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The role of endogenous lipids in the emulsifying properties of cocoa

Joanne M. Gould[1*], Samuel Furse[1], Bettina Wolf[1]

[1]School of Bioscience, University of Nottingham, United Kingdom

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Author contribution statement

BW wrote the original grant proposal. JG and BW conceived the research project and designed surface tension experiments. JG carried out all surface tension experiments and isolated lipid samples. SF supervised lipid isolation, ran NMR spectroscopy experiments and analysed NMR data. JG drafted the manuscript; all authors were involved in revising it; BW and SF supervised its preparation. All authors (JG, BW, SF) have approved and are accountable for the final version of the manuscript submitted.

Keywords

Oil-in-water emulsions, Cocoa, Emulsifier, Phospholipids, pickering

Abstract

Word count: 174

This paper describes a study in which the emulsifying properties of cocoa material with and without its lipid fraction were explored. This study was motivated by the commercial interest in naturally-occurring particulate emulsifiers as opposed to the chemically modified emulsifying particles presently available for commercial use. The hypothesis was that endogenous lipids from cocoa were responsible for driving the formation of stable oil-in-water (o/w) emulsions. The data presented includes relative quantification of phospholipids from different commercially available cocoa material using 31P NMR spectroscopy and analyses of the emulsifying power of delipidified cocoa material. The commercially available cocoa material comprised several phospholipids, with phosphatidylcholine being the most abundant in all samples. Dispersions of delipidified cocoa material were found to drive the formation of o/w emulsions despite the absence of lipids. We therefore concluded that the emulsifying behaviour of cocoa material is not entirely reliant upon the endogenous lipids. This suggests that cocoa material may have a new and potentially widespread use in industrial food preparation and may inform manufacturing strategies for novel food grade emulsifiers.

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Ethics statement

(Authors are required to state the ethical considerations of their study in the manuscript including for cases where the study was exempt from ethical approval procedures.)

Did the study presented in the manuscript involve human or animal subjects: No

The role of endogenous lipids in the emulsifying properties of cocoa

Joanne Gould*, Samuel Furse and Bettina Wolf

School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK.

Abstract

This paper describes a study in which the emulsifying properties of cocoa material with and without its lipid fraction were explored. This study was motivated by the commercial interest in naturally-occurring particulate emulsifiers as opposed to the chemically modified emulsifying particles presently available for commercial use. The hypothesis was that endogenous lipids from cocoa were responsible for driving the formation of stable oil-in-water (o/w) emulsions. The data presented includes relative quantification of phospholipids from different commercially available cocoa material using ^{31}P NMR spectroscopy and analyses of the emulsifying power of delipidified cocoa material. The commercially available cocoa material comprised several phospholipids, with phosphatidylcholine being the most abundant in all samples. Dispersions of delipidified cocoa material were found to drive the formation of o/w emulsions despite the absence of lipids. We therefore concluded that the emulsifying behaviour of cocoa material is not entirely reliant upon the endogenous lipids. This suggests that cocoa material may have a new and potentially widespread use in industrial food preparation and may inform manufacturing strategies for novel food grade emulsifiers.

Keywords

Oil-in-water emulsions, Cocoa, Emulsifier, Phospholipids, Pickering

Introduction

Emulsions comprising oil as the dispersed liquid fraction and water as the continuous one are found across a considerable variety of foods, including soups, salad dressings, mayonnaise, sauces and the majority of dairy products. The emulsification of oil into water to produce such oil-in-water (o/w) emulsion based consumer goods requires the addition of an emulsifying agent. Such agents facilitate emulsion processing through reduction in interfacial tension and contributing to emulsion stability during shelf-life by counteracting thermodynamically driven instability mechanisms. Typical emulsifying agents in foods are amphiphilic proteins and surfactants such as lecithin, although, in recent years, the use of solid particles as emulsifiers has attracted significant research interest (Berton-Carabin and Schroën, 2015, Dickinson, 2012, Lam et al., 2014). Solid particles have been reported to impart a higher emulsion stability with respect to proteins and surfactants, as their energy of desorption from the interface is several orders of magnitude higher than in the case of proteins and surfactants (Hunter et al., 2008, Aveyard et al., 2003). Thus, coalescence and Ostwald

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46 ripening are less favoured, imparting prolonged microstructure stability which in
47 turn increases the shelf-life of edible emulsion based consumer goods. This
48 property also facilitates formation of stable multiple emulsions, a system that is
49 sought-after for low fat food formulations (Dickinson, 2011, Lobato-Calleros et
50 al., 2008), and for the encapsulation of bioactive species for targeted release
51 (McClements, 2015, Lamba et al., 2015). An additional attraction of formulating
52 food emulsions with particulate material is that artificial surfactants are not
53 required. However, to date, the application has been limited due to the scarcity of
54 food grade particulate emulsifier ingredients.
55
56 Recently, we demonstrated that o/w emulsions comprising only sunflower oil,
57 water and a particulate material from several parts of the Theobroma cacao bean
58 showed no evidence of emulsion instability in form of droplet coalescence (≥100
59 days) or free oil (≥2 years) (Gould et al., 2013) for storage periods well above the
60 requirements of most emulsion based manufactured foods. While the use of food
61 grade particles is an obvious requirement for application in the food industry,
62 cocoa particles are not only food grade but can be classified as natural
63 emulsifying food particles as there is no requirement for chemical modification to
64 impart emulsifying ability. This is in contrast to hydrophobised starches (Yusoff
65 and Murray, 2011); the only particulate emulsifier ingredient applied in the food
66 industry to date.
67
68 The efficacy of cocoa material as an emulsifier (Gould et al., 2013) raises the
69 question of which molecular species is/are responsible for it. It has been
70 understood for some time that cocoa comprises up to around 0.4% (w/w)
71 phospholipids (Parsons et al., 1969, Knapp, 1937). Phospholipids, as a class of
72 biomolecules, are well-established emulsifying and surface-active agents in the
73 context of food (Pichot et al., 2013, Singh et al., 2009, Guzey and McClements,
74 2006). The presence of such species in this quantity is consistent with the
75 commonly used food emulsifier lecithin, which contains a mixture of
76 phospholipids, and can drive the formation of o/w emulsions at concentrations as
77 low as 0.5% (w/w) (Pan et al., 2002). The presence of these species in all of the
78 cocoa material therefore led us to the hypothesis that the cocoa material used
79 previously (Gould et al., 2013) was an emulsifying agent because of the presence
80 of phospholipids.
81
82 In order to determine the contribution of such endogenous lipids to the kinetic
83 stability of the microstructure of o/w emulsions with cocoa material, the
84 phospholipid fraction of four different types of cocoa was profiled using ^{31}P
85 NMR. Surface tension and emulsion assays were used to determine the interfacial
86 functionality of both the untreated and the delipidified cocoa material and, by
87 comparison, indicate what contribution non-lipid species make to the emulsifying
88 power of cocoa material.
89

90 **Materials and methods**

91 **Cocoa material**

92 Cocoa powders (CPs) were a gift from Barry Callebaut (Banbury, Oxfordshire,
93 UK) and cocoa fibre (CF) was donated by Food Ingredient Technology (Sandy,
94 Bedfordshire, UK). Data including the total lipid content are given in Table 1.

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The samples codes used in a previous study are included for comparison (Gould et al., 2013).

Lipid extraction

Extraction solvents (petroleum ether, chloroform, ethanol and triethlyamine) were HPLC grade (Sigma-Aldrich, Gillingham, Dorset, UK) and used without further purification. CUBO solvent for NMR was prepared freshly before use following published protocol (Bosco et al., 1997, Culeddu et al., 1998, Cremonini et al., 2004) using guanidinium chloride (Fisher scientific, Loughborough, UK), deuteriated dimethylformamide (d_7-DMF) (Sigma-Aldrich, Gillingham, Dorset, UK) and triethlyamine. NMR tubes were obtained from Wilmad (Vineland, NJ, USA). To extract the triglyceride fraction, a 50 g sample of cocoa was mixed with petroleum ether (300 mL) and agitated (1 h at 4 °C). The remaining solid was isolated by centrifugation (11,400 × g, 1 h, 4 °C; J2-21M Induction Drive Centrifuge, Beckman, High Wycombe, Buckinghamshire, UK). The solid was re-suspended in CET (Furse et al., 2013, Furse et al., 2015a) (chloroform: ethanol: triethylamine, 3:1:1, 300 mL) and agitated (1 h at 4 °C) to extract the phospholipid fraction. The organic solutions were concentrated to dryness separately under reduced pressure. The solids were suspended in CET for a second time, agitated and dried; however no further lipid was extracted. All traces of solvent were removed by drying the delipidified cocoa (40 °C, 4 days, Vacuum Oven, Weiss Gallenkamp, Leicester, UK). Three independent extractions were carried out for each type of cocoa material.

Profiling of lipid fractions

Lipid isolates (80 mg) were dissolved in 200 µL CUBO solvent and after agitation centrifuged at 21,100 × g for 30 min at 4 °C (Heraeus Fresco 21 Microcentrifuge, Thermo Corporation, Waltham, USA). The organic solution was then transferred to a 5 mm NMR tube and diluted (CUBO solvent, overall sample volume 500 µL). The ^{31}P NMR spectra were obtained from a Brucker AV400 spectrometer (Brucker, Coventry, UK). The phospholipids were identified using the reported shifts of phosphorous resonances (Bosco et al., 1997, Culeddu et al., 1998, Cremonini et al., 2004), which were 5.12 ppm for phosphatidic acid (PA), 1.25 ppm and 1.21 ppm for phosphatidylglycerol (PG), 1.07 ppm for phosphatidylinositol (PI), 0.48 ppm and 0.44 ppm for lyso-phosphatidylcholine (LPC) and phosphatidylcholine (PC) was calibrated to 0.00 ppm.

Emulsion preparation

The oil phase of the emulsions consisted of commercially available sunflower oil (J Sainsbury Plc, London, UK) and double distilled water. Surface active impurities present in sunflower oil were removed by adding magnesium silicate (4% (w/w), Sigma-Aldrich, Dorset, UK) followed by stirring (30 min, 600 rpm, RCT Basic, IKA – Werke GmbH & Co, Staufen, Germany). The magnesium silicate was removed by centrifugation (2,700 × g, 30 min, Jouan CR3i multifunction Centrifuge, Thermo Fisher Scientific, Massachusetts, USA). Absence of surface active molecules in the purified oil was confirmed by measuring interfacial tension against water using the pendant drop technique (see method below) verifying that interfacial tension at 20°C was constant at a value of 27.3 mN.m^{-1} ± 1.5 mN.m^{-1}. It remained unchanged for at least 29 days of storage at room temperature in the dark which was the longest storage period for purified

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145 oil used for emulsion preparation in this study. Sodium azide (Sigma-Aldrich,
146 Gillingham, Dorset, UK) was added as anti-microbial agent to all aqueous
147 emulsion phases at a final concentration of 0.02% (w/w). Emulsions (o/w, 20%
148 (w/w) oil) were produced on a 100 g scale. Water (75.2 g) and cocoa material
149 (4.8 g) were placed in a glass beaker (250 mL) and mixed briefly by hand to
150 produce an aqueous dispersion of 6% (w/w) cocoa material. Sunflower oil (20 g)
151 was added to the dispersion prior to homogenisation using a high shear overhead
152 mixer (L5M Series fitted with emulsor screen, Silverson, Chesham, Hertfordshire,
153 UK) operating at 8,000 rpm for 2 min.
154
155 **Characterisation of emulsions**
156 Particle size analysis was used to assess the coalescence stability of the emulsions;
157 no significant increase in droplet size over the storage period deemed a stable
158 emulsion in this study. Size distributions of prepared emulsions were measured
159 with a low angle laser diffraction particle size analyser (LS 13 320, Beckman
160 Coulter, High Wycombe, UK) fitted with an aqueous dispersion cell (Universal
161 liquid module, LS13 320, Beckman Coulter, High Wycombe, Buckinghamshire,
162 UK). Data was analysed using the Fraunhofer approximation optical model from
163 the instrument's software. Graphical representations of the surface area based
164 mean, $d_{3,2}$, are presented. Three independent measurements of each sample were
165 used to calculate the values given.
166
167 **Interfacial tension and surface tension measurement**
168 A drop shape tensiometer (PAT-1, Sinterface, Berlin, D) was used to quantify
169 interfacial tension and surface tension of samples. Interfacial tension measurement
170 was used to confirm the absence of surface active impurities in the oil, whereas
171 the surface tension of the aqueous dispersions of cocoa material was used to
172 assess the contribution of the lipid fraction to the surface activity of cocoa. All
173 measurements were taken at 20 °C and values recorded for 600 s after drop
174 formation. The values reported in the results section represent an average of the
175 equilibrium surface tension, recorded at 600 s. Three independent measurements
176 of each sample were used. For interfacial tension measurement at the purified
177 sunflower oil/water interface a straight capillary with a diameter of 2 mm (outer
178 diameter) was used to dose a water drop of 35 mm^3 constant volume into the oil
179 phase contained in a quartz glass cuvette. The surface tension of aqueous
180 dispersions of 6% (w/w) cocoa material was measured using the same straight
181 capillary and constant drop volume of 35 mm^3. Due to the low surface tension of
182 the 6% (w/w) aqueous dispersion of cocoa fibre the volume of the droplet was
183 reduced to 25 mm^3 for this sample. It was previously verified that reducing the
184 droplet volume did not change the result.
185
186 **Statistical analysis**
187 Mean droplet size and standard deviation are reported based on three independent
188 samples. Whether or not significant increases in emulsion droplet diameter
189 occurred over storage was determined using an ANOVA and Tukey's statistical
190 test was carried out. In order to compare the mean droplet diameter of emulsions
191 stabilised by the same type of cocoa material either untreated or delipidified, a t-
192 Test was used to assess difference between the two samples. The level of
193 significance was set at $p = 0.05$ for both statistical tests.

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Results

The cocoa used in this study reflected the cocoa material from different parts of the cocoa bean, and different treatments during isolation. The latter represent the range of sizes of the lipid fraction in commercially available cocoa and are low, medium and high lipid and denoted CP-l, CP-m and CP-h, respectively. The fourth cocoa material is powdered cocoa fibre (CF).

Endogenous lipid composition

A sequential extraction approach (Furse et al., 2015a) was adopted in order to separate the triglyceride (TG) fraction and the phospholipid (PL) fractions clearly. This approach has been shown to remove more than 99.99% of the TGs and thus gives practically no contamination of the PL fraction (Furse et al., 2013). The triglyceride fraction of the cocoa materials was therefore isolated using petroleum ether, after which the PL fraction was isolated using the CET solvent system (Furse et al., 2013) The size of the TG and PL fractions was assessed gravimetrically (Figure 1) and the profile of the PL fraction determined using ^{31}P NMR spectroscopy (Figure 2).

The overall size of phospholipid fraction of the cocoa varied between 0.9% (w/w) and 2.4% (w/w) for the CP/s with the lowest (CP-l) and the highest (CP-h) lipid content, respectively.

^{31}P NMR spectroscopy showed that the same five phospholipids PA, PG, PI, LPC and PC are present in all cocoa material, regardless of lipid content or origin (Figure 2). Signals were identified using literature values for chemical shift (Furse et al., 2013, Furse et al., 2015a, Murgia et al., 2003). Phosphatidyl ethanolamine and phosphatidyl serine have been previously identified in cocoa beans (Parsons et al., 1969) but were not found in the cocoa material tested in this study.

The relative proportions of the phospholipids were determined by integrating the resonances of the different phosphorus environments. This indicated the composition of the phospholipid fraction of each cocoa material (Figure 3). Phosphatidylcholine (PC) dominated in each type of cocoa material. Not all of the signals could be identified, however, unidentified signals contributed less than 10% of the total phospholipid for the cocoa powders. ^{31}P NMR spectra of all samples showed additional peaks at chemical shifts of 0.18 ppm and 0.7 ppm. The peak at 0.75 ppm may be evidence for the presence of cardiolipin (Culeddu et al., 1998), which was present in a much higher concentration for CF (24%). The unknown signals (0.18, 1.4, 3.7 and 4.8 ppm) may be ascribed to small molecules, e.g. glycerol phosphate. Inorganic phosphates such as sodium phosphate, and pyrophosphates such as ADP can be ruled out as they do not dissolve in the solvent system used to disperse lipids samples for NMR spectroscopy. Full structural determination is required for unambiguous identification of these phosphorylated species.

Characterisation of the emulsifying ability of delipidified cocoa material

All types of delipidified cocoa material stabilised o/w emulsions. Figure 4 shows that there was no significant increase in droplet size of the emulsions stabilised with untreated or delipidified cocoa material measured over 100 days of storage at 20 °C ($p<0.05$). In addition there was no evidence of a coalesced oil layer after 2

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244 years of storage at 20 °C. This indicates that the removal of lipid did not affect the
245 coalescence stability of cocoa stabilised o/w emulsions.
246
247 While none of the emulsions showed coalescence, in the case of the cocoa
248 powders with medium and high lipid content (CP-m and CP-h, respectively) the
249 removal of the lipid fraction had a significant impact on the mean droplet
250 diameter of the emulsions. In both cases it was significantly lower following lipid
251 extraction compared to the mean diameter of the emulsions prepared with the
252 original cocoa material ($p=0.01$ and $p=0.02$, respectively). As emulsion droplet
253 size has a linear relationship with the size of the particles stabilising the interface
254 (Binks and Lumsdon, 2001), the particle size of the cocoa material was evaluated.
255 Particle size measurement of the cocoa material, shown in Table 2, confirms that
256 lipid extraction caused a significant reduction in particle size of cocoa materials
257 CP-m and CP-h. There was no significant change in particle size of CP-1 or CF.
258

259 **Surface tension of delipidified cocoa material**

260 The surface tension of the aqueous dispersions of the four cocoa materials was
261 measured to assess whether the phospholipid fraction contributes to the interfacial
262 activity of cocoa as phospholipids are known to facilitate emulsion formation by
263 reducing the interfacial tension. The surface tension of the aqueous dispersion of
264 the cocoa material analysed after 600 s are shown in Table 3.
265
266 These data indicate that lipid extraction significantly decreased the surface tension
267 of the cocoa material except in the case of the cocoa powder with the smallest
268 proportion of lipid (CP-l). Surface tension measurements of the aqueous
269 dispersions of the cocoa material indicated that the phospholipids contribute to the
270 surface activity of the dispersions as removal of the lipid fraction decreased the
271 surface tension.
272

273 **Discussion**

274 In this study the contribution of the endogenous lipid of cocoa material was
275 evaluated with respect to the emulsifying ability of cocoa. The phospholipid
276 fraction, quantified by mass after extraction using organic solvents, was consistent
277 with supplier specifications and removal of the entire lipid fraction from the cocoa
278 material. Notably, the phospholipid fraction (0.9 – 2.4% (w/w)) was higher than
279 the 0.3% - 0.4% (w/w) previously reported for cocoa beans (Parsons et al., 1969,
280 Knapp, 1937). However, the techniques used previously were based on
281 chromatographic rather than spectroscopic methods and thus did not produce
282 structural data to support identification of the compounds present. The
283 comparative advantage of the ^{31}P NMR spectroscopy is that it is a high resolution,
284 quantitative technique that gives structural data. Furthermore, the method used to
285 isolate the lipid fraction in the current study was designed to isolate lipids with a
286 variety of head groups. Earlier methods were less general (Furse et al., 2015a,
287 Rydhag and Wilton, 1981). This may be why previously unreported
288 phosphorylated species (unknown species, Figure 3) were observed.
289
290 ^{31}P NMR spectroscopy of the cocoa indicated the presence of phospholipids in all
291 types of cocoa material tested with five well-known phospholipids (PA, PG, PI,
292 LPC and PC) identified in all samples. The proportion of each of phospholipid
293 varied between the types of cocoa material although PC was the major

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294 phospholipid (25 – 57% (w/w)) in all samples. The same high concentration of
295 PC was previously reported for cocoa beans where PC was found to contribute
296 36% – 40% of the total phospholipid (Parsons et al., 1969). The presence of PC
297 was of particular interest as PCs are a major component of lecithin; a commonly
298 used emulsifier (Whittinghill et al., 2000, Pichot et al., 2013).
299
300 The contribution of the (whole) lipid fraction to the emulsifying ability of the
301 cocoa material was quantified by a comparison of the emulsions generated by
302 cocoa before and after extraction of the lipid fractions. All four cocoa materials
303 were found to be able to stabilise an o/w emulsion after lipid extraction. There
304 was no significant increase in emulsion droplet diameter over a period of 100 days
305 which indicates that emulsions were stable to droplet coalescence. Lipid
306 extraction did affect the droplet size of two of the emulsion samples prepared
307 from cocoa materials of originally medium and high lipid content. This may be
308 ascribed to the change in the size distribution of the cocoa material to smaller
309 diameters following lipid extraction, as shown in Table 2, as smaller particle
310 diameters are known to enable stabilisation of smaller droplets (Binks and
311 Lumsdon, 2001, Luo et al., 2011). The shift in size distribution of the particles
312 may be due to removal of surface lipid that promotes particle aggregation.
313
314 The role of the lipid fraction on the interfacial properties of commercially
315 available cocoa material was quantified by analysis of the surface tension of
316 aqueous dispersions of both untreated and delipidified cocoa material. We found
317 that the lipid present in cocoa contributed to the surface activity of the dispersions.
318 This is consistent with the established behaviour of phospholipid as a surfactant.
319 However, extracting the lipids decreased the surface tension to values comparable
320 to the cocoa powder with the originally smallest lipid fraction, (CP-l), which was
321 still capable of driving the formation of an emulsion. The aqueous dispersions of
322 delipidified cocoa material were still surface active which may explain why
323 emulsions could be formed by the delipidified cocoa. However, the stability of
324 particle stabilised emulsions is not reliant upon particle adsorption at the interface
325 impacting the interfacial tension as dispersions with known emulsifying ability
326 have been shown not to decrease interfacial tension (Rana et al., 2012, Tzoumaki
327 et al., 2011).
328

329 **Conclusion**

330 The evidence from this study indicates that several phospholipids found in lecithin
331 and other known surfactant mixtures are present in cocoa material. Crucially,
332 there is evidence that the formation of o/w emulsions based on cocoa material is
333 not only driven by these molecules but also by other biomolecules that do not
334 dissolve in organic solvents or water. This observation raises a number of
335 questions. Naturally, what this component is and how it works are important but
336 also where else such components are found and how they may be developed for
337 commercial use. Further research is required to characterise this emulsifier
338 properly. Cocoa is a heterogeneous material containing lipids, polyphenol,
339 proteins, starch and lignin, all of which have known emulsifying ability. We
340 suggest that the next step in elucidating the behaviour of these systems is to
341 evaluate role of these components on the emulsifying ability of the cocoa
342 material. The results of such a study would shape efforts in identifying other

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naturally occurring material with emulsifying ability as well as preparing emulsifying material from natural material.

Conflict of Interest

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Acknowledgements

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Author Contributions

BW wrote the original grant proposal. JG and BW conceived the research project and designed surface tension experiments. JG carried out all surface tension experiments and isolated lipid samples. SF supervised lipid isolation, ran NMR spectroscopy experiments and analysed NMR data. JG drafted the manuscript; all authors were involved in revising it; BW and SF supervised its preparation. All authors (JG, BW, SF) have approved and are accountable for the final version of the manuscript submitted.

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Table 1: Cocoa material investigated for impact of lipid composition on emulsifying ability. The sample code signifies whether the cocoa material is a cocoa powder (CP) or a cocoa fibre (CF) with the lipid content indicated for the CP/s as l = low, m = medium and h = high. The second column shows the sample coding used in previous work (Gould et al., 2013).

Sample code	Sample description	Total lipid fraction according to supplier specification (% (w/w))	Equivalent sample in (Gould et al., 2013)
CP-l	Fat-reduced alkalised cocoa powder	<1	CP1(1)
CP-m	Medium brown alkalised cocoa powder	10-12	CP5(10-12)
CP-h	Medium brown alkalised cocoa powder	20-22	CP10(20-22)
CF	Cocoa fibre	5	CF(5)

Table 2: Particle size ($d_{3,2}$) of aqueous dispersions of untreated and delipidified cocoa material. CP-l, m and h indicates the low, medium and high lipid content of the original cocoa powders respectively. The effect of lipid extraction on particle size was statistically tested per cocoa material type. The presence of an asterisk (*) indicates the particle size of the delipidified material was significantly different ($p<0.05$) to the untreated material. Differences between the types of cocoa material were not evaluated.

Type of cocoa material	**Particle size ($d_{3,2}$) (µm)**	
	untreated	**delipidified**
CP-l	9.58 ± 0.24	9.90 ± 0.37
CP-m	10.58 ± 0.13	8.02 ± 0.76*
CP-h	13.66 ± 1.03	8.27 ± 0.31*
CF	16.87 ± 0.57	18.28 ± 1.69

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Table 3: Surface tension of aqueous dispersions of untreated and delipidified cocoa material. CP-l, m and h indicate the low, medium and high lipid content of the original cocoa powders respectively. Mean surface tension values after 600 s of measurement at 20 °C are presented. The different letters represent a significant difference ($p<0.05$).

Type of cocoa material	Surface tension ($mN.m^{-1}$)
CP-l	47.82 ± 0.13^c
delipidified CP-l	48.84 ± 0.00^c
CP-m	44.43 ± 0.73^b
delipidified CP-m	50.35 ± 0.66^c
CP-h	40.78 ± 0.59^a
delipidified CP-h	50.05 ± 0.91^c
CF	37.99 ± 0.87^a
delipidified CF	48.34 ± 2.59^c

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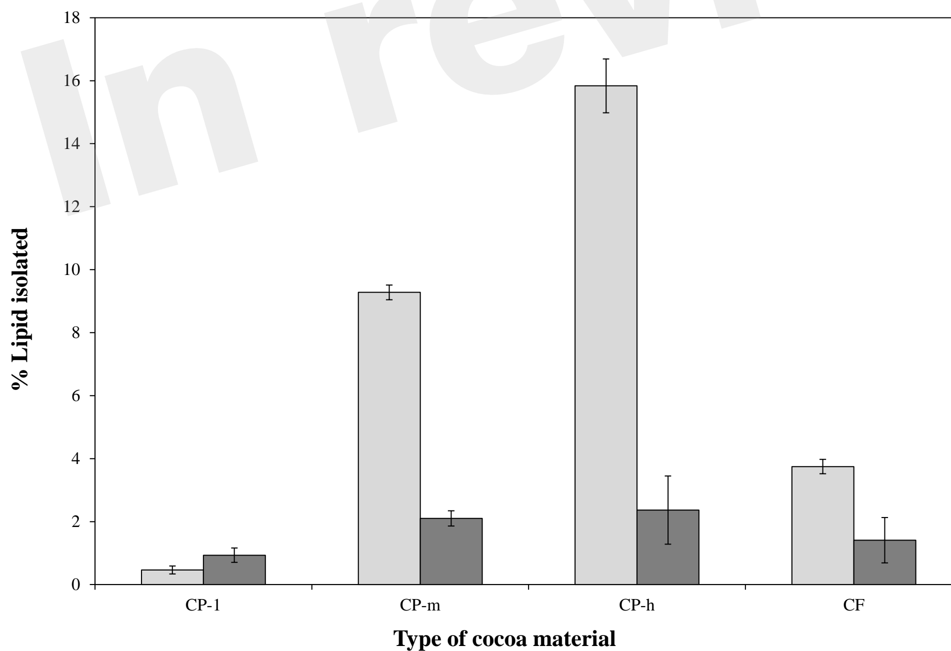

Figure 1: Relative sizes of the phospholipid and triglyceride fractions of four cocoa materials. The percentage of lipid recovered was based on initial dry sample weight and the triglyceride (TAG, ☐) and phospholipid (■) proportions of the lipid recovered are shown. CP-l, m and h indicate the low, medium and high lipid content of the original cocoa powders, respectively. The error bars represent standard deviation.

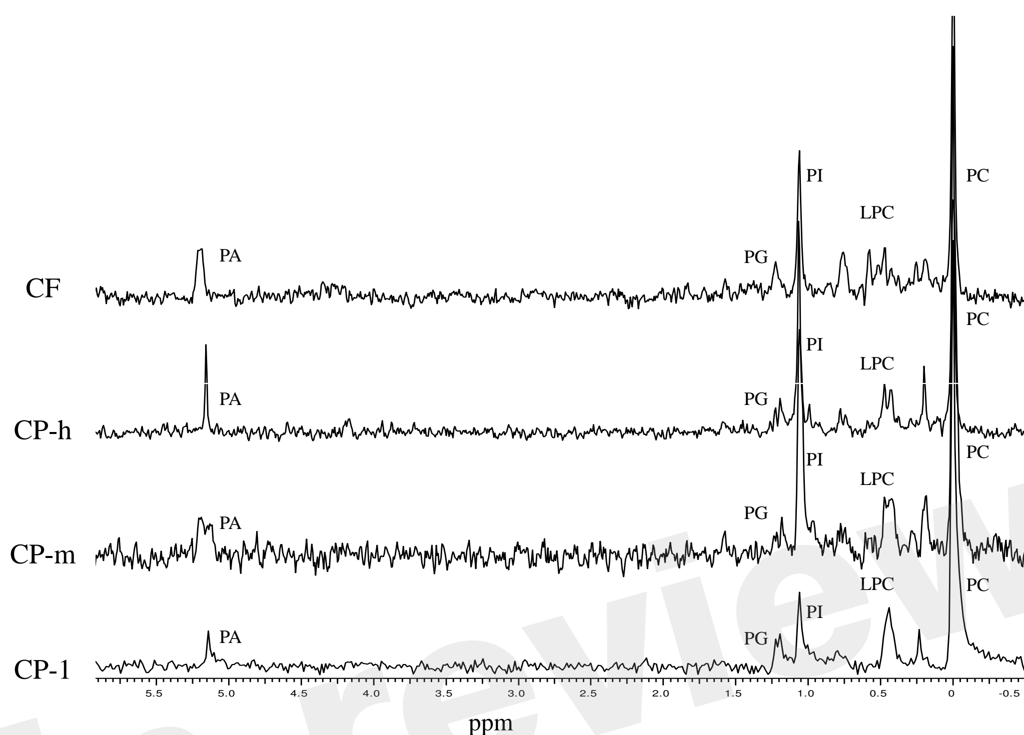

Figure 2: ^{31}P NMR spectra of the phospholipid fraction of the cocoa lipid extracts acquired by CET (chloroform, ethanol and triethylamine) solvent extraction. The identity of the phospholipids was established using reported shifts of phosphorous resonances for phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylinositol (PI), lyso-phosphatidylcholine (LPC) and phosphatidylcholine (PC). Assignments made by assigning the PC resonance to 0·00 ppm and measuring the relative shift of each resonance and comparing with literature values (Furse et al., 2013, Cremonini et al., 2004, Furse et al., 2015b). CP-l, m and h indicate the low, medium and high lipid content of the original cocoa powders respectively.

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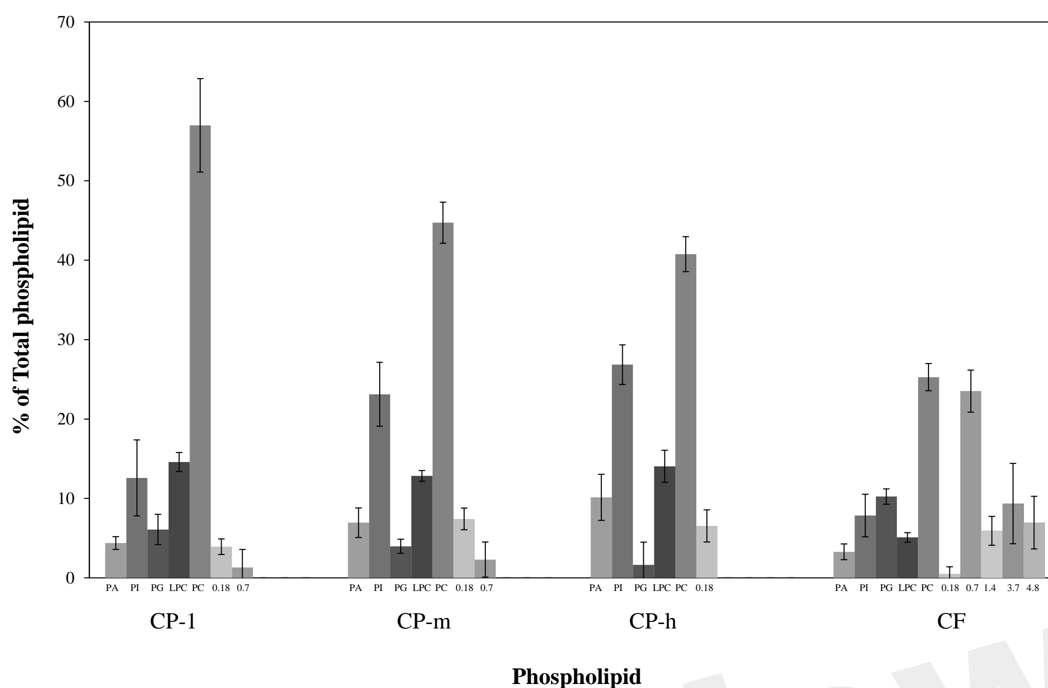

Figure 3: Profiling of phospholipid fraction of four cocoa materials using ^{31}P NMR. Abbreviations: Phosphatidic acid (PA), Phosphatidylglycerol (PG), Phosphatidylinositol (PI), Lyso-phosphatidylcholine (LPC) and Phosphatidylcholine (PC). The contribution of the total phospholipid which was not identified is presented by the chemical shift (ppm). CP-l, m and h indicate the low, medium and high lipid content of the original cocoa powders respectively. Error bars denote standard deviation.

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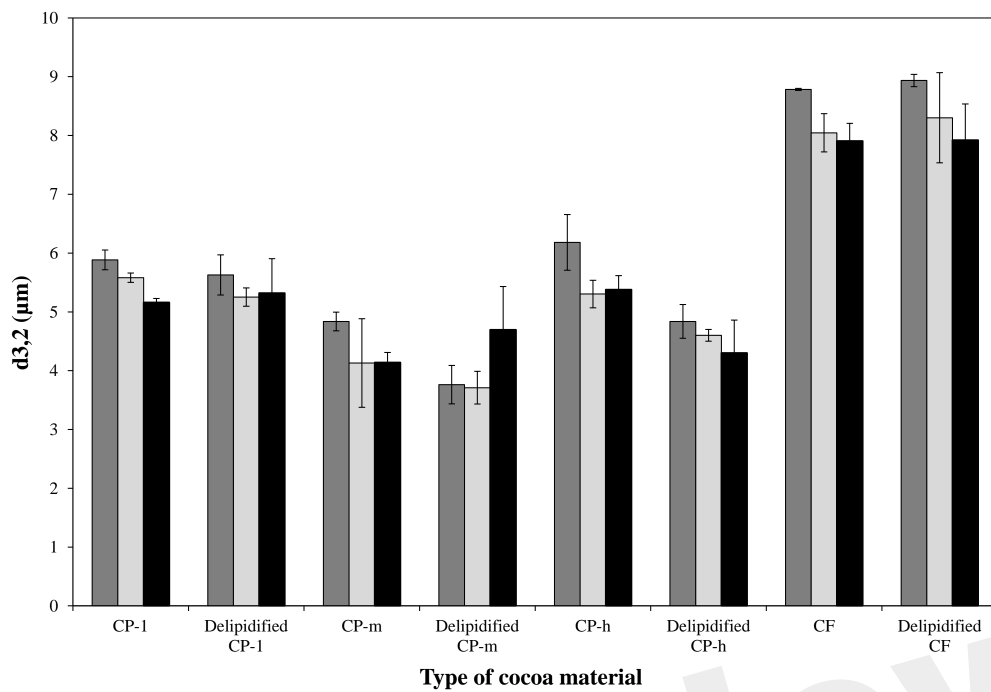

Figure 4: Droplet diameter of emulsions stabilised with untreated and delipidified cocoa material measured after 1 day (■), 53 days (□) and 100 days (■) of storage at 20 °C. CP-l, m and h indicate the low, medium and high lipid content of the original cocoa powders respectively. The error bars denote standard deviation.

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