# PROPERTIES OF W<sub>1</sub>/O/W<sub>2</sub> EMULSIONS AS POTENTIAL FAT REPLACERS IN MEAT PRODUCTS

R. Bou<sup>1</sup>, S. Cofrades<sup>1</sup> and F. Jiménez-Colmenero<sup>1</sup>

<sup>1</sup>Institute of Food Science Technology, and Nutrition (ICTAN-CSIC). 28040-Madrid, Spain. (e-mail: ricard\_bou@ictan.csic.es)

Abstract – The aim of this study was to characterize and evaluate the possibility of using a food-grade  $W_1/O/W_2$  multiple emulsion as a low-fat food ingredient for the meat industry. Two lipid sources (pork lard and olive oil) were used as the oil phase and the resulting double emulsions (DEs) were subjected to conventional thermal treatment (70 °C for 30 min), and storage at chilling temperatures (4 °C for 1, 6 and 10 days) to determine their influence on oil droplet particle size (d<sub>32</sub>), physical stability and instrumental colour. Results showed that the d<sub>32</sub> was slightly higher when olive oil was used. In addition, the DEs containing olive oil were less stable. The thermal treatment did not affect d<sub>32</sub> although it reduced stability. The physical stability of DEs was mainly affected by storage at chilling stable temperatures. Overall, DEs are to environmental stresses typically occurring in the food industry.

Key Words – double emulsion, multiple emulsions, thermal treatment, stability, physical parameters.

# I. INTRODUCTION

Emulsions have been used in various cosmetic, pharmaceutical and food applications for many years [1]. More recently, series of more complex and structured emulsions have been developed for a more specific and controlled functionalization. One of these is a water-in-oil-in-water ( $W_1/O/W_2$ ) double emulsion (DE) consisting of a water-in-oil ( $W_1/O$ ) emulsion dispersed as droplets within an aqueous phase ( $W_2$ ) [2,3].

This type of emulsion may offer some advantages for food applications. For instance, it has been found to be a potentially useful strategy in masking off-flavours and controlling the release and protection of labile ingredients [4-6]. Moreover, a DE emulsion can be used to reduce the fat content, since part of the lipid material is replaced by water particles dispersed inside it. Therefore, it is possible to use DEs as fat replacers in reformulation processes to produce low-fat (low-calorie) foods with similar physicochemical and sensory properties to full-fat products [2,7,8]. Several foods, such as meat products, contain animal fats (e.g. pork backfat) in their formulation, and this can be replaced in part by a DE in which the same fat is used as the lipid phase. In this way it can be used as a low-fat food ingredient (fat replacers), thus making for healthier meat products. In addition, DEs can be used to modify qualitative aspects of the lipid material in foods by providing healthier fatty acid profiles. Thus, the choice of the most suitable lipid phase in the DE is a promising approach to develop food products more in line with health recommendations.

Several vegetable oils (rapeseed, soybean, corn, etc.) have been used as oil phases to produce foodgrade  $W_1/O/W_2$  emulsions. Of these, olive oil is particularly promising [9,10]. It has a high biological value, attributed to a high ratio of vitamin E to unsaturated fatty acids [11]. It also has beneficial effects on postprandial lipid metabolism and thrombosis and inhibits LDL oxidation [12].

However, DEs are known to be unstable systems with a strong tendency of coalescence, flocculation and creaming [2,4,6]. There is a need to address the characteristics and behaviour of DEs as affected by different processing conditions. This type of information is useful since their physicochemical characteristics affect quality properties of reformulated products.

The objectives of this study, then, were: a) to examine the physicochemical parameters of DEs as affected by two lipid phases (pork lard and olive oil); and b) to examine the effects of conventional thermal processing on physicochemical parameters of those DEs.

# II. MATERIALS AND METHODS

*Preparation of DEs.* A simple two stage procedure was used as reported elsewhere [10]. Briefly, the inner  $(W_1)$  phase consisted of 0.1 M aqueous NaCl.

The outer  $(W_2)$  phase was prepared by dispersing 0.1 M NaCl plus 0.5% sodium caseinate in distilled water. Thereafter, 0.02% sodium azide was added. The oil phase (O), consisted of olive oil or pork lard at 94% plus the lipophilic surfactant polyglycerol polyricinoleate (PGPR) at 6%. A coarse primary emulsion (W<sub>1</sub>/O) was prepared by drop-wise addition of the inner aqueous phase (20%) to the lipid phase (80%). The resulting coarse emulsion was then passed twice through a two-stage high pressure homogenizer at 550/70 bar (Panda Plus 1000, GEA NiroSoavi, Parma, Italy). The DEs were prepared by gradually adding the W<sub>1</sub>/O fine pork lard and olive oil emulsions (40%) to the  $W_2$  phase (60%) mixed in a Thermomix food processor set at 37 °C and 700 rpm. The resulting coarse  $W_1/O/W_2$ emulsions were passed twice through a two-stage high pressure homogenizer at 150/30 bar and, immediately, stored overnight at 4 °C.

*Microscopy*. Optical microscopy was used to examine the emulsion morphology using a Reichert microscope (Munich, Germany) at 40X magnification.

*Particle size characteristics.* Mean particle size  $(d_{32})$  was determined with a Malvern Mastersizer 2000 laser diffraction particle size analyser (Malvern Inst. Ltd, Worcestershire, U.K.) [10].

*Storage stability.* Aliquots were placed in tubes. The gravitational separation of DEs was recorded during storage in terms of phase separation and expressed as % of initial sample height.

*Thermal stability.* Samples were heated in a water bath at 70 °C for 30 minutes and stability was measured as described in storage stability.

*Colour measurement.* Surface colour of the double emulsions was measured by determining L\*, a\* and b\* using a CIELab scale, on a CR-400 Chroma Meter (Konica Minolta Inc., Tokyo, Japan).

*Statistical analysis.* The effects of oil phase lipid source, thermal treatments and chilling storage of DEs on  $d_{32}$ , storage stability, thermal stability and colour were analysed using a multifactor ANOVA. When the effects of treatments were significant

(P<0.05), the Tukey-HSD test was used. The statistical analysis was carried out using IBM SPSS Statistics 21.

# III. RESULTS AND DISCUSSION

The morphology of DEs containing olive oil and pork lard is shown in Fig. 1. DEs with olive oil seemed to contain smaller  $W_1$  droplets at day 1 than after 10 days of storage. The diameter of the  $W_1$  droplets increased but without greatly affecting the oil globule size, probably due to coalescence of the  $W_1$  droplets between them.

The thermal treatment applied (70 °C for 30 min) emulsion caused no apparent changes in  $W_1$  droplets, which may be resistant to the thermal stresses typically occurring in meat products.

As for the DE containing pork lard, there were almost no differences between 1 and 10 days of storage, which suggests good stability. This was expected since the fabrication process and the emulsifiers used were optimized for extended DE stability [10,13]. However, DEs with pork lard displayed less tendency to aggregate (Fig. 1). The coalescence of the  $W_1$  droplets and the aggregation of oil globules decreased in the DEs with pork lard, possibly as a consequence of the lower melting point of this lipid.



Figure 1. Optical microscopy images of DE prepared with olive oil (A) or pork lard (B) after chilling storage at 4 ° C for 10 days and then heating for 30 min at 70 °C

In general, both DEs presented unimodal distributions (ranging from 0.5 to 10  $\mu$ m). However, bimodal distributions were also occasionally recorded in those measurements carried out over the course of storage (results not shown).

The  $d_{32}$  size for the different studied factors is shown in Table 1. The  $d_{32}$  of the pork lard DE oil globule was slightly lower than that of the olive oil DE (Fig. 1). In other DEs that presented a unimodal distribution, the droplet sizes were about 2-fold greater [14]. However, the  $W_1$  of this DE contained 5% gelatine and the lipid phase consisted of various mixtures of vegetable fats (with different solid fat contents). The diameters found are in agreement with other very similar DEs that presented bimodal or trimodal distributions [9,13,15].

The average oil globule size was unaffected by the thermal treatment (Table 1), indicating that the DE concerned can resist conventional thermal stresses occurring in the food industry. Thus, it is reasonable to suppose that these treatments not only do not change the lipid size of the oil globules but also do not cause DE droplet aggregation.

The same concentration of salt was added to both aqueous phases to minimize water transport. However, an increase of the  $d_{32}$  was observed after 10 days of storage at 4 °C. Various authors have also reported increased globule size after storage when using PGPR as an emulsifier [10,16]. In these experiments PGPR was used as an emulsifier and was reported to provide an expandable and compressible interfacial layer, thus allowing the shrinkage or swelling of the droplets upon storage [16]. It is possible that the PGPR concentration combined with the type of the oil used, among other factors, could affect the stability of the DE differently.

The storage stability of the DEs was studied up to 10 days under refrigeration, which is as long as meat products with a relatively short shelf-life can be used (Table 1). Overall, olive oil and pork lard DEs showed small signs of creaming when stored at 4 °C. Moreover, the appearance of the separated lower phase was hard to distinguish from the upper phase. Phase separation was less clear in the emulsions containing pork lard than in the ones containing olive oil. This is probably because the  $d_{32}$  size was greater in the DE made with olive oil.

The thermal treatment reduced the stability of the DEs (Table 1). However, the overall stability of DEs subjected to the thermal treatment was over 90%, and therefore they may still be suitable for some meat products which exhibit high viscosity, minimizing the separation of phases.

DE phase separation increased with longer storage times (Table 1). After 10 days of storage at 4 °C the separation was about 15%, which suggests that this factor is crucial in determining stability. Looking at Fig. 1, it is reasonable to assume that

the structure of DEs is maintained in spite of this partial separation. Frasch-Melnik *et al.* [16] reported DE emulsion creaming and coalescence leading to globule size increases after 6 weeks of storage. Despite that, when there was no osmotic pressure gradient between aqueous phases the DE structure was retained. Therefore, the viscosity of many food products may play a key role in minimizing this gravitational instability.

Table 1 Effect of the oil phase used, thermal treatment, and storage time at 4 °C on particle size  $(d_{32})$  and stability

Factor		d <sub>32</sub>	Stability (%)
Lipid source	Olive oil	2.10 b	89.3 a
	Pork lard	1.85 a	96.7 b
	SEM	0.014	0.27
Thermal treatment	Before	1.97	95.0 b
(70 °C, 30 min)	After	1.98	91.0 a
	SEM	0.014	0.27
Storage time	1 day	1.97 b	98.5 c
	6 days	1.89 a	94.8 b
	10 days	2.06 c	85.8 a
	SEM	0.017	0.33

The oil used influenced  $L^*$ ,  $a^*$  and  $b^*$  of the DE (Table 2). The thermal treatment did not affect  $L^*$ , but it did cause changes in both  $a^*$  and  $b^*$ . Olive oil contains carotenoids, which are extremely susceptible to isomerizing and oxidizing conditions [17]. Hence, the decrease in redness can be explained by carotenoid loss as a result of the heating process. Likewise, degradation of pigments such as chlorophylls due to the thermal treatment may lead to decreased yellowness.

Table 2 Effect of the oil phase used, thermal treatment, and storage time at 4 °C on colour

Factor		Instrumental colour		
Factor		L*	a*	b*
Lipid source	Olive oil	64.87 a	-1.63 a	9.21 b
	Pork lard	66.35 b	0.12 b	1.08 a
	SEM	0.354	0.023	0.031
Thermal treatment	Before	65.97	-0.88 b	5.27 b
(70 °C, 30 min)	After	65.25	-0.62 a	5.01 a
	SEM	0.354	0.023	0.031
Storage time	1 day	65.64	-0.87 b	5.63 b
	6 days	65.73	-1.02 c	6.18 c
	10 days	65.46	-0.36 a	3.61 a
	SEM	0.433	0.028	0.038

Although lightness was not affected, a\* and b\* decreased after 10 days of storage at chilling temperature (Table 2). Therefore, if food colour is to be maintained, chromophores need to be stable or to be protected, especially during storage.

# IV. CONCLUSION

It is possible to fabricate DEs as low-fat food ingredients for use in the food industry. The stability of DEs is mainly affected by the storage time, although minimal creaming was observed in DEs stored for 10 days at 4 °C. In addition, the experimental DEs showed sufficient stability to conventional thermal treatments. Also, the nature of the oil was found to affect the droplet size, which in turn could explain the increased stability to creaming of DEs with pork lard.

# ACKNOWLEDGEMENTS

R. Bou was supported by a contract from the JAEpostdoctoral (CSIC) Program. This research was supported by projects AGL 2011-29644-C02-01 and Consolider-Ingenio 2010:CARNISENUSA (CSD2007-00016).

# REFERENCES

- Jiménez-Colmenero, F. (2007). Healthier lipid formulation approaches in meat based functional foods. Technological options for replacement of meat fats by non-meat fats. Trends in Food Science & Technology 18:567-578.
- Garti, N. (1997). Progress in stabilization and transport phenomena of double emulsions in food applications. Lebensmittel-Wissenschaft und Technologie 30:222–235.
- McClements, D. J. (2012). Advances in fabrication of emulsions with enhanced functionality using structural design principles. Current Opinion in Colloid & Interface Science 17: 235-245.
- 4. Benichou, A., Aserin, A., & Garti, N. (2004). Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters. Advances in Colloid & Interface Science 108:29-41.
- Muschiolik, G. (2007). Multiple emulsions for food use. Current Opinion Colloid & Interface Science 12:213-220.

- 6. Dickinson, E. (2011). Double emulsions stabilized by food biopolymers. Food Biophysics 6:1-11.
- McClements, D. J., Demetriades, K. (1998). An Integrated Approach to the Development of Reduced-Fat Food Emulsions. Critical Reviews in Food Science & Nutrition 38:511–536.
- 8. Jiménez-Colmenero, F. (2013). Potential applications of multiple emulsions in the development of healthy and functional foods. Food Research International 52:64–74.
- Bonnet, M., Cansell, M., Berkaoui, A., Ropers, H. M., Anton, M., & Leal-Calderon, F. (2009). Release rate profiles of magnesium from multiple W/O/W emulsions. Food Hydrocolloids 23:92-101.
- Cofrades, S., Antoniou, I., Solas, M. T., Herrero, A. M., & Jiménez-Colmenero, F. (2013). Preparation and impact of multiple (water-in-oil-in-water) emulsions in meat systems. Food Chemistry. http://dx.doi.org/1016/j.foodchem.2013.02.097.
- Bloukas, J. G., Paneras, E. D., Fournitzis, G. C. (1997). Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. Meat Science 45:133-144.
- 12. Lurueña-Martinez, M. A., Vivar-Quintana, A. M., Revilla, I. (2004). Effect of locust bean/xanthan gum addition and replacement of pork fat with olive oil on the quality characteristics of low-fat frankfurters. Meat Science 68:383-389.
- Su, J., Flanagan, J., Hemar, Y., & Singh, H. (2006). Synergistic effect of polyglycerol ester polyricinoleic acid and sodium caseinate on the stabilization of water-oil-water emulsions. Food Hydrocolloids 20:261–268.
- Weiss, J., Scherze, I., & Muschiolik, G. (2005). Polysaccharide gel with multiple emulsions. Food Hydrocolloids 19:605–615.
- O'Reagan, J., & Mulvihill, D. M. (2009). Water soluble inner aqueous phase markers as indicators of encapsulation properties of water-in-oil-in-water emulsions stabilized with sodium caseinate. Food Hydrocolloids 23:2339-2345.
- 16. Frasch-Melnik, S., Spyropoulos, F., Norton, I. T. (2010).  $W_1/O/W_2$  double emulsions stabilised by fat crystals. Formulation, stability and salt release. Journal of Colloid & Interface Science 350:178–185.
- 17. Mínguez-Mosquera, M. I., Hornero-Méndez, D. & Pérez-Gálvez, A. (2002). Carotenoids and provitamin A in functional foods. In: Jeffrey Hurst, Methods of Analysis for Functional Foods and Nutraceuticals. Boca Raton: CRC Press.