SİDAS MEDYA
Akademik Gıda®
ISSN Print: 1304-7582, Online: 2148-015X
http://www.academicfoodjournal.com

Akademik Gıda 13(1) (2015) 22-34

Research Paper / Araştırma Makalesi

Physical Properties of Olive Oil in Water Model Emulsion: Effect of Aqueous and Oil Phase Concentration and Homogenization Types

Aslı Zungur[1], Mehmet Koç[2,] ✉, Figen Kaymak Ertekin[1]

[1]Ege University, Faculty of Engineering, Department of Food Engineering, 35100, Bornova, Izmir, Turkey
[2]Adnan Menderes University, Faculty of Engineering, Department of Food Engineering, 09100, Aydın, Turkey

Received (Geliş Tarihi): 10.02.2015, Accepted (Kabul Tarihi): 23.03.2015
✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): mehmetkoc@adu.edu.tr (M. Koç)*
+90 256 213 75 03 +90 256 213 66 86

ABSTRACT

In this study, olive oil in water emulsions with different oil contents (30, 40 and 50% w/w on dry matter basis) were prepared for further encapsulation processes. Whey protein isolate and maltodextrin were used as encapsulating agents at different dry matter contents (30, 40 and 50%), and Tween 20 was used at 1% as a stabilizer. Emulsions were produced by a rotor-stator (classic) or an ultrasonic homogenizer. The effect of dry matter content, composition of aqueous phase containing encapsulating agents and oil content on the emulsion stability, rheological properties, droplet size, and microscopy images were determined. The water phase of emulsions containing maltodextrin was more viscous that resulted in better emulsion stability. Moreover the viscosity of emulsions increased with increasing dry matter content and decreasing oil content. The droplet size of emulsions ($D_{[4,3]}$) prepared by ultrasonic homogenization were lower (0.390-1.974 µm) than that by classic homogenization (1.003-5.205 µm).

Keywords: Olive oil; emulsion; whey protein isolate; maltodextrin; ultrasonic homogenization

Suda Zeytin Yağı Model Emülsiyonunun Fiziksel Özellikleri: Su ve Yağ Fazı Konsantrasyonu ile Homojenizasyon Tiplerinin Etkisi

ÖZ

Bu çalışmada enkapsülasyon denemesi için farklı yağ içeriklerine (%30, %40, %50 w/w kuru temelde) sahip su içinde zeytinyağı emülsiyonları hazırlanmıştır. Kaplama materyali olarak farklı oranlardaki peyniraltı suyu protein izolatı ve maltodekstrin (%30, %40, %50 w/w kuru temelde), stabilizatör olarak %1 oranında Tween 20 kullanılmıştır. Emülsiyonlar klasik ve ultrasonik homojenizatör ile hazırlanmıştır. Emülsiyon stabilitesi, reolojik özellikler, damlacık çapı ve mikroskopisi üzerine kuru madde içeriğinin, enkapsüle edici ajanları içeren sulu fazın kompozisyonu ve yağ miktarının etkisi incelenmiştir. Sulu fazında maltodekstrin içeren emülsiyonlar daha viskoz yapıda bulgulanmış ve emülsiyon stabilitesi bakımından daha iyi sonuç vermiştir. Ayrıca emülsiyonların viskozitesi kuru maddenin artışı ile artmış, yağ içerinin artışı ile azalmıştır. Ultrasonik homojenizasyon yöntemi ile hazırlanan emülsiyonların damlacık çapı (0.390-1.974 µm) klasik yöntem ile hazırlananlara (1.003-5.205 µm) oranla daha düşük bulunmuştur.

Anahtar Kelimeler: Zeytinyağı; emülsiyon; peyniraltı suyu protein izolatı; maltodekstrin; ultrasonik homojenizasyon

INTRODUCTION

Oil-in-water emulsion has very important place for food industry. Because many natural and processed foods including milk, cream, butter, margarine, fruit beverages, soups, cake batters, mayonnaise, cream liqueurs, sauces, desserts, and salad creams contain emulsion forms either partly or wholly [1-5]. McClements [6]

defined the food emulsions as compositionally, structurally, and dynamically complex materials because of water and oil-soluble components in the aqueous and oil phases This complex matrix of food emulsions such as viscosity of the continuous phase, the volume fraction of the dispersed phase, and the size and distribution of the droplets [7] have an effect on their stability and rheology.

The stability and rheology of the emulsion were also changed with oil and water composition and the homogenization method [8]. Rotor-stator systems (classic), ultrasonic homogenizers, high shear dispersers, and high pressure homogenizers are the most common homogenization techniques. Dalmazzone [9] reported that generally two homogenization techniques are combined together to obtain finer emulsions. Abismail et al. [10] compared two homogenization methods, ultrasonic and rotor-stator systems, and found out that the droplet size of emulsions produced in ultrasonic homogenization was smaller than rotor-stator homogenization method.

The stability of an emulsion is mentioned as the resistance to changes in the characteristics of the emulsion. Creaming, which is one of the properties of emulsions, is used to define emulsion stability. The creaming index and rate are very important parameters for stability and rheology of emulsion to define the quality of the emulsion [11]. The degree of droplet aggregation in an emulsion could be understood by the creaming index and rate. The rate at which an emulsion breaks down, and the mechanism by which this process occurs, depends on its composition and microstructure [8, 12], as well as on the environmental conditions (e.g., temperature variations, mechanical agitation, and storage conditions) [6]. Compositional and structural complexity of an emulsion causes difficulty to predict its stability quantitatively. Bergenstahl [13] found out that the stability and rheology of an emulsion largely depend upon the interactions between the emulsion droplets, and these in turn depend on the interfacial composition.

The emulsion properties, especially stability and rheology, are also important in the encapsulation process because the preparation of emulsion is basic and the first step in oil encapsulation technology. The food industry applies the encapsulation technology for stabilization and controlled release of core material (oil, colour pigment, aroma components, etc.), masking unpleasant tastes and smells, and protecting core material against oxidation [14]. Encapsulation of vegetable and aromatic oils, the type of coating materials and their concentrations affect the stability, viscosity, and droplet size of the emulsion. Soottitantawat et al. [15, 16] reported that the retention of oils during microencapsulation by spray-drying could be enhanced by reducing the mean particle size of the dispersed core material during emulsification.

In the encapsulation process, a single encapsulating agent does not possess all required characteristics, and efforts to improve encapsulation properties have been achieved by blending two or more encapsulating agents; for instance, proteins and polysaccharides at different proportions [17, 18]. Both proteins and polysaccharides play prominent roles in the formulation of food emulsions. The droplets of an emulsion were protected by proteins, which work as emulsifier and stabilizer in emulsion against aggregation and coalescence. Moreover high-molecular weight-polysaccharides enhanced the stability of emulsions by keeping droplets separately after their formation and thus protect them against creaming, flocculation, and coalescence [18]. In addition, homogenization conditions such as type of method (ultrasonic or classic homogenizer), rotational speed and temperature also affect the emulsion properties and the final product quality. The total solids concentration, viscosity, stability, and droplet size of emulsion have also effects on the encapsulation efficiency. The smaller emulsion droplet size is a better retention of oil in encapsulation processes. Jafari et al. [19] also reported that if an emulsion is formed from smaller droplet sizes, the final encapsulated product has less extractable surface oil. Because finer emulsions are more stable in terms of emulsion stability compared with the emulsions comprised of large droplets. Besides being stable enough, an emulsion should have a sufficiently low viscosity in order to be pumpable [20] and dryable [21]. However emulsions with low viscosity may increase surface oil content in dried particles. On the other hand, an increase in the viscosity of the feeding emulsion should help oil retention because of the reduction of internal circulations in the droplets and rapid semi-permeable membrane formation [19]. But it should not be ignored that viscous emulsions could clog up the atomizer or nozzle during spray dry process and drying could not be adequate as expected because of large droplets formation.

Da Fonseca et al. [22] reported that proteins combined with polysaccharides can improve the emulsifying properties of the emulsions by influencing the thickness of the interfacial aqueous film, which covers the oil droplets. The optimum viscosity of the emulsion should be certainly specific depending on the properties and the proportion of the aqueous phase of an emulsion [23]

Consequently, regarding rheological properties and the stability of emulsions, it is important to achieve the encapsulation process. Therefore, for further encapsulation study of olive oil, this study was aimed to investigate the influence of type and concentration of whey protein isolate and maltodextrin as encapsulating agents in emulsion, total dry matter and olive oil content of the emulsion on emulsion stability, viscosity and rheological behaviour of the emulsion, droplet size and size distribution, and microscopy of emulsions.

MATERIAL and METHODS

Materials

Extra virgin olive oil (refractive index=1.47, ρ=910 kg/m^3, μ=0.083 Pa.s) was purchased from a local grocery store in Turkey. The whey protein isolate (WPI) was supplied by Ak Gıda San. Tic. A.Ş. and maltodextrin (MD, DE19)

was provided by Qinhuangdao Starch Co Ltd. Tween 20 (Merck, Darmstadt, Germany) was used as stabilizer.

Preparation of olive oil in water emulsions

The maltodextrin (MD) and whey protein isolate (WPI) solutions were prepared by dissolving the powder in distilled water with magnetic stirring for 90 min at room temperature (25ºC) and were kept overnight at room temperature for complete dissolution. In order to obtain olive oil in water emulsions (o/w), the required amount of olive oil was poured drop wise into the required amount of encapsulating agent solution (MD or WPI or MD+WPI). Total solid concentration of the emulsions (encapsulating agent + olive oil) ranged between 30% to 50%. The olive oil concentration in the emulsions was between 30% and 50% of the dry matter. Tween 20 was added to the emulsions as a stabilizer at 1% (w/w). The weight ratios of protein (WPI) to carbohydrate (MD) in the emulsions were 0:1, 1:0, and 1:1. The formulations of 27 different emulsions are given in Table 1.

Table 1. Composition of the emulsions with different oil contents

Emulsion Number	Dry Matter content (%, wb)	Oil content in DM (%, db)	Water (%,wb)	Oil (%,wb)	MD (%,wb)	WPI (%,wb)	MD:WPI ratio
1	30	30	70	9	20	0	1:0
2	30	40	70	12	17	0	1:0
3	30	50	70	15	14	0	1:0
4	40	30	60	12	27	0	1:0
5	40	40	60	16	23	0	1:0
6	40	50	60	20	19	0	1:0
7	50	30	50	15	34	0	1:0
8	50	40	50	20	29	0	1:0
9	50	50	50	25	24	0	1:0
10	30	30	70	9	0	20	0:1
11	30	40	70	12	0	17	0:1
12	30	50	70	15	0	14	0:1
13	40	30	60	12	0	27	0:1
14	40	40	60	16	0	23	0:1
15	40	50	60	20	0	19	0:1
16	50	30	50	15	0	34	0:1
17	50	40	50	20	0	29	0:1
18	50	50	50	25	0	24	0:1
19	30	30	70	9	10	10	1:1
20	30	40	70	12	8.5	8.5	1:1
21	30	50	70	15	7	7	1:1
22	40	30	60	12	13.5	13.5	1:1
23	40	40	60	16	11.5	11.5	1:1
24	40	50	60	20	9.5	9.5	1:1
25	50	30	50	15	17	17	1:1
26	50	40	50	20	14.5	14.5	1:1
27	50	50	50	25	12	12	1:1

*: DM: Dry matter, MD: Maltodextrin, WPI: Whey protein isolate

To prepare oil-in-water emulsions, two different homogenization techniques were used namely rotor-stator (classic) and ultrasonic homogenization. Classic homogenization was performed with an IKA Ultra Turrax T25 homogenizer at 20000 rpm for 5 min for 100 g mixture. In the ultrasonic homogenization method, the mixture (100 g) was mechanically aggregated with an Ultra Turrax at 10000 rpm for 3 min and then was immediately homogenized at 24 kHz for 2 min through an ultrasonic homogenizer (Hielscher UP400S) with H14 titanium Sonotrode (diameter= 14 mm, length=100 mm). Each emulsion listed in Table 1 was prepared in 2 replicates using both homogenization methods. The emulsions were maintained in an iced water bath during the homogenization process to keep the temperature below 25ºC.

Analyses

Creaming Stability

Each emulsion (15 mL) was poured into cylindrical plastic test tubes (diameter: 1.6 cm, height:12.5 cm) sealed with a plastic cap and stored at 25ºC for 15 days. The emulsion stability was measured by the change in the height of the bottom serum phase (H_s) within time and was compared with the total height of the emulsion (H_E) [24]. The creaming index (CI) was determined according to Eq. (1).

$$\text{Creaming index } \% = 100 \times \frac{H_S}{H_E} \qquad (1)$$

The analyses were carried out in triplicates.

Rheological Measurements

A controlled stress rheometer (Haake viscotester 550, Karlsruhe, Germany) with standard MV DIN sensor (radius 19.36 mm, length 58.08 mm, gap 1.64 mm, sample volume 50 cm^3) was used for performing the viscosity measurement. The measurements with fresh emulsions were made in duplicate at 25ºC. The samples were first placed in the temperature controlled measurement vessel until equilibration at 25ºC. The flow curves (Shear stress, τ (Pa) as a function of shear rate, γ (s^{-1})) of emulsions were obtained by applying an increasing shear rate from 2 s^{-1} to1032 s^{-1}.

The experimental data were fitted to Bingham plastic (Eq. (2)) and Herschel-Bulkley (Eq. (3)) models to obtain rheological (τ_0, K and n) and statistical (R^2) parameters;

$\tau = \tau_0 + K\gamma$ (2)
$\tau = \tau_0 + K\gamma^n$ (3)

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), K is the consistency coefficient (Pa.s^n), and n is the flow behaviour index (dimensionless).

Measurement of Emulsion Droplet Size and Size Distribution

The size distribution of oil droplets in the emulsions was measured by laser light diffraction (Malvern Mastersizer Model 2000, Malvern Instruments Ltd., Worcestershire, UK). To prevent multiple scatting effects, the emulsions were diluted with distilled water. The refractive index used for lipid drops was 1.47 and the density of olive oil was 910 kg/m^3. Determinations were done in triplicates and data was reported as average.

The mean diameter of the oil droplets was expressed as the volume mean diameter ($D_{[4,3]}$) and the droplet size distribution was also reported according to Eq (4).

$$D_{[4,3]} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \qquad (4)$$

where n_i is the number of particles of diameter d_i.

The particle size distribution of the powder was measured as the span, which is defined as

$$span = \frac{d_{90} - d_{10}}{d_{50}} \qquad (5)$$

where d_{90}, d_{10}, and d_{50} are the equivalent volume diameters at 90, 10, and 50% cumulative volume, respectively.

Microscopic Images of Emulsions

The microstructure of the emulsions was observed using a trinocular microscope (Olympus CX31) with an attached digital camera (Olympus Digital SLR E330). The samples were poured onto microscope slides, covered with glass cover slips, and observed with the x100 objective lense. For a better view of globules in emulsions, 0.1% fat soluble dye, Sudan I was added to emulsions.

Statistical Analysis

All measurements were made in triplicates. Results are expressed as mean ± standard deviation. The results were evaluated by analysis of variance (ANOVA) test. The statistical analyses were carried out using the SPSS version 15.0 Windows software program (SPSS Inc., Chicago, IL).

RESULTS and DISCUSSION

Emulsion Stability

The creaming index values of the olive oil in water emulsions with different dry matter contents (30, 40, 50 % w/w) and olive oil contents in DM (30, 40, 50 % w/w) were given in Table 2. Creaming is one of the emulsion instabilities, which were caused by gravity [25] Emulsions with low creaming index and low creaming rate display good creaming behaviour and emulsion stability [11]. The creaming index values of emulsions prepared with classic and ultrasonic homogenization techniques, changed between 20.77 and 86.26 % and 15.63 to 91.55 %, respectively. In general terms, the results showed that the emulsions prepared with ultrasonic emulsification are more stable than those prepared with the classic homogenization method. Similar behaviour was obtained by Abismail et al. [26].

As shown in Table 2, the creaming index values of emulsions prepared with two different homogenization methods decreased appreciably with increasing dry matter content of the emulsions. This result demonstrated that the emulsion stability gets better with high dry matter content. According to the ANOVA results (Table 3), emulsion stability was significantly affected by the dry matter and olive oil content of the emulsions (p<0.01). The composition of continuous phase (MD and/or WPI content) had also a significant (p<0.01) effect on the stability of the emulsion.

The lowest creaming index value (20.77 %) was observed in the emulsion prepared with classic homogenization with 50% dry matter and containing only WPI solution as continuous phase (Table 2). At higher WPI concentrations, i.e. greater than 30% in the emulsions, a slightly lower creaming index was observed, possibly due to the unabsorbed WPI in the continuous phase. Papalamprou et al. [27] and Sun and Gunasekaran [28] explained that high WPI concentrations in continuous phase may be adsorbed by the interface of oil droplets, consequently increase the

density of the oil droplets, and decrease the creaming index. In addition, the surfaces of oil droplets are covered well with increasing WPI concentration. In this way the flocculation of oil droplets was prevented and creaming possibility of emulsion was decreased [29]. As a result, an increase in the WPI concentration resulted in a more stable emulsion (Table 2). This circumstance was also observed in the emulsions containing only MD or MD+WPI mixtures. The creaming of the emulsion with low concentrations of MD/WPI was attributed to the insufficient amount of coating materials for covering the surfaces of the oil droplets. In addition, due to the adsorption of WPI to the interface of oil droplets, phase separation was not observed in emulsions prepared with ultrasonic homogenization.

Table 2. Creaming index (%) of emulsions prepared with classic and ultrasonic homogenization.

Emulsion Number	Classic homogenization	Ultrasonic homogenization
	Creaming index (%)	Creaming index (%)
1	80.85±0.51	74.56±1.35
2	79.56±0.34	-*
3	78.67±0.33	-*
4	80.35±1.12	43.78±0.47
5	62.72±1.35	29.69±1.35
6	62.17±0.16	38.98±2.48
7	42.09±1.22	17.01±1.86
8	39.02±1.36	18.36±2.83
9	38.83±1.39	15.63±2.41
10	85.15±0.43	91.55±0.32
11	81.78±0.69	-*
12	76.93±3.21	79.70±0.30
13	82.77±0.17	-*
14	78.50±0.84	-*
15	71.38±0.74	-*
16	-*	-*
17	20.77±2.36	-*
18	32.16±0.48	-*
19	86.26±4.01	81.45±2.14
20	85.68±3.91	79.27±1.72
21	83.12±2.41	73.62±1.22
22	82.92±2.69	41.52±3.01
23	70.61±0.86	41.35±1.39
24	68.07±0.86	38.83±2.20
25	49.62±0.10	21.61±3.31
26	47.69±0.64	22.67±1.67
27	32.16±1.68	20.20±1.06

*Phase separation was unobserved.

The results showed that mass fraction of olive oil plays an important role in creaming of the emulsion (Table 2). With increased olive oil content in the dry matter of the emulsion, emulsion stability showed an increase. Increasing oil-phase mass fraction in an emulsion resulted in improvement of emulsion stability due to the increase in packing fraction of oil droplets as stated by Dickinson and Golding [30], which enhanced the emulsion viscosity and lowered the creaming rate.

Rheological Behaviour of Emulsions

The flow curves for the emulsions prepared by classic and ultrasonic homogenization are given in Figs. 1 and 2, respectively. All the emulsions were found to be near Newtonian fluids, with low behaviour indexes within a range of 0.780 to 1.000 (Table 4). The viscosity of the emulsions, homogenized either with classic or ultrasonic homogenization methods, decreased when the oil content was increased.

The emulsions prepared with the classic homogenization method, containing only maltodextrin or a mixture of MD+WPI at 50% DM content (Samples 7, 8, 9, 25, 26, 27), showed Herschel-Bulkley type flow behaviour. However, this behaviour was not observed for emulsions containing WPI at 50% DM content, except for the emulsion with low oil content (Sample 16). Similar behaviour was observed for the emulsions prepared with the ultrasonic method (samples 4, 5, 7, 8, 9, 16, 17, 25, 26, 27). All emulsions at low DM content (30%), homogenized either with the classic or ultrasonic method, showed Newtonian type behaviour regardless of the olive oil content. For the emulsions, increase in the DM content especially containing maltodextrin as a continuous phase leads to changes in the type of the flow behaviour varying from Bingham plastic to Herschel-Bulkley type behaviour (Table 4). Dokic et al. [31] also reported that maltodextrin content directly affects the viscosity of the sunflower oil emulsions. Last of all, the viscosity of the continuous phase was the

main factor affecting the flow properties of olive oil emulsions containing maltodextrin (Figs. 1 and 2).

The K value was increased with increasing dry matter content and decreasing olive oil content in the emulsions prepared either by classic or ultrasonic homogenization. The ANOVA results were inconsistent with these findings (Table 3). The highest K value was found for emulsions with 50% DM content and 9% oil content (lowest oil content) (Table 4). McClements [6] also reported that the consistency coefficient increased with increasing dry matter content in the emulsions. When the olive oil content increased from 30 to 50% in DM, the K value slightly decreased due to formation of larger droplets increasing oil amount in emulsion. The attraction forces among the droplets became weaker and thus sensitive to lower shearing forces, giving lower yield stress values. The increase in consistency in most emulsions could be related to an increase in their viscous nature due to weaker attraction forces among the droplets [32.] As a result, the viscosity of the obtained emulsions was defined by the viscosity of the continuous phase. Maltodextrin, which leads to more viscous aqueous phase, was found to be more efficient for the production of finer emulsions

Table 3. ANOVA results of stability (%), rheological consistency (K, Pa*s^n) and oil droplet size ($D_{4,3}$, µm) of emulsions

		Classic Homogenization								Ultrasonic Homogenization							
		Emulsion Stability (%)			$D_{4,3}$ (µm)			K (Pa*s^n)		Emulsion Stability (%)			$D_{4,3}$ (µm)			K (Pa*s^n)	
	Source	df	Mean square	p-value†	df	Mean square	p-value†	df	Mean square	df	Mean square	p-value†	df	Mean square	p-value†	df	Mean square	p-value†
Dry matter (%)	Between Groups	2	12749.91	0.000**	2	38.435	0.000**	2	0.034	2	18253.23	0.000**	2	1.671	0.000**	2	0.210	0.000**
	Within Groups	75	50.851		78	0.521		78	0.001	53	27.853		78	0.131		78	0.005	
	Total	77			80			80		55			80			80		
Oil (%)	Between Groups	5	3705.409	0.000**	5	4.341	0.008**	5	0.005	5	4428.143	0.000**	5	0.275	0.147	5	0.030	0.008**
	Within Groups	72	149.814		75	1.278		75	0.001	50	316.84		75	0.162		75	0.009	
	Total	77			80			80		55			80			80		
MD (%)	Between Groups	17	855.574	0.000**	17	4.514	0.000**	17	0.006	16	2260.99	0.000**	17	0.661	0.000**	17	0.044	0.000**
	Within Groups	60	246.148		63	0.647		63	0.000	39	46.33		63	0.050		63	0.001	
	Total	77			80			80		55			80			80		
WPI (%)	Between Groups	16	1229.538	0.000**	17	2.976	0.002**	17	0.002	11	2180.314	0.000**	17	0.557	0.000**	17	0.014	0.116
	Within Groups	61	158.05		63	1.062		63	0.001	44	318.165		63	0.065		63	0.009	
	Total	77			80			80		55			80			80		

*: Significant at p<0.05; **: significant at p<0.01.

Microstructure and Droplet Size of the Emulsions

A relevant functional property of the microcapsules in practical applications is their ability to form stable emulsions when re-dispersed in water. The droplet size distribution of the emulsion prepared with the classic and ultrasonic homogenization methods was found to be bimodal but comparatively different of their maximum and secondary maximum (Fig. 3). Hayati et al. [32] explained that a bimodal distribution could simply be caused by the short time of homogenization and the wrong type of homogenizer employed. In this study the emulsions prepared with the classic homogenization method a mechanical homogenizer was used, which was only capable of generating fairly low energy inputs to disrupt and mix the oil and water phases, and therefore is incapable of producing small droplet sizes [33]. On the other hand, the ultrasonic homogenization led to a bimodal distribution due to insufficient duration of homogenization since the temperature of the emulsions increases with higher durations of applying homogenization.

The size distribution, $D_{[4,3]}$, span and uniformity values of the emulsions were given in Table 5. It was found that the oil phase particle size, $D_{[4,3]}$ of emulsion prepared with classic homogenization altered between 1,003 and 5,205 µm. The dry matter and oil content of the emulsions prepared with a classic homogenizer significantly (p<0.05) (Table 4) affected the oil phase particle size. The emulsion particle sizes obtained by ultrasonic homogenization are smaller than those obtained by the classic homogenization method (Table 5). They varied between 0,390 and 1,974 µm. Ultrasonic treatment has also been considered as an effective method for the reduction of oil droplet size [34, 35].

Regardless of the oil content, droplet size of the emulsions decreased with increasing dry matter content. In contrast, increased oil fraction in an emulsion led to a gradual increase in the droplet size ($D_{[4,3]}$) (Table 5). As already observed by Christensen et al. [36] and Turchiuli et al. [8] the emulsion droplet size was also found to depend on the oil content, with smaller oil contents in the same dry matter leading to smaller emulsion droplet size. It can be attributed to the lower olive oil fraction, which inhibits droplet aggregation and coalescence. As a result, a minimum droplet size was obtained for the emulsion, which had a 50% DM with 30% oil in DM and contained only MD as an aqueous phase (Sample 7) with the classic homogenization method (Table 5). On

the contrary, in the ultrasonic homogenization method, the emulsion prepared with a 50% DM with 30% oil in DM and containing only WPI as an aqueous phase, had the minimum droplet size (Table 5).

Table 4. Rheological parameters for olive oil emulsions prepared by classic and ultrasonic homogenization methods with MD and WPI

Emulsion Number	Classic homogenization				Emulsion Number	Ultrasonic homogenization			
	τ_0 (Pa)	K (Pa.s^n)	n	R^2		τ_0 (Pa)	K (Pa.s^n)	n	R^2
1*	0.000	0.016	1.000	0.931	1*	0.000	0.020	1.000	0.952
2*	0.000	0.014	1.000	0.937	2*	0.000	0.018	1.000	0.947
3*	0.000	0.012	1.000	0.950	3*	0.000	0.016	1.000	0.929
4**	0.286	0.024	1.000	0.999	4***	1.841	0.074	0.887	0.999
5**	0.255	0.022	1.000	0.994	5***	2.092	0.053	0.902	0.999
6*	0.000	0.020	1.000	0.975	6*	0.000	0.020	1.000	0.975
7***	4.358	0.136	0.926	0.999	7***	0.031	0.393	0.824	1.000
8***	4.250	0.130	0.897	0.999	8***	7.641	0.329	0.789	0.999
9***	4.066	0.091	0.902	0.999	9***	8.095	0.220	0.804	0.999
10*	0.000	0.011	1.000	0.953	10*	0.000	0.010	1.000	0.987
11*	0.000	0.010	1.000	0.947	11*	0.000	0.011	1.000	0.955
12*	0.000	0.010	1.000	0.950	12*	0.000	0.011	1.000	0.956
13*	0.000	0.015	1.000	0.926	13*	0.000	0.016	1.000	0.947
14*	0.000	0.015	1.000	0.926	14*	0.000	0.016	1.000	0.936
15*	0.000	0.015	1.000	0.928	15*	0.000	0.015	1.000	0.953
16***	1.673	0.028	0.986	0.999	16***	1.723	0.078	0.857	0.999
17**	0.797	0.022	1.000	0.997	17***	2.468	0.061	0.875	0.999
18*	0.000	0.021	1.000	0.988	18*	0.000	0.021	1.000	0.994
19*	0.000	0.013	1.000	0.923	19*	0.000	0.041	1.000	0.951
20*	0.000	0.014	1.000	0.925	20*	0.000	0.015	1.000	0.942
21*	0.000	0.013	1.000	0.941	21*	0.000	0.015	1.000	0.942
22*	0.000	0.020	1.000	0.967	22**	1.318	0.022	1.000	0.993
23**	0.421	0.020	1.000	0.978	23**	1.698	0.021	1.000	0.989
24*	0.000	0.018	1.000	0.955	24**	0.549	0.019	1.000	0.980
25***	1.732	0.110	0.890	1.000	25***	3.013	0.175	0.834	1.000
26***	2.496	0.100	0.870	1.000	26***	5.183	0.213	0.783	0.999
27***	2.190	0.050	0.932	0.999	27***	4.456	0.152	0.793	0.999

*Newtonian type flow; ** Bingham plastic type flow; *** Herschel–Bulkley type flow

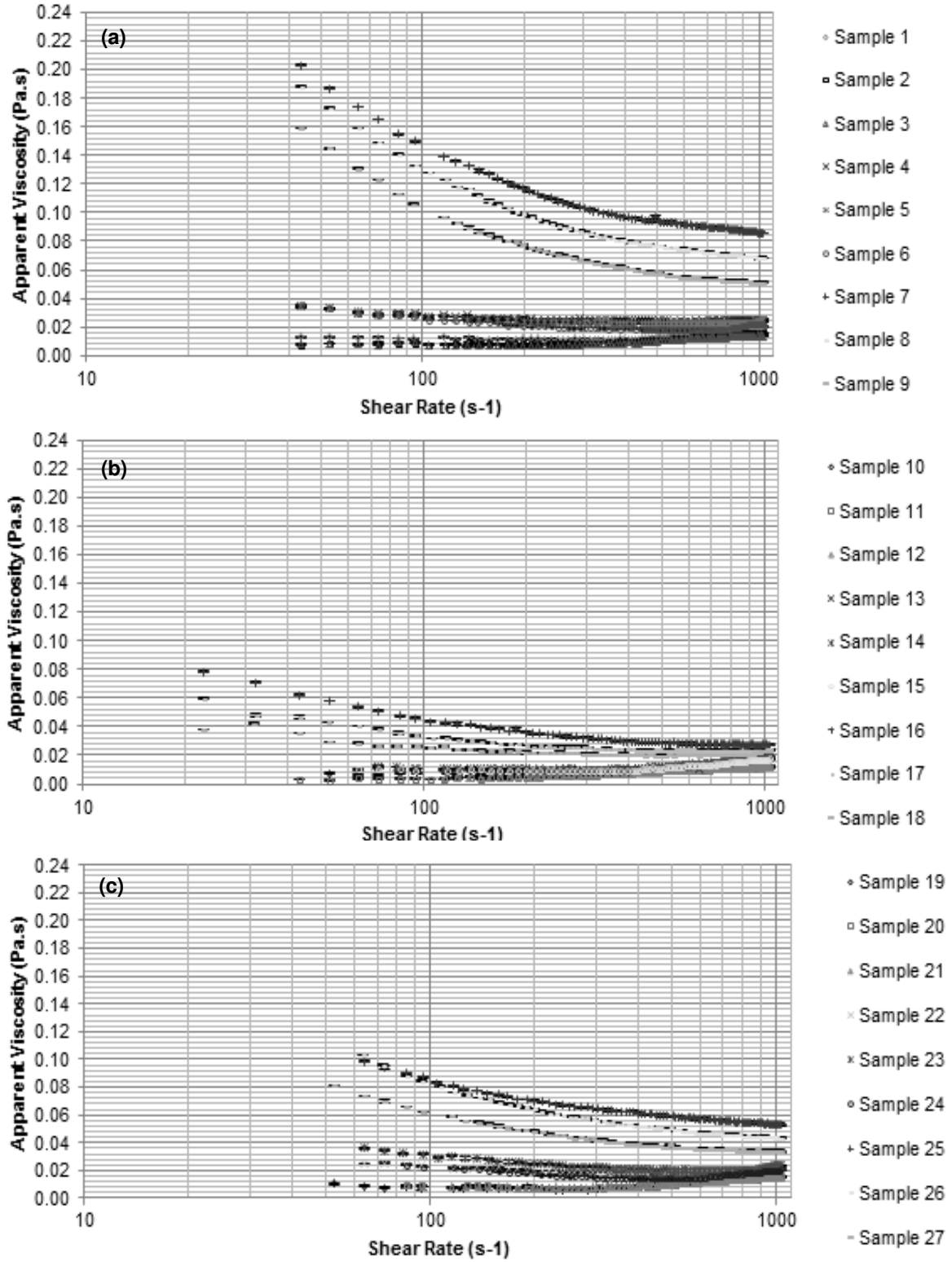

Figure 1. Flow curves for the emulsions obtained by classic homogenization (a) MD as a continuous phase, (b) WPI as a continuous phase and (c) mixture of WPI/MD as a continuous phase with different oil contents.

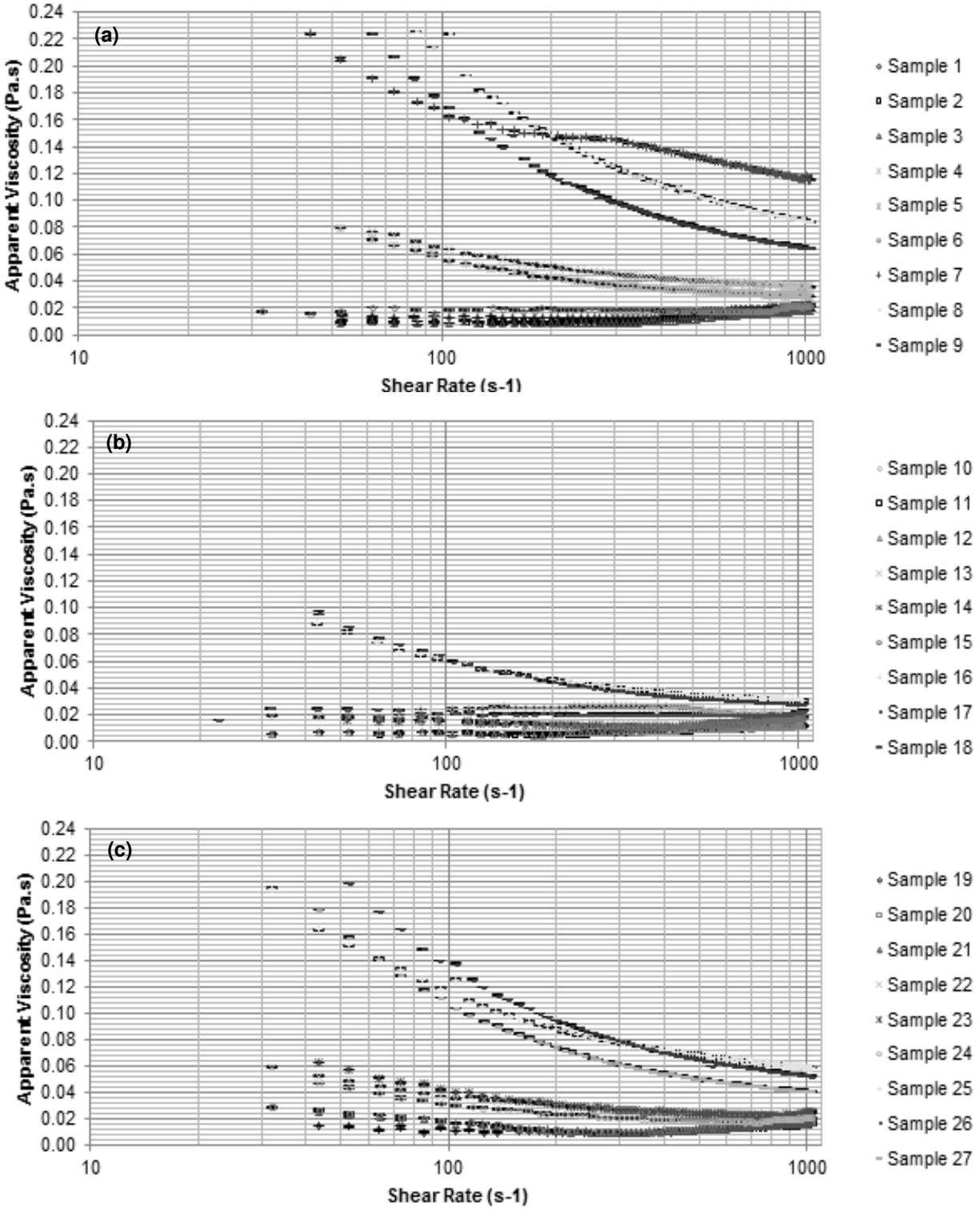

Figure 2. Flow curves for the emulsions obtained by ultrasonic homogenization (a) MD as a continuous phase, (b) WPI as a continuous phase and (c) mixture of WPI/MD as a continuous phase with different oil contents.

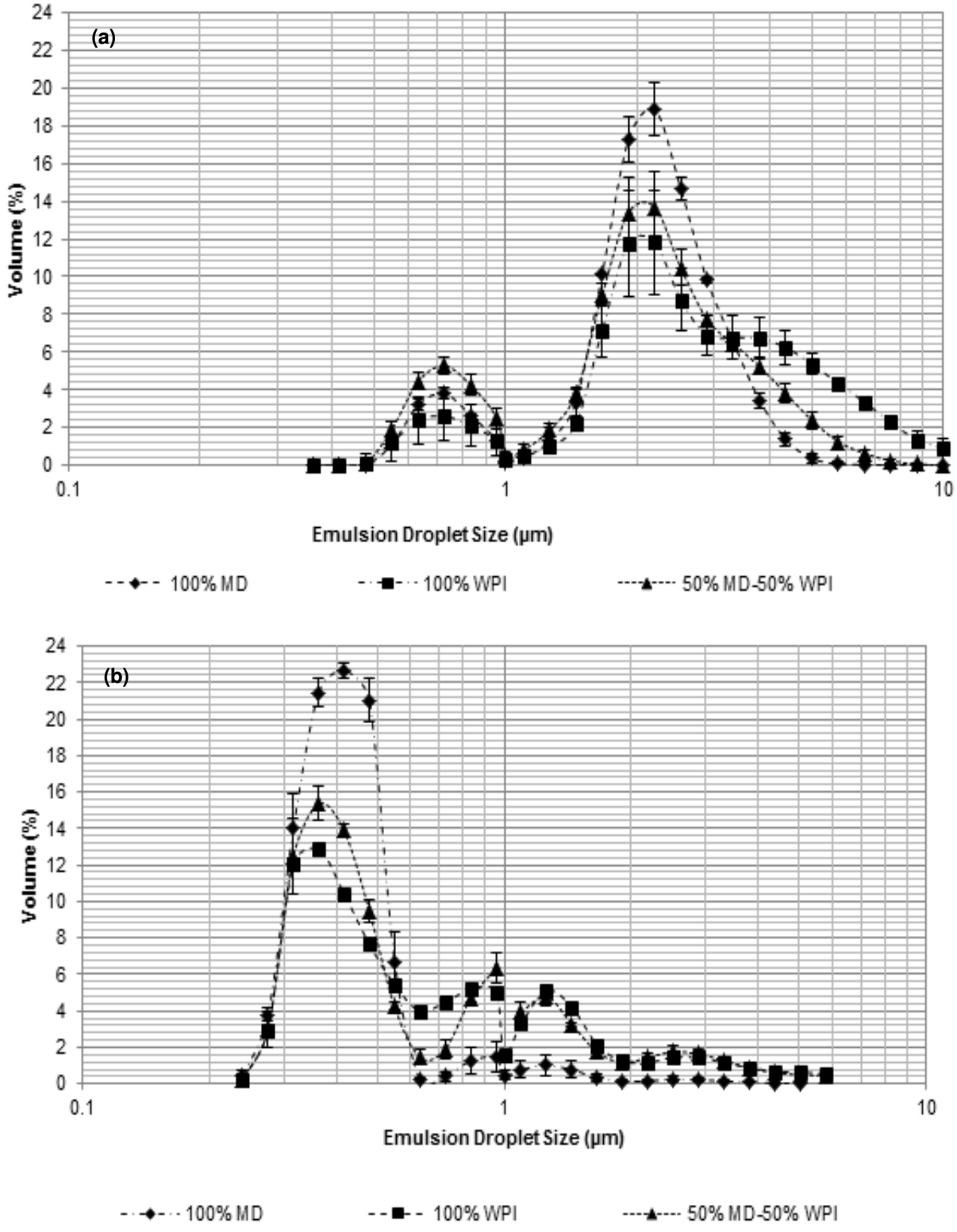

Figure 3. Droplet size distribution of emulsions prepared by (a) classic homogenization method (b) ultrasonic homogenization method with MD, WPI and their mixture, having 40% dry matter with 9% oil (w/w) content.

Table 5. Particle size ($D_{[4,3]}$ (µm)), span and uniformity values of emulsions prepared with classic and ultrasonic homogenization with different oil contents.

Emulsion Number	Classic Homogenization			Ultrasonic Homogenization		
	$D_{[4,3]}$ (µm)	Span	Uniformity	$D_{[4,3]}$ (µm)	Span	Uniformity
1	5.205±0.580	2.973±0.200	0.944±0.085	0.938±0.078	1.883±0.119	0.638±0.075
2	3.529±0.123	2.582±0.059	0.764±0.030	0.960±0.020	1.928±0.006	0.665±0.007
3	4.717±4.717	2.836±0.113	0.910±0.027	1.051±0.003	1.807±0.035	0.597±0.012
4	2.033±0.021	1.149±0.036	0.316±0.020	0.390±0.003	0.484±0.060	0.138±0.001
5	2.400±0.104	1.330±0.202	0.400±0.063	0.543±0.023	1.940±0.123	0.659±0.072
6	2.566±0.043	1.397±0.152	0.421±0.033	0.818±0.030	1.465±0.503	0.530±0.188
7	1.003±0.170	1.527±0.523	0.446±0.165	1.339±0.018	1.245±0.192	0.432±0.080
8	1.503±0.016	1.040±0.089	0.350±0.054	1.357±0.054	3.778±0.830	1.203±0.231
9	1.894±0.047	1.041±0.081	0.278±0.030	1.052±0.013	4.560±0.235	1.260±0.141
10	3.466±0.231	2.927±0.564	0.881±0.200	1.289±0.025	1.793±0.068	0.552±0.022
11	4.400±0.072	3.684±0.511	1.117±0.159	1.338±0.024	1.547±0.145	0.486±0.023
12	4.904±0.370	3.357±0.541	1.044±0.161	1.738±0.031	1.745±0.139	0.563±0.042
13	3.714±0.819	2.119±0.082	0.659±0.018	0.958±0.097	3.661±0.264	1.587±0.223
14	4.033±0.297	3.279±0.488	0.975±0.110	1.037±0.107	2.217±0.236	0.821±0.008
15	3.510±0.153	2.299±0.070	0.706±0.026	1.073±0.047	2.013±0.041	0.669±0.008
16	2.094±0.141	1.190±0.096	0.332±0.030	1.018±0.188	5.130±0.909	2.207±0.270
17	2.152±0.034	1.259±0.055	0.338±0.016	1.072±0.020	2.444±0.229	0.947±0.190
18	2.252±0.047	1.119±0.177	0.328±0.037	1.094±0.052	2.387±0.895	1.330±0.053
19	2.258±0.039	1.590±0.053	0.444±0.020	1.546±0.041	1.423±0.109	0.477±0.048
20	3.873±0.049	2.419±0.074	0.740±0.013	1.729±0.054	1.420±0.104	0.451±0.025
21	4.757±0.222	2.531±0.185	0.814±0.048	1.974±0.024	1.480±0.051	0.443±0.021
22	2.087±0.022	1.440±0.112	0.406±0.043	0.790±0.012	2.576±0.347	1.056±0.087
23	2.702±0.041	1.697±0.014	0.498±0.005	1.035±0.114	3.451±0.924	1.493±0.211
24	3.221±3.221	2.031±0.009	0.630±0.006	1.441±0.195	1.694±0.074	0.590±0.051
25	1.445±0.026	1.036±0.070	0.394±0.024	0.362±0.016	0.399±0.025	0.127±0.012
26	1.817±0.037	1.034±0.195	0.284±0.074	1.477±0.159	5.574±0.342	1.388±0.420
27	1.474±0.018	0.946±0.073	0.329±0.046	1.575±0.759	5.006±0.229	1.643±0.266

Microscopy images of the emulsions also showed that the particle size of the emulsions prepared with ultrasonic homogenization was smaller than those prepared with classic homogenization (Figure 4). The surface properties of the oil droplets in emulsion with low concentration of MD/ MD+WPI also have effect on creaming by the coalescence or flocculation of the oil droplets.

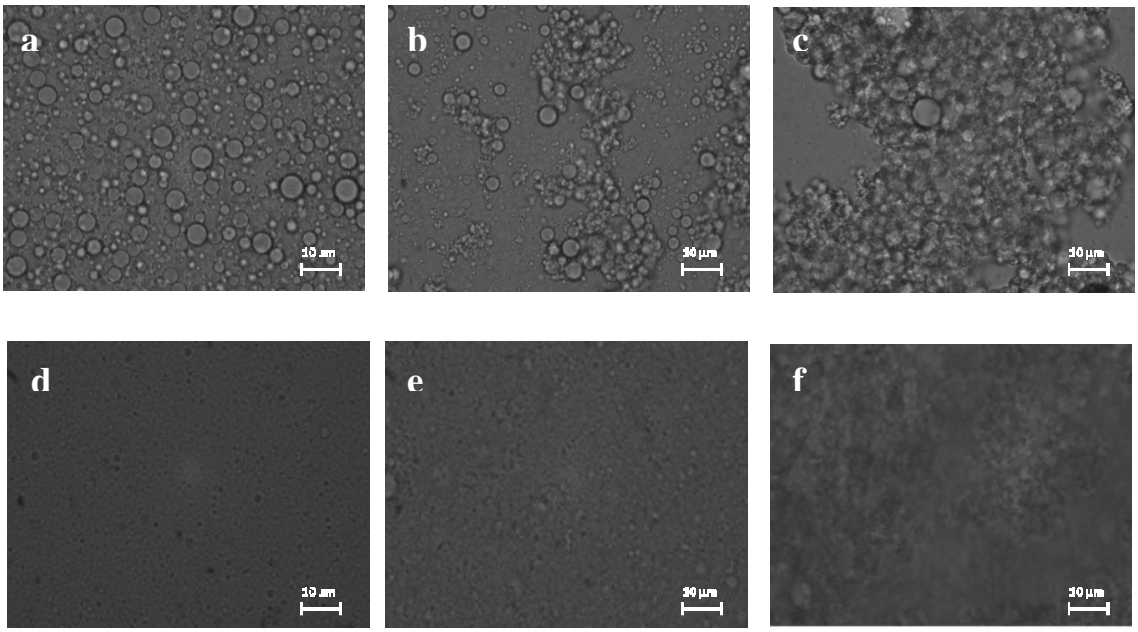

Figure 4. The microscopy images (at 100xmagnification) of containing 40 % (wb) dry matter and 9 % (wb) oil emulsions prepared by classic homogenization method with (a) WPI, (b) MD, (c) WPI-MD mixture (1:1); and prepared by ultrasonic homogenization method with (d) WPI (e) MD (f) WPI-MD mixture (1:1)

CONCLUSION

One of the most critical steps for an effective encapsulation process is the preparation phase of an emulsion. For this purpose, the examination of the homogenization method and the prepared emulsion stability, rheology, and droplet morphology are quite important. Results showed that suitable MD and WPI

compositions and the homogenization method for preparing water-olive oil emulsions possess an excellent capability to form stable emulsions which might be appropriate for further encapsulation. In addition to emulsion stability, the K values representing viscosity of the emulsions were influenced by the dry matter and olive oil contents. The viscosity of the emulsions homogenized either with the classic or ultrasonic method decreased when the oil content was increased. The particle size of the emulsion obtained with ultrasonic homogenization is smaller than those obtained with classic homogenization. Particle size distribution is also related to creaming rate, the smaller emulsion droplets are more physically stable than the larger emulsion droplets. Oil content of the emulsion significantly affected the droplet size. In general, the droplet size increased with increasing oil content.

ACKNOWLEDGEMENTS

The authors acknowledge TUBITAK-TOVAG (Project Number: 111 O 345), Ege University, Council of Scientific Research Projects (Project Number: BAP 2010/MÜH/011) and EBILTEM (Project Number: 12-BIL-018) for financial support.

REFERENCES

[1] Friberg S., Larsson K., 1997. Food Emulsions, 3rd ed., New York: Marcel Dekker.

[2] Krog M.J., Riisom T.H., Larsson K., 1983. Applications in the food industry. In: Becker P and Dekker M (Eds.), Encyclopedia of Emulsion Technology Volume 2 (chapter 5), New York: 270 Madison Ave.

[3] Jaynes E.N., 1983. Applications in the food industry. In: Becker P and Dekker M (Eds.), Encyclopedia of Emulsion Technology Volume 2 (chapter 6), New York: 270 Madison Ave.

[4] Dickinson E., Stainsby G., 1982. Colloids in Foods. London: Applied Science Publishers, pp. 107-195.

[5] Swaisgood H.E.,1996. Characteristics of milk. In: Fox PF (Eds.), Food Chemistry 3rd ed. New York: Marcel Dekkel, Inc., pp. 841-878.

[6] McClements D.J., 1999. Food Emulsions; Principles, Practice, and Techniques. Bocs Raton, FL: CRC Press.

[7] Pal R., Yan Y., Masliyah J.H., 1992. Rheology of emulsions. In: Schramm LL (Eds.), Emulsions: Fundamentals and Applications in the Petroleum Industry Washington, D.C.: American Chemical Society, pp. 131-170.

[8] Turchiuli C., Lemarié N., Cuvelier M.E., Dumoulin E., 2012. Production of fine emulsions at pilot scale for oil compounds encapsulation. *Journal of Food Engineering* 115(4): 452-458.

[9] Dalmazzone C., 2000. Génération mécanique des emulsions. *Oil & Gas Science and Technology-Revue De L Institut Francais Du Petrole* 55(3): 281-305.

[10] Abismail B., Canselier J.P., Wilhelm A.M., Delmas H., Gourdon C., 2000. Emulsification processes: On-line study by multiple light scattering measurements. *Ultrasonics Sonochemistry* 7: 187-192.

[11] Mirhosseini H., Tan C.H., Hamid N.S., Yusof S., 2008. Optimization of the contents of arabic gum, xanthan gum and orange oil affecting turbidity, average particle size, polydispersity index and density in orange beverage emulsion. *Food Hydrocolloids* 22: 1212-1223.

[12] Dickinson E., 1992. Introduction to Food Colloids. Oxford: Oxford University Press.

[13] Bergenstahl G., 1997. Physiochemical aspects of an emulsifier functionality, food emulsifiers and their applications. In: Hasenhuettl G.L., Hartel R.W. (Eds.), Food Emulsifiers and Their Applications. New York: Chapman and Hall, pp. 173-194.

[14] Arshady R., George M.H., 1993. Suspension, dispersion, and interfacial polycondensation: A methodological survey. *Polymer Engineering and Science* 33(14): 865-876.

[15] Soottitantawat A., Yoshii H., Furuta T., Ohkawara M., Linko P.,2003. Microencapsulation by spray drying: Influence of emulsion size on the retention of volatile compounds. *Journal of Food Science* 68: 2256-2262.

[16] Soottitantawat A., Bigeard F., Yoshii H., Furuta T., Ohkawara M., Linko P., 2005. Influence of emulsion and powder size on the stability of encapsulated D-limonene by spray drying. *Innovative Food Science & Emerging Technologies* 6: 107-114.

[17] Bhandari B.R., Dumoulin H.M.J., Richard H.M.J., Noleau I., Lebert A.M., 1992. Flavor encapsulation by spray drying: Application to citral and linalyl acetate. *Journal of Food Science* 57: 217-221.

[18] Dickinson E., 1993. Protein–polysaccharide interactions in food colloids. In: Dickinson E., Walstra P. (Eds.), Food Colloids and Polymers: Stability and Mechanical Properties. Cambridge: Royal Society of Chemistry, pp. 77-93.

[19] Jafari S.H., Assadpoor E., He Y., Bhandari B., 2008. Re-coalescence of emulsion droplets during high-energy emulsification. Food Hydrocolloids 22: 1191-1202.

[20] Thies C., 2004. Microencapsulation: What it is and purpose. In: Vilstrup P (Eds.), Microencapsulation of Food Ingredients Leatherhead, UK: Leatherhead Publishing, (pp 1-30).

[21] Rosenberg M., Kopelman I.J., Talmon Y., 1990. Factors affecting retention in spray-drying microencapsulation of volatile materials. *Journal of Agricultural and Food Chemistry* 38(5): 1288-1294.

[22] Da Fonseca V.C., Haminiuk C.W.I, Izydoro D.R., Waszczynskyj N., Scheer A.P., Sierakowsk M.R., 2009. Stability and rheological behaviour of salad dressing obtained with whey and different combinations of stabilizers. *International Journal of Food Science and Technology* 44: 777-783.

[23] Laine P., Toppinen E., Kivelä R., Taavitsainen V., Knuutila O., Sontag-Strohm T., Jouppila K., Loponen J., 2011. Emulsion preparation with modified oat bran: Optimization of the emulsification process for microencapsulation purposes. *Journal of Food Engineering* 104: 538-547.

[24] Klinkesorn U., Sophanodora P., Chinachoti P., McClements D.J., 2004. Stability and rheology of

corn oil-in-water emulsions containing maltodextrin. *Food Research International* 37: 851-859.

[25] Tadros T., 2004. Application of rheology for assessment and prediction of the long term physical stability of emulsions. *Advances in Colloid and Interface Science* 108–109, 227–258.

[26] Abismail B., Canselier J.P., Wilhelm A.M., Delmas H., Gourdon C., 1999. Emulsification by ultrasound: Drop size distribution and stability. *Ultrasonics Sonochemistry* 6: 75-83.

[27] Papalamprou E.M., Makri E.A., Kiosseoglou V.D., Doxastakis G.I., 2005. Effect of medium molecular weight xanthan gum in rheology and stability of oil-in- water emulsions stabilized with legume proteins. *Journal of the Science of Food and Agriculture* 85: 1967–1973.

[28] Sun C., Gunasekaran S., 2009).Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. *Food Hydrocolloids* 23(1): 165-174

[29] Sun C., Gunasekaran S., Richards M.P., 2007. Effect of xanthan gum on physicochemical properties of whey protein isolated stabilized oil-in-water emulsions. *Food Hydrocolloids* 21(4): 555-564.

[30] Dickinson E., Golding M., 1997. Rheology of sodium caseinate stabilized oil-in water emulsions. *Journal of Colloid and Interface Science* 191: 166-176.

[31] Dokic P.P., Dokic L.P., Sovilj V.J., Katona J.M., 2004. Influence of maltodextrin dextrose equivalent value on rheological and dispersion properties of sunflower oil in water emulsions. *APTEFF* 35: 17-24.

[32] Hayati I.N., Man Y.B.C., Tan C.P., Aini I.N., 2009. Droplet characterization and stability of soybean oil/palm kernel olein O/W emulsions with the presence of selected polysaccharides. *Food Hydrocolloids* 23: 233-243.

[33] Walstra P., 1983. Formation of emulsion. In: Becker P and Dekker M (Eds.), Encyclopedia of Emulsion Technology Volume 1 New York, pp. 87-127.

[34] Schmidt H.W., 1985. Untersuchungen zur ultraschall-homogenisierung von milchproben. Lebensmittelindustr 32: 173-175.

[35] Martinez F.E., Desai I.D., Davidson A.G.F., Nakai S., Radcliffe A., 1987. Ultrasonic homogenization of expressed human milk to prevent fat loss during tube feeding. *Journal of Pediatric Gastroenterology and Nutrition* 6:593-597.

[36] Christensen K.L., Pedersen G.P., Kristensen H.G., 2001. Preparation of redispersible dry emulsions by spray drying. *International Journal of Pharmaceutics* 212: 187-194.