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STRUCTURE AND OXIDATIVE STABILITY OF OIL IN WATER EMULSIONS AS AFFECTED BY RUTIN AND HOMOGENIZATION PROCEDURE

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Abstract

The structural properties of oil-in-water (O/W) emulsions, as well as their oxidative stability upon storage at 50ºC, were studied. Eight different formulations were prepared with the aim of studying the effect of three variables - the composition of the oil phase, the presence of the flavonoid rutin and the homogenization procedure - on the structure and the oxidative stability. It was found that high pressure homogenization, through droplet size reduction, stabilized the emulsions both against creaming and oil oxidation. The interfacial protein was also partially replaced by rutin, further improving the stability of the emulsions, whereas purification of the oil phase had hardly any effect. Thus, the structural and oxidative stability of emulsions was controlled by the size of the droplets and improved by the addition of rutin.

Keywords: oil oxidation, O/W emulsions, sunflower oil, rutin, flavonoid

1. INTRODUCTION

Oil-in-water (O/W) emulsions constitute a very common structure in food products, including sauces, soups and beverages (Cheng, Y., Xiong, Y.L., & Chen, J., 2010). Being rich in oxidizable lipids, emulsions are prone to lipid oxidation, which is frequently addressed by the incorporation of antioxidant agents. The choice of an appropriate antioxidant compound to be used in this type of system is not easy, since their effectiveness in emulsions has proven to be very different than in bulk oils (Dimattia, C.D., Sacchetti, G., Mastrocola, D., & Pittia, P., 2009). This fact has been attributed to the occurrence of interfacial phenomena (Frankel, E., Huang, S. W., Kanner, J., & German, J. B.,

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1994). Whereas in bulk oils hydrophilic antioxidants locate preferentially at the oil-air interface and better protect the oil from oxidation, in O/W emulsions lipophilic antioxidants concentrate at the oil–water interface and inhibit lipid oxidation more effectively than hydrophilic antioxidants that partition into the water phase (Porter, W.L., 1993; Porter, W. L., Black, E. D., & Drolet, A. M., 1989). Further examples illustrating this so-called “polar paradox” can be found in Frankel, E.N., & Meyer, A.S. (2000).

According to Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F., & Liu, F. (2010), synthetic antioxidants have widespread use as food additives in many countries. However, natural antioxidants are now preferred over synthetic, the latter imposing potential health problems (Hou, D. X., 2003; Prior, R. L., 2004). In the recent years, natural compounds with antioxidant capacity, such as tocopherols and flavonoids, have attracted an increasing interest (Erkan, N., Ayranci, G., & Ayranci, E., 2008). Rutin is a flavonoid comprised of the flavonol quercetin and the disaccharide rutinose, whose antioxidant activity in vitro is comparable to that of ascorbic acid (Yang, J., Guo, J., & Yuan, J., 2008) and higher than that of alpha-tocopherol (Frankel, E.N., & Meyer, A.S., 2000). Furthermore, this flavonoid has been described as having “good emulsifying activity”, meaning that rutin particles stabilized Pickering emulsions in which the individual droplets were too small to distinguish by the naked eye and no observable oiling off occurred after one week storage (Luo, Z., Murray, B.S., Yusoff, A., Morgan, M.R.A., Povey, M.J.W., & Day, A.J., 2011).

A wide range of factors, other than the presence of antioxidant agents, have been signalled as affecting the lipid oxidation process in O/W emulsions. According to Lethuaut, L., Metro, F., & Genot, C. (2002), lipid oxidation in emulsified systems is mainly influenced by the properties of the interface. Apart from the characteristics of the interfacial layer (composition, thickness and charge), the concentration and physical state of the dispersed phase, as well as the size of droplets determine the extent of the oxidation process (Frankel, E.N., & Meyer, A.S., 2000; Lethuaut, L., Metro, F., & Genot, C., 2002; McClements, D. J., & Decker, E. A., 2000). Other factors to be taken into consideration are the composition of the oil phase (greatly determining its liability to peroxidation) and the presence of proteins and peptides, which have shown to inhibit oxidation in this type of systems (Elias, R.J., McClements, D.J., & Decker, E.A., 2007). According to Hu, M., McClements, D. J., & Decker, E. A. (2003), whey proteins have been found to inhibit lipid oxidation in O/W emulsions when they are either at the emulsion droplet surface or in the aqueous phase. A better understanding of

how whey protein can influence oxidative reactions could help in the development of new antioxidant strategies for O/W emulsions.

The aim of this study was the evaluation of the structure and oxidative stability of O/W emulsions formulated with whey protein and sunflower oil. The process variables were the purification of the oil phase, the presence of the flavonoid rutin and the homogenization procedure, which directly affects the droplet size distribution.

2. MATERIALS AND METHODS

2.1. Materials

Sunflower oil, of a brand known to lack added antioxidants, was purchased in a local market. Commercial whey protein isolate (BiPro) was obtained from Davisco Foods (MN, USA) and contained 97.7% protein, 0.3% fat, 1.9% ash and 4.8% moisture. Rutin (HPLC grade), alumina, isooctane, acetic acid glacial, potassium iodide, ethanol and thiobarbituric acid were obtained from Sigma-Aldrich. Aldrich supplied the 1,1,3,3 tetraethoxypropane. Sodium thiosulfate and starch solution were purchased from Fluka. BDH provided the trichloroacetic acid (TCA). Acros Organics supplied the potassium iodate and Fisher provided methanol, hexane, potassium hydroxide and hydrochloric acid. FSA supplies provided NaCl and KH_2PO_4. Riedel de Heen supplied the Na_2HPO_4. Xanthan gum (KELTROL, food grade) was obtained from CP Kelco (USA).

2.2. Removal of natural antioxidants in the sunflower oil

In order to test the role of the natural antioxidants in sunflower oil against the progression of rancidity, some of the emulsions were prepared with purified sunflower oil (PSO). This purification was attained by forcing the oil through an open column containing aluminium oxide which had been previously dried at 200ºC for 5 hours. The flow was accelerated with compressed air. The same procedure has been utilised for the purification of sunflower oil (Almajano, M.P., Delgado, M.E., & Gordon, M.H., 2007; Yoshida, H., 1993; Kiokias, S., & Oreopoulou, V., 2006) and others (Kiokias, S., & Gordon, M. 2003).

2.3. Preparation of the O/W emulsions

The aqueous phases of all emulsions were based on buffered phosphate solutions (pH = 7) prepared with Milli-Q water. When added, rutin (1 mM in the aqueous phase) was dispersed using an ULTRA-TURRAX T25 mixer (Janke & Kunkel, IKA-Labortechnik) at 13500 rpm for 3 min. Whey protein (1.5 % in the aqueous phase) was dissolved in buffer by magnetic stirring. Sunflower oil (either untreated or purified) was used to prepare O/W emulsions with an oil: aqueous volume ratio of 1:4. Two different homogenization procedures were performed. Coarse emulsions were prepared also by means of the ULTRA-TURRAX mixer, working at 13500 rpm for 3 min. Fine emulsions were prepared by passing coarse emulsions through a high pressure jet homogenizer (Burgaud, I., Dickinson, E., & Nelson,P.V., 1990) operating at 300 bar. Table 1 summarizes the experimental design followed and the codes used for all emulsions.

2.4. Structural studies: droplet size characterization, physical stability and confocal microscopy

In order to assess the effect of the three variables on the emulsion microstructure, the droplet-size distributions of the emulsions were measured by static multiangle light scattering via a Mastersizer Hydro 2000 (Malvern Instrument, Malvern, UK). Average droplet sizes were characterized in terms of the Sauter mean diameter d_{32} or volume mean diameter d_{43} defined by:

$$d_{ab} = \frac{\sum_i n_i d_i^a}{\sum_i n_i d_i^b} \qquad \text{Equation 1}$$

where n_i is the number of the droplets of diameter d_i. All measurements were made in triplicate at room temperature. The refractive indices of water and sunflower oil were taken as 1.330 and 1.429, respectively.

One month after their preparation, images of test tubes filled with the emulsions were obtained with a Canon EOS 400D Digital SLR camera, operated without flash and in close up mode. For microscopy of the emulsions, a Leica TCS SP2 confocal laser scanning microscope (CLSM), mounted on a Leica Model DM RXE microscope base, was operated in fluorescence mode. Approximately 80 µL of sample were placed into a laboratory made welled slide, filling it completely. A

coverslip (0.17 mm thickness) was placed on top of the well, ensuring that there was no air gap (or bubbles) trapped between the sample and coverslip. The samples were scanned at 24 ºC, using 10X or 40X oil-immersion objective lenses, of numerical apertures of 0.3 and 1.25, respectively, approximately 10 to 20 μm below the level of the coverslip, in order to minimize hydrodynamic (and other) interactions with the coverslip. Fluorescence from the sample was excited with the 488 nm Ar and 633 nm HeNe laser lines. Images were recorded at a resolution of 1024 X 1024 pixels. In most cases up to 0.05 wt.% xanthan gum was added to the samples prior to microscopy to try and prevent creaming of the larger droplets during the time-scale of the examination.

2.5. Studies on oxidative stability

Immediately after preparation, the emulsions were poured into sealed glass jars and stored in an incubator at 50°C to accelerate their oxidative rancidity. In order to assess this chemical process, it is recommended to use at least two different types of assay (Moon, J.K., & Shibamoto, T., 2009): one to monitor the early stage of peroxidation (primary products) and other to monitor the final state (secondary products). Every 7 days, the extent of primary oxidation was evaluated by performing a iodometric assay. After one month storage period, the extent of secondary oxidation was quantified by determining the content in thiobarbituric acid reactive substances (TBARS).

2.5.1. Iodometric assay

The progression of the primary stage of oil oxidation was monitored by determining the peroxide value (PV) using a modification of the method described in the norm UNE 55-023 (Atarés, L., Bonilla, J., & Chiralt, A., 2010). Before sampling, the emulsions were shaken vigorously manually then a 2.5g sample of emulsion was weighed out. Each sample was dissolved in 20ml of a 60:40 (v/v) mixture of acetic acid glacial and isooctane. An aliquot (500μl) of saturated KI solution was added, and after one minute the sample was titrated with sodium thiosulfate solution (0.002 mol dm^{-3}), using starch solution as indicator. The sodium thiosulfate solution was previously standardized as described by the method. All emulsions were tested in triplicate, and the PV was expressed as mEq of oxygen per kilogram of emulsion.

2.5.2. TBARS

The method described by Dimattia, C.D., Sacchetti, G., Mastrocola, D., & Pittia, P. (2009) was slightly modified for the quantification of the 2-thiobarbituric reactive substances (TBARS) in the emulsions at the end of the storage period. Three 1ml aliquots per emulsion were mixed with 2ml of TBA reagent (15%w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25M HCl) in test tubes. These were incubated in a boiling water bath for 30 minutes and cooled down under running tap water for 5 additional minutes. A clear liquid phase was obtained by centrifugation (Allegra X-22 Series centrifuge, Beckman Coulter, Inc., Fullerton, CA, USA) at 4200rpm for 30 min. Finally, the absorbance at 532nm was measured against TBA reagent as the blank, using a spectrophotometer (CECIL, CE 3021, Cecil Instruments, UK). The concentration of TBARS was determined from a standard curve prepared with 1,1,3,3 tetraethoxypropane in concentrations ranging between 2.96 $x10^{-8}$ and 2.96 $x10^{-6}$ g ml^{-1}. The results are reported as concentrations of malonaldehyde (MA) in μM.

2.6. Statistical analysis

The statistical analysis of the data was performed through analysis of variance (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, Md.) Fisher's least significant difference (LSD) procedure was used.

3 RESULTS AND DISCUSSION

3.1. Structural studies: droplet size characterization, physical stability and confocal microscopy

Figure 1 shows typical droplet size distributions of all eight emulsions, and Table 1 gives the mean diameters d_{32} and d_{43}, averaged from at least four measurements. The droplet size distributions of the coarse emulsions overlap and all show a large peak at a size of around 70μm (similar to the $d_{4,3}$ values in Table 1). A smaller population of particles with peak at around 10μm was also observed in the coarse emulsions. This latter peak probably corresponds to free rutin particles, since the Mastersizer cannot distinguish between particles of different nature. The fine emulsions produced via the jet homogenizer showed a particle size distribution centred in a much smaller size range, due to the much higher shear forces during homogenization. Similar results were found by Perrier-Cornet,

J.M., Marie, P., & Gervais, P. (2005) when investigating the effect of different homogenization procedures on the microstructure of their emulsions. The particle size distributions for the fine emulsions were broader than those found for the coarse emulsions. However, this again may be due to the inability to distinguish unequivocally between droplets and rutin particles if the sizes of the droplets and the rutin particles overlap more.

The results of the average diameters d_{32} and d_{43} were consistent with the size distribution curves. When submitted to a multifactorial ANOVA analysis, it was found that both d_{32} and d_{43} were significantly affected by the homogenization procedure ($p<0.05$), whereas the presence of rutin did not affect these parameters significantly ($p>0.05$). Consequently, it may be concluded that the dominant emulsifying component is the whey protein and not the rutin. This makes sense because the protein is present at a higher concentration than the rutin and protein molecules will tend to adsorb more quickly than the much larger rutin particles.

As a direct consequence of the larger droplet size in the coarse emulsions, these creamed significantly within just a few minutes after preparation or manual shaking. In contrast, the fine emulsions showed no creaming and remained structurally stable over the period of oxidation (one month). Figure 2 shows the appearance of all eight formulations one month after their preparation, after the accelerated rancidity test was over. The greater droplet size reduction accomplished with high pressure homogenization resulted in significant whitening of fine emulsions as compared to the coarse emulsions. The natural colour of rutin imparts a yellowish-green colour, especially noticeable in the lower aqueous phase of the coarse formulations. Rutin is only very slightly soluble in water and in the coarse emulsions some flavonoid particles could be seen to settle to the bottom of the aqueous phase. For the most part, purification of the oil had no significant effect on d_{32} or d_{43}. The exception is the case of fine emulsions prepared with added rutin where smaller droplets were obtained with the purified oil. The purification procedure used will remove any low molecular weight polar species such fatty acids and monoglycerides that also tend to have some surface activity. At low to intermediate levels, such species can compromise the ability of proteins to act as stabilizers by causing their partial displacement from the O-W interface (Murray, B. S., Færgemand, M., Trotereau, M. & Ventura, A., 1998; Mackie, A. & Wilde, P., 2005). This may possibly explain the slightly more efficient emulsification here for the purified oil, in terms of slightly lower droplet size. However, there could also be competition between such species and proteins for adsorption to the rutin particles. How this might

affect the state of dispersion of the rutin particles and subsequent stabilization of the oil droplets has not yet been investigated.

Whey protein proved to be effective in stabilizing all emulsions against coalescence, with and without rutin, since oil separation was never observed, at least within the time-scale of the experiment (1 month), despite the large size of some of the oil droplets and their rapid creaming. The coarse emulsions containing rutin gave slightly thicker cream layers than those without the flavonoid, probably due to the slightly smaller droplets formed when rutin was present (see Table 1), indicating that the presence rutin did exert some effect on the emulsion stability. At least some rutin adsorption might be expected to take place, since once it is adsorbed at O-W interface it is very difficult to remove from it and very coarse emulsions stabilized by rutin alone can be very stable to flocculation and coalescence, as shown by Luo, Z., Murray, B.S., Yusoff, A., Morgan, M.R.A., Povey, M.J.W., & Day, A.J., (2011). These authors measured the octanol-water partition coefficients (*P*) of several flavonoids and found a $\log_{10}P$ value of −0.27 for rutin, partly explaining why rutin particles might have amphiphilicity and therefore some surface activity, whereas flavonoids with very high or low $\log_{10}P$ would tend to be very oil-soluble or water-soluble, respectively, and therefore not surface active. On the other hand, it was also shown that there are exceptions to this rule, partly because molecular solubility (as indicated by $\log_{10}P$ values) is not necessary a good indicator of the hydrophobicity of *particles* of insoluble flavonoid. Recent work (Luo, Z., Murray, B.S., Ross, A.-L., Povey, M.J.W., Morgan, M.R.A., & Day, A.J., in press) has shown that other factors such as pH and salt concentration can have significant effects on the emulsion-stabilizing properties of rutin and other flavonoids of widely varying $\log_{10}P$ values.

In order to corroborate the hypothesis that at least some significant degree of rutin adsorption takes place, even in the presence of whey protein, detailed CSLM examination of the samples was carried out. Figure 3 shows images of emulsions prepared with SO once the oxidation experiment was over (corresponding images of emulsions prepared with PSO were very similar). The brightness in the images is due to the autofluorescence of the flavonoid. Figure 3a (SO/R/F) shows how the fine oil droplets were surrounded by a dense layer of rutin particles, confirming the preferential location of the flavonoid at the O-W interface. In addition, some rutin particles were dispersed in the aqueous phase. Some droplets also appeared as not completely spherical, which is another sign of aged

238 particle-stabilized (Pickering) emulsions, where the particle layer is very rigid (Dickinson, E., 2010;
239 Murray, B. S., Durga, K., Yusoff, A. & Stoyanov, S. D., 2011).

240 Figure 3c for the coarse emulsions (SO/R/C) reveals a large number of very bright larger
241 objects, most likely rutin particles that have not been well dispersed, whilst oil droplets do not show
242 very clearly. Some weak intensity around the perimeter of the droplets may be due to
243 autofluorescence of adsorbed protein and/or scattering of light from the droplets, as distinct from the
244 very much brighter perimeter with the fine emulsions due to an adsorbed layer of rutin particles, which
245 will also be much thicker. Passage through the jet homogenizer will have the effect of breaking up
246 both the oil and the flavonoid into smaller particles than in coarse emulsification and this will aid the
247 formation of adsorbed rutin layers as the droplets are formed. It is even possible that the whey protein
248 adsorbs to the rutin particles (see above) and high pressure homogenization aids the formation of a
249 finer dispersion of rutin particles that has a greater capability for competing with or co-absorbing with
250 protein for the O-W interface. Either way, coverage of droplets by rutin is clearly more efficient when
251 higher shear is applied.

252 In the case of emulsions without rutin, only a weak intensity around the droplets is again
253 observed (due to adsorbed protein autofluorescence) because there is no layer of adsorbed rutin
254 particles to highlight the interface. The appearance of the fine emulsions in the absence of rutin
255 (Figure 3b, SO/-/F) is in particular in marked contrast to that of the fine emulsion in the presence of
256 rutin (Figure 3a, SO/R/F). The dark regions are xanthan-rich regions, which slowly phase separate
257 from the emulsion phase on standing (Moschakis, T., Murray, B. S. & Dickinson, E., 2005). In the
258 case of the coarse emulsions without rutin (Figure 3d, SO/-/C) hardly any oil droplets visible, due to
259 their rapid creaming to the top of the sample well even in the presence of xanthan as thickening
260 agent.

261

262 **3.2. Studies on oxidative stability: iodometric assay and TBARS**

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264 Figure 4 shows the increase of PV of the emulsions prepared with sunflower oil (Figure 4a)
265 and purified sunflower oil (Figure 4b) as the storage time progressed. Figure 5 shows the standard
266 curve of the TBARS test where a close linear fit (R^2>0.999) of A_{532} *versus* concentration of 1,1,3,3

267 tetraetoxypropane can be observed. The final values of TBARS of the emulsions (expressed as μM
268 malonaldehyde) are also shown.

269 According to these data, lipid oxidation occurred to a higher extent in the coarse emulsions as
270 compared with the fine emulsions, a tendency that was noticeable both in the presence and in the
271 absence of rutin. At a fixed oil concentration, total droplets surface increases as each droplet diameter
272 decreases, and therefore the rate of lipid oxidation is expected to increase (Nakaya, K., Ushio, H.,
273 Matsukawa, S., Shimizu, M., & Ohshima, T., 2005). This general trend is justified by Azuma, G.,
274 Kimura, N., Hosokawa, M., & Miyashita, K. (2009) through an increase in the opportunity for the
275 attack by oxidation inducers such as free radicals or metal ions on the lipids at the interface. However,
276 this tendency can be modified, or even inverted, owing to the specific characteristics of the emulsion
277 and the protective ability of the interface against oxidation, as found in a number of studies. Azuma,
278 G., Kimura, N., Hosokawa, M., & Miyashita, K. (2009) found both (opposite) types of behaviour with
279 two different types of O/W emulsions (formulated with soybean and fish oils), and attributed the
280 results to the differences in the interfacial conformation of lipids. Nakaya, K., Ushio, H., Matsukawa,
281 S., Shimizu, M., & Ohshima, T. (2005) studied the oxidation process of O/W emulsions and also found
282 that the fine emulsions were more stable to oxidation. They suggested that the location of emulsifier
283 molecules at the O/W interphase may influence the mobility of the lipid molecules and may
284 consequently improve oxidative stability. They estimated that the actual concentration of emulsifier on
285 smaller droplets was 10 times higher than that on the larger droplets and consequently, the
286 concentration of unsaturated oil in a smaller droplet becomes lower and therefore lipids in the
287 emulsion become more stable against oxidation. Thus, the emulsifier plays a key role when it comes
288 to the effect of droplet size on oxidation kinetics. Lethuaut, L., Metro, F., & Genot, C. (2002) prepared
289 O/W emulsions stabilized by bovine serum albumin and found that fine emulsions suffered faster
290 oxidation, i.e. this protein did not have a protective effect on the oil. In the present study, whey protein
291 may have been an active protective agent against lipid oxidation, as previously observed by Hu, M.,
292 McClements, D. J., & Decker, E. A. (2003) and Tong, L. M., Sasaki, S., McClements, D. J., & Decker,
293 E. A. (2000) on salmon oil-in-water emulsions.

294 In order to corroborate our qualitative observations, the set of data obtained at week four
295 (both of PV and TBARS) were submitted to a factorial ANOVA analysis. Amongst the three
296 independent variables (droplet size, rutin and purification of the sunflower oil), droplet size was the

297 factor having the most significant effect on PV ($p<0.05$), followed by the presence of rutin. The oil
298 purification did not have any significant impact on the results and no significant interactions between
299 the variables were observed in the case of PV results, whereas for TBARS quantification, a
300 statistically significant interaction ($p<0.05$) between the droplet size and the presence of rutin was
301 found.

302 Emulsions with rutin were better protected against lipid oxidation than those without the
303 flavonoid, as shown in Figures 4 and 5. The antioxidant properties of rutin *in vitro* were studied
304 through different assays by Yang, J., Guo, J., & Yuan, J. (2008). They found that rutin exhibited
305 strong DPPH radical scavenging activity, similar to that of ascorbic acid. They used egg yolk
306 homogenates as lipid-rich media and performed the TBARS assay to find that the inhibition of lipid
307 oxidation increased with the increased concentration of rutin. The percentage inhibition in lipid
308 peroxidation caused by 0.5mg/ml rutin was 69% whereas that of ascorbic acid at the same
309 concentration was only 26%. Although no studies have been found where this flavonoid has been
310 utilised in O/W emulsions with the aim of protecting them from lipid oxidation, it seems plausible that
311 the antioxidant behaviour observed *in vitro* be the mechanism behind our results for emulsions.
312 Furthermore, as described in section 3.1., rutin tends to accumulate on the oil-water interface,
313 providing a particularly effective protective barrier against lipid oxidation. Tests on the effect of
314 homogenizing the rutin itself suggest no other change in its properties other than a temporary
315 decrease in the rutin particle size, before it slowly re-aggregates (Luo, Z., Murray, B.S., Ross, A.-L.,
316 Povey, M.J.W., Morgan, M.R.A., & Day, A.J., in press). However, creation of finer emulsions via the
317 jet homogenizer in the presence of rutin produces droplets better covered in rutin, which are therefore
318 better protected.

319

320 **4. CONCLUSIONS**

321

322 The properties of O/W emulsions formulated with sunflower oil and whey protein were
323 significantly affected by the homogenization procedure. Coarse emulsions showed creaming, but
324 were structurally stable over one month storage at 50ºC. Emulsions stabilized by whey protein and
325 rutin seemed to be particularly stable to coalescence. For such systems CLSM images revealed that
326 a significant proportion of the rutin became adsorbed to the O-W interface, either partially replacing

the protein or co-adsorbing with it. Higher shear emulsification increased the tendency for rutin particle adsorption. Purification of the oil phase prior to emulsification did not affect the stability of the emulsions significantly. Fine emulsions in the absence of rutin were more resistant to lipid oxidation than coarse emulsions, possibly due to the specific protective characteristics of interfacial whey protein. However, the higher stability for fine emulsions of purified oil was even more marked when rutin was also present, possibly due to improved coverage of the droplets with particles of the antioxidant flavonoid. Oxidation was always lower in the presence of rutin for the other emulsions, although the difference was not always statistically significant. Nevertheless, this initial study suggests that one might be able to control the oxidative stability of vegetable oil O/W emulsions by adjusting the size of the droplets plus the addition of rutin, or particles of some similar flavonoid. This is therefore deserving of further, more detailed study.

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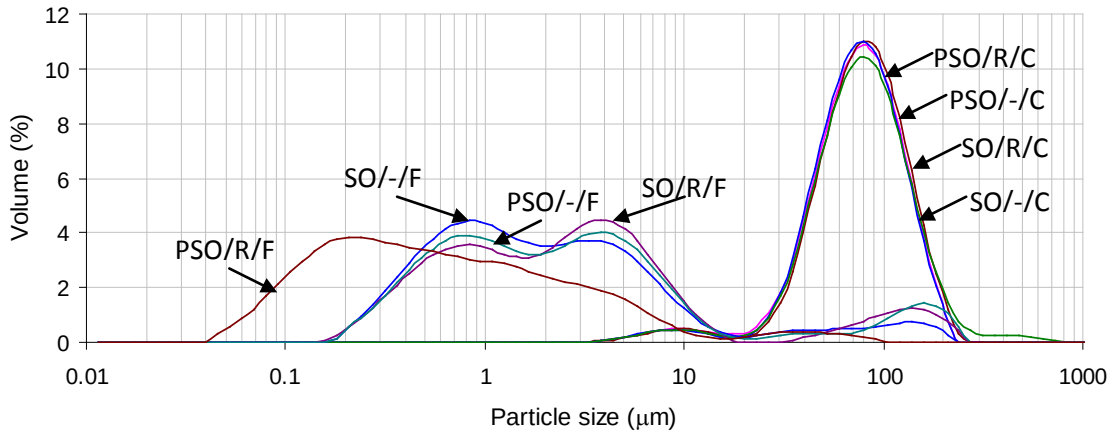
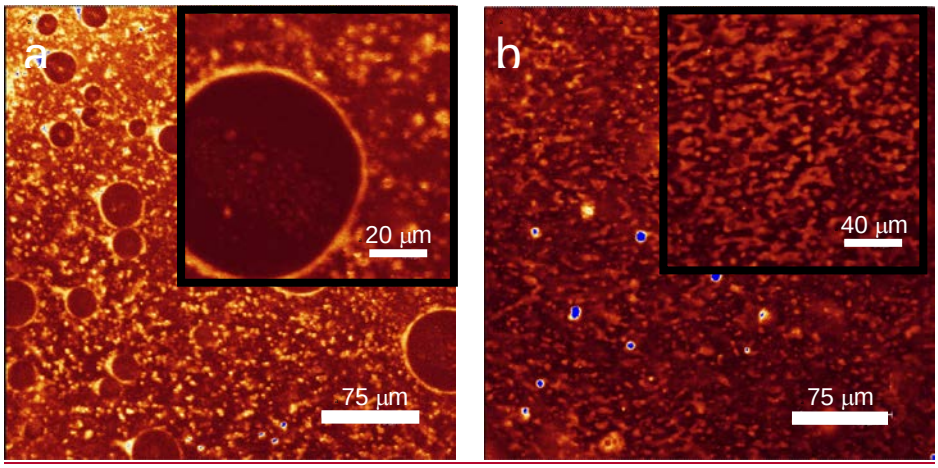


Figure 1: Oil droplet distribution of all emulsions. SO = Sunflower Oil, PSO = Purified Sunflower Oil, R = Rutin, C = Coarse, F= Fine.

Figure 2: Emulsions prepared with sunflower oil (left) and with purified sunflower oil (right) one month after being prepared. SO = Sunflower Oil, PSO = Purified Sunflower Oil, R = Rutin, C = Coarse, F= Fine.



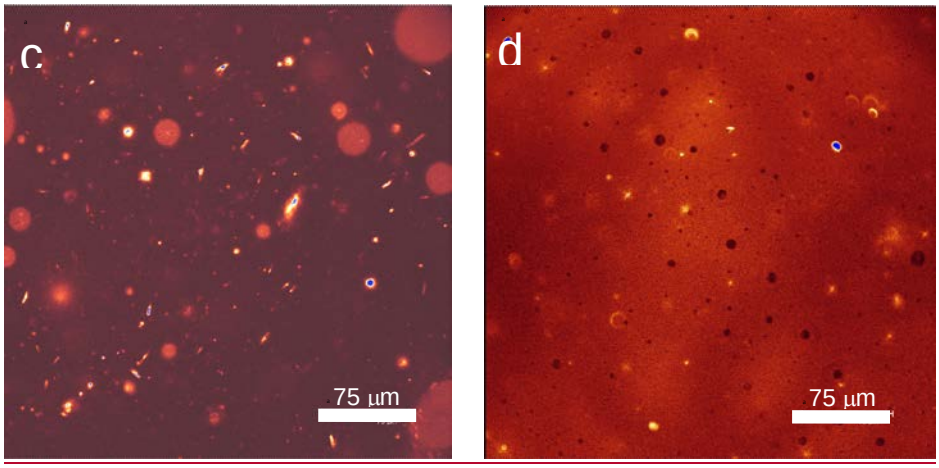


Figure 3: CSLM images of the emulsions made with SO. (a) SO/R/F, with inset at higher magnification; (b) SO/-/F, with inset at higher magnification; (c) SO/R/C; (d) SO/-/C. Blue regions are out of focus and should be ignored.

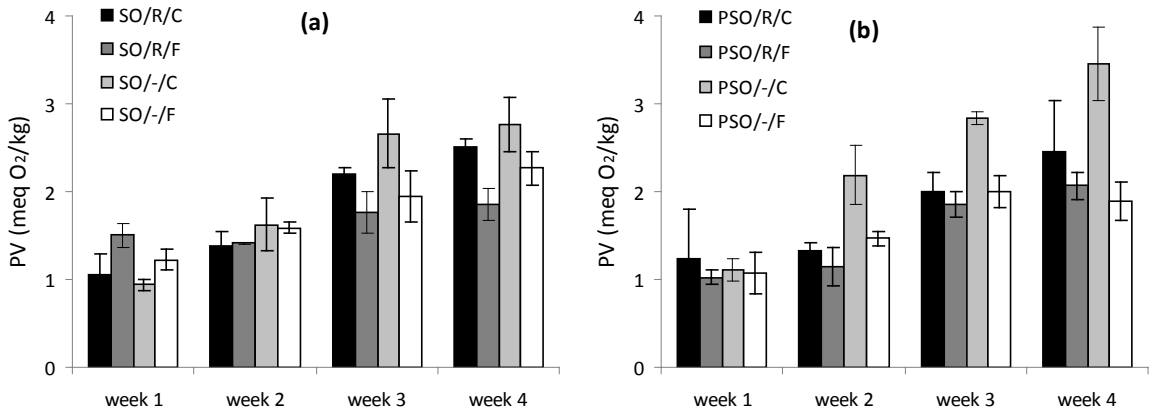


Figure 4: Progression of the peroxide value (meq O_2 per kg emulsion) over storage at 50ºC of emulsions prepared with sunflower oil (a) and purified sunflower oil (b). SO = Sunflower Oil, PSO = Purified Sunflower Oil, R = Rutin, C = Coarse, F= Fine.

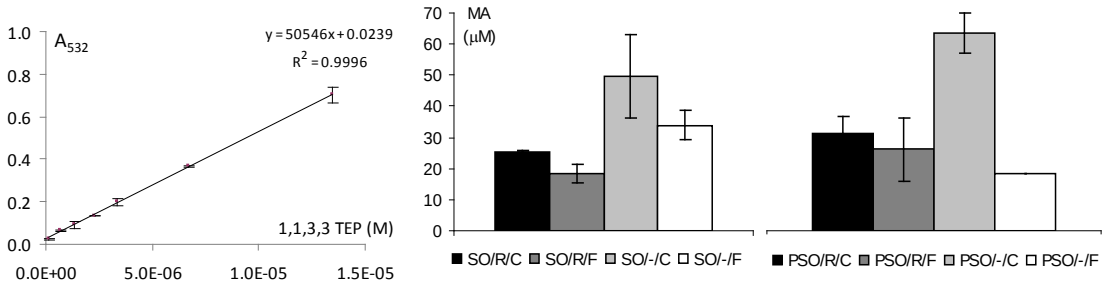


Figure 5: Standard curve for TBARS assay (left), where the absorbance at 532 nm is plotted versus the concentration of the standard 1,1,3,3 tetraetoxypropane (TEP). TBARS content (right), expressed as malonaldehyde (MA) concentration in μM, of the emulsions after 4 weeks storage at 50ºC. SO = Sunflower Oil, PSO = Purified Sunflower Oil, R = Rutin, C = Coarse, F= Fine.

Table 1: Code used to identify the emulsions on the basis of their oil phase, presence of rutin and homogenization procedure. Diameters $d_{3,2}$ and $d_{4,3}$ of the eight emulsions. Average values and standard deviations in brackets. SO = Sunflower Oil, PSO = Purified Sunflower Oil, R = Rutin, C = Coarse, F= Fine.

Code	Oil phase	Rutin	Homogenization	$d_{3,2}$ (μm)	$d_{4,3}$ (μm)
SO/R/C	Sunflower Oil	Yes	Coarse	48 (4)^{b}	77 (9)^{a}
SO/R/F	Sunflower Oil	Yes	Fine	1.10(0.04)^{d}	15 (6)^{c}
SO/-/C	Sunflower Oil	No	Coarse	45 (6)^{c}	68 (7)^{b}
SO/-/F	Sunflower Oil	No	Fine	1.2 (0.2)^{d}	17 (10)^{c}
PSO/R/C	Purified Sunflower Oil	Yes	Coarse	51 (1)^{a}	77 (1)^{a}
PSO/R/F	Purified Sunflower Oil	Yes	Fine	0.27 (0.01)^{d}	1.9 (0.5)^{d}
PSO/-/C	Purified Sunflower Oil	No	Coarse	51 (2)^{a}	77 (3)^{a}
PSO/-/F	Purified Sunflower Oil	No	Fine	1.1 (0.1)^{d}	14 (8)^{c}