Formulation engineering of water-in-oil-in-water emulsions for salt reduction with sucrose oleate as a PGPR-alternative lipophilic emulsifier

Zhang, Liling; Gould, Joanne; Wolf, Bettina

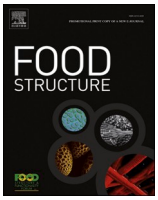
DOI:
10.1016/j.foostr.2023.100309

Food Structure 35 (2023) 100309

Contents lists available at ScienceDirect

Food Structure

journal homepage: www.elsevier.com/locate/foostr



Formulation engineering of water-in-oil-in-water emulsions for salt reduction with sucrose oleate as a PGPR-alternative lipophilic emulsifier

Liling Zhang [a,b], Joanne Gould [a,b], Bettina Wolf [b,*]

[a] Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK
[b] School of Chemical Engineering, University of Birmingham, Birmingham B15 2TT, UK

ARTICLE INFO

Keywords:
Salt release
Salt encapsulation
W/o emulsion
W/o/w emulsion
Starch emulsifier
Lipophilic emulsifier
in vitro salt release

ABSTRACT

Sucrose oleate was assessed as an alternative lipophilic emulsifier to polyglycerol polyricinoleate (PGPR) for the stabilisation of the internal aqueous phase of a water-in-oil-in-water emulsion formulation designed for salt release from the internal aqueous phase during oral processing. A water-in-oil emulsion (30 g water/100 g oil), containing an internalised salt solution (1.5 g salt/100 g), was successfully incorporated as droplets into a salt containing external aqueous phase (0.5 g salt/100 g) with *in-situ* gelatinised waxy rice starch (WRS) stabilising the oil droplet interface. The droplets of the sucrose ester stabilised water-in-oil emulsion were aggregated, and this microstructure carried over into the water-in-oil-in-water emulsion. The PGPR stabilised water-in-oil emulsion showed no evidence of aggregation, and the primary droplet size was smaller. Mean oil droplet size was comparable, slightly increasing for the sucrose ester containing formulation over a 3-months observation period. Nevertheless, salt encapsulation efficiency, reducing by around 10% over 3-months, as well as *in vitro* salt release, reducing by 20%, were comparable. This study demonstrated that sucrose ester SE O-170 is a viable alternative for PGPR in w/o/w emulsions designed for salt release during oral processing.

1. Introduction

It has been well-documented that lowering dietary salt intake would result in a major improvement in population health due to reducing the incidence of hypertension and cardiovascular disease caused by salt overconsumption (Engstrom et al., 1997; He & MacGregor, 2010; Wenstedt et al., 2020). However, the reported habitual daily salt intake in most countries around the world continues to significantly exceed the WHO recommendation of 5 g salt per day (Härtl, 2013). WHO Member States have agreed on a voluntary global health strategy for a 30% reduction by 2025 in mean population salt intake (Santos et al., 2021). In most developed countries, the challenge to lower the dietary salt intake lies in reducing salt content of processed food, shown to be the largest source of salt in the daily diet (up to 75–80%) (Dötsch et al., 2009; Kloss et al., 2015) although many food and beverage companies have pledged to reduce the level of salt by reformulating existing products (Dötsch et al., 2009; Jo et al., 2020; Kloss et al., 2015; Yach et al., 2010). Salt reduction strategies include stepwise reduction to adjust consumer's expectation of the saltiness of a product, substitution of salt with non-sodium salts or taste enhancers, maximising saltiness perception by increasing salt delivery efficiency from the food surface to the tongue (Kloss et al., 2015), or by delivering a temporal change in salt concentration during oral processing (Kloss et al., 2015). The latter involves compartmentalisation of salt within the food designed to release the salt in a temporal fashion. Thereby saltiness perception is enhanced compared to delivering the same (reduced) amount of salt all at once upon ingestion without compromising consumer acceptability (Kloss et al., 2015).

Compartmentalisation of salt in liquid foods is challenging due to the fact that salt is water soluble. However, one of us has previously demonstrated that this challenge can be overcome by encapsulating salt in the internal aqueous phase of a water-in-oil-in-water ($w_1/o/w_2$) emulsion (Kasprzak et al., 2019). The internal aqueous phase (w_1), comprising a higher level of salt than the external aqueous phase (w_2), was emulsified into sunflower oil containing the lipophilic emulsifier polyglycerol polyricinoleate (PGPR). The resulting w_1/o was submitted to a second emulsification step to create a $w_1/o/w_2$ emulsion stabilised by non-chemically modified waxy rice starch. The non-chemical modification of the waxy rice starch involved the gelatinisation of the starch ingredient *via* the frictional heat input during the secondary emulsification step using a high shear overhead mixer. This procedure was previously found to provide long-term stability to o/w emulsions

* Corresponding author.
E-mail address: b.wolf@bham.ac.uk (B. Wolf).

https://doi.org/10.1016/j.foostr.2023.100309
Received 10 October 2022; Received in revised form 4 January 2023; Accepted 5 January 2023
Available online 7 January 2023

(Kasprzak et al., 2019).

The presence of salt in w_2 delivers saltiness upon first ingestion of the emulsion-based food, whereas the salt in w_1 is released due to the action of salivary amylase-mediated emulsion destabilisation during the oral processing stages between ingestion and swallow.

One of the hurdles to apply this salt reduction strategy in commercial formulations is the reliance on PGPR as the lipophilic emulsifier. It is widely used in research for the stabilisation of the internal interface of w/o/w emulsions, or w/o emulsions as it generally creates a fine internal droplet size spectrum, that is stable over time (Choi et al., 2017; Cofrades et al., 2014; Matos et al., 2014; Muschiolik & Dickinson, 2017). Without a stable internal w_1/o emulsion, the targeted salt reduction strategy will not work. However, there are drawbacks to the use of PGPR such as off-taste, legal restrictions of PGPR (ADI of 25 mg/kg body weight per day) (Akhtar & Dickinson, 2001; Balcaen et al., 2017; Mortensen et al., 2017) and the synthetic connotation limits consumer acceptability. PGPR has the E-number E476.

In this study, sucrose oleate SE O-170 was evaluated as an alternative lipophilic emulsifier to PGPR to stabilise the internal w_1/o emulsion in the aforementioned $w_1/o/w_2$ emulsion system introduced by Kasprzak et al. (2019). The chemical structures of both emulsifiers are provided in Fig. 1. Sucrose esters comprise a sucrose molecule esterified with fatty acids and in the case of sucrose oleate, the main acid is oleic acid (Nelen et al., 2015). Oleic acid is widely found in natural cruciferous oils such as rapeseed and mustard seeds (Glatter et al., 2001; Gupta et al., 1983; MCF, 2020). Sucrose esters are widely used in foods (E-number E473), cosmetics, and pharmaceuticals (Baker et al., 2000). Legal restrictions for use in foods also apply to sucrose ester (ADI of 40 mg/kg body weight per day) (Younes et al., 2018), indifferent to type of sucrose ester (varying fatty acid chain length and, thus, HLB value).

PGPR was not simply replaced by SE O-170 reproducing all other steps of emulsion preparation reported by Kasprzak et al. (2019). Specifically, the gelatinisation step of the waxy rice starch was decoupled from the second emulsification step to research a process that could in future be applied to formulations with temperature sensitive ingredients

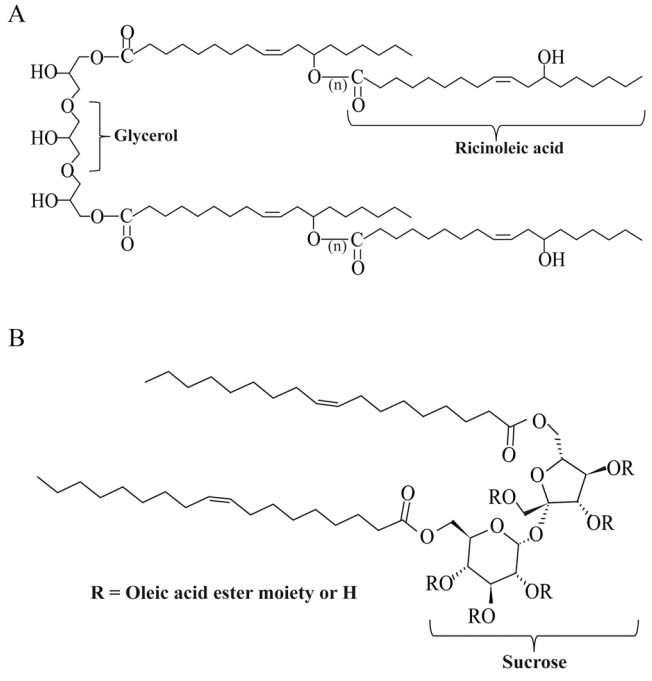


Fig. 1. Molecular structure of polyglycerol polyricinoleate (PGPR) (A). The polyglycerol chain can have more or fewer than three glycerol units. "n" in the polyricinoleate chain indicates that several ricinoleic acids can be included, the terminal fatty acid can be a different one, *e.g.*, stearic acid (Price et al., 2022); and sucrose oleate (B).

or additives in any of the three emulsion phases. Further, the high shear processing protocol of the primary w_1/o emulsion was optimised for droplet size and w_1/o emulsion stability. Smaller w_1 droplets are less likely to be "lost" into the external aqueous phase during the second emulsification step (Garti & Aserin, 1996) and primary w_1/o emulsion stability was reported to be closely linked to the stability of the final $w_1/o/w_2$ emulsion (Leister & Karbstein, 2020). Finally, salt encapsulation efficiency and *in vitro* salt release of optimised emulsion formulations stabilised with SE O-170 were compared to PGPR stabilised emulsions to evaluate the true potential of SE O-170 as an alternative to PGPR for salt reduction of liquid foods *via* a complex emulsion approach.

2. Materials and methods

2.1. Materials

Sunflower oil purchased from a local supermarket was used as the continuous phase of the w_1/o emulsions. Both aqueous phases in the $w_1/o/w_2$ emulsions were Milli-Q water (conductivity $\leq$ 0.5 μs/cm) containing 0.02 g sodium azide/100 g (Sigma-Aldrich, Gillingham, UK) to prevent microbial spoilage during sample storage and sodium chloride (Fisher Scientific, Loughborough, UK). Polyglycerol polyricinoleate (GRINDSTED PGPR90, HLB 1–2) and sucrose ester O-170 (sucrose oleate, HLB 1, 70% oleic acid and 100% di-, tri- and poly ester) were donated by DuPont Danisco (Kettering, UK) and Mitsubishi-Kagaku Food Corp (Tokyo, Japan), respectively. Waxy rice starch (WRS, amylose 6.1 (± 0.1) g/100 g, moisture 13.0 (± 0.4) g/100 g and protein 0.66 (± 0.03) g/100 g) was provided by Synergie Nutrylon, Urlick and Short Ltd (Pontefract, UK). For the *in vitro* digestion assay, porcine pancreas amylase (Type VI-B, activity $\geq$ 10 units/mg solid), sodium phosphate dibasic anhydrous (NaH_2PO_4), sodium phosphate monobasic (Na_2HPO_4), hydrochloride acid solution (HCl) and sodium hydroxide solution (NaOH) were acquired from Sigma-Aldrich (Gillingham, UK).

2.2. Emulsion preparation

2.2.1. Primary emulsions

The primary w_1/o emulsion was prepared by initially mixing the oil phase (o) containing 2.86 g PGPR or SE O-170 per 100 g sunflower oil at 500 rpm for 2 h. The choice of lipophilic emulsifier concentration is based on our earlier work where we reported that at lower concentrations of PGPR external aqueous phase solution w_2 was partially incorporated into the oil phase during the second emulsification step (Kasprzak et al., 2019). The inner water phase (w_1) comprised a solution of 1.5 g salt/100 g water. The w_1/o emulsions (30 g w_1/100 g emulsion) were prepared using one of two high shear overhead mixers, a Silverson LR4 (Chesham, UK) fitted with an emulsor screen or an Ultra-Turrax T25 (IKA-Werke GmbH & Co, Staufen, Germany) fitted with an S25N-10 G dispersing tool, following protocols from literature with slight modifications (Chiu et al., 2017). The dimensions of the two devices and the operating conditions as well as the emulsion temperatures at the end of the processing are provided in Table 1. Using the Silverson, the aqueous phase was added to the oil phase prior to processing. Using the Ultra-Turrax, the aqueous phase was added dropwise to the oil phase during 2 min while mixing at 5000 rpm followed by the processing condition stated in Table 1. The prepared w_1/o emulsions were stored at ambient temperature (~ 20 °C) in 100 ml glass vials until used for analysis or preparation of $w_1/o/w_2$ emulsions.

2.2.2. Final emulsions

The first step of the creating the $w_1/o/w_2$ emulsions comprised the preparation of the aqueous starch dispersion used as the external water phase w_2. 4 g of waxy rice starch (WRS) and 66 g of 0.5 g salt/100 g water dispersion was processed in a beaker of 250 ml using the Silverson LR4 at 8000 rpm for 11 ± 2 min, with the temperature reaching 88 ± 3 °C. Afterwards, the starch dispersion was covered and kept in a

Table 1
Characteristics of the mixing devices and processing conditions to prepare the w_1/o emulsions and final emulsion temperature.

Device	Mixed head diameter (m)	Speed (rpm)	Time (min)	Angular velocity (rad/s)	Tangential velocity (m/s)	Emulsion temperature (°C)
Silverson LR4	0.04	8000	2	837.8	16.8	65 ± 3
Ultra-Turrax T25	0.01	20,000	10	2094.4	10.5	45 ± 3

water bath at 90 °C for 20 min with constant stirring at 300 rpm on a magnetic stirrer to complete the gelatinisation of the WRS. Finally, the starch dispersion/w_2 phase was allowed to naturally cool to ambient temperature (~ 20 °C). To prepare the $w_1/o/w_2$ emulsion, the beaker with the w_2 phase was placed into an ice bath and 30 g of w_1/o emulsion was incorporated by mixing with the Silverson at 8000 rpm for 3 min. The temperature of the final emulsions at the end of processing was 24 ± 3 °C. The ratio of the different phases in the $w_1/o/w_2$ emulsions was w_1:o:w_2 = 9:21:70 and contained 4 g of WRS per 100 g emulsion, varying levels of PGPR or SE O-170 (0.15, 0.3, 0.6 g per 100 g emulsion) and a total of 0.465 g salt (0.135 g from w_1 and 0.33 g from w_2) per 100 g of emulsion. The prepared $w_1/o/w_2$ emulsions were stored at room temperature (~ 20 °C) until further use.

2.3. Characterisation of emulsion microstructure

The microstructure of both types of emulsions was visualised immediately after preparation using an optical microscope (EVOS FL, Cell Imaging System, ThermoFisher, UK), after dilution with salt solution (0.5 g salt/100 g water) in case of $w_1/o/w_2$ emulsions and oil containing either SE O-170 or PGPR (2.86 g/100 g) in case of the w_1/o emulsions. Also imaged were the $w_1/o/w_2$ emulsions following *in vitro* digestion, which were already diluted during the digestion assay. The slides for w_1/o emulsion were prepared by placing a small drop of diluted emulsion and carefully sliding a cover slip over the sample. Cover slips were not used for $w_1/o/w_2$ emulsions as the large size of some of the oil droplets meant that they deformed upon applying a cover slip. All images were taken at ~ 20 °C.

The internal microstructure of the oil droplets was assessed by employing a cryo-focused ion beam scanning electron microscope (FEI Quanta 200 3D, FEI, Portland, OR) equipped with a Quorum PPT 2000 cryo-stage (Quorum Technologies Ltd, Loughton, UK) and an INCA Energy 250 microanalysis system (Oxford Instruments, Oxford, UK). The ion milling was done at current between 1 nA and 50 pA. The frozen and milled surfaces were further sectioned by a nanomanipulator to reveal the sectional structure of the double emulsion. Images were acquired 2 days after emulsion preparation at an accelerating voltage of 10 kV.

2.4. Determination w1/o emulsion stability

The stability of w_1/o emulsions stabilised either with PGPR or SE O-170 (2.86 g/100 g oil) was assessed using a Turbiscan^{Lab} Expert Stability Analyser (Formulation, Toulouse, France). 20 ml of w_1/o emulsion was poured into a measuring tube immediately after preparation, up to the recommended sample height of 42 mm. The emulsions were scanned immediately and then at regular intervals for a period of 7 days, taken from storage at ~ 20 °C. The transmitted light (T) was negligible in most parts of all samples and is therefore not reported. The raw data of the backscattered light (BS) was analysed in the Delta mode (ΔBS) to improve the signal to noise ratio. The first BS scan for each w_1/o emulsion sample was taken as the reference data to build the ΔBS graph.

2.5. Determination of w1/o/w2-emulsion droplet size distributions

Droplet size distributions of $w_1/o/w_2$ emulsions were acquiring using a laser diffraction particle size analyser (LS 13 320, Beckman Coulter, High Wycombe, UK) fitted with a micro volume liquid module cell containing reverse osmosis water as the dispersing medium. Data was analysed using the Mie theory, where 1.54 and 1.33 were pre-set as the refractive indices for the dispersed oil phase and the continuous aqueous phase, respectively. Three independent measurements were taken on each sample and results are reported as averaged volume-based mean droplet size $d_{[4,3]}$ with standard deviation.

2.6. Measurement of salt encapsulation efficiency

Salt encapsulation efficiency was defined, see Eq. (1), and measured following the same principle as in previously referenced work (Kasprzak et al., 2019). Using a conductivity meter (Mettler Toledo Ltd, Leicester, UK) fitted with a 4-pole platinum conductivity probe with a chemical resistant glass body (inLab 710, 0.01–500 ms/cm, Mettler Toledo Ltd, Leicester, UK), the conductivity of the $w_1/o/w_2$ emulsion was measured at 20 °C while stirring on a magnetic stirrer at 500 rpm, taking readings every second for 1 min. Once the conductivity reading varied by less than 5% within 5 s, that data point was chosen as the conductivity value of emulsion. To convert conductivity into quantity of salt in w_2, calibration curves were constructed by adding varying salt quantities (0 – 0.5 g) to 100 g double emulsion samples prepared in the absence of salt in either aqueous phase with w_1 stabilised by SE O-170 or PGPR at any of the three levels applied. Conductivity measurements were conducted in triplicate directly after emulsion preparation and after a 3-months storage, and EE calculated using Eq. (1). It is worth noting that based on emulsion composition and assuming no transfer of salt from w_1 to w_2 or *vice versa*, the value for EE would be 29%.

$$EE(\%) = \frac{Saltg/100gemulsion - Saltinw_2g/100gemulsion}{Saltg/100gemulsion} \times 100\% \quad (1)$$

2.7. In vitro salt release assessment

Amylase-mediated release of salt from w_1 into w_2 was measured using the same *in vitro* digestion model set-up as in our earlier work (Kasprzak et al., 2019). 10 g of emulsion was gently stirred on a magnetic stirrer at 300 rpm and 37 °C, 10 ml of α-amylase in phosphate buffer solution (100 U/1 ml enzyme level, pH 7, 0.01 M) added and conductivity recording started for 30 s. The sodium phosphate buffer was prepared by placing 4.68 g of sodium phosphate monobasic and 8.662 g of sodium phosphate dibasic in a 1000 ml volumetric flask. Then, the volumetric flask was filled with deionised water up to 1 litre, followed by stirring at 500 rpm for 30 min until fully dissolved, corresponding to a 0.1 M sodium phosphate buffer. The pH value of the sodium phosphate buffer solution was adjusted to 7.0 with hydrochloride acid solution (0.02 M) and sodium hydroxide solution (0.02 M). Then, the 0.1 M sodium phosphate buffer was diluted 10 times to obtain a 0.01 M sodium phosphate buffer (pH 7.0). Afterwards, 1 g of porcine amylase was dispersed in 100 ml of 0.01 M sodium phosphate buffer. Consequently, the enzyme activity of the final amylase mediated *in-vitro* oral digestion buffer was approximately 100 U/1 ml which is considered to be close to the average level of human salivary amylase activity at 92.5 U/1 ml (Mandel et al., 2010).

To convert conductivity reading into quantity of salt in w_2, calibration curves were acquired as above but with digestion buffer added. Results are presented as the quantity of salt in w_2 relative to the quantity of salt in the total emulsion system (0.465 g salt/100 g emulsion), and the symbol SC_{w2} (%) was used. If the $w_1/o/w_2$ emulsion collapsed completely during *in vitro* oral, releasing all of w_1 into w_2, SC_{w2} would equal 100%. Finally, salt release (%) was calculated as the difference between the quantity of salt in w_2 before and after *in vitro* digestion.

3. Results and discussions

3.1. Primary w_1/o emulsion design

The efficacy of SE O-170 compared to PGPR to stabilise water-in-oil emulsions containing salt (2.86 g SE O-170 or PGPR/100 g oil, 1.5 g salt/100 g water (w_1), 30 g w_1/ 100 g emulsion) was evaluated based on micrographs and Turbiscan data. Emulsions were processed using one of the two protocols summarised in Table 1, from here on referred to as processed with the Silverson or processed with the Ultra-Turrax. Fig. 2 shows micrographs acquired on SE O-170 stabilised emulsions, and Fig. 3 concerns PGPR stabilised emulsions.

The w/o emulsions stabilised with SE O-170 were characterised by the presence of large droplet aggregates (Fig. 2A, C), and a primary emulsion droplet size cannot be sensibly estimated from these images. Similar microstructures have been reported in literature for w/o emulsions stabilised with lecithin, sorbitan esters or sucrose esters dissolved in sunflower oil or olive oil as the continuous emulsion phase (Balcaen et al., 2017; Mazo Rivas et al., 2016; Ushikubo & Cunha, 2014). According to Ushikubo and Cunha (2014) and Mazo Rivas et al. (2016), the formation of droplet aggregates in sucrose ester stabilised w/o emulsions can be attributed to the self-assembly of the sucrose ester molecules dissolved in oil forming an entangled dynamic network and thus promoting aggregation in oil-based emulsions. Nevertheless, the aggregates could be somewhat disintegrated by shearing on a magnetic stirrer as evidenced in Fig. 2B and C for the Silverson and Ultra-Turrax processed SE O-170 stabilised emulsion, respectively. A similar observation was previously made by Knoth et al. (2005).

The micrographs acquired on the emulsions stabilised with PGPR (Fig. 3) show no evidence of droplet aggregation and a primary droplet size of less a few μm can be estimated from the micrographs. This difference in behaviour between sucrose ester and PGPR has previously been reported by Balcaen et al. (2017) suggesting that the polymeric side chain of a PGPR molecule compared to the monomeric side chain of a sucrose ester might be responsible. In any case, PGPR is a well-known emulsifier for w/o emulsions, imparting long-range steric forces and repulsive barriers between adjacent droplets overcoming classical emulsion droplet instability phenomena such as droplet coalescence and flocculation/aggregation (Mazo Rivas et al., 2016; Middendorf et al., 2015). Another observation was that the PGPR stabilised droplets were much smaller than the SE O-170 stabilised droplets, so small indeed that it is not possible to ascertain whether the emulsion processed with the Silverson (Fig. 3A) or the Ultra Turrax (Fig. 3B) had the finer droplet size spectrum. As for the SE O-170 stabilised emulsions, based on the micrographs (Fig. 2) alone it is also not possible to determine which of the two overhead mixers delivers a more stable emulsion. Hence, the stability of the w/o emulsions was assessed using a Turbiscan and the results are reported in Fig. 4 as delta backscattered (ΔBS) light intensity.

A small negative ΔBS peak at the bottom of the sample tube (<1 mm tube length) observed for all four emulsions is caused by a volume of free water, indicating that a small degree of droplet coalescence occurred in all w/o emulsions. The ΔBS < 1 mm data was unchanged over the 7 days observation period suggesting that no further bulk phase separation took place.

Emulsions stabilised with SE O-170 showed a negative ΔBS between 3 and 35 mm (Fig. 4A, B) which would be caused by an increase in droplet size due to coalescence and/or flocculation (Márquez et al., 2007; Pan et al., 2002). The data suggest that the SE O-170 emulsion processed with the Ultra-Turrax (Fig. 4B) showed a higher stability against coalescence and/or flocculation (Fig. 4A) as the ΔBS was slightly less negative over the 7-day observation period. A reduction in ΔBS at the top of the sample tube (35 – 40 mm) indicates the sedimentation of water droplets and is typically correlated with an increase in ΔBS at 1 – 3 mm. The reduction in ΔBS at the top is evident for all four emulsions although in the case of the SE O-170 stabilised emulsions droplet sedimentation is either slowed or fully suppressed compared to the PGPR stabilised emulsions. This statement is supported by the observation of a positive ΔBS peak between 1 and 3 mm in case of PGPR but not SE O-170. The cause for this difference in behaviour would be sample microstructure (Figs. 2, 3) with the aggregated droplet structure in the SE O-170 locking the droplets in place.

Both PGPR stabilised emulsions showed gravitational separation as indicated by the increase in ΔBS at 1 – 3 mm over the 7-day observation period (Fig. 4C, D). The increase, and the accompanying decrease in ΔBS

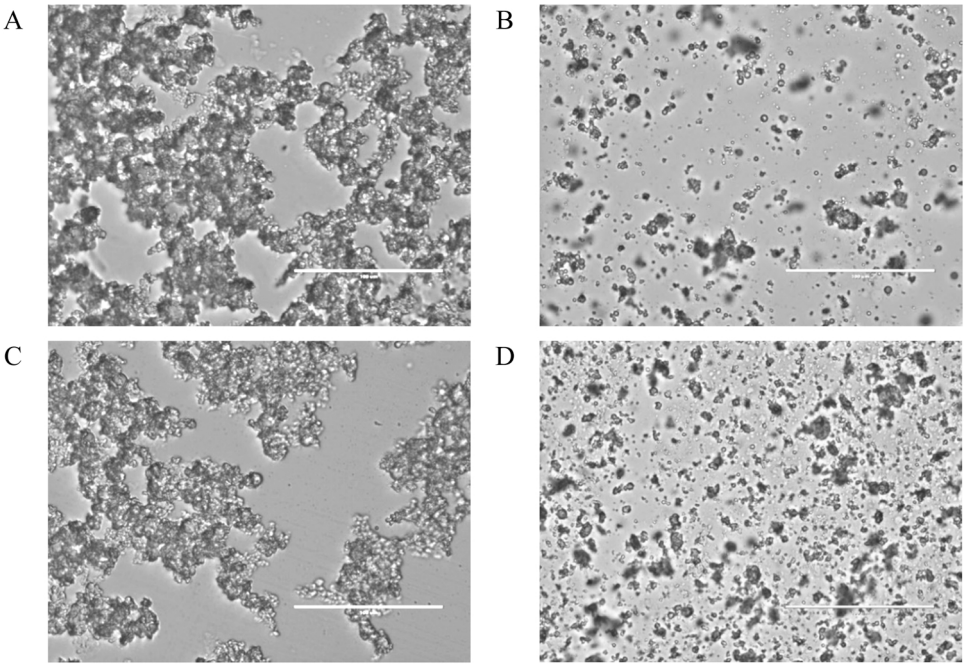


Fig. 2. Microstructure of w/o emulsions containing 30 g aqueous phase/100 g emulsion stabilised with 2.86 g SE O-170/100 g oil and containing 1.5 g salt/100 g water. Emulsions were imaged immediately after processing with the Silverson (A) or the Ultra-Turrax (C). Emulsions were also imaged immediately after breaking up the clusters on a magnetic stirrer operating at 500 rpm (B, D). Scale bar = 100 μm.

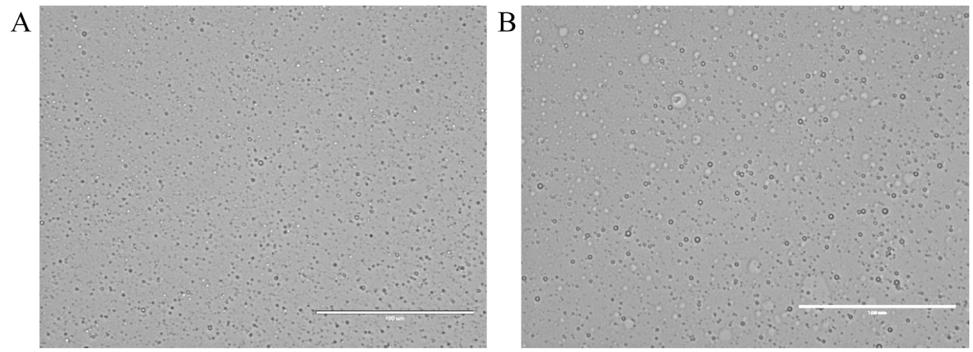


Fig. 3. Microstructure of w/o emulsions containing 30 g aqueous phase/100 g emulsion stabilised with 2.86 g PGPR/100 g oil and containing 1.5 g salt/100 g water. Emulsions were imaged immediately after processing with the Silverson (A) or the Ultra-Turrax (B). Scale bar = 100 μm.

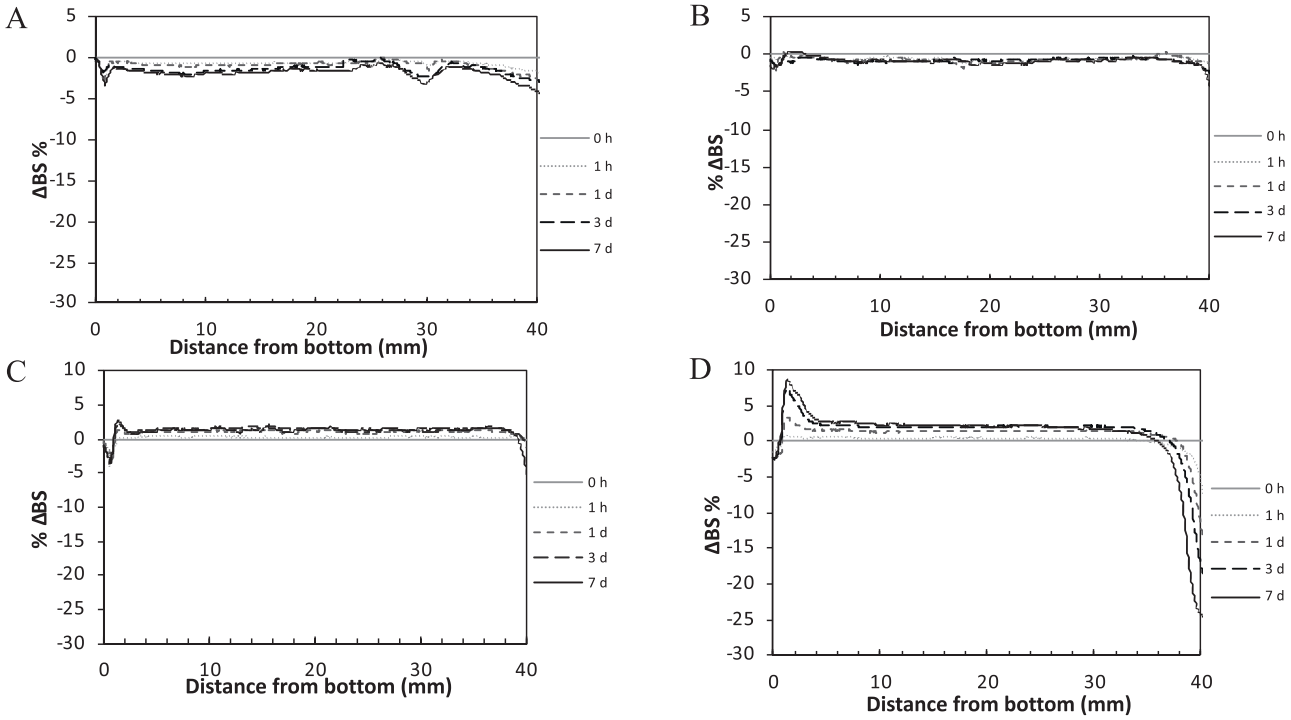


Fig. 4. Delta Backscattering (% ΔBS) profiles of w_1/o emulsions containing 30 g aqueous phase/100 g emulsion stabilised with 2.86 g SE O-170 (A, B) or PGPR (C, D)/100 g oil and containing 1.5 g salt/100 g water. Emulsions were imaged over a period of 7 days (immediately – 0 h, 1 h, 1 day, 3 days, 7 days) after processing with the Silverson (A, C)) or the Ultra-Turrax (B, D). Scale bar = 100 μm.

at the top, is much more pronounced for processing with the Ultra-Turrax (Fig. 4D) than the Silverson (Fig. 4C) suggesting a finer droplet size spectrum and thus enhanced stability for the Silverson processed emulsion. At this point it is worth restating that the stability of the primary w_1/o emulsion has previously been linked to the stability of the final $w_1/o/w_2$ emulsion (Leister & Karbstein, 2020).

So, moving onto the preparation of the final $w_1/o/w_2$ emulsion, the SE O-170 stabilised w_1/o emulsion was processed with the Ultra-Turrax device/protocol and the PGPR stabilised w_1/o emulsion with the Silverson device/protocol.

3.2. Final $w_1/o/w_2$ emulsion microstructure and salt encapsulation efficiency

3.2.1. Microstructure and droplet size

The microstructure of the two final prepared $w_1/o/w_2$ emulsions immediately after processing is depicted in Fig. 5. To facilitate comparison of emulsion microstructure before and after *in vitro* digestion discussed later, micrographs of the microstructure after *in vitro* digestion are included in Fig. 5. The dark appearance of the oil droplets (Fig. 5) provides evidence for the successful entrapment of w_1 droplets and thus the formation of a w/o/w emulsion (Garti et al., 1994; Kasprzak et al., 2019; Pawlik et al., 2010). Oil droplets with a light appearance signify a less successful incorporation of w_1 droplets or no incorporation at all, as is for example the case for a droplet located just above the centre of Fig. 5A.

The mean droplet diameter droplet $d_{[4,3]}$ 1 day and 3 months after processing was 17.21 (± 1.36) μm and 20.95 (± 1.09) μm, respectively, for the SE O-170 stabilised emulsion, and 16.52 (± 1.11) μm and 16.24 (± 0.76) μm, respectively, for the PGPR stabilised emulsion. Interestingly, the mean droplet size of both emulsions 1 day after processing was comparable, despite the clear difference in the microstructure of the primary w_1/o emulsions (Figs. 3, 4) although the aggregated microstructure of the SE O-170 stabilised w_1 droplets was carried over into the processed $w_1/o/w_2$ emulsion as demonstrated in the cryo-SEM images shown in Fig. 6.

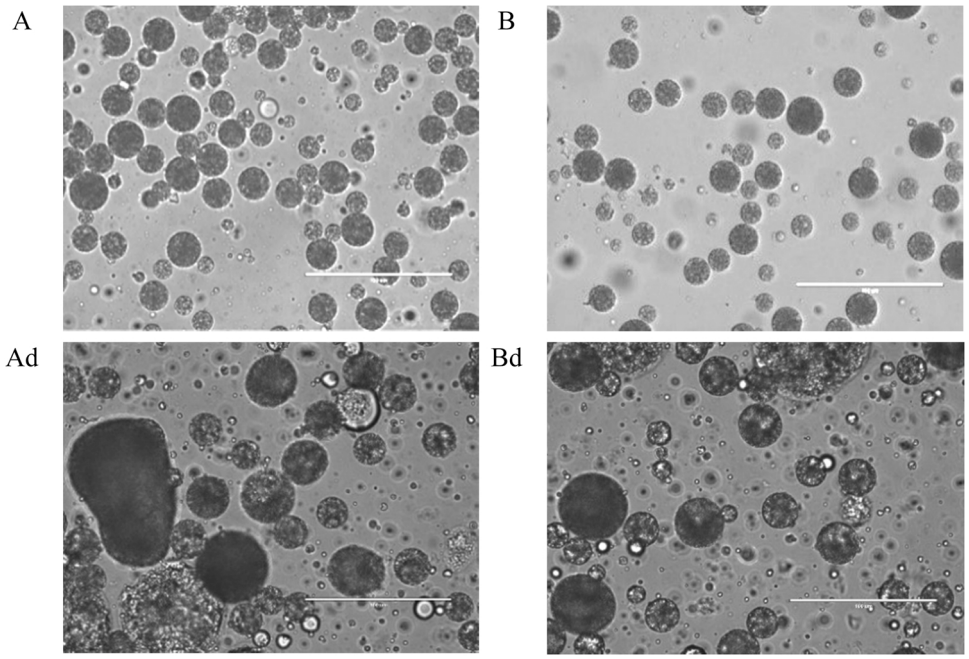


Fig. 5. Microstructure of $w_1/o/w_2$ emulsions with SE O-170 (A, Ad) or PGPR (B, Bd) as lipophilic emulsifier imaged immediately after processing (A, B) and after *in vitro* digestion 1 day after processing (Ad, Bd). 100 g emulsion contained 0.135 g salt in w_1, 0.33 g salt and 4 g WRS in w_2, and 0.6 g lipophilic emulsifier (2.86 g/100 g oil). Scale bar = 100 μm.

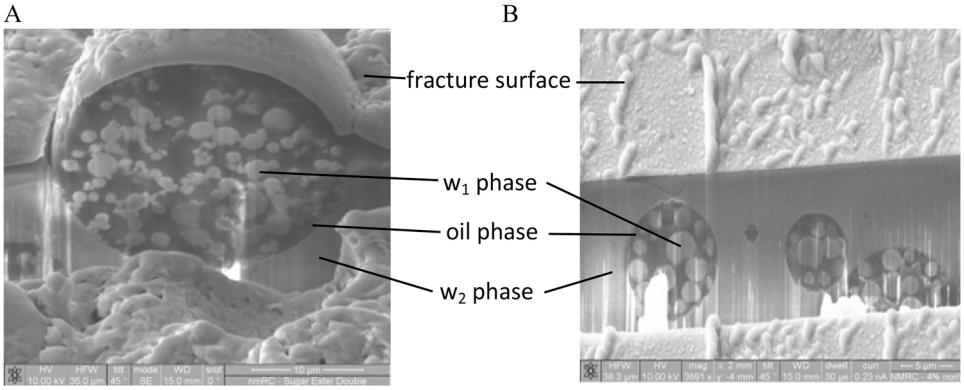


Fig. 6. Cryo-SEM images of $w_1/o/w_2$ emulsions containing 30 g w_1/o emulsion per 100 g emulsion. 100 g emulsion contained 0.135 g salt in w_1, 0.33 g salt and 4 g WRS in w_2, and 0.6 g lipophilic emulsifier (2.86 g/100 g oil). Images were acquired within 2 days of preparation. (A) relates to SE O-170 as lipophilic emulsifier and (B) to PGPR.

It appears though that the internal emulsion microstructure affected the storage stability, although the increase of the mean droplet diameter of the $w_1/o/w_2$ emulsion with SE O-170 as the lipophilic emulsifier by around 3 μm over a 3-months observation period (see above) is probably not of any consequence in practical application. The reason for this minimal increase in droplet size is unlikely to be due to droplet coalescence since it was not observed for the $w_1/o/w_2$ emulsions with PGPR as the lipophilic emulsifier. Long term stability of o/w emulsions stabilised with native starches, including WRS, against coalescence (o-o coalescence) and Ostwald ripening (o-o diffusion) has previously been reported (Gomez-Luria et al., 2019; Kasprzak et al., 2019; Villamonte et al., 2016; Zhao et al., 2017). It can therefore be assumed that diffusion processes between the two aqueous phases have occurred, which, due to the only minimal increase in droplet size may not be reflected in a change in salt encapsulation efficiency over storage.

3.2.2. Salt encapsulation efficiency and in vitro salt release

The salt encapsulation efficiency of the $w_1/o/w_2$ emulsion containing PGPR as the lipophilic emulsifier 1 day after processing was 39.0 (± 1.9) % compared to 34.6 (± 1.3) % using SE O-170 as the lipophilic emulsifier. EE values measured after the 3-months observation period were 39.7 (± 1.2) % and 33.3 (± 3.9) %, respectively. Compared to our earlier work on the PGPR containing emulsion system (Kasprzak et al., 2019) a ~4% higher EE value was found here, assumed to be due to the amended processing protocol. All four values reported here were higher than the theoretical value of 29%, indicating that a portion of w_2 was incorporated into the oil droplets during the secondary emulsification step.

Both $w_1/o/w_2$ emulsions were subjected to amylase-mediated *in vitro* digestion for 30 s followed by determining salt release defined as the change in the quantity of salt in w_2. Analysed 1 day after emulsion processing, the salt release values were 16.7 (± 1.5) % and 13.2 (± 1.0) % applying PGPR and SE O-170 as the lipophilic emulsifier, respectively. The corresponding values after the 3-months observation period were 14.9 (± 0.7) % and 12.9 (± 2.0) %, respectively. As aforementioned, micrographs of the microstructure of the digested emulsions were

included in Fig. 5. The droplets were visibly larger hinting at *in vitro* digestion assay-induced droplet coalescence. As observed in our earlier work (Kasprzak et al., 2019), a large proportion of the imaged droplets still appeared dark suggesting that the release of w_2 into w_1 during the coalescence process was incomplete. The salt release of the PGPR containing $w_1/o/w_2$ emulsion recorded here was also comparable to what we found previously (Kasprzak et al., 2019). Formulating with SE O-170 as the lipophilic emulsifier resulted in a lower value for salt release although, as was the case for using PGPR, the 3-months observation period did not affect the value. Reasons for the lower salt release in case of SE O-170 as the lipophilic emulsifier may be the lower salt encapsulation efficiency, the aggregated network structure of the w_2 droplets (Fig. 6) which might need to be broken up by mechanical forces (see Fig. 2) before w_1-w_2 coalescence can occur, and/or a stronger interfacial film caused by lipophilic and hydrophilic (gelatinised WRS) emulsifier interaction.

4. Conclusions

Sucrose ester SE O-170 can be utilised as lipophilic emulsifier to stabilise the internal water-in-oil emulsion of water-in-oil-in-water emulsions, with the internal aqueous phase containing a higher amount of dissolved salt (sodium chloride) than the external aqueous phase. Here, the oil/water interface was stabilised by gelatinised waxy rice starch as the emulsions were formulated for the release of the encapsulated salt during oral processing *via* amylase-mediated enzymatic destabilisation of the emulsion microstructure. Processing of the emulsion microstructure in a high shear batch process was optimised for internal w/o emulsion microstructure, targeting smallest water droplet size and least aggregation. Even though, compared to the widely successfully applied PGPR as the lipophilic emulsifier in these formulations, at the same level, the water droplets were aggregated and larger in size. The aggregated microstructure carried forward into the w/o/w emulsion and the mean droplet diameter droplet $d_{[4,3]}$ was similar to that of the PGPR containing w/o/w emulsion but increased slightly (from around 17 μm to around 21 μm) over the 3-months observation period. Salt encapsulation efficiency, decreasing by around 10% (from around 40–36%) over the 3-months observation period, did not differ between the two formulations. The values are higher than the theoretical encapsulation efficiency revealing that external aqueous phase was incorporated into either oil phase during the second emulsification step (processing the o/w emulsion into the external aqueous phase). *In vitro* assessed salt release was slightly higher from the PGPR containing formulation but similar after the 3-months observation period, during which the values decreased by around 20% (from around 17–13% and 15–13%, respectively). To conclude, sucrose ester SE O-170 is a viable replacement for PGPR in w/o/w emulsions designed for salt release during oral processing. Since the data for this study were acquired, Balcaen et al. (2021) have reported on phosphatidylcholine-depleted lecithin as a clean-label low-HLB emulsifier to replace PGPR in w/o and w/o/w emulsions. It would be interesting to assess their PGPR-alternative emulsifier in our salt-release formulations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

The authors wish to thank Chris Parmenter from the Nanoscale and Microscale Research Centre, University of Nottingham, for the acquisition of the cryo-SEM images.

References

Akhtar, M., & Dickinson, E. (2001). Water-in-oil-in-water multiple emulsions stabilized by polymeric and natural emulsifiers. In E. Dickinson, & R. Miller (Eds.), *Food colloids: Fundamentals of formulation* (pp. 133–143). The Royal Society of Chemistry. https://doi.org/10.1039/9781847550842-00133.

Baker, I. J. A., Matthews, B., Suares, H., Krodkiewska, I., Furlong, D. N., Grieser, F., & Drummond, C. I. (2000). Sugar fatty acid ester surfactants: Structure and ultimate aerobic biodegradability. *Journal of Surfactants and Detergents, 3*(1), 1–11. https://doi.org/10.1007/s11743-000-0107-2

Balcaen, M., Steyls, J., Schoeppe, A., Nelis, V., & Van der Meeren, P. (2021). Phosphatidylcholine-depleted lecithin: A clean-label low-HLB emulsifier to replace PGPR in w/o and w/o/w emulsions. *Journal of Colloid and Interface Science, 581*, 836–846. https://doi.org/10.1016/j.jcis.2020.07.149

Balcaen, M., Vermeir, L., & Van der Meeren, P. (2017). Influence of protein type on polyglycerol polyricinoleate replacement in W/O/W (water-in-oil-in-water) double emulsions for food applications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 535*, 105–113. https://doi.org/10.1016/j.colsurfa.2017.09.034

Chiu, N., Tarrega, A., Parmenter, C., Hewson, L., Wolf, B., & Fisk, I. D. (2017). Optimisation of octinyl succinic anhydride starch stablised w1/o/w2 emulsions for oral destablisation of encapsulated salt and enhanced saltiness. *Food Hydrocolloids, 69*, 450–458. https://doi.org/10.1016/j.foodhyd.2017.03.002

Choi, H., Kim, S.-J., Lee, S.-Y., & Choi, M.-J. (2017). Effect of abalone hydrolysates encapsulated by double emulsion on the physicochemical and sensorial properties of fresh cheese. *Korean Journal for Food Science of Animal Resources, 37*(2), 210–218. https://doi.org/10.5851/kosfa.2017.37.2.210

Cofrades, S., Santos-López, J., Freire, M., Benedí, J., Sánchez-Muniz, F., & Jiménez-Colmenero, F. (2014). Oxidative stability of meat systems made with W1/O/W2 emulsions prepared with hydroxytyrosol and chia oil as lipid phase. *LWT-Food Science and Technology, 59*(2), 941–947. https://doi.org/10.1016/j.lwt.2014.06.051

Dötsch, M., Busch, J., Batenburg, M., Liem, G., Tareilus, E., Mueller, R., & Meijer, G. (2009). Strategies to reduce sodium consumption: a food industry perspective. *Critical Reviews in Food Science and Nutrition, 49*(10), 841–851. https://doi.org/10.1080/10408390903044297

Engstrom, A., Tobelmann, R. C., & Albertson, A. M. (1997). Sodium intake trends and food choices. *The American Journal of Clinical Nutrition, 65*(2 Suppl), 704s–707s. https://doi.org/10.1093/ajcn/65.2.704S

Garti, N., Aserin, A., & Cohen, Y. (1994). Mechanistic considerations on the release of electrolytes from multiple emulsions stabilized by BSA and nonionic surfactants. *Journal of Controlled Release, 29*(1), 41–51. https://doi.org/10.1016/0168-3659(94)90120-1

Garti, N., & Aserin, A. (1996). Double emulsions stabilized by macromolecular surfactants. *Advances in Colloid and Interface Science, 65*, 37–69. https://doi.org/10.1016/0001-8686(95)00289-8

Glatter, O., Orthaber, D., Stradner, A., Scherf, G., Fanun, M., Garti, N., Clément, V., & Leser, M. E. (2001). Sugar-ester nonionic microemulsion: structural characterization. *Journal of Colloid and Interface Science, 241*(1), 215–225. https://doi.org/10.1006/jcis.2001.7670

Gomez-Luria, D., Vernon-Carter, E. J., Alvarez-Ramirez, J., & Cruz-Sosa, F. (2019). Insights of the ability of gelatinized fractions from non-chemical modified corn, rice, wheat, and waxy corn starches to stabilize O/W emulsions. *[Article] Food Hydrocolloids, 89*, 726–734. https://doi.org/10.1016/j.foodhyd.2018.11.045

Gupta, R. K., James, K., & Smith, F. J. (1983). Sucrose esters and sucrose ester/glyceride blends as emulsifiers. *Journal of the American Oil Chemists' Society, 60*(4), 862–869. https://doi.org/10.1007/BF02787451

Härtl, G. (2013). WHO issues new guidance on dietary salt and potassium. Retrieved September 18, 2022 from https://pubmed.ncbi.nlm.nih.gov/23741892/.

He, F. J., & MacGregor, G. A. (2010). Reducing population salt intake worldwide: from evidence to implementation. *Progress in Cardiovascular Diseases, 52*(5), 363–382. https://doi.org/10.1016/j.pcad.2009.12.006

Jo, N., Bethany, K., & Zachery, R. (2020). Salt reduction targets for 2024. PHE publications Retrieved September 18, 2022 from https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/915406/2024_salt_reduction_targets_070920-FINAL-1.pdf.

Kasprzak, M. M., Wilde, P. J., Hill, S. E., Harding, S., Ford, R., & Wolf, B. (2019). Non-chemically modified waxy rice starch stabilised wow emulsions for salt reduction. *Food & Function, 10*(7), 4242–4255. https://doi.org/10.1039/C8FO01938J

Kloss, L., Meyer, J. D., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union—A review. *NFS Journal, 1*, 9–19. https://doi.org/10.1016/j.nfs.2015.03.001

Knoth, A., Scherze, I., & Muschiolik, G. (2005). Stability of water-in-oil-emulsions containing phosphatidylcholine-depleted lecithin. *Food Hydrocolloids, 19*(3), 635–640. https://doi.org/10.1016/j.foodhyd.2004.10.024

Leister, N., & Karbstein, H. (2020). Evaluating the stability of double emulsions–A review of the measurement techniques for the systematic investigation of instability mechanisms. *Colloids and Interfaces, 4*, 8. https://doi.org/10.3390/colloids4010008

Mandel, A. L., des, Gachons, Plank, C. P., Alarcon, S, K. L., & Breslin, P. A. S. (2010). Individual differences in AMY1 gene copy number, salivary alpha-amylase levels, and the perception of oral starch. *PLoS One, 5*(10), 9. Article e13352. https://doi.org/10.1371/journal.pone.0013352.

Márquez, A., Palazolo, G., & Wagner, J. (2007). Water in oil (w/o) and double (w/o/w) emulsions prepared with spans: Microstructure, stability, and rheology. *Kolloid-*

Zeitschrift und Zeitschrift für Polymere, 285(10), 1119–1128. https://doi.org/10.1007/s00396-007-1663-3

Matos, M., Gutiérrez, G., Coca, J., & Pazos, C. (2014). Preparation of water-in-oil-in-water (W1/O/W2) double emulsions containing trans-resveratrol. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 442*, 69–79. https://doi.org/10.1016/j.colsurfa.2013.05.065

Mazo Rivas, J. C., Schneider, Y., & Rohm, H. (2016). Effect of emulsifier type on physicochemical properties of water-in-oil emulsions for confectionery applications. *International Journal of Food Science & Technology, 51*(4), 1026–1033. https://doi.org/10.1111/ijfs.13063

MCF. (2020). Ryoto Sugar Ester. Mitsubishi-Kagaku Foods Corporation. Retrieved September 18, 2022 from http://www.mfc.co.jp/english/seihin.htm.

Middendorf, D., Juadjur, A., Bindrich, U., & Mischnick, P. (2015). AFM approach to study the function of PGPR's emulsifying properties in cocoa butter based suspensions. *Food Structure, 4*, 16–26. https://doi.org/10.1016/j.foostr.2014.11.003

Mortensen, A., Aguilar, F., Crebelli, R., Di Domenico, A., Dusemund, B., Frutos, M. J., Galtier, P., Gott, D., Gundert-Remy, U., Leblanc, J. C., Lindtner, O., Moldeus, P., Mosesso, P., Parent-Massin, D., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R. A., Wright, M., & Lambré, C. (2017). Re-evaluation of polyglycerol polyricinoleate (E 476) as a food additive. *EFSA Journal, 15*(3). n/a-n/a. https://doi.org/10.2903/j.efsa.2017.4743.

Muschiolik, G., & Dickinson, E. (2017). Double emulsions relevant to food systems: Preparation, stability, and applications. *Comprehensive Reviews in Food Science and Food Safety, 16*(3), 532–555. https://doi.org/10.1111/1541-4337.12261

Nelen, B. A. P., Bax, L., & Cooper, J. M. (2015). *Sucrose Esters. Emulsifiers in Food Technology* (second ed., pp. 147–180). John Wiley & Sons Ltd. <Go to ISI>://WOS:000361324300008.

Pan, L. G., Tomas, M. C., & Anon, M. C. (2002). Effect of sunflower lecithins on the stability of water-in-oil and oil-in-water emulsions. *Journal of Surfactants and Detergents, 5*(2), 135–143. https://doi.org/10.1007/s11743-002-0213-1

Pawlik, A., Cox, P. W., & Norton, I. T. (2010). Food grade duplex emulsions designed and stabilised with different osmotic pressures. *Journal of Colloid and Interface Science, 352*(1), 59–67. https://doi.org/10.1016/j.jcis.2010.08.049

Price, R., Gray, D., Watson, N., Vieira, J., & Wolf, B. (2022). Linking the yield stress functionality of polyglycerol polyricinoleate in a highly filled suspension to its molecular properties (Article) *Lwt-Food Science and Technology, 165*(9), Article 113704. https://doi.org/10.1016/j.lwt.2022.113704.

Santos, J. A., Tekle, D., Rosewarne, E., Flexner, N., Cobb, L., Al-Jawaldeh, A., Kim, W. J., Breda, J., Whiting, S., Campbell, N., Neal, B., Webster, J., & Trieu, K. (2021). A systematic review of salt reduction initiatives around the world: A midterm evaluation of progress towards the 2025 global non-communicable diseases salt reduction target. *Advances in Nutrition, 12*(5), 1768–1780. https://doi.org/10.1093/advances/nmab008

Ushikubo, F. Y., & Cunha, R. L. (2014). Stability mechanisms of liquid water-in-oil emulsions. *Food Hydrocolloids, 34*, 145–153. https://doi.org/10.1016/j.foodhyd.2012.11.016

Villamonte, G., Jury, V., & de Lamballerie, M. (2016). Stabilizing emulsions using high-pressure-treated corn starch. *Food Hydrocolloids, 52*, 581–589. https://doi.org/10.1016/j.foodhyd.2015.07.031

Wenstedt, E. F. E., Olde Engberink, R. H., Rorije, N. M. G., van den Born, B. H., Claessen, N., Aten, J., & Vogt, L. (2020). Salt-sensitive blood pressure rise in type 1 diabetes patients is accompanied by disturbed skin macrophage influx and lymphatic dilation-a proof-of-concept study. *Translational Research, 217*, 23–32. https://doi.org/10.1016/j.trsl.2019.12.001

Yach, D., Khan, M., Bradley, D., Hargrove, R., Kehoe, S., & Mensah, G. (2010). The role and challenges of the food industry in addressing chronic disease. *Globalization and Health, 6*(1), 10. https://doi.org/10.1186/1744-8603-6-10

Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., Filipic, M., Frutos, M. J., Galtier, P., Gott, D., Gundert-Remy, U., Kuhnle, G. G., Lambre, C., Lillegaard, I. T., Moldeus, P., Mortensen, A., Oskarsson, A., Stankovic, I., Waalkens-Berendsen, I., Woutersen, R. A., & Nutr, E. P. F. A. (2018). Refined exposure assessment of sucrose esters of fatty acids (E 473) from its use as a food additive [Article]. Article 5087 *EFSA Journal, 16*(1), Article 22. https://doi.org/10.2903/j.efsa.2018.5087.

Zhao, Y., Khalid, N., Shu, G., Neves, M. A., Kobayashi, I., & Nakajima, M. (2017). Formulation and characterization of oil-in-water emulsions stabilized by gelatinized kudzu starch. *International Journal of Food Properties, 20*(sup2), 1329–1341. https://doi.org/10.1080/10942912.2017.1347675