population implying that within a 3 % OSA-starch, there will be granules with greater than 3 % OSA and others with less or no modification [34-36]. OSA-starch treatment is limited to 3 % OSA modification of starch for food use and OSA loading has been shown to be proportional to resistance to digestion in a suspended (non-emulsified) state [37, 38]. The presented results confirm the digestibility of interfacially adsorbed commercially relevant OSA-starch, NC46, on a timescale appropriate to the consumption of emulsion based foods.

Sodium release

The rate of sodium release from the complex emulsions *in vitro* is shown in Figure 4 for emulsions originally prepared with w_2 not containing any sodium. The detection

of sodium indicates that during emulsion preparation some of the internal sodium containing water phase was released into the external water phase.

Sodium was rapidly released from the NC46 stabilised emulsion when in the presence of amylase, the NC46 emulsion was stable without the enzyme and the PPI stabilised emulsion was stable both with and without the enzyme. This supports the data presented previously that partial release of sodium can be achieved through enzymatic digestion. It is expected that the *in vivo* release would be greater although this cannot be verified within the current experimental design. The release of encapsulated sodium causes a difference in sodium concentration in the continuous phase overtime.

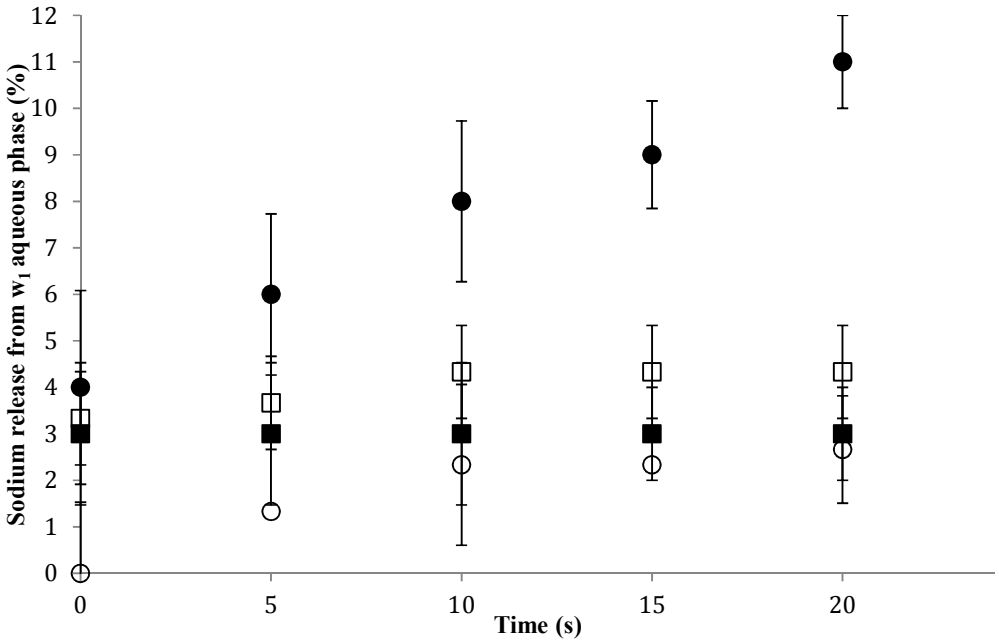

Figure 4 Sodium release from w_1 phase, initially containing 0.171 mol/L salt and w_2 not containing any salt, following the addition of α-amylase to the emulsion stabilised with NC46 or PPI and holding for 20 s at 37 °C. NC46 stabilised emulsion with (●) and without (○) α-amylase enzyme, PPI stabilised emulsion with (■) and without (□) α-amylase enzyme.

Saltiness perception

To validate the proposed oral destabilisation concept for enhancing saltiness perception, paired comparison tests were conducted. The results are presented in

Table 2. Complete removal of the internal sodium within the stable PPI emulsions had no impact on saltiness perception as revealed by Test 1. NC46 stabilised emulsions were perceived as saltier when compared directly to PPI stabilised emulsions containing equivalent external and internal salt concentrations as illustrated by the results of Test 2. This supports the previous result showing a loss of emulsion integrity during oral processing of the NC46 stabilised emulsion (Figure 3).

The higher perceived saltiness of the NC46 stabilised emulsion in Test 2 demonstrates potential to reduce the sodium concentration in the emulsion to achieve similar saltiness to the PPI stabilised emulsion. This is confirmed by the results of Test 3 where the NC46 stabilised emulsion of the pair contained 18.2 % less salt in w_2 compared to the PPI stabilised emulsion. Overall, this equates to a salt reduction of 23.7 % without comprising saltiness perception. Not unexpectedly, if both of these emulsions were formulated with zero salt in the included water phase, the PPI emulsion was perceived as saltier than the NC46 stabilised emulsion because of the higher salt content in the former as shown in Test 4. It should be noted that the concentrations of salt in both aqueous phases of the NC46 stabilised emulsion included in Tests 3 and 4 appear random. However, they are based on various combinations tested in preliminary research on starch stabilised wow emulsion strategy for salt reduction.

330 Table 2 Saltiness perception using paired comparison tests: Emulsion composition,
331 pairs and saltiness scores.

Test	Emulsifier	NaCl in w_1 (mol/ L)	NaCl in w_2 (mol/ L)	Total NaCl (g/ 100g emulsion)	No. of panellists selecting sample to be saltier	Result
1	PPI	0.171	0.171	0.650	62	similar[##]
	PPI	0	0.171	0.500	58	
2	PPI	0.171	0.171	0.650	41	
	NC46	0.171	0.171	0.650	79	saltier[#]
3	PPI	0.171	0.171	0.650	59	similar[##]
	NC46	0.100	0.140	0.496	61	
4	PPI	0	0.171	0.500	108	saltier[#]
	NC46	0	0.140	0.409	12	

332 [#] Samples perceived to be significantly saltier (p<0.05).
333 [##]Similarity concluded between the 2 samples (95 % confidence interval, p_d 30 %).

Conclusions

Utilising a combined approach of *in vitro*, *in vivo* and sensory analysis has revealed that it is possible to enhance saltiness perception from emulsions comprising an encapsulated aqueous salt phase provided it is released during oral processing. These emulsions programmed for oral breakdown were of the wow emulsion type where the oil/water interface was stabilised through a commercial emulsifying OSA-starch. The oil phase with the included droplets of aqueous salt solution was stabilised with PGPR. Comparing salt release and saltiness perception to wow emulsions formulated with a protein instead of starch, as well as quantifying the breakdown of starch, clearly validated the hypothesis that a stabilising system susceptible to degradation in contact with salivary enzymes releases encapsulated tastant. The time scale of release was found to be in the order of a typical oral residence time of liquid and semi-liquid food during eating. While saltiness perception was enhanced, *in vitro* data suggest that only a limited amount of tastant was released which may be due to the type of observed microstructure breakdown, as partial coalescence rather than complete breakdown of the wow emulsion microstructure was observed. Nevertheless, based on a commercial OSA-starch it was possible to decrease the total salt content of the emulsion from 0.65 to 0.496 g/100g emulsion, equating to 23.7 % salt reduction, without compromising saltiness perception.

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