

# Generation of magnesium enriched water-in-oil-in-water food emulsions by stirred cell membrane emulsification

Pu, Xiaolu; Wolf, Bettina; Dragosavac, Marijana

DOI:

[10.1016/j.jfoodeng.2018.11.022](https://doi.org/10.1016/j.jfoodeng.2018.11.022)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Pu, X, Wolf, B & Dragosavac, M 2019, 'Generation of magnesium enriched water-in-oil-in-water food emulsions by stirred cell membrane emulsification', *Journal of Food Engineering*, vol. 247, pp. 178-187.  
<https://doi.org/10.1016/j.jfoodeng.2018.11.022>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**

Checked for eligibility 07/01/2019

<https://doi.org/10.1016/j.jfoodeng.2018.11.022>

**General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

**Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

1 **Generation of water-in-oil-in-water food emulsions enriched with magnesium by stirred cell**  
2 **membrane emulsification**

3  
4 Xiaolu Pu<sup>1</sup>, Bettina Wolf<sup>1\*</sup>, Marijana Dragosavac<sup>2</sup>

5 <sup>1</sup>Division of Food Sciences, School of Biosciences, University of Nottingham,

6 Sutton Bonington Campus, Loughborough LE12 5RD, UK

7 <sup>2</sup>Chemical Engineering Department, Loughborough University,

8 Loughborough LE11 3TU, UK

9 \*present address: School of Chemical Engineering, University of Birmingham, Birmingham B15 2TT

10 **Highlights**

- 11 • PGPR was used to stabilise internal water droplet interface  
12 • Starch and pea protein isolate were used to stabilise  $w_1/o$  droplet interface  
13 • Low energy membrane emulsification process generated stable  $w_1/o/w_2$  emulsions  
14 • Variation of shear stress and injection rate produced drops between 35 and 320  $\mu\text{m}$   
15 • Complex food emulsifiers delayed release of  $\text{Mg}^{2+}$  ions for up to 2 weeks

16  
17 **Abstract**

18 This study has for the first time shown that complex food emulsifiers such as starch and protein can  
19 be applied to produce stable  $w/o/w$  emulsions with the membrane emulsification technology. Using  
20 a microporous metal membrane with a 20  $\mu\text{m}$  pore size, 2% of polyoxyethylene (20) sorbitan  
21 monolaurate (Tween 20), 4% of octenyl succinic anhydride (OSA) starch or 1.5% of pea protein isolate  
22 (PPI) in the external water phase respectively was the minimum concentration necessary to stabilise  
23 the  $w/o/w$  droplets. Uniform with a span as low as 0.45 and for at least 13-day stable  $w/o/w$

24 emulsions of droplets between 35 and 320 $\mu$ m were obtained. The release of a magnesium tracer  
25 from the internal water phase of xanthan gum-thickened w/o/w emulsions, when OSA starch and PPI  
26 were used, was found to be limited to around 3% after 13-day storage. However, w/o/w emulsions  
27 stabilised with Tween 20 were less stable with magnesium showing a release of 27% on day 13.

28

29 **Keywords:** membrane emulsification; w/o/w emulsion; food; OSA starch; pea protein; delayed  
30 magnesium release.

## 31 **1 Introduction**

32 Water-in-oil-in-water (w/o/w) emulsions are aqueous emulsions where the included oil droplet  
33 phase contains small water droplets in a water-in-oil emulsion. Such emulsion microstructure offers  
34 the opportunity to entrap in a food systems materials for targeted release in the internal aqueous  
35 phase, for example, micronutrients such as metal supplements, flavours and vitamins during  
36 consumption (Herzi and Essafi, 2018, Manickam et al., 2018). The release profiles of those  
37 components will depend on the oils and surfactants used as well as the droplet size of the w/o/w  
38 emulsion (Leadi Cole and L. Whateley, 1997, Oppermann et al., 2018, Schuch et al., 2014, Schuch et  
39 al., 2013). Lower encapsulation efficiency of the inner water phase in w/o/w emulsions stabilised  
40 with polyglycerol polyricinoleate (PGPR) and egg yolk powder were found to correlate with smaller  
41 double emulsion droplet size independent of two emulsification methods (Schuch et al., 2014). On  
42 the contrary, Oppermann et al. (2018) showed that greater encapsulation efficiency of the inner  
43 water phase in w/o/w emulsions was correlated to smaller double emulsion droplet size. Tween 20,  
44 sodium caseinate and Whey protein isolate were used as stabilizers of the external water phase.  
45 Consequently, it is appropriate to seek a tool to control the droplet size of w/o/w emulsions  
46 independent of the hydrophilic emulsifier type and to investigate the impact of the hydrophilic  
47 emulsifier alone on encapsulation efficiency.

48 w/o/w emulsions are usually manufactured using a conventional two-step emulsification method  
49 based on high-pressure or high shear. However, these conventional methods rely on high energy  
50 input to disrupt the dispersed phase and form droplets (Schubert et al., 2003). The mechanical stress  
51 during processing tends to disrupt the emulsion droplets leading to a reduction in the encapsulation  
52 efficiency of the w/o/w emulsions (Kim et al., 2017). In contrast to this top-down processing  
53 approach, bottom-up processing technologies such as membrane emulsification and microchannel  
54 emulsification have been described in the literature as ways of obtaining a controllable droplet size  
55 while processing at much lower mechanical stress input (Schröder et al., 1998, Walstra and  
56 Smulders, 1998, Joscelyne and Tragardh, 2000, Schubert and Ax, 2003, Spyropoulos et al., 2014).  
57 Others often cited the advantages of bottom-up or mild emulsification processes to include  
58 increased energy efficiency as less energy is lost as frictional energy (Walstra, 1993, Joscelyne and  
59 Tragardh, 2000) and prevention of degradation or loss of functionality of heat and shear sensitive  
60 ingredients used to stabilise the emulsions, for example starch and protein (van der Graaf et al.,  
61 2005). In this research membrane emulsification, specifically stirred cell membrane emulsification  
62 (Kosvintsev et al., 2005, Dragosavac et al., 2008), was investigated as a process to generate similarly  
63 sized w/o/w emulsions of narrow droplet size distribution stabilised with different food emulsifiers.

64 PGPR, oil soluble surfactant, is commonly used in the oil phase of  $w_1/o/w_2$  emulsions to stabilize the  
65 internal water phase ( $w_1$ ) via top-down processing (Silva et al., 2018, Chen et al., 2018). The primary  
66 emulsion ( $w_1/o$ ) is then applied to further top-down or alternatively bottom-up processing to create  
67 the final w/o/w emulsion where water soluble surfactant (most commonly Tween 20) must be  
68 present in the outer water phase ( $w_2$ ). Another group recently reported on Tween 20 applied in the  
69 external aqueous emulsion phase to successfully stabilise w/o/w emulsions with encapsulated garlic  
70 extract via stirred cell membrane emulsification (Ilić et al., 2017, Nikolovski et al., 2018). Tween 20 is a  
71 small molecular weight surfactant with higher mobility compared to the macromolecules octenyl succinic  
72 anhydride starch (OSA) and pea protein isolate (PPI). OSA starch is native starch, often of the waxy type,

73 i.e., majorly consisting of amylopectin, that has been chemically modified to contain the anionic and  
74 nonpolar group – octenyl succinic anhydride. PPI mainly contains two globular proteins, legumin and  
75 vicilin (O' Kane et al., 2005). Globular proteins are rigid molecules and rearrange at the interface slowly  
76 (Stauffer, 1999). The starch and protein sorb slower at the droplet surface compared to Tween 20 but  
77 develop a thick and viscoelastic layer and stabilise the droplets through steric and electrostatic repulsion  
78 (Bhosale and Singhal, 2006, Dickinson, 2010). Therefore, comparison of drop stabilisation and  
79 encapsulation/release properties of starch, protein and Tween 20 would be beneficial.

80 However, to the best of our knowledge, there are no publications on the use of complex food  
81 emulsifiers such as starches and proteins to stabilise w/o/w emulsions via membrane emulsification.

82 We were particularly interested in designing process conditions that would impart a comparable and  
83 narrow droplet size spectrum for both types of hydrophilic emulsifier, to then independently assess  
84 the release of magnesium encapsulated in the internal water phase. Magnesium was selected for  
85 convenient detection of release following previously published method (Bonnet et al., 2009). The  
86 emulsions, generated by stirred cell membrane emulsification, were thickened with the hydrophilic  
87 food hydrocolloid xanthan gum post emulsification to alleviate the impact of creaming on the results  
88 of the release measurement. Based on predictive modelling (Dragosavac et al., 2012), a formulation  
89 and processing protocol enabling the independent study of the impact of the choice of hydrophilic  
90 emulsifier on the release properties of a w/o/w emulsion, applicable to a broader choice of  
91 encapsulates than just magnesium, provided they will not alter the physico-chemical properties of  
92 the emulsion system, is introduced.

93

## 94 2 Materials and methods

### 95 2.1 Materials and emulsion phases

96 All used materials were food grade and were used without modifications. To match the osmotic  
97 pressure NaCl (Fisher Scientific, Loughborough, UK) was used both in the internal ( $w_1$ ) and the  
98 external water phase ( $w_2$ ) of  $w_1/o/w_2$  emulsions. NaCl was selected as it enhances the adsorption of  
99 PGPR at the oil-water interface thus providing superior stability (Pawlik et al., 2010). NaCl, within the  
100 internal water phase ( $w_1$ ), was replaced with  $MgCl_2 \cdot 6H_2O$  (Sigma Aldrich, Dorset, UK) for easier and  
101 accurate detection of encapsulation efficiency or release. Internal water droplets ( $w_1$ ) were  
102 stabilised in the oil phase (sunflower oil, purchased from local supermarket) with PGPR (PGPR 90;  
103 DuPont Danisco, Kettering, UK). Tween 20 (Sigma Aldrich, Dorset, UK), octenyl succinic anhydride  
104 (OSA) starch (N-creamer 46, Univar, Widnes, UK) and pea protein isolate (PPI) (MyProtein,  
105 Northwich, UK) were applied as a hydrophilic emulsifier. Xanthan gum (CP Kelco, San Diego, USA)  
106 was used as a thickening agent. Deionized (DI) water, produced on site, was used throughout this  
107 study, and sodium azide (Sigma Aldrich, Dorset, UK) was added to all aqueous phases to suppress  
108 microbial spoilage. Acetone (Sigma Aldrich, Dorset, UK) was used as a solvent for a membrane  
109 wetting agent (Micropore Technologies Ltd., Redcar, UK). All concentrations are provided on a  
110 weight by weight basis, unless stated otherwise.

111 The external water phases ( $w_2$ ) were prepared by mixing the appropriate amount of hydrophilic  
112 emulsifier with 0.1M NaCl solution. For investigating the impact of emulsifier concentration on  
113 stirred cell membrane emulsification 0.5%, 1%, 2% and 4% Tween 20; 2% and 4% OSA starch; and  
114 0.5%, 1.5%, 3% and 6% PPI were applied.

115 For encapsulation efficiency and release measurement, 1600 ppm  $Mg^{2+}$  ( $MgCl_2 \cdot 6H_2O$ , vacuum-dried  
116 overnight at 95°C to remove free moisture), was dissolved in water to constitute the internal  
117 aqueous phase ( $w_1$ ) of the  $w_1/o/w_2$  emulsions instead of 0.1 M NaCl. The outer water phase ( $w_2$ )  
118 consisted of 0.5% xanthan gum and 2% Tween 20, 4% OSA starch or 1.5% PPI. To maintain the

119 osmotic pressure balance between two aqueous phases of the w/o/w emulsions,  $Mg^{2+}$   
120 concentration was calculated according to Equation 1:

$$121 \quad C_{Mg^{2+}} + 2C_{Cl^-} = C_{Na^+} + C_{Cl^-} = 2C_{NaCl} = 3C_{MgCl_2} = 0.2 \text{ M} \quad \text{Eq.1}$$

122 where  $C_{Mg^{2+}}$ ,  $C_{Cl^-}$ ,  $C_{Na^+}$ ,  $C_{NaCl}$  and  $C_{MgCl_2}$  are molar concentrations of  $Mg^{2+}$ ,  $Cl^-$ ,  $Na^+$  ions, NaCl and  $MgCl_2$   
123 present in  $w_1$ . It was checked that the addition of  $MgCl_2$  to the w/o/w emulsions instead of NaCl had  
124 no influence on the microstructure and droplet size distribution. The oil phase contained 4% PGPR  
125 and was prepared by stirring for at least 30 min on a magnetic stirrer at room temperature.

126 The  $w_1/o$  emulsions, as the internal emulsion phase of the w/o/w emulsions, were produced by slow  
127 addition of internal water phase ( $w_1$ ) into the oil phase containing 4% PGPR under high shear mixing  
128 (Ultra Turrax, model T25, IKA Works, Staufen, Germany) operating at 24000 rpm for 5 min.  
129 Emulsification was performed in an ice bath to avoid overheating. These process conditions have  
130 previously been reported to generate a droplet size of around 0.5  $\mu m$  (Vladisavljevic and Schubert,  
131 2003). Final concentration of internal water phase ( $w_1$ ) within the oil phase was 40%.

132

## 133 2.2 Stirred cell membrane emulsification

134 For the preparation of the  $w_1/o/w_2$  emulsions stirred cell membrane emulsification was used. A  
135 hydrophilic nickel membrane with 4 cm diameter (Micropore Technologies Ltd., Redcar, UK),  
136 containing uniform straight through 20  $\mu m$  cylindrical pores with 200  $\mu m$  pore spacing, was used  
137 (see Figure A1 in the Appendix). Based on these two parameters, the porosity of the membrane  
138 (Dragosavac et al., 2008) was calculated to be 0.91%. To increase the hydrophilicity of the  
139 membrane and to avoid the spreading of the dispersed phase (w/o emulsions) over the membrane  
140 surface, the membrane was pre-soaked for 30 min in 2% wetting agent (Micropore Technologies Ltd.,

141 Redcar, UK). For a set-up the membrane was placed in the base of the Dispersion Cell (Micropore  
142 Technologies Ltd., Redcar, UK) filled with continuous phase.

143 After preparation of the base, a cylinder glass cell (125 cm<sup>3</sup> volume) was fitted over the membrane  
144 and filled with continuous phase (outer water phase (w<sub>2</sub>)). A two-blade paddle stirrer, driven by a  
145 24V DC motor and power supply (INSTEK Model PR 3060, UK), was fixed on the top of the cell.  
146 Maximum shear stress was controlled by rotational speed and ranged between 200 and 1500 rpm  
147 corresponding to a maximum shear stress at the membrane surface between 1 and 51 Pa depending  
148 on a continuous phase used. The dispersion phases (primary w<sub>1</sub>/o emulsions) were injected through  
149 the microporous membrane surface using a syringe pump (AL-1000, World Precision Instrument,  
150 Hitchin, UK) fitted with a glass syringe of 29 mm inner diameter at constant injection rate in the  
151 range of 1 to 15 ml min<sup>-1</sup> corresponding to a transmembrane flux between 70 and 1150 L h<sup>-1</sup> m<sup>-2</sup>. The  
152 experiments were continued until the dispersed phase volume fraction reached 10 or 30 vol.%. Once  
153 the desired amount of the w<sub>1</sub>/o emulsion had passed through the membrane, the pump and the  
154 stirrer were switched off followed by transferring the w/o/w emulsion into a glass beaker (100 ml of  
155 w/o/w emulsion was prepared). Finally, 1 ml aqueous sodium azide solution was added to w/o/w  
156 emulsions to obtain a final sodium azide concentration of 0.02% to prevent microbial spoilage. The  
157 beaker was then covered with cling film and stored at room temperature (21 ± 5 °C) until further  
158 analysis.

159 After each use, the membrane was cleaned for 1 min with detergent solution in an ultrasonic bath  
160 followed by cleaning with acetone and DI water before drying using compressed air.

161 Injection speed and maximum shear stress applied to the membrane surface was varied depending  
162 whether the impact of formulation (type and concentration of hydrophilic emulsifier) or processing  
163 parameters on emulsion characteristics was evaluated. Emulsions were also produced to assess



164 their microstructure stability and encapsulation or release properties. Parameter settings are  
165 evident from the presentation of the results.

166

### 167 2.3 Methods for acquisition of parameters required for the droplet diameter predictive model

168 To predict the droplet diameter ( $x$ ) produced with the Dispersion Cell, a conventional shear force  
169 model (Kosvintsev et al., 2005, Dragosavac et al. 2008) based on the balance between the capillary  
170 force (function of equilibrium interfacial tension ( $\gamma$ ) and pore size ( $r_p$ )) and the drag force (function of  
171 a maximum shear stress ( $\tau_{max}$ ) and the droplet size ( $x$ )) acting on a strongly deformed droplet at a  
172 single membrane pore was applied. The droplet diameter can be estimated according to Equation 2.

$$173 \quad x = \frac{\sqrt{18\tau_{max}^2 r_p^2 + 2\sqrt{81\tau_{max}^4 r_p^4 + 4r_p^2 \tau_{max}^2 \gamma^2}}}{3\tau_{max}} \quad \text{Eq.2}$$

174 Thus, to calculate the predicted droplet diameter, the interfacial tension between the  $w_1/o$  phase  
175 and  $w_2$  phases, the viscosity and the density of  $w_2$  were measured as follows. All samples for these  
176 analyses were prepared in triplicate and analysed once.

177 **Equilibrium interfacial tension** ( $\gamma$ ) data at the interface between all the external aqueous emulsion  
178 phases and the  $w_1/o$  emulsion was measured with a force tensiometer (DB2KS, White Electric  
179 Instrument, Malvern, UK) using the Du Nouy ring method at room temperature ( $21 \pm 5$  °C). The  
180 **viscosity** (20°C) of all the external aqueous emulsion phases was measured using a rotational  
181 rheometer (MCR 301, Anton Paar, Graz, Austria) fitted with a concentric cylinder double gap  
182 geometry (DG26.7/T200). Shear rate was stepped up at 5 points/decade between 0.1 and 1000  $s^{-1}$   
183 and a total number of 21 points were acquired every 5 s. The **density** of external aqueous emulsion  
184 phases was measured using a density meter (DMA 5000, Anton Paar, Graz, Austria).

185

186 2.4 Analysis of emulsion characteristics

187 The visual microstructure appearance and droplet size distribution of the produced emulsions were  
188 analysed up to 13 days after processing (immediately after production; on day 1, 2, 6 and 13) to gain  
189 insight into their microstructure stability.

190 The **microstructure** of the w/o/w emulsions was visualised using an epifluorescence microscope  
191 (L3201LED, GT Vision Ltd., Suffolk, UK) operated in bright field illumination mode. Slides were  
192 prepared by pipetting a few drops of the continuous phase ( $w_2$ ) first, to reduce the influence of the  
193 surface tension on drops, and then a few drops of emulsion onto a glass slide followed by carefully  
194 sliding over a glass cover slip. At least three randomly selected areas of each slide were imaged at a  
195 lower and a higher magnification (x4 and x20 objective) and three slides were prepared for each  
196 emulsion.

197 The **droplet size distributions** were analysed with a laser diffraction particle size analyser (Malvern  
198 Mastersizer 2000, Malvern Panalytical Ltd, Malvern, UK). The Dispersion cell was filled with  
199 deionized water as the dispersing medium. Measurement set up and analysis was controlled by the  
200 instrument's software package. The refractive index of the dispersion medium (water) and the  
201 dispersed phase (oil) was set to 1.33 and 1.47, respectively. The absorption value of the dispersed  
202 phase was set to zero. Once the emulsion was dispersed in the water, three measurements were  
203 taken, and the raw data was fitted with a general model. Measurement was carried out in triplicate.

204

205 2.5 Preparation of xanthan gum thickened emulsions

206 To prevent creaming during encapsulation or release measurements, xanthan gum was added to the  
207 emulsion after manufacturing. 1% xanthan gum solution was prepared by dispersing the xanthan  
208 gum powder into water pre-heated to 80°C, while mixing at 1500 rpm with an overhead mixer

209 (RW20 fitted with a 4-bladed propeller stirrer, IKA, Staufen, Germany) for 1 h. The solution was left  
210 overnight to cool down to room temperature ( $21 \pm 5$  °C) and to reach complete hydration before use.  
211 70 g of xanthan gum solution was added to 100 g of emulsion and mixed at 600 rpm on a magnetic  
212 stirrer for 30 min obtaining a final xanthan gum concentration in the external aqueous phase of the  
213 w/o/w emulsions of 0.5%. Using the particle sized analyser and microscope, it was confirmed that  
214 the droplet size and their distribution of the w/o/w emulsions did not change due to these mixing  
215 conditions.

216

## 217 2.6 Assessing magnesium ( $Mg^{2+}$ ) encapsulation and release

218 An Atomic Absorption Spectrophotometer (Spectra AA-200 Varian, UK), operating at the wavelength  
219 of 285.2 nm, was used to detect  $Mg^{2+}$  concentration during the encapsulation and release study.  
220 Standard calibration curves with the  $Mg^{2+}$  concentration as a function of the measurement signal  
221 (absorbance) for different  $w_2$  solutions are shown in Figure A2 in the Appendix. The absorption  
222 obtained from the spectroscopy increased with increasing magnesium concentration. The  
223 relationships were linear and repeatable.

224 To assess  $w_2$  for leakage of  $w_1$  and magnesium into  $w_2$ , the concentration of magnesium in  $w_2$  was  
225 calculated based on the standard calibration curve. Magnesium release percentage was calculated  
226 as follows (Bonnet et al. 2009):

$$227 \quad Mg (\%) = (C_{Mg} \cdot \varphi_{w_2}) / (C_t \cdot \varphi_{w_1}) * 100 \quad \text{Eq.3}$$

228 where  $C_{Mg}$  is the magnesium concentration in  $w_2$ , which was calculated from the corresponding  
229 calibration curves, made for each release media used.  $\varphi_{w_2}$  is the volume fraction of  $w_2$  in final  
230  $w_1/o/w_2$  emulsion (0.8),  $\varphi_{w_1}$  is the volume fraction of  $w_1$  in  $w_1/o$  emulsion (0.4) and  $C_t$  is the total

231  $Mg^{2+}$  concentration initially added in the internal water phase (1600 ppm). From the amount of  $Mg^{2+}$   
232 released in the  $w_2$  phase immediately after production (Figure 5; day 0) it is also possible to estimate  
233 Magnesium encapsulation efficiency ( $EE$ )  $EE (\%) = 100 - (C_{Mg} / C_i) \cdot (1 - \varphi_{w_2}) / \varphi_{w_1} \cdot \varphi_{w_2}$  (Dragosavac et  
234 al., 2012).

235 To prepare the samples for release analysis, a  $w_1/o/w_2$  emulsion was centrifuged for 30 min at 3500  
236 rpm (Heraeus Labofuge 400R, Thermo Scientific, Germany). The bottom layer was then carefully  
237 taken out by pipette and centrifuged again at the same conditions to ensure that  $w_2$  was void of oil  
238 droplets. Via microscopic observation and droplet size analysis of the creamed emulsion droplets it  
239 was verified that the chosen centrifugation conditions had not changed the droplet size distribution.  
240 All measurements were taken over 13 days at the same days as emulsion droplet appearance was  
241 checked.

242

### 243 3 Results and discussion

#### 244 3.1 Effect of emulsifier concentration

245 The effect of the surfactant concentration (Tween 20, OSA starch and PPI) and maximum shear  
246 stress on the  $w/o/w$  emulsions droplet size and span have been jointly reported in Figure 1. Having  
247 in mind that the model used to predict the droplet size using the Eq. 1 does not take into  
248 consideration the injection rate, the experimental data are shown for the injection rate of  $1 \text{ ml min}^{-1}$   
249 corresponding to the lowest meaningful injection rate applicable in the experimental set-up.  
250 Increasing emulsifier concentration led to a decrease in droplet size for the larger molecular weight  
251 emulsifiers PPI and OSA starch, but not for Tween 20. At the same time, droplet size decreased  
252 considerably when the maximum shear stress was stepped up from a low level (1 Pa) to a mid and  
253 high level (6 and 20 Pa), where the droplet size was comparable. These findings were independent of

254 emulsifier type. In the case of the Tween 20 stabilised w/o/w emulsions (Figure 1A), the increase in  
255 emulsifier concentration from 0.5% to 4% had little impact on the droplet size, as could be expected  
256 based on the much lower literature value for this emulsifier's CMC reported in Table 2. On the other  
257 hand, the increase in Tween 20 concentration led to an improvement in the span for the  
258 intermediate maximum shear stress (6 Pa). This could be due to the presence of excess emulsifier  
259 molecules in the continuous emulsion phase protecting the formed droplets against coalescence. In  
260 literature, 2% Tween 20 is often reported for the production of uniform and stable w/o/w emulsions  
261 (Pawlik and Norton 2012, Dragosavac et al., 2012 and Pradhan et al., 2014), and was therefore  
262 chosen as a constant in the investigation of the other processing parameters on emulsion  
263 microstructure. For OSA starch stabilised w/o/w emulsions (Figure 1B), the droplet size decreased  
264 when increasing OSA starch concentration from 2% to 4%. This was accompanied with a span  
265 reduction to 0.53 for maximum shear stress of 51 Pa. Further increase in starch concentration did  
266 not allow the formation of uniformly sized w/o/w emulsions, potentially due to the associated large  
267 increase in external phase viscosity. Therefore 4% OSA starch was used in further experiments. For  
268 PPI stabilised w/o/w emulsions (Figure 1C), a decrease in the droplet size was observed with  
269 increasing PPI concentration from 0.5% to 1.5%. Once the PPI concentration was above 1.5%, no  
270 further decrease of the droplet size, while span increased, was observed. Thus, 1.5% PPI was  
271 selected further on.

272 It is worth noting that the Tween 20 stabilised w/o/w emulsions had a smaller droplet size and  
273 slightly better emulsion uniformity (lower span) compared to the OSA starch and PPI stabilised  
274 emulsions. This can be explained by the higher surface activity of this low molecular weight  
275 emulsifier, as reported in Table 2, and the faster adsorption rate at the interface compared to the  
276 complex emulsifiers starch and protein (Bos and van Vliet, 2001, Kralova and Sjöblom, 2009).  
277 Nevertheless, values of span never exceeded 1 when complex food emulsifiers were used.

278

### 279 3.2 Effect of maximum shear stress and injection rate

280 Both injection rate (1-15 ml min<sup>-1</sup>) and maximum shear stress (1-51 Pa) have been proven in  
281 literature to influence the mean droplet size and uniformity of w/o/w emulsions. Therefore, their  
282 joint influence was studied experimentally within the Dispersion cell. Concentration of emulsifiers  
283 was optimised and 2% Tween 20, 4% OSA starch and 1.5% PPI was used to evaluate the maximum  
284 shear stress and injection rate influence. Produced emulsions showed the characteristic appearance  
285 of a w/o/w emulsion, namely dark appearance of the dispersed droplets. For illustration, one  
286 representative image of one emulsion each stabilised with Tween 20, OSA starch and PPI at the  
287 lowest and the highest maximum shear stress is shown in Figure 2.

288 Mean droplet size and span of the emulsions are presented in Figure 3 along with the model  
289 predictions for droplet size (Equation 1). The experimental droplet sizes were larger than the  
290 predicted data but followed the same decreasing trend with increasing maximum shear stress. As  
291 expected, experimental data was closest to the model prediction at the lowest injection rate of 1 ml  
292 min<sup>-1</sup>, and findings agree with literature (Vladisavljevic and Schubert, 2003, Dragosavac et al., 2012,  
293 Holdich et al., 2010).

294 When 2% Tween 20 was used as emulsifier, drops between 50 and 250 µm were produced with a  
295 span below 0.7. At the low maximum shear stress (1 Pa),  $d_{4,3}$  was larger than 200 µm, which is larger  
296 than the spacing between the pores. This could mean that the newly formed emulsion droplets built  
297 up at the membrane surface rather than immediately detached. Possibly, the small shear force  
298 applied with the paddle led to the formation of a droplet layer on the membrane surface, which then  
299 slowly dispersed into the bulk (Pawlik and Norton, 2012). Besides, it could be that not all of the  
300 membrane pores were used to produce droplets during emulsification, providing more space for  
301 droplets to grow on the membrane (Vladisavljevic and Schubert, 2002). When the lowest injection

302 rate of  $1 \text{ ml min}^{-1}$  was applied, uniform emulsion droplets with a span between 0.4 and 0.6 could be  
303 obtained. This also suggests that not all membrane pores were active. If all membrane pores were  
304 active to produce droplets, two neighbouring droplets would limit the droplet growth to interpore  
305 distance leading to a lower span due to the additional push off force (Kosvintsev et al. 2005). The  
306 lowest span for the Tween 20 stabilised system was 0.49 and recorded for  $1 \text{ ml min}^{-1}$  injection rate  
307 and 10 Pa maximum shear stress. The highest span of approximately 0.65 was found when the  
308 highest injection rate of  $15 \text{ ml min}^{-1}$  and the extreme cases of the low (1 Pa) and high (20 Pa) end of  
309 the shear stress range was applied, which suggests fewer uniform droplets. This could be due to  
310 some large droplets being broken up by the paddle stirrer at the high maximum shear stress and  
311 droplets creaming at the low maximum shear stress or the highest injection rate (Dragosavac et al.,  
312 2012, Thompson et al., 2011).

313 When PPI was used to stabilise the w/o/w emulsions (Figure 3B) drops between 300 and  $60 \mu\text{m}$  were  
314 produced with spans below 0.85. For the OSA starch as emulsifier (Figure 3C) drops between 350 and  
315  $65 \mu\text{m}$  were produced with spans below 1. The viscosity of the OSA starch solution was roughly 10x  
316 greater compared to the viscosity of the Tween and PPI solutions. Therefore, the greater span and  
317 larger droplet size of the emulsions stabilised with starch can be explained with the lower diffusivity  
318 of the molecules and longer time for drop stabilisation leading eventually to coalescence. As found  
319 for the Tween 20 stabilised system, when the lowest injection rate of  $1 \text{ ml min}^{-1}$  was applied, narrow  
320 droplet size distributions were generally produced with spans around 0.6 for the OSA starch and PPI  
321 stabilised systems. The lowest span for the OSA starch stabilised system was 0.4 when processed at  
322  $1 \text{ ml min}^{-1}$  injection rate and 5 Pa maximum shear stress. The lowest span for the PPI stabilised  
323 emulsions was 0.4 when processed at  $10 \text{ ml min}^{-1}$  injection rate and 1 Pa maximum shear stress.

324 The predicted droplet diameter decreased with increasing maximum shear stress for all emulsifiers  
325 (model line within Figure 3). As expected based on the interfacial tension values (see Table 2), the

326 smallest droplet diameter was predicted for the Tween 20 (Figure 3A) stabilised emulsion, followed  
327 by PPI (Figure 3B) and then OSA starch (Figure 3C) stabilised systems, at all maximum shear stress  
328 values. The maximum shear stress range was extended to higher values for the OSA starch stabilised  
329 w/o/w emulsion due to its around tenfold higher viscosity of the continuous emulsion phase  
330 compared to the other two systems (see Table 2). The maximum shear stress range of the predicted  
331 droplet diameter curve for the Tween 20 and the PPI stabilised systems were very similar.

332 A relatively high maximum shear stress in the present set-up (14-51Pa) combined with a low  
333 injection rate (i.e. 1 ml min<sup>-1</sup>) yielded w/o/w emulsions for all three emulsifiers with comparable  
334 droplet size of around 60-70 µm. As our intention for the Mg<sup>2+</sup> encapsulation/release tests was to  
335 investigate the influence of emulsifier independently of droplet size (to keep the surface area for the  
336 release constant) droplets with a diameter of roughly 60 µm were produced according to the  
337 conditions from Figure 3.

338

### 339 3.3 Mid-term microstructure stability of the w/o/w emulsions

340 The coalescence stability of the w/o/w emulsions stabilised with 2% Tween 20, 4% OSA starch and  
341 1.5% PPI manufactured at 1ml min<sup>-1</sup> injection rate and the three maximum shear stress levels (low,  
342 mid and high) was investigated for up to 13 days after processing.

343 Figure 4 shows the corresponding droplet size distributions and micrographs. For each emulsion, the  
344 droplet size distributions showed no difference over 13 days, which suggests these w/o/w emulsions  
345 were stable against coalescence independent of emulsifier type and sample age. Although all w/o/w  
346 emulsions creamed by visual observation, the micrographs show that there was no apparent change  
347 in microstructure and no emptying out for any of the emulsions over the 13 day period of  
348 observation. As it can be seen from Figure 4, even on day 13, the emulsion droplets had a dark  
349 appearance, which demonstrates that there was little or no loss of the inner water droplets from the



350 oil droplets of the w/o/w emulsions.

351

#### 352 3.4 Effect of continuous phase ( $w_2$ ) on $Mg^{2+}$ release and encapsulation

353 Magnesium release was tracked over a period of 13 days to explore encapsulation efficiency of  
354 magnesium or the diffusion of the internal water phase ( $w_1$ ) to the external water phase ( $w_2$ ) of the  
355  $w_1/o/w_2$  emulsions. These emulsions had xanthan gum added post emulsification to eliminate the  
356 impact of creaming on the release data. According to section 3.2, similarly sized uniform droplets  
357 (roughly 60  $\mu m$  diameter), characterised by a low span, independent of emulsifier type were  
358 obtained when a low injection rate ( $1 \text{ ml min}^{-1}$ ) was combined with the maximum shear stress of 14,  
359 16 and 36 Pa for Tween 20, PPI and OSA starch (see Figure 3). For production of w/o/w emulsions for  
360 the release measurement sodium chloride was substituted for magnesium as a more convenient  
361 marker molecule (see section 2.6). To maximise the observation window, the volume fraction of  
362  $w_1/o$  in w/o/w emulsions was increased from 10 vol.% to 30 vol.%. So, initially it was ascertained  
363 through microscopic inspection and acquisition of droplet size distribution data that these two  
364 formulation changes had no impact on the microstructure of the w/o/w emulsions. There was no  
365 apparent change in the microstructure of the w/o/w emulsions when using  $Mg^{2+}$  instead of NaCl in  
366  $w_1$  compared to the respective microstructure shown in Figure 3 on the day of emulsion processing  
367 and on day 13 (micrographs omitted for sake of brevity).

368 Figure 5 shows the release of magnesium from  $w_1$  into  $w_2$  of the xanthan gum thickened w/o/w  
369 emulsions over 13 days. It has been widely reported that an increase in the viscosity of aqueous  
370 phases in w/o/w emulsions by the addition of thickening and gelling agents leads to an improvement  
371 in the encapsulation efficiency of w/o/w emulsions (Kim et al., 2017, Oppermann et al., 2018).  
372 Although viscosity change induced by xanthan gum was expected to play a significant role on the  
373 encapsulation efficiency, there were differences found in the released amount of magnesium from

374 all xanthan gum added w/o/w emulsions depending on emulsifier type. Encapsulation efficiency  
375 immediately after production was 100% for the OSA starch and PPI stabilised w/o/w emulsions. The  
376 OSA starch and PPI stabilised w/o/w emulsions showed some release only between day 3 and day 6  
377 after emulsion preparation. Approximately 1% of magnesium were detected in  $w_2$  on day 6. Release  
378 continued at a slow rate and reached roughly 3% on day 13. So, these two types of emulsions  
379 appeared relatively stable against magnesium release from the encapsulated water phase, thus it is  
380 assumed that there was limited diffusion of  $w_1$  into  $w_2$  setting on only between 3 and 6 days after  
381 emulsion generation.

382 The Tween 20 stabilised w/o/w emulsion was less stable against magnesium release. 5% magnesium  
383 release was noted on the day of emulsion processing meaning that encapsulation efficiency of 2%  
384 Tween was 95%. This could be indicative of a rapid setting on of diffusion of  $w_1$  into  $w_2$ , or loss of  $w_1$   
385 into  $w_2$  during the emulsification process. Magnesium continuously leaked into the external water  
386 phase albeit at decreasing rate over time. Similar observations for Tween 20 stabilised w/o/w  
387 emulsions, but manufactured at a higher injection speed (about  $5 \text{ ml min}^{-1}$ ), so having a larger  
388 droplet size ( $d_{3,2} = 107 \text{ }\mu\text{m}$ ), and encapsulating copper in  $w_1$ , have previously been reported  
389 (Dragosavac et al., 2012). In that case around 50% of the encapsulated copper was released and  
390 w/o/w drops appeared clear within 13 days of emulsion generation. In the current study, there was  
391 no apparent change in the droplet appearance of Tween 20 stabilised w/o/w emulsions after 13-day  
392 storage. However, a loss of 27% of internal water phase ( $w_1$ ) into  $w_2$  by day 13 has been detected.  
393 Nevertheless, this loss might not be enough to visibly change the appearance of the droplets, but  
394 diffusion of  $w_1$  into  $w_2$  might still have occurred. Water and water soluble material transport in  
395 w/o/w emulsions can be explained either by a swelling-breakdown mechanism or diffusion and/ or  
396 permeation through the oil film (Cheng et al., 2007). Specifically, mechanisms behind diffusion and/  
397 or permeation including an osmotic pressure gradient between two aqueous phases (Matsumoto et  
398 al., 1980), the thin lamellae of surfactant which partially form in the oil layer due to fluctuations in its

399 thickness (Jager-Lezer et al., 1997, Garti, 1997b), or reverse micelles in the oil phase (Sela et al.,  
400 1995) have previously been reported. Since the osmotic pressure was balanced in this study, water  
401 transport between two aqueous phases and release of magnesium might result from the thin  
402 lamellae of surfactant forming in the oil film and the PGPR micelles and/or Tween 20 reverse micelles  
403 in the oil phase.

404

#### 405 4 Conclusions

406 This research has for the first time shown that complex food emulsifiers such as starch and protein  
407 can be applied to produce stable w/o/w emulsions with the technology of stirred cell membrane  
408 emulsification. One should consider though that stabilisation with a low molecular surfactant such as  
409 Tween 20 would allow formation of slightly more uniform droplet size distributions (lower span)  
410 with a lower mean diameter. For the release of magnesium from the internal water phase to the  
411 external water phase, OSA starch and PPI stabilised w/o/w emulsions thickened by xanthan gum  
412 showed a better stability against release than Tween 20 stabilised ones. The results reported in this  
413 study enabled the production of uniformly sized w/o/w emulsions with similar average droplet  
414 diameters and high encapsulation efficiency using complex food emulsifiers. Immediately after  
415 production encapsulation efficiency for OSA starch and PPI was 100% while for Tween it was 97%.  
416 Delayed release was obtained when complex food emulsifiers (starch and protein) were used with  
417 almost no release up to 2 days. After 13 days, the emulsions stabilised with Tween 20 had released  
418 almost 30% of  $Mg^{2+}$  and for those stabilised with starch and protein  $Mg^{2+}$  leakage was less than 4%.  
419 This study has introduced a pathway, beneficial for food and pharmaceutical applications, to  
420 enhance the stability and encapsulation efficiency of w/o/w emulsions based on the appropriate  
421 selection of the hydrophilic emulsifier. Low energy membrane emulsification process proved to be a  
422 worthy tool to control as desired, both the droplet size of w/o/w emulsions independent of the

423 hydrophilic emulsifier. Future work will focus on incorporation of volatile flavours within the  
424 emulsion matrix stabilised by complex food emulsifiers (PPI and starch).

425

426 Acknowledgement

427 XP acknowledges the scholarship from China Scholarship Council (CSC).

428

429 References

- 430 BAHTZ, J., GUNES, D. Z., SYRBE, A., MOSCA, N., FISCHER, P. & WINDHAB, E. J. (2016). Quantification  
431 of spontaneous w/o emulsification and its impact on the swelling kinetics of multiple w/o/w  
432 emulsions. *Langmuir*, 32, 5787-5795.
- 433 BHOSALE, R. & SINGHAL, R. 2006. Process optimization for the synthesis of octenyl succinyl  
434 derivative of waxy corn and amaranth starches. *Carbohydrate Polymers*, 66, 521-527.
- 435 BONNET, M., CANCELL, M., BERKAOUI, A., ROPERS, M. H., ANTON, M. & LEAL-CALDERON, F. 2009.  
436 Release rate profiles of magnesium from multiple W/O/W emulsions. *Food Hydrocolloids*, 23,  
437 92-101.
- 438 BOS, M., A. & VAN VLIET, T. 2001. Interfacial rheological properties of adsorbed protein layers and  
439 surfactants: a review. *Advances in Colloid and Interface Science*, 91, 437-471.
- 440 CHEN, X., MCCLEMENTS, D. J., WANG, J., ZOU, L., DENG, S., LIU, W., YAN, C., ZHU, Y., CHENG, C. & LIU,  
441 C. 2018. Coencapsulation of (-)-Epigallocatechin-3-gallate and Quercetin in Particle-  
442 Stabilized W/O/W Emulsion Gels: Controlled Release and Bioaccessibility. *Journal of*  
443 *Agricultural and Food Chemistry*.
- 444 CHENG, J., CHEN, J.-F., ZHAO, M., LUO, Q., WEN, L.-X. & PAPADOPOULOS, K. D. 2007. Transport of  
445 ions through the oil phase of W-1/O/W-2 double emulsions. *Journal of Colloid and Interface*  
446 *Science*, 305, 175-182.
- 447 COTTRELL, T. & VAN PEIJ, J. (2015). Sorbitan esters and polysorbates. In: NORN, V. (ed.) *Emulsifiers in*  
448 *food technology*. West Sussex, UK: Wiley Blackwell.
- 449 DICKINSON, E. 2010. Flocculation of protein-stabilized oil-in-water emulsions. *Colloids Surf B*  
450 *Biointerfaces*, 81, 130-40.
- 451 DRAGOSAVAC, M. M., HOLDICH, R. G., VLADISAVLJEVIĆ, G. T. & SOVILJ, M. N. 2012. Stirred cell  
452 membrane emulsification for multiple emulsions containing unrefined pumpkin seed oil with  
453 uniform droplet size. *Journal of Membrane Science*, 392-393, 122-129.
- 454 DRAGOSAVAC, M. M., SOVILJ, M. N., KOSVINTSEV, S. R., HOLDICH, R. G. & VLADISAVLJEVIĆ, G. T.  
455 2008. Controlled production of oil-in-water emulsions containing unrefined pumpkin seed oil  
456 using stirred cell membrane emulsification. *Journal of Membrane Science*, 322, 178-188.
- 457 GARTI, N. 1997b. Progress in stabilization and transport phenomena of double emulsions in food  
458 applications. *Lebensm.-Wiss.u.-Technol*, 30, 222-235.
- 459 GHARSALLAOUI, A., CASES, E., CHAMBIN, O. & SAUREL, R. (2009). Interfacial and emulsifying  
460 characteristics of acid-treated pea protein. *Food Biophysics*, 4, 273-280.

461 HERZI, S. & ESSAFI, W. 2018. Different magnesium release profiles from W/O/W emulsions based on  
462 crystallized oils. *Journal of Colloid and Interface Science*, 509, 178-188.

463 HOLDICH, R. G., DRAGOSAVAC, M. M., VLADISAVLJEVIĆ, G. T. & KOSVINTSEV, S. R. 2010. Membrane  
464 emulsification with oscillating and stationary membranes. *Industrial & Engineering  
465 Chemistry Research*, 49, 3810-3817.

466 ILIĆ, J. D., NIKOLOVSKI, B. G., PETROVIĆ, L. B., KOJIĆ, P. S., LONČAREVIĆ, I. S. & PETROVIĆ, J. S. 2017.  
467 The garlic (*A. sativum* L.) extracts food grade W1/O/W2 emulsions prepared by  
468 homogenization and stirred cell membrane emulsification. *Journal of Food Engineering*, 205,  
469 1-11.

470 JAGER-LEZER, N., TERRISSE, I., BRUNEAU, F., TOKGOZ, S., FERREIRA, L., CLAUSSE, D., SEILLER, M. &  
471 GROSSIORD, J. L. 1997. Influence of lipophilic surfactant on the release kinetics of water-  
472 soluble molecules entrapped in a W/O/W multiple emulsion. *Journal of Controlled Release*,  
473 45, 1-13.

474 JOSCELYNE, S. M. & TRAGARDH, G. 2000. Membrane emulsification - a literature review. *Journal of  
475 Membrane Science*, 169, 107-117.

476 KASPRZAK, M. M., MACNAUGHTAN, W., HARDING, S., WILDE, P. & WOLF, B. 2018. Stabilisation of  
477 oil-in-water emulsions with non-chemical modified gelatinised starch. *Food Hydrocolloids*, 81,  
478 409-418. KIM, Y.-L., MUN, S., RHO, S.-J., DO, H. V. & KIM, Y.-R. 2017. "Influence of  
479 physicochemical properties of enzymatically modified starch gel on the encapsulation  
480 efficiency of W/O/W emulsion containing NaCl". *Food and Bioprocess Technology*, 10, 77-88.

481 KOSVINTSEV, S. R., GASPARINI, G., HOLDICH, R. G., CUMMING, I. W. & STILLWELL, M. T. 2005.  
482 Liquid-liquid membrane dispersion in a stirred cell with and without controlled shear.  
483 *Industrial & Engineering Chemistry Research*, 44, 9323-9330.

484 KRALOVA, I. & SJÖBLÖM, J. 2009. Surfactants used in food industry: a review. *Journal of Dispersion  
485 Science and Technology*, 30, 1363-1383.

486 KRSTONOŠIĆ, V., DOKIĆ, L. & MILANOVIĆ, J. (2011). Micellar properties of OSA starch and interaction  
487 with xanthan gum in aqueous solution. *Food Hydrocolloids*, 25, 361-367.

488 LEADI COLE, M. & L. WHATELEY, T. 1997. Release rate profiles of theophylline and insulin from stable  
489 multiple w/o/w emulsions. *Journal of Controlled Release*, 49, 51-58.

490 MANICKAM, S., MUTHOSAMY, K. & RAVIADARAN, R. 2018. Simple and Multiple Emulsions  
491 Emphasizing on Industrial Applications and Stability Assessment. *Food Process Engineering  
492 and Quality Assurance*. Apple Academic Press.

493 MATSUMOTO, S., INOUE, T., KOHDA, M. & IKURA, K. 1980. Water permeability of oil layers in  
494 W/O/W emulsions under osmotic pressure gradients. *Journal of Colloid and Interface Science*,  
495 77, 555-563.

496 NIKOLOVSKI, B. G., BAJAC, J. D., MARTINOVIC, F. L. & BOGUNOVIĆ, N. 2018. Optimizing stirred cell  
497 membrane emulsification process for making a food-grade multiple emulsion. *Chemical  
498 Papers*, 72, 533-542.

499 O' KANE, F. E., VEREIJKEN, J. M., GRUPPEN, H. & VAN BOEKEL, M. 2005. Gelation behavior of protein  
500 isolates extruded from 5 cultivars of *Pisum sativum* L. *Journal of Food Science*, 70, C132-C137.

501 OBRADOVIĆ, S. & POŠA, M. (2017). The influence of the structure of selected Brij and Tween  
502 homologues on the thermodynamic stability of their binary mixed micelles. *The Journal of  
503 Chemical Thermodynamics*, 110, 41-50.

504 OPPERMAN, A. K. L., NOPPERS, J. M. E., STIEGER, M. & SCHOLTEN, E. 2018. Effect of outer water  
505 phase composition on oil droplet size and yield of (w1/o/w2) double emulsions. *Food  
506 Research International*, 107, 148-157.

507 PAWLIK, A., COX, P. W. & NORTON, I. T. 2010. Food grade duplex emulsions designed and stabilised  
508 with different osmotic pressures. *J Colloid Interface Sci*, 352, 59-67.

- 509 PAWLIK, A. K. & NORTON, I. T. 2012. Encapsulation stability of duplex emulsions prepared with SPG  
510 cross-flow membrane, SPG rotating membrane and rotor-stator techniques-A comparison.  
511 *Journal of Membrane Science*, 415, 459-468.
- 512 PRADHAN, R., KIM, Y.-I., JEONG, J.-H., CHOI, H.-G., YONG, C. S. & KIM, J. O. 2014. Fabrication,  
513 Characterization and Pharmacokinetic Evaluation of Doxorubicin-Loaded Water-in-Oil-in-  
514 Water Microemulsions Using a Membrane Emulsification Technique. *Chemical and  
515 Pharmaceutical Bulletin*, 62, 875-882.
- 516 SCHRÖDER, V., BEHREND, O. & SCHUBERT, H. 1998. Effect of Dynamic Interfacial Tension on the  
517 Emulsification Process Using Microporous, Ceramic Membranes. *Journal of Colloid and  
518 Interface Science*, 202, 334-340.
- 519 SCHUBERT, H. & AX, K. 2003. Engineering food emulsions. In: MCKENNA, B. M. (ed.) *Texture in food,*  
520 *volume 1: semid-solid foods*. 1st Edition ed. Boca Raton, FL, USA: CRC Press.
- 521 SCHUBERT, H., AX, K. & BEHREND, O. 2003. Product engineering of dispersed systems. *Trends in  
522 Food Science & Technology*, 14, 9-16.
- 523 SCHUCH, A., DEITERS, P., HENNE, J., KOHLER, K. & SCHUCHMANN, H. P. 2013. Production of W/O/W  
524 (water-in-oil-in-water) multiple emulsions: droplet breakup and release of water. *Journal of  
525 Colloid and Interface Science*, 402, 157-64.
- 526 SCHUCH, A., WRENGER, J. & SCHUCHMANN, H. P. 2014. Production of W/O/W double emulsions.  
527 Part II: Influence of emulsification device on release of water by coalescence. *Colloids and  
528 Surfaces A: Physicochemical and Engineering Aspects*, 461, 344-351.
- 529 SELA, Y., MAGDASSI, S. & GARTI, N. 1995. Release of markers from the inner water phase of W/O/W  
530 emulsions stabilized by silicone based polymeric surfactants. *Journal of controlled release*, 33,  
531 1-12.
- 532 SHOGREN, R. L., VISWANATHAN, A., FELKER, F. & GROSS, R. A. (2000). Distribution of octenyl  
533 succinate groups in octenyl succinic anhydride modified waxy maize starch. *Starch - Stärke*,  
534 52, 196-204.
- 535 SILVA, W., TORRES-GATICA, M. F., OYARZUN-AMPUERO, F., SILVA-WEISS, A., ROBERT, P., COFRADES,  
536 S. & GIMÉNEZ, B. 2018. Double emulsions as potential fat replacers with gallic acid and  
537 quercetin nanoemulsions in the aqueous phases. *Food Chemistry*, 253, 71-78.
- 538 SPYROPOULOS, F., LLOYD, D. M., HANCOCKS, R. D. & PAWLIK, A. K. 2014. Advances in membrane  
539 emulsification. Part A: recent developments in processing aspects and microstructural  
540 design approaches. *Journal of the Science of Food and Agriculture*, 94, 613-627.
- 541 STAUFFER, C. E. 1999. *Emulsifiers*, St. Paul, Minnesota, Egan Press.
- 542 THOMPSON, K. L., ARMES, S. P. & YORK, D. W. 2011. Preparation of Pickering Emulsions and  
543 Colloidosomes with Relatively Narrow Size Distributions by Stirred Cell Membrane  
544 Emulsification. *Langmuir*, 27, 2357-2363.
- 545 USHIKUBO, F. Y. & CUNHA, R. L. (2014). Stability mechanisms of liquid water-in-oil emulsions. *Food  
546 Hydrocolloids*, 34, 145-153.
- 547 VAN DER GRAAF, S., SCHROËN, C. G. P. H. & BOOM, R. M. 2005. Preparation of double emulsions by  
548 membrane emulsification—a review. *Journal of Membrane Science*, 251, 7-15.
- 549 VLADISAVLJEVIC, G. T. & SCHUBERT, H. 2002. Preparation and analysis of oil-in-water emulsions with  
550 a narrow droplet size distribution using Shirasu-porous-glass (SPG) membranes. *Desalination*,  
551 144, 167-172.
- 552 VLADISAVLJEVIC, G. T. & SCHUBERT, H. 2003. Influence of process parameters on droplet size  
553 distribution in SPG membrane emulsification and stability of prepared emulsion droplets.  
554 *Journal of Membrane Science*, 225, 15-23.
- 555 WALSTRA, P. 1993. Principles of emulsion formation. *Chemical Engineering Science*, 48, 333-349.
- 556 WALSTRA, P. & SMULDERS, P. E. A. 1998. Emulsion formation. In: BINKS, B. P. (ed.) *Modern aspects  
557 of emulsion science*. 1st Edition ed. Cambridge, UK: The Royal Society of Chemistry.

558  
559

560 List of tables

561

562 Table 1: Averaged interfacial tension, viscosity (at  $10\text{ s}^{-1}$ ) and density data acquired at  $20\text{ }^{\circ}\text{C}$ .

563

564 Table 2: Physicochemical properties of emulsifiers used in this study. CMC: critical micelle  
565 concentration.

566

567

568



569 Table 1: Averaged interfacial tension, viscosity (at  $10 \text{ s}^{-1}$ ) and density data acquired at  $20 \text{ }^\circ\text{C}$

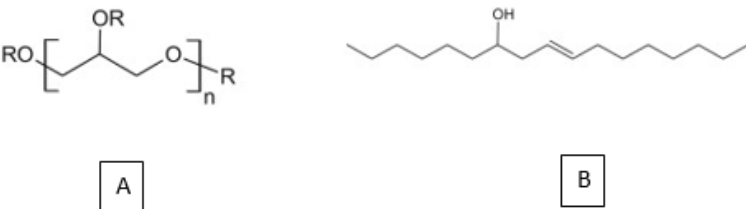
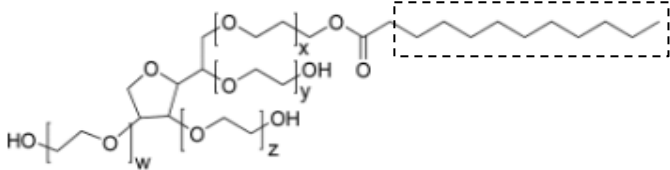
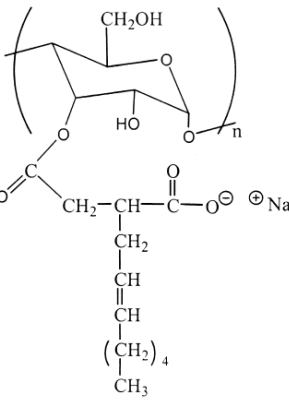
$w_2$	interfacial tension at $w_1/o$ interface (mN/m)	viscosity (mPa.s)	density ( $\text{g}/\text{cm}^3$ )
2% Tween 20 in 0.1 M NaCl	$5.9 \pm 0.4$	$1.07 \pm 0.01$	$1.0050 \pm 0.0000$
4% OSA starch in 0.1 M NaCl	$13.7 \pm 0.2$	$11.57 \pm 0.12$	$1.0173 \pm 0.0000$
1.5% PPI in 0.1 M NaCl	$10.5 \pm 0.4$	$1.26 \pm 0.05$	$1.0065 \pm 0.0002$

570

571

572

573 Table 2: Physicochemical properties of emulsifiers used in this study. CMC: critical micelle  
 574 concentration .

Emulsifier	Approximate molecular weight (g/mol)	Approximate CMC	Structural formula
PGPR	3000 (Ushikubo and Cunha, 2014)	1.8 (% w/w) at 20 °C (Bahtz et al., 2016)	 <p>A) chemical structure of PGPR. R is a hydrogen, ricinoleic acid or polyricinoleic acid. The average value of n is about 3.        B) chemical structure of ricinoleic acid. (Ushikubo and Cunha, 2014)</p>
Tween 20	1228 (Obradović and Poša, 2017)	0.07 (% w/w) at 25°C (Cottrell and Van Peij, 2015)	 <p>Dotted box notes the alkyl chain. (Obradović and Poša, 2017)</p>
OSA starch	470000 (Kasprzak et al., 2018)	0.05 (% w/v) at 25°C (Krstonošić et al., 2011)	 <p>(Shogren et al., 2000)</p>
PPI	Main components (O' Kane et al., 2005): legumin, 380000 g/mol; vicilin, 150000 g/mol.	0.04 (% w/w) at 20 °C (Gharsallaoui et al., 2009)	-

575

576

577