Hittite Journal of Science and Engineering, 2019, 6 (2) 91-98
ISSN NUMBER: 2148-4171
DOI: 10.17350/HJSE19030000133

Effects of Water Soluble Materials on Water-in-oil Primary Food Emulsion Stability

Hulya Cakmak[1,2] **Seher Kumcuoglu**[2], **Sebnem Tavman**[2]
[1]Hitit University, Food Engineering, Corum/Turkey
[2]Ege University, Food Engineering, İzmir/Turkey

Article History:
Received: 2018/05/01
Accepted: 2019/06/17
Online: 2019/06/30

Correspondence to: Hulya Cakmak,
Hitit University, Food Engineering,19030,
Corum, TURKEY
E-Mail: hulyacakmak@hitit.edu.tr
Phone: +90 364 227 45 33
Fax: +90 364 227 45 35

ABSTRACT

Water-in-oil (w/o) primary food emulsions are often used for encapsulation of bioactive food ingredients or preparing multiple emulsions, however there is a lack of study about evaluation of stability of these emulsions by adding food materials in the dispersed phase. In this study, water-in-olive oil emulsions were prepared with using different dispersed phases; maltodextrin (MD), whey protein isolate (WPI) and maltodextrin+whey protein isolate (MD+WPI) mixture (1:1 w/w) solutions were used at different rates to determine the effects of dissolved material on physical, chemical, rheological and thermal properties of the emulsions. The kinetic stability of emulsions were significantly improved with increasing rates of soluble materials ($p<0.05$), however the improvement was much clearer in emulsions with MD and WPI. The electrical conductivity of emulsions gradually decreased with the increasing amounts of soluble materials and the lowest conductivity was observed for the emulsion with 16% MD+WPI. Newtonian flow behaviour was observed for all of the emulsions, and the viscosity decreased with increasing amounts of dissolved material. The freezing and melting profiles of emulsions were found different than pure olive oil. The average freezing peak temperatures as well as the freezing and melting enthalpies of emulsions were higher than olive oil, but there was no clear difference observed depending on the amount or type of dissolved material. Average d43 values were found changing between 0.855-2.793 µm and the lowest polydispersity was obtained when MD+WPI mixtures were used as the dispersed phase at 16% level.

Keywords:
W/O; Stability; Electrical conductivity; Viscosity; Freezing.

INTRODUCTION

Emulsions are member of disperse systems and they are classified into two main groups as water-in-oil (w/o) or oil-in-water (o/w) depending on disperse or continuous phases. One of two immiscible liquids (oil or water) are dispersed as small spherical droplets in the continuous phase by incorporation of a surfactant (emulsifier) which helps to decrease the interfacial tension between these phases [1, 2]. However, the formed emulsion is thermodynamically unstable, and has a heterogeneous structure; therefore understanding stability mechanisms of emulsion is crucial for determining the shelf life stability of emulsion [1]. Forming of stable emulsion with smaller droplet diameter require high energy mixing or incorporating higher amounts of surfactant [3]. Therefore increasing emulsion stability by using new ingredients is gaining attention nowadays.

Emulsion properties can be characterized by using different experimental procedures; such as storage stability tests (accelerated or regular) and instrumental tests (microscopic analysis, electrical conductivity, droplet size measurement, thermal analysis, droplet charge etc.). There are several studies on evaluation of the stability of double emulsions; however there is a lack of knowledge on w/o primary food emulsion stability depending on the soluble protein and polysaccharide ratio found in dispersed phase of the emulsions.

Proteins and polysaccharides are two main food components used for controlling emulsion stability, microstructure and its texture as well [4]. The amphiphilic and surface-active nature of proteins especially milk proteins may improve the stability of emulsion when it is used as a surfactant [5, 6]. Besides, when two amphiphilic materials such as proteins and stabilizers are used together, they have synergistic effect against the rupture of formed interfacial film [7]. Proteins can form viscoelastic films at the oil-water interface and maintain the electrostatic and steric stability [8]. Viscosity of the dispersed phase with addition of soluble solids may approach to the continuous phase of emulsion and thus phase separation is reduced [9]. It was stated that, increasing polysaccharide ratio generates a high viscosity droplet network and helps to prevent phase separation [4]. Surfactants can interact with the polysaccharides and proteins which will then change conformation or association, while altering their functional properties such as; rheology, appearance and phase separation [1]. Protein and polysaccharide complexes and conjugates are used as stabilizers for encapsulation purposes [10]. The addition of polysaccharides improves elasticity of the emulsion by forming intermolecular complexes between the proteins and polysaccharides [11]. Utilization of protein and polysaccharide complexes or conjugates for improving o/w emulsion stability is extensively studied, however using of polysaccharide alone and together with the protein addition in w/o emulsion is not yet studied in the literature.

The main goal of this study is to evaluate kinetic stability of water-in-olive oil food emulsion by incorporation of whey protein isolate (WPI), maltodextrin (MD) and their 1:1 mixtures (WPI+MD) into the dispersed phase of emulsion. Physical, chemical, rheological and thermal stability of w/o emulsions were discussed thoroughly based on increasing amounts of soluble materials.

MATERIAL AND METHODS

Refined olive oil (average density: 908 kg/m^3, refractive index: 1.47, viscosity: 53.52 mPa.s, surface tension: 32.9 mN/m, electrical conductivity: 13.5 pS/m at 25°C) and polyglycerol polyricinoleate (PGPR) with of HLB 1.5-2.0 was kindly supplied by TARIS Zeytin ve Zeytinyagi Tarim Satis Kooperatifleri Birligi (Izmir) and Elvan Gida San. ve Tic. A.S. (Istanbul), respectively. Whey protein isolate (Hipro Isowhey, Hardline Nutrition, Istanbul) and maltodextrin with dextrose equivalent 5-7 (Paselli™ MD6, Avebe Nisasta Ltd., Izmir) were purchased from local suppliers.

Emulsion Preparation

The emulsions were prepared depending on the formulations given in Table 1, to wit 80% (w/w) oil phase, 20% (w/w) water phase were homogenized with 5% PGPR (of oil basis) regarding the previous study of the authors [12]. The water phase was composed of pure distilled water for control emulsion, while 1-2-4-8-16% (w/w) maltodextrin (MD), whey protein isolate (WPI) or maltodextrin+ whey protein isolate (1:1) mixture (MD+WPI) were incorporated into water phase as given in Table 1. W/o emulsions were homogenised by using rotor-stator homogenizer (IKA, T25 Ultra-turrax, Germany) equipped with a stainless steel 18G dispersing tool. Water phase of emulsions including either MD, WPI or MD+WPI solutions were prepared with tridistilled water and mixed for 1 h with a magnetic stirrer (Stuart CB162, UK) before using as dispersed phase of the w/o emulsion. Depending on the emulsion formulation, the dispersed phase was added into continuous phase (oil+emulsifier) and homogenized at 8000 rpm for 5 min as it was stated in the study of Cakmak et al. [12].

Table 1. Emulsion formulations

Emulsion type	*Oil (g)*	*Water (g)*	*PGPR (g)*	*MD (g)*	*WPI (g)*	*MD+WPI (1:1) (g)*
0%	*24*	*6.00*	*1.5*	*0*	*0*	*0*
1%	*24*	*5.94*	*1.5*	*0.06*	*0.06*	*0.06*
2%	*24*	*5.88*	*1.5*	*0.12*	*0.12*	*0.12*
4%	*24*	*5.76*	*1.5*	*0.24*	*0.24*	*0.24*
8%	*24*	*5.52*	*1.5*	*0.48*	*0.48*	*0.48*
16%	*24*	*5.04*	*1.5*	*0.96*	*0.96*	*0.96*

Determination of Separated Oil Phase and Viscosity of Emulsions

Emulsions kinetic stability was tested with an accelerated shelf-life test procedure as stated in the study of Wardhono [13] and Cakmak et al. [12]. 15 ml aliquots of emulsion in glass test tubes were stored at 54°C for 14 days in a vacuum oven (WiseVen WOV-30, Daihan Scientific Instruments, Korea). The height of separated oil phase in test tubes was measured daily over 14 days and separated oil phase percent (v/v) was calculated according to the following equation;

$$\text{sep. oil \%} = \frac{V_{\text{oil, t}}}{V_{\text{oil,t0}}} \times 100 \qquad (1)$$

here $V_{oil,t}$ represents the volume of separated oil at t=t$_1$, and V_{oil},t_0 represents the volume of oil at initial state (t=0). Accelerated shelf-life tests were performed as three parallels and two replicates.

Viscosity of emulsions was measured at 25°C with a stress-controlled rheometer (DHR3, TA Instruments, USA) equipped with concentric cylinder (bob diameter: 27.99 mm, cup diameter: 30 mm). 25 ml of emulsion was placed into cup, and was conditioned at 25°C for 1 min, and steady pre-shear was applied at a shear rate of 15 rad/s (217.76 1/s) for 2 min in order to eliminate prior stresses. The emulsion was equilibrated for another 1 min at 25°C before application of flow-ramp test, and test was applied between 0.01 to 200 1/s shear rate. Apparent viscosity values (mPa.s) of these emulsions were obtained from the slope of graph of shear rate (1/s) versus shear stress (mPa), since Newtonian flow behaviour was observed for the emulsions ($R^2>0.99$).

Electrical Conductivity and Surface Tension of the Emulsions

Electrical conductivity of the olive oil and emulsions were measured with a hand held conductivity meter (Stanhope-Seta, JF 1A-HH, Surrey, UK) at 25°C working within the range of 0-2000 pS/m and in accordance with ASTM D2624 standard test method. In addition, electrical conductivity of the dispersed phases was measured with Mettler-Toledo Seven Excellence S700 analytical meter with INlab 731 ISM probe (Schwerzenbach, Switzerland). This probe allows measurement of electrical conductivity within the range of 0.01-1000 mS/cm.

The surface tension of oil or emulsions against air interface was measured at 25°C according to Wilhelmy plate method by using Krüss K20 Easy dyne (Krüss Gmbh, Germany) tensiometer. The measurement was performed in triplicate and average values were reported.

Droplet Diameter and Thermal Analyses of the Emulsions

Droplet diameter distribution of the emulsions were determined by static light scattering method using Mastersizer 2000 (Malvern Instruments, UK) particle analyser equipped with liquid cell (Hydro 2000S) as stated in the study of Tontul and Topuz [14]. Refractive index of olive oil measured with a digital refractometer (RFM 330, Bellingham+Stanley Ltd., Kent, UK) and found as 1.47 and 1.33 for water. The mean droplet diameter was expressed as volume-weighted mean diameter (d_{43}), and surface-weighted mean diameter (d_{32}) given in the below equations;

$$d_{43} = \frac{\sum_{i=1} n_i d_i^{\,4}}{\sum_{i=1} n_i d_i^{\,3}} \tag{2}$$

$$d_{32} = \frac{\sum_{i=1} n_i d_i^{\,3}}{\sum_{i=1} n_i d_i^{\,2}} \tag{3}$$

here n_i is the number of droplet and d_i is the droplet diameter. For polydisperse emulsions, d_{43} diameter is indicated to be more sensitive to the presence of large particles (coalescence and/or flocculation) than d_{32}; therefore the average droplets size was discussed based on the d_{43} values [1, 15]. The width of droplet diameter distribution was determined by calculation of span values from the following equation;

$$span = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \tag{4}$$

In this equation, $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ are the diameters of 10, 50 and 90% volume percentiles of the droplets smaller or equal to these values.

Thermal properties of the pure olive oil and the emulsions were performed using Q2000 differential scanning calorimeter (DSC Q2000, V24.11, TA Instruments, USA) by using the method stated in the study of Zafimahova-Ratisbonne et al. [16]. 10 mg of sample were weighed into aluminium pan by an electronic balance (Mettler Toledo, MS205DU, Switzerland) with a resolution of ±0.01 mg, and the cover was hermetically sealed. An empty hermetically sealed aluminium pan was used as a reference pan. Nitrogen gas with 99% purity was used as the purge gas at 50 ml/min steady flow rate. Samples were equilibrated at 20°C for 5 min, and cooled to -60°C at the rate of 2.5°C/min. Then the sample held at -60°C for 5 min before heating from -60°C to 20°C at the same flow rate. The thermograms were analysed with TA Universal Analysis software (Version V4.5A, TA Instruments), and freezing and melting enthalpies (ΔH_{fr} and ΔH_m, J/g), onset (T_{on}, °C) and offset (T_{off}, °C) transition temperatures and peak temperatures (T_p, °C) were recorded.

Statistical Analyses

The differences between the results were analysed using SPSS version 20.0. They were compared using analysis of variance (ANOVA) by Duncan's multiple range test with a significance level of 95%.

RESULTS AND DISCUSSION

Properties of Emulsions with MD

The physical, chemical and electrical properties of MD including emulsions are given in Table 2A. The separated oil phase percent decreased with increasing amounts of soluble solids and therefore the kinetic stability of the w/o emulsion was further improved. This could be due to the gradual decrease of volume of water in the emulsion or increase in the viscosity of the water phase. As stated in the study of Cakmak et al. [12], the increase in the dispersed phase fractions (Φ) leads to the coalescence and phase separation. Maltodextrins are not surface-active compounds, and they can improve emulsion stability by viscosity modification, acting as a thickening agent and gelatinization of continuous water phase [17].

Viscosity of the emulsions with MD were statistically in the same group up to 4% ratio ($p>0.05$), however the viscosity of emulsion decreased in the latter ratios of MD ($p<0.05$).

Table 2A. Properties of MD including emulsions.

Maltodextrin amount (%, w/w)	Sep. oil % (v/v)	Viscosity (mPa.s)	Electrical conductivity (pS/m)	Surface tension (mN/m)
0	21.46^{d}	113.64^{e}	-	33.3^{a}
1	12.47^{c}	112.54^{d}	1947.7^{e}	33.3^{a}
2	$12.03^{b,c}$	$111.85^{c,d}$	1701.2^{d}	33.3^{a}
4	$10.34^{a,b}$	111.19^{c}	1506.9^{c}	33.2^{a}
8	$10.42^{a,b}$	109.48^{b}	1309.7^{b}	33.3^{a}
16	9.73^{a}	107.68^{a}	1159.6^{a}	33.4^{a}

Table 2B. Properties of WPI including emulsions.

Maltodextrin amount (%, w/w)	Sep. oil % (v/v)	Viscosity (mPa.s)	Electrical conductivity (pS/m)	Surface tension (mN/m)
0	21.46^{d}	113.64^{d}	-	33.3^{a}
1	12.44^{c}	113.19^{d}	1935.9^{e}	33.2^{a}
2	$11.52^{b,c}$	112.74^{d}	1797.3^{d}	33.2^{a}
4	$10.75^{a,b}$	111.60^{c}	1362.3^{c}	33.2^{a}
8	9.61^{a}	109.03^{b}	1132.3^{b}	33.1^{a}
16	9.37^{a}	107.93^{a}	908.4^{a}	33.1^{a}

Table 2C. Properties of MD+WPI including emulsions.

Maltodextrin amount (%, w/w)	Sep. oil % (v/v)	Viscosity (mPa.s)	Electrical conductivity (pS/m)	Surface tension (mN/m)
0	21.46^{b}	113.64^{c}	-	33.3^{a}
1	10.84^{a}	111.58^{b}	1656.7^{e}	33.1^{a}
2	9.49^{a}	111.64^{b}	1597.1^{d}	33.3^{a}
4	9.35^{a}	111.60^{b}	1389.6^{c}	33.2^{a}
8	9.08^{a}	111.61^{b}	1053.2^{b}	33.2^{a}
16	9.03^{a}	107.59^{a}	838.9^{a}	33.2^{a}

$^{a-e}$ Different letters in the same column are statistically different ($p<0.05$).

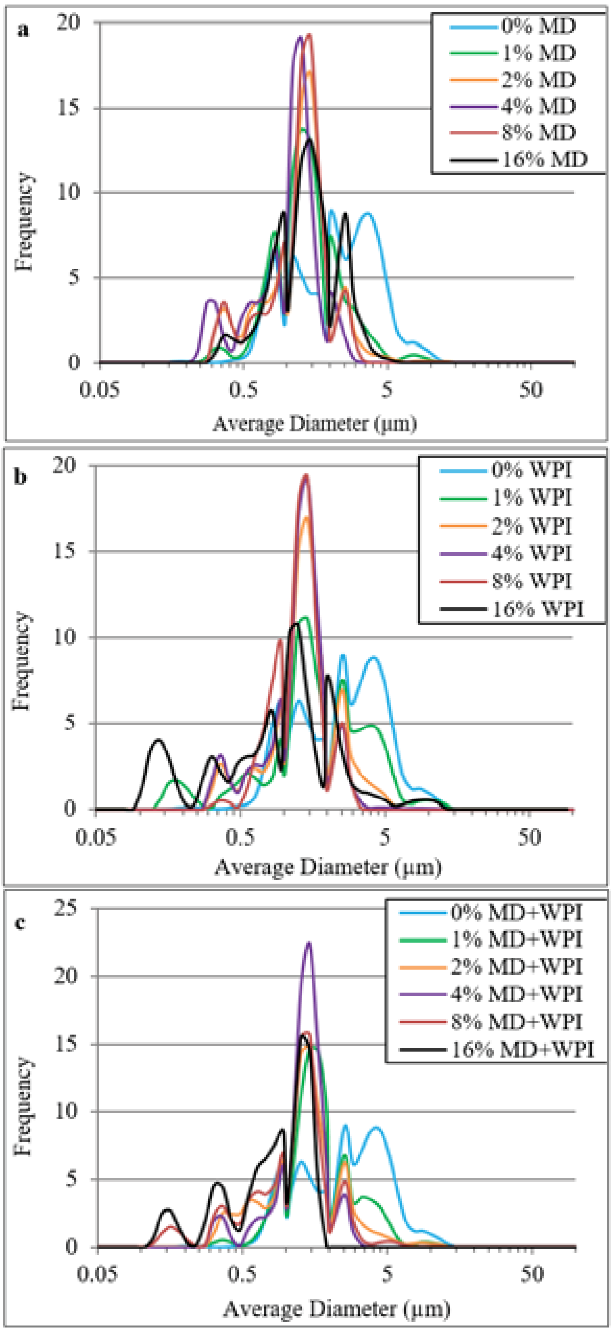

Figure 1. Average particle diameter distribution of emulsion with MD (a); emulsion with WPI (b); emulsion with MD+WPI (c)

Even though the food emulsions are generally known to be having non-Newtonian flow behaviour, the results of the present study are contrary to this knowledge. Similar to this present study, Ushikubo and Cunha [18] have stated that water-in-soybean oil emulsions prepared with PGPR at 70:30 (oil:water) ratio had a Newtonian flow behaviour; however it was stated that at 60:40 ratio emulsions had a shear-thinning behaviour.

The increment in the ratio of MD further increases the dispersed phase viscosity, however the formed emulsions had lower viscosity than the control emulsion (0% MD). Polydisperse emulsions can be packed more efficiently compared to the monodisperse emulsions, so the viscosity of a polydisperse emulsion may be less than the monodisperse emulsions [1]. As shown in Fig. 1A, the studied emulsions had a polydisperse size distribution apart from the MD ratio in the dispersed phase. Depending on the equation derived by Einstein, the viscosity of an emulsion (suspension) is correlated with the viscosity of the continuous phase [1]. However this equation is valid when the continuous phase is Newtonian, dispersed particles are rigid with spherical shape and in case of no particle-particle interaction is observed. For most of the food emulsions, the viscosity of dispersed phase are rather has no importance because of the droplets acts as rigid spheres [1].

The electrical conductivities of the emulsions are found

Table 3A. Average particle diameter and span values of emulsion with MD.

MD amount (%, w/w)	d_{32} (µm)	d_{43} (µm)	span
0	$1.748^{d;C}$	$2.793^{d;D}$	1.757
1	$1.247^{c;B}$	$1.678^{c;C}$	1.487
2	$0.942^{a,b;A}$	$1.294^{b;B}$	1.225
4	$0.900^{a;A}$	$1.175^{a;A}$	1.023
8	$0.932^{a,b;A}$	$1.182^{a;A}$	1.027
16	$0.966^{b;A}$	$1.344^{b;B}$	1.482

Table 3B. Average particle diameter and span values of emulsion with WPI.

MD amount (%, w/w)	d_{32} (µm)	d_{43} (µm)	span
0	$1.748^{c;C}$	$2.793^{e;E}$	1.757
1	$1.023^{b;B}$	$2.037^{d;D}$	2.597
2	$1.070^{b;B}$	$1.440^{c;C}$	1.416
4	$0.988^{b;B}$	$1.272^{a,b;A,B}$	1.072
8	$1.074^{b;B}$	$1.230^{a,b;A}$	0.941
16	$0.423^{a;A}$	$1.344^{b;B,C}$	1.722

Table 3C. Average particle diameter and span values of emulsion with MD+WPI.

MD amount (%, w/w)	d_{32} (µm)	d_{43} (µm)	span
0	$1.748^{e;E}$	$2.793^{f;F}$	1.757
1	$1.354^{d;D}$	$1.831^{e;E}$	1.713
2	$0.988^{c;C}$	$1.450^{d;D}$	1.567
4	$1.028^{c;C}$	$1.265^{c;C}$	0.905
8	$0.748^{b;B}$	$1.153^{b;B}$	1.292
16	$0.574^{a;A}$	$0.855^{a;A}$	1.259

$^{a\text{-}f}$Different letters in the same column are statistically different ($p<0.05$).
$^{A\text{-}F}$Different letters in the same column are statistically different ($p<0.01$).

to be gradually decreasing with increasing amounts of MD, which directly corresponds to the gradual decrease of volumetric amount of water in the dispersed phase. Phase separation (gravitational) or phase inversion can be determined by measuring electrical conductivity [1, 6, 19]. The sharp decrease in electrical conductivity occurs when the o/w emulsion inverts to w/o emulsion [19]. The electrical conductivity of continuous oil phase was 13.5 pS/m and pure water having of comparably higher electrical conductivity, the emulsion conductivity was therefore closer to the continuous phase. But if there was a phase separation depending on the instability of the emulsion, we could observe that the electrical conductivity of the emulsions might be much higher, even getting closer to the water phase. The dispersed phase conductivity with increasing amount of MD dissolved was increased in spite of the opposite trend was observed for the emulsion conductivity. 16% MD solution had an average electrical conductivity of 34.5±0.0 mS/m.

The surface tension of the emulsion were not statistically different ($p>0.05$) depending on the MD amount dissolved. Moreover, the surface tension of the pure oil (32.9 mN/m) was also similar to these emulsions.

Particle size distribution and average particle diameter values of MD including emulsion are shown in Fig. 1A and Table 3A. Multimodal droplet diameter distribution was observed for all MD including emulsions. However, depending on the increase in the MD amount dissolved, the polydispersity of droplet diameter decreased and narrow diameter distributions with much smaller diameters were observed. The smallest d_{43} values were observed for 4% and 8% MD emulsions, but the smallest span value was observed for 4% MD emulsion. Higher span values are associated with a more polydisperse and therefore a lower stability of the emulsion [20]. If the phase separation values and average droplet diameter values were analysed together, the smallest average diameter were not observed in the lowest phase separated values. Small droplet diameter values are desired for increasing the emulsion stability; however decrease in the droplet size induces Brownian motion which may promote destabilization of emulsion by flocculation mechanism [21].

Properties of Emulsions with WPI

The physical, chemical and electrical properties of emulsions with WPI are given in Table 2B. Separated oil phase of the WPI emulsions were found exponentially decreasing with the amount of WPI dissolved ($R^2=0.9767$), similar to the viscosity ($R^2=0.9429$) and electrical conductivity change ($R^2=0.9794$). Milk proteins and especially whey proteins are often used with surfactants for increasing the emulsion stability by contributing steric stabilization against flocculation and coalescence [9]. Proteins are able to cover the oil droplets as a monolayer during homogenization and thus it lowers the interfacial tension and prevent droplet coalescence in o/w emulsions [22, 23]. The increment in the WPI content resulted in a similar behaviour with the MD including emulsions, so that the lower separated oil phase, viscosity and electrical conductivity was observed in higher dissolved material concentrations. However, the electrical conductivity of 16% WPI emulsion was statistically lower than the 16% MD emulsion ($p<0.05$). This result is contrary to the electrical conductivity of the solutions with MD and WPI. 16% MD solution had an average electrical conductivity of 34.5±0.0 mS/m while 16% WPI emulsion had an average conductivity of 195.2±0.3 mS/m. As stated by McClements [22], surfactants may affect the protein conformation and the interactions; such as non-ionic surfactants may bind to the protein molecules with hydrophobic interactions. Also the change in molecular characteristics of the globular proteins (such as WPI) may then alter the interfacial properties of the emulsion.

The surface tension of the emulsions with WPI were found again similar to the oil, and there was no change observed depending on the amount of material dissolved (p>0.05).

Particle size distribution and average particle diameter values of WPI including emulsion are shown in Fig. 1B and Table 3B. Average particle diameters were in accordance with the separated oil phase amount, however the smallest d_{43} values were observed in the emulsion with 8% WPI. In addition, the particle diameter distribution is shifted towards left, which demonstrates the narrower particle diameter with smaller average diameters were observed depending on the increment in the WPI amount. 16% WPI emulsion had a lot more number of particles smaller than 0.5 μm compared to the other emulsions, and these results in accordance with the surface-weighted mean diameter. Moreover the type of the dissolved material did not have any significant effect on the kinetic stabilities of the emulsions, but on microscopic level differences were revealed by the droplet diameter distributions.

The stability of emulsions with proteins can be further improved with addition of polysaccharides for controlling rheology, and hence the phase separation and gravity induced creaming is delayed [9]. The use of proteins together with the polysaccharides provides some advantages such as; improvement of the physicochemical and storage stability as well as textural and mouthfeel properties [23]. Ercelebi and Ibanoğlu [24] stated that, the creaming instability of the protein stabilized emulsions were decreased by the use of polysaccharides (pectin vs. guar gum), and WPI-guar gum complex produced the more stable emulsions compared to the WPI-pectin complexes.

Properties of Emulsions with MD+WPI Mixture

The kinetic stability of the MD+WPI emulsions were found statistically in the same group (p>0.05), which was not in accordance with the general trend observed in MD or WPI emulsions. Protein and polysaccharide conjugates improve the emulsion stability better than protein used alone [5]. But there was no data about the improvement of emulsion stability when protein and polysaccharide is used as a solution instead of conjugate. As shown in Table 2C, the kinetic stability of emulsion with 1:1 mixture of MD+WPI was further improved even at 1% incorporation level. Although the dispersed phase fraction was similar for each dispersed material type (protein, polysaccharide or their mixture), the viscosities of 1-2-4 and 8% MD+WPI were found in the same group as well (p>0.05). These findings support our claim that not only conjugates but the solution of maltodextrin with whey protein isolate at 1:1 level may improve the stability of w/o emulsions. Also, the electrical conductivity of the MD+WPI solution were found between the MD and WPI solutions alone, however the conductivity of 16% MD+WPI mixture was statistically lower compared to the 16% MD and 16% WPI emulsions (data not shown). This data also was consistent with the previous findings, and the interfacial tension between water and oil phase of the emulsion with 16% MD+WPI mixture might possibly decreased.

The droplet diameter distributions and the average particle diameters of the MD+WPI emulsions are given in Fig. 1C and Table 3C, respectively. The diameter distribution of the emulsions gets narrower with increasing amount of dissolved material, and there was no particle observed above 2 μm diameter for 16% MD+WPI. The degree of polydispersity was reduced, and even better results were obtained compared to WPI emulsions when the MD+WPI mixtures were used as the dispersed phase at 16% incorporation level. So, MD+WPI solutions at 1:1 level may further be used to improve the stability of w/o emulsions.

Thermal Properties of the Emulsions

DSC analysis provides practical information about the thermodynamic changes occurred during phase transition of edible oils, and these thermodynamic characteristics may help to identify the chemical composition, phase separation (instability of emulsion) as well as identification or detection of the adulteration of these edible oils [25-28]. Thermal properties of the emulsions between freezing and melting processes are shown in Table 4. During freezing, a major exothermic peak around -40°C and a smaller peak around -10°C was observed in the thermograms of pure olive oil and 1% WPI including emulsion (data not shown). Two endothermic peaks, one of them having a much steeper peak around -5°C was observed during melting. For extra virgin olive oil, a minor peak at -13°C and a major peak at -38°C was observed during freezing as it was stated in the study of Chiavaro et al. [26]. Similar to this present study, Calligaris et al. [29] stated that a major peak between -18°C and 0°C with a minor peak between 0-10°C was observed during melting of extra virgin olive oil. These distinct differences in two peaks are related with the chemical composition of the oil; since saturated fatty acids (triacylglycerol) crystallize at higher temperatures and melt at higher temperatures compared to the unsaturated fatty acids [26, 28-30].

Melting and freezing enthalpies of the emulsions were

Table 4. Freezing and melting properties of emulsions.

Sample	$T_{fr,on}$ (°C)	$T_{fr,p}$ (°C)	$T_{fr,off}$ (°C)	ΔH_{fr} (J/g)	$T_{m,on}$ (°C)	$T_{m,p}$ (°C)	$T_{m,off}$ (°C)	ΔH_m (J/g)
Olive oil	-35.67±1.12	-40.14±0.15	-51.22±0.22	34.39±0.40	-19.24±0.04	-4.01±0.05	9.98±0.04	67.91±0.84
0%	-35.09±0.09	-38.14±0.03	-44.52±0.27	63.59±1.11	-15.77±0.09	0.64±0.03	8.99±0.14	106.35±1.35
1% MD	-34.72±0.29	-38.40±0.01	-45.41±0.38	62.75±1.09	-16.61±0.47	0.61±0.08	9.41±0.34	105.20±1.80
1% WPI	-34.94±0.14	-38.20±0.01	-43.81±0.09	64.29±0.10	-16.55±0.24	0.62±0.01	9.54±0.17	109.35±0.05
1% MD+WPI	-34.73±0.36	-38.33±0.03	-44.47±0.29	62.08±0.65	-16.32±0.15	0.62±0.01	9.21±0.07	105.55±1.75
16% MD	-34.96±0.16	-38.78±0.05	-45.10±0.05	53.90±0.07	-14.90±0.16	0.45±0.05	8.98±0.00	91.14±0.18
16% WPI	-33.34±0.16	-38.77±0.02	-43.05±0.05	60.45±0.20	-16.57±0.00	0.34±0.01	9.95±0.11	100.40±0.30
16% MD+WPI	-34.30±0.06	-38.67±0.02	-43.84±0.20	57.06±0.18	-17.05±0.60	0.27±0.00	10.12±0.15	97.76±0.40

significantly higher than the olive oil itself, since water and emulsifier (PGPR) addition to oil contributed to this increase as similarly stated by the literature [16]. However, there is no clear difference in the melting or freezing enthalpies observed depending on the type of material dissolved (either protein or polysaccharide) or the amount of material in the dispersed phase. The droplet diameter also is an important parameter on freezing temperature, such as the emulsion with a smaller mean diameter have a lower freezing temperature [16], however there was no significant difference observed in the freezing temperatures of the emulsions depending on the average droplet diameter in this present study.

CONCLUSION

The physical and chemical stability of the emulsions can be determined with various methods; however the effects of soluble solid type and the amount of soluble materials not yet discussed. In this study, whey protein isolate and maltodextrin were incorporated individually or together into the dispersed water phase of the w/o emulsion and depending on the obtained results; MD+WPI mixtures at 1:1 rate could successfully increase the kinetic, chemical and thermal stability of w/o food emulsions. Especially 16% MD+WPI incorporation level have significantly reduced the average particle diameter (d_{43}). The kinetic stability, and viscosity of MD and WPI incorporated emulsions was in the same group with the MD+WPI emulsion for 16% level, however the electrical conductivity of the emulsion with MD+WPI was significantly lower than both of the emulsion with MD and WPI. Further studies may focus on testing the stability of primary w/o emulsions with respect to homogenization conditions or effect of different protein and polysaccharide sources on emulsion stability may be tested.

ACKNOWLEDGEMENT

This study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK) with the Grant No. 214O405 and by Ege University Science and Technology Project Commission (BAP) with the Grant No. 15-BIL-024. Authors also would like to thank Dr. Ismail Tontul and Prof. Dr. Ayhan Topuz for their help in droplet diameter measurements, Dr. A. Evren Yavuz for his help in surface tension measurement, G. Ela Gurpuz and Neslihan Bozdogan for their help in some of experimental studies. Some parts of this study was presented in the 21st National Thermal Science and Technology Congress (2017) held in Çorum.

References

1. McClements D.J. Food Emulsions: Principles, Practices, and Techniques, 2nd ed., CRC Press, Boca Raton, p. 609, 2005.
2. Pichot R. Stability and Characterisation of Emulsions in the Presence of Colloidal Particles and Surfactants, PhD Thesis, University of Birmingham, UK, p. 204., 2010.
3. Tadros T.F. Emulsion Science and Technology, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, p. 344, 2009.
4. Herrera M.L. Analytical Techniques for Studying the Physical Properties of Lipid Emulsions, Springer, New York, p. 61, 2012.
5. Akhtar M., Dickinson E. Whey protein–maltodextrin conjugates as emulsifying agents: an alternative to gum arabic. Food Hydrocolloids 21(4) (2007) 607-616.
6. Tavernier I., Wijaya W., Van der Meeren P., Dewettinck K., Patel A. R. Food-grade particles for emulsion stabilization. Trends in Food Science & Technology 50 (2016) 159-174.
7. Aserin A. Multiple Emulsions: Technology and Applications, John Wiley & Sons Inc., Hoboken, p. 326, 2008.
8. Lam R.S., Nickerson M.T. Food proteins: a review on their emulsifying properties using a structure–function approach. Food Chemistry 141(2) (2013) 975-984.
9. Dickinson E. Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. Soft Matter 4(5) (2008) 932-942.
10. Evans M., Ratcliffe I., Williams, P.A. Emulsion stabilisation using polysaccharide–protein complexes. Current Opinion in Colloid & Interface Science 18(4) (2013) 272-282.
11. Derkach S.R. Rheology of emulsions. Advances in Colloid and Interface Science 151(1-2) (2009) 1-23.
12. Cakmak H., Gurpuz G.E., Bozdogan N., Kumcuoglu S., Tavman S.

Dispersed Phase and Emulsification Conditions on the Stability of Water-in-Oil Emulsion. Food Studies 7(1) (2017) 29-37.

13. Wardhono E.Y. Optimization of Concentrated W/O Emulsions: Stability, Trapping and Release of Polysaccharides, PhD Thesis, Université de Technologie Compiégne, France, p. 168, 2014.
14. Tontul, I., Topuz, A. Mixture design approach in wall material selection and evaluation of ultrasonic emulsification in flaxseed oil microencapsulation. Drying Technology 31(12) (2013) 1362-1373.
15. Komaiko J., Sastrosubroto A., McClements, D.J. Formation of oil-in-water emulsions from natural emulsifiers using spontaneous emulsification: sunflower phospholipids. Journal of Agricultural and Food Chemistry, 63(45) (2015) 10078-10088.
16. Zafimahova-Ratisbonne A., Wardhono E.Y., Lanoisellé J.L., Saleh K., Clausse D. Stability of W/O emulsions encapsulating polysaccharides. Journal of Dispersion Science and Technology 35(1) (2014) 38-47.
17. Silva K.A., Rocha-Leão M.H., Coelho M.A.Z. Evaluation of aging mechanisms of olive oil–lemon juice emulsion through digital image analysis. Journal of Food Engineering, 97(3) (2010) 335-340.
18. Ushikubo F.Y., Cunha R.L. Stability mechanisms of liquid water-in-oil emulsions. Food Hydrocolloids 34 (2014) 145-153.
19. Maali A., Mosavian, M.H. Preparation and application of nanoemulsions in the last decade (2000–2010). Journal of Dispersion Science and Technology 34(1) (2013) 92-105.
20. Eisinaite V., Juraite D., Schroën K., Leskauskaite, D. Preparation of stable food-grade double emulsions with a hybrid premix membrane emulsification system. Food Chemistry 206 (2016) 59-66.
21. Tadros T., Izquierdo P., Esquena J., Solans, C. Formation and stability of nano-emulsions. Advances in Colloid and Interface Science 108 (2004) 303-318.
22. McClements D.J. Protein-stabilized emulsions. Current Opinion in Colloid & Interface Science 9(5) (2004) 305-313.
23. Sun C., Gunasekaran S., Richards, M.P. Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. Food Hydrocolloids 21(4) (2007) 555-564.
24. Ercelebi E.A., Ibanoğlu, E. Influence of hydrocolloids on phase separation and emulsion properties of whey protein isolate. Journal of Food Engineering 80(2) (2007) 454-459.
25. Tan C.P., Man, Y.C. Differential scanning calorimetric analysis of edible oils: comparison of thermal properties and chemical composition. Journal of the American Oil Chemists' Society 77(2) (2000) 143-155.
26. Chiavaro E., Vittadini E., Rodriguez-Estrada M.T., Cerretani L., Bendini A. Differential scanning calorimeter application to the detection of refined hazelnut oil in extra virgin olive oil. Food Chemistry 110(1) (2008) 248-256.
27. Fasina O.O., Craig-Schmidt M., Colley Z., Hallman H. Predicting melting characteristics of vegetable oils from fatty acid composition. LWT-Food Science and Technology 41(8) (2008) 1501-1505.
28. Jafari M., Kadivar M., Keramat, J. Detection of adulteration in Iranian olive oils using instrumental (GC, NMR, DSC) methods. Journal of the American Oil Chemists' Society 86(2) (2009) 103-110.
29. Calligaris S., Sovrano S., Manzocco L., Nicoli, M.C. Influence of crystallization on the oxidative stability of extra virgin olive oil. Journal of Agricultural and Food Chemistry 54(2) (2006) 529-535.
30. Barba L., Arrighetti G., Calligaris S. Crystallization and melting properties of extra virgin olive oil studied by synchrotron XRD and DSC. European Journal of Lipid Science and Technology 115(3) (2013) 322-329.