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Oxidative Stability and Rheological Properties of Oil-In-Water Emulsions with Walnut Oil

Kremena Nikovska
University of Food Technologies, Plovdiv, 26 Maritza Blvd, Bulgaria

Abstract: The oxidative stability of walnut oil and oil-in-water (O/W) emulsions with walnut oil stabilized by soy protein isolate (SI) and Whey Protein Isolate (WPI) was evaluated. The food emulsions were more stable than walnut oil, as indicated by measuring the formation of primary and secondary oxidation products. It was shown that the emulsions with WPI had a better oxidative stability than the emulsions with SI, probably due to the ability of whey proteins to inactivate peroxil radicals. In addition, the basic rheological characteristics: apparent and plastic viscosities, yield stress, consistency coefficient, and flow behavior index of the evaluated O/W emulsions, were determined. The rheological behavior and flow curves were analyzed using a fitted power law and Herschel-Bulkley model. The emulsions with SI showed increased viscosity in comparison to WPI emulsions. Moreover, soy protein formed a continuous network, and emulsions were more stable to creaming.

Key words: Food emulsions, oxidation, protein emulsifiers, rheology

INTRODUCTION

The oil-in-water (O/W) food emulsions are the basis of many food products and their properties define food quality to a great extent. As a consequence, interest in the theory and practice of food emulsions has been increasing. Scientific studies are oriented more and more toward different aspects of the characteristics, formation, behavior, and application of these food emulsions.

Theoretical and technological fundamentals of food emulsions are described in great detail in monographs by Becher (1965) and Sherman (1968), as well as in other scientific articles and books. A lot of information has been published on the production of oil-in-water emulsion systems, too. A number of studies are concerned with basic characteristics and factors which influence the behavior, quality and properties of food emulsions.

The most important emulsion properties are emulsion stability, rheological behavior, interfacial properties and interactions, texture, and flavor. The factors that have influence on these properties can be generalized as follows: type and concentration of emulsifiers and oil phase, the method of homogenization and emulsifying, and contents of the continuous phase. Regarding food emulsions technologies, it could be suggested that the emphasis should be on the use of natural emulsifying and stabilizing compositions, as well as on the incorporation of nutritional oils with beneficial fatty acids contents. Some scientific studies also emphasize the rheological properties and oxidative stability of food emulsions.

Recently, a great deal of research has been focused on oxidation in oil-in-water emulsions (Coupland and McClements, 1996; German, 1999; Hu *et al.*, 2003). The reason is the availability of many emulsified food products. Lipid oxidation has been extensively studied in bulk fats and oils, as well as in emulsified lipids (Frankel *et al.*, 1994; Chaiyasit *et al.*, 2007). The various molecules in an emulsion distribute themselves between three different regions (the interior of a droplet, the continuous phase, and the interfacial region) according to their polarity and surface activity (Huang *et al.*, 1997; Deis, 2002). The factors that affect the oxidative stability of O/W emulsions are chemical structure of lipids, interfacial characteristics, droplet characteristics (concentration, size), and interactions with aqueous phase components (salt, sugars, proteins) (Coupland and McClements, 1996; Hu *et al.*, 2002).

The process of lipid oxidation depends on the type of emulsifier and emulsified oil (Frankel, 1991; Mei *et al.*, 1999). In many of the studies of oxidation in an O/W emulsions model, emulsions have been prepared with synthetic surfactants (Brij, Tween), whereas natural proteins are recommended for food use.

Regarding oils, a wide variety of different types of oils have traditionally been used in food emulsions, including soybean, corn, canola, olive, safflower, and sunflower oils (Hui, 1992). The trend has been to replace traditional oils with more health-promoting oils, such as polyunsaturated lipids. Walnut oil is one of these beneficial oils.

Walnut oil is high in omega-3 fats, which have been linked to a variety of health benefits, including reducing the risk of dementia and heart disease (Patel, 2005). The problem associated with incorporating polyunsaturated lipids into food is their high susceptibility to oxidation (Popov and Yanishlieva, 1976).

The objective of this study is to perform measurements of oxidative stability and rheological behavior of oil-in-water emulsions with walnut oil and natural protein emulsifiers.

MATERIALS AND METHODS

Materials: The study was conducted in May 2009 at the University of Food Technologies, Bulgaria. Unrefined cold-pressed walnut oil was provided by an oil-manufacturing plant in Bulgaria. Soy protein isolate (protein, wt91.1%; moisture, wt4.5%) was obtained from Solae Belgium N.V. Whey protein isolate (protein, wt90%; moisture, wt4%; ash, wt5 %; carbohydrates, wt1 %) was obtained from F.I.A. Food Ingredients Anthes GmbH (Germany). Sodium benzoate, obtained from Qingdao Rich Trading Co.Ltd (China), was used as an antimicrobial agent.

Preparation of oil-in-water emulsions: Thirty and seventy percent v/v oil-in-water emulsions were prepared as follows: WPI and SI was first dispersed in the water phase to form a solution of 4, 6, and 8% w/v. Emulsions were prepared by slowly adding 30 ml and 70 ml olive oil into the 70 ml WPI and 30 ml SI solutions respectively. Emulsification was performed by a Taurus homogenizer at 6000 rpm for 2 min. Sodium benzoate was added at a concentration of 0.1 w/v in the continuous phase. The pH values of the final 12 O/W emulsion samples ranged between 3.8 and 4.1.

Oxidative stability evaluation: Experimental design: Duplicate samples of each emulsion and walnut oil were placed in watch glasses in a thin layer (1 mm) and held at 40ºC. Measurements were performed every day.

Analytical determination: The progress of lipid oxidation was assessed by measuring Peroxide Value (PV), p-Anisidine Value (AV), Total Oxidation Value (TOTOX), Conjugated Dienes (CD), Conjugated Trienes (CT).

The AV test was modified according to the British Standard Method (Rossell, 1986): 0.5-4 ml emulsions were added into a 25 ml volumetric flask and made up to the mark with isooctane. The test tubes were vortexed two times for 10 sec each. After centrifugation for 10 min at 5000 rpm, the absorbance (A_1) of the samples was measured at 350 nm against a pure isooctane blank using a spectrophotometer. 5 ml aliquots or 5 ml isooctane (as blank) was then transferred to 10 ml test tubes and 1 ml para-anisidine solution (0.25 % w/v solution in glacial acetic acid) was added. After vortexing 10 s and standing for 10 min, its absorbance (A_2) was measured at 350 nm against the isooctane blank containing p-anisidine.

$$AV = 25(1.2xA_2-A_1)/m$$

where; m is the sample mass.

The traditional method of determining PV, expressed as milliequivalents of active oxygen per kilogramme of oil (meq/kg), involves a titration of the oil containing potassium iodide in a chloroform-acetic acid mixture. The hydroperoxides oxidise the iodide to iodine, which can be determined by titration with sodium thiosulphate (AOAC official method 965.33). The analysis was performed according to a modified method (Popov and Yanishlieva, 1976) the oil content in the emulsion sample was 0.5 g, and the blank sample contained water with volume equivalent to the water in the emulsion sample.

The determination of the absorbance in the UV spectrum of the samples was measured at 232 nm as conjugated dienes and at 268 nm as conjugated trienes according to standard methods (IUPAC, 1987), with minor modifications. The model emulsions (0.150 g) were dissolved in methanol (20-25 ml). The samples were centrifuged at 12 000 rpm for 5 min, and the absorbance of the supernants was measured. Totox value was calculated as 2PV+AV (Rossell, 1986).

Rheological studies: Emulsion viscosity was measured at 20±0.1ºC over a shear rate-range of 13.5 to 364.5 s^{-1}, using a rotational viscometer RV-2. The shear data were then analyzed according to the power-low equation $\tau = K \cdot y^n$ to obtain the consistency index (K) and the flow behavior index (n) of the food emulsions. The flow curves giving viscosity η (mPa.s) as a function of shear rate γ (s^{-1}) were characteristic of shear-thinning behavior. For non-ideal plastics, the flow curves were fitted according to the Herschel-Bulkley model and the equation:

$$\tau = \tau_0 + K\,\gamma^n$$

where; τ_0 is the yield stress (Pa).

The values of the plastic viscosities were determined by means of a graphical fit to the flow curves. The thixotropy was evaluated by means of the loop area between the upward and downward flow curves.

Emulsion stability studies: The model emulsions were centrifuged at 3000 rpm for 15 min in order to accelerate the creaming test. Three different layers were observed and measured: a lower droplet-depleted “serum” layer, an intermediate “emulsion” layer, and a droplet-rich “creamed” upper layer (Smith and Mitchell, 1976; Sherman, 1995).

Statistical analysis: All experiments were performed on duplicate samples. Statistical analysis was conducted with a software package implementing the one-way ANOVA method. Significant differences between means (at the level of $p<0.05$) were determined by means of an LSD test.

RESULTS AND DISCUSSION

Oxidative stability of model food emulsions: The walnut oil was rapidly oxidized because of its unsaturated character. Such oil could be used as the dispersed phase in O/W emulsions, which could possibly cause food quality deteriorations that include undesirable changes of flavor, texture, and nutritional profile. On the other hand, the incorporation of walnut oil in food products could increase their nutritional value, if implemented safely.

Table 1 presents the fatty acid composition of walnut oil in comparison with the kind of sunflower oil most commonly used for food emulsions, as well as with olive oil.

The walnut oil contained alpha-linolenic acid, as well as unsaturated palmitoleic acid. The content of saturated fatty acids in the studied walnut oil was 12.3%, that of monounsaturated acids was 26.6%, and that of polyunsaturated acids was 61.1%. According to data from the United States Department of Agriculture (USDA), the composition of fatty acids was as follows: 9.1% saturated fatty acids, 22.8% monounsaturated fatty acids, and 63.3% polyunsaturated fatty acids. The walnut oil was found also to be rich in neutral lipids (96.9% of total lipids), and low in polar lipids (3.1% of total lipids); the unsaturated fatty acids were found to be as high as 85%, whereas the percentage of saturated fatty acids was found to be 15% (Tsamouris *et al.*, 2002).

It is known that the oxidation products of oils display characteristic spectra in the ultraviolet region. The increase of the conjugated dienes values of the walnut oil (absorbance, 232 nm) during the first days was insignificant (Table 2). Extremely large increase of the diene conjugation rate began during the eighth day, and reached the level of 1.25. The vegetable oils with a concentration of polyenic fatty acids above 30% (walnut oil contains 61.1%) and the absorbance at 232 nm in the rage of 1-1.25 were considered to be oxidative (Popov and Yanishlieva, 1976). The proportional increase of the conjugated dienes showed significant correlation with the accumulation of hydroperoxides during the initial oxidation period. Hydroperoxides were measured to determine the initial rate of oxidation, because they are accepted to be the first product formed by oxidation (Rossell, 1986).

The absorbance at 268 nm was also increased during the storage period (0.05-0.29). The formation of conjugated trienes was due to a reaction of decomposition, and resulted in the generation of a wide variety of different molecules, including aldehydes, ketones, alcohols (data not shown).

Proteins are the most widely used emulsifiers in food. They form a stable emulsion and change the interfacial membranes. There are some scientific studies that suggest

Table 1: Fatty acid compositions of walnut, sunflower and olive oil

Fatty acids	Walnut oil	Sunflower Oil[1]	Olive Oil[1]
Myristic $C_{14:0}$	0.3	<1	0.1-1.2
Palmitic $C_{16:0}$	9	2-10	7-16
Palmitoleic $C_{16:1}$	1.7	<1	-
Stearic $C_{18:0}$	2.8	1-10	1-3
Oleic $C_{18:1}$	24.9	14-65	65-85
Linoleic $C_{18:2}$	54.6	20-75	4-15
Linolenic $C_{18:3}$	6.5	<1.5	<1.5
Arachidonic $C_{20:4}$	0.2	-	-

[1]: Source: Welch, Holme and Cllark Co., Inc.

that protein emulsifiers may influence the lipid oxidation processes in oil-in-water emulsions (Frankel *et al.*, 1994; Fomuso *et al.*, 2002; Hu *et al.*, 2003; Hannah and Akoh, 2004). The reasons for that could be: 1) formation of a thick droplet membrane which acts like a physical barrier and limits the interactions between lipid hydroperoxides and prooxidants; 2) chelation of proorxidative metals; 3) inactivation of free radicals through different amino acids; and 4) formation of cationic charges on the surface of emulsions droplets which repeal transition metal.

Comparative studies on the level of conjugated dienes (absorbance 232 nm) in walnut oil and model food emulsions with different dispersed phase (30 and 70%) and different protein emulsifiers concentration (4, 6, 8%) - Soy Isolate (SI) and Whey Protein Isolate (WPI) are shown at Table 2.

The emulsions with 30% dispersed phase and SI had higher CD values than the emulsions with the same oil content and WPI. The walnut oil had lower absorption than the emulsions with 6 and 8% WPI, and higher than those with 4% WPI. Regarding the emulsions with 70% dispersed phase, the emulsions with both protein emulsifiers showed CD values higher than those for walnut oil. The comparison between the CD values of O/W emulsions indicated that those with WPI demonstrated lower level of absorption than those with SI.

Figure 1 represents the changes of Peroxide Value (PV) in walnut oil and model O/W emulsions with 30% dispersed phase and different protein emulsifiers concentration (4, 6, 8%) - Soy Isolate (SI) and Whey Protein Isolate (WPI).

Comparative analyses according to rate of hydroperoxide formation confirmed the tendency described above. The emulsions with 30% dispersed phase and 6 and 8% WPI exhibited better oxidative stability. Oxidation was more rapid in the walnut-oil sample due to the availability of polyunsaturated alpha-linolenic acid in it.

Results from the present study and analysis of the PV, AV, TOTOX, CD, and CT values showed that the emulsions with WPI were more resistant to oxidation than the emulsions with SI. These differences were probably due to the interfacial membrane, which acts as a barrier and separates the lipid substrate and prooxidants in the

Table 2: Conjugated diene (CD) values for walnut oil and O/W food emulsions with protein emulsions stored at 40ºC

		Conjugated diene values (absorbance, 232 nm)							
		Day							
Oil phase	Emulsifier	1	2	3	4	5	6	7	8
Walnut oil									
		$0,36^{a}\pm0,014$	$0,42^{a}\pm0,02$	$0,46^{c}\pm0,02$	$0,50^{c}\pm0,02$	$0,53^{c}\pm0,02$	$0,58^{b}\pm0,01$	$0,71^{b}\pm0,007$	$1,25^{a}\pm0,014$
Emulsions with soy isolate									
Oil (%)	Soy isolate (%)								
30	4	$0,38^{a}$	$0,45^{a}$	$0,61^{a}$	$0,68^{a}$	$0,85^{a}$	$0,93^{a}$	$0,98^{a}$	$1,37^{a}$
	6	$0,37^{a}$	$0,47^{a}$	$0,57^{a}$	$0,59^{b}$	$0,63^{b}$	$0,72^{b}$	$0,84^{b}$	$1,32^{a}$
	8	$0,31^{b}$	$0,39^{b}$	$0,47^{c}$	$0,49^{c}$	$0,54^{c}$	$0,67^{b}$	$0,80^{b}$	$1,28^{a}$
70	4	$0,52^{c}$	$0,58^{d}$	$0,62^{a}$	$0,85^{d}$	$1,24^{d}$	$1,35^{c}$	$1,38^{c}$	$1,77^{b}$
	6	$0,49^{c}$	$0,53^{c}$	$0,60^{a}$	$0,83^{d}$	$1,05^{e}$	$1,22^{d}$	$1,29^{c}$	$1,62^{c}$
	8	$0,48^{c}$	$0,51^{c}$	$0,57^{a}$	$0,79^{e}$	$1,03^{e}$	$1,11^{e}$	$1,25^{c}$	$1,55^{c}$
Emulsions with whey protein isolate									
Oil (%)	Whey protein isolate (%)								
30	4	$0,45^{a}$	$0,49^{a}$	$0,62^{a}$	$0,72^{a}$	$0,75^{a}$	$0,81^{a}$	$1,15^{a}$	$1,30^{a}$
	6	$0,31^{b}$	$0,37^{b}$	$0,38^{b}$	$0,42^{c}$	$0,45^{c}$	$0,51^{b}$	$0,59^{d}$	$1,13^{e}$
	8	$0,34^{b}$	$0,43^{a}$	$0,45^{c}$	$0,51^{c}$	$0,55^{c}$	$0,60^{c}$	$0,65^{d}$	$1,17^{e}$
70	4	$0,46^{c}$	$0,57^{d}$	$0,75^{d}$	$0,83^{d}$	$1,00^{d}$	$1,05^{e}$	$1,21^{c}$	$1,73^{b}$
	6	$0,42^{c}$	$0,48^{a}$	$0,55^{a}$	$0,60^{b}$	$0,83^{e}$	$0,88^{a}$	$1,05^{a}$	$1,51^{d}$
	8	$0,45^{c}$	$0,52^{c}$	$0,59^{a}$	$0,78^{e}$	$0,91^{d}$	$0,96^{e}$	$1,18^{a}$	$1,53^{d}$

Values are means for duplicate analyses of 2 replicates. Standard deviation values are given for walnut oil. Statistical difference was determined by ANOVA followed by t-test and was accepted at $p<0.05$. Values in the same vertical column with different superscripts are significantly different.

Table 3: Rheological characteristics of O/W emulsions with SI – plastic viscosity, yield stress, consistence coefficient (K), and flow behavior index (n)

Emulsions with:		Rheological characteristics:				
Dispersed phase (%)	Soy protein isolate (%)	Plastic viscosity (mPa.s)	τ_0 (Pa)	K (Pa.s)	n	r^2
30	6	-	-	0.06	0.74	0.99
	4	250	-	0.28	0.64	0.98
	6	430	1.0	0.29	0.79	0.99
50	8	760	4.0	0.44	0.74	0.99
70	6	760	14.3	8.37	0.48	0.96

aqueous phase. Moreover, WPI could be used as an antioxidant, and other authors have reported similar findings, too (Hannah and Akoh, 2004; Hu *et al.*, 2002; Tong *et al.*, 2000).

Rheological properties: The basic rheological properties of the model oil-in-water emulsions with Soy Isolate (SI) and Whey Protein Isolate (WPI) were examined. The studied emulsions with SI exhibited high viscosity. These emulsions showed the highest values of rheological characteristics in comparison with other similarly studied emulsions that contained egg yolk protein and sodium caseinate (Nikovska, 2008).

The viscosity (η) of all investigated model emulsions increased with the increasing volume of the dispersed phase and emulsifier concentration. The influence of the dispersed phase volume was more significant. It was shown that this increase in viscosity was slower in the emulsions with lower emulsifier concentration. Other studies on the influence of oil concentration confirm our results (Ma and Barbosa-Cánovas, 1995). According to these authors, a compact three-dimensional network forms between the protein molecules and absorbed droplets was formed.

The rheological characteristics were calculated as a function of dispersed phase volume and emulsifier concentration. The flow curves of the emulsions (shear stress versus shear rate) are shown in Fig. 2.

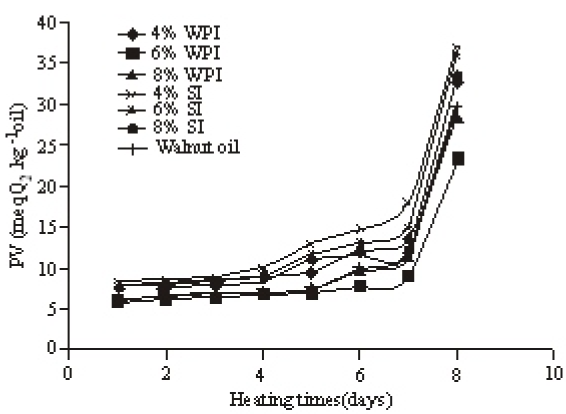

Fig. 1: Peroxide values of walnut oil and food emulsions (Dispersed phase 30%)

The flow curves demonstrate the rheological behavior of the samples. When the shear rate increases, the values of the viscosity decreases, and this tendency is more strongly exhibited in the emulsions with higher emulsifier concentration and dispersed-phase volume. This rheological behavior could be described as typical for a non-Newtonian, pseudoplastic fluid.

Table 4: Apparent viscosities of the emulsions as a function of emulsifier concentration and dispersive-phase volume

		Apparent viscosity η (mPa.s) at γ (s^{-1})				
Dispersivephase, (%)	Emulsifier, (%)	13.5	24.3	40.5	121.5	218.7
		Soy Protein Isolate (SI)				
30	4	27.0	25.5	18.5	9.2	8.3
	6	36.7	28.5	20.1	16.7	15.5
	8	42.5	30.5	20.8	18.3	16.8
70	4	1072.7	641.8	430.1	310.9	247.7
	6	2846.9	1948.4	1363.5	590.6	410.2
	8	3218.2	2085.9	1771.9	1132.0	765.6
		Whey Protein Isolate (WPI)				
30	4	16.6	14.8	9.9	2.6	2.6
	6	17.5	15.2	10.9	3.1	2.6
	8	18.7	17.1	12.9	3.6	2.7
70	4	789.0	547.2	419.4	256.7	190.3
	6	982.4	691.4	634.5	301.3	221.1
	8	1542.0	1126.0	854.0	456.6	328.8

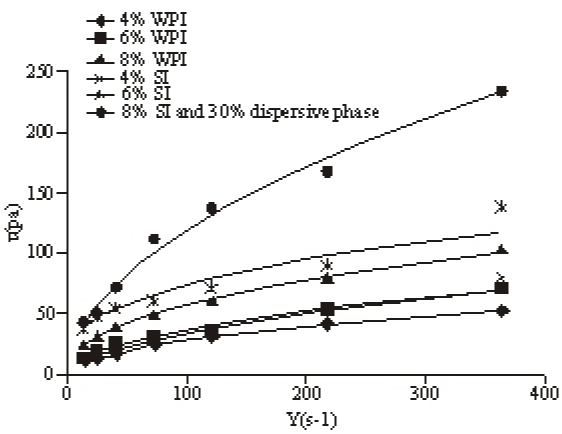

Fig. 2: Flow curves of food emulsions

As can be seen in Table 3, the rheological properties of the samples with SI were in a close agreement with the Herschel-Bulkley model (r^2 = 0.96-0.99). The higher values of rheological characteristics of model O/W emulsions were mainly due to high oil concentration. Some of the investigated samples showed a thixotropy hysteresis loop area. The higher thixotropy was measured in more viscous emulsions.

The impact of the two factors, emulsifier concentration and dispersed-phase volume, on the rheological properties of emulsions with WPI were similar.

Apparent viscosities of the emulsions with different WPI and SI concentrations and dispersive phases and at different shear rates are shown in Table 4.

Furthermore, data on Emulsion Stability (ES) was collected and analyzed. It was established that ES was dependent on the emulsifier concentration and dispersive-phase volume (DP). Emulsions with 0.5% SI concentration and 30, 50, and 70% DP exhibited only a 5-10% intermediate, not separated “emulsion” layer after centrifugation, whereas the emulsions with 1% SI had 40-85% stable “emulsion” layer.

The main factor influencing ES was established to be the increase of DP. The most stable emulsion against creaming was the O/W emulsion with 70% DP. This observation concerning the stability of model emulsions was confirmed by the experimental result from emulsions with WPY. The stability of emulsions with high oil concentration should probably be attributed to the formation of a droplet network and the resulting greater number of interdroplet interactions within that network.

CONCLUSION

It can be concluded that the lipid oxidation of walnut oil and emulsions with walnut oil occurred rapidly because of their content of polyunsaturated fatty acids. The higher oxidative stability of emulsions than that of walnut oil was partly due to the barrier effect of the protein film around the oil droplets and properties of the interfacial region. Whey protein isolate formed walnut food emulsions that were somewhat more unsusceptible to lipid oxidation than the emulsions with soy isolate, and this seems to be due to certain antioxidative properties of WPI.

The rheological properties of the investigated emulsions showed dependency on the type and concentration of the used emulsifiers, as well as on the volume of the dispersive phase. These factors influenced rheological behavior, leading to substances ranging from low viscosity fluids to plastic materials.

Emulsion stability of model emulsions with high concentration of proteins and high dispersed-phase volume can be explained by the interaction between proteins present in the dispersed phase, and by the presence of oil droplets entrapped in a network of proteins.

Further methods to improve the oxidation stability of emulsions with walnut oil should be investigated in the future, too.

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