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Study of olive oil-in-water emulsions with protein emulsifiers

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Abstract

The rheological properties and oxidative stability of oil-in-water emulsions (O/W) with olive oil stabilized by soy protein isolate (SI) and whey protein isolate (WPI) were investigated. The emulsions were prepared at oil phase volume concentrations ranging from 30 to 70% v/v and emulsifier concentrations ranging from 4 to 8 wt%. The emulsions exhibited Newtonian or pseudoplastic behaviour at lower oil and emulsifier concentrations and plastic behaviour at higher concentrations. In addition, the emulsion stability against creaming over storage period was determined. It was established that the emulsions at lower oil concentration were less stable. The creaming stability correlated well with the viscosity of dispersive systems. The differences in the oxidation processes were evaluated by measuring the formation of primary and secondary oxidation products. It was shown that the emulsions with lower olive oil volume concentration were less stable than the emulsions with higher one. The bulk olive oil was more stable than the O/W emulsions both with SI and WPI.

Key words: Oil-in-water emulsions, Olive oil, Oxidation, Protein emulsifiers, Rheology

Introduction

Olive oil is a beneficial food product that finds wide application in food industry. Its fatty acids content is very valuable, because of its high concentration of unsaturated linoleic and oleic fatty acids. It is also possible to use olive oil as natural juice, as well as in food oil-in-water emulsions.

There are many scientific studies concerned with different aspects of food emulsions: their characteristics, formation, behavior, and application (Becher, 1965; Deis, 2002; McClements, 2005). The production methods and technology of food emulsions has been investigated, too. Most often, the focus has been the vegetable oils used, as well as on the emulsifiers and stabilizers employed. The most important characteristics of food emulsions are their stability, rheological properties, droplet size distribution, appearance, and flavor. The oxidative stability of dispersive phase is also an important factor for the quality of emulsions.

Recently, interest on oxidation of O/W emulsions has increased (Coupland, 1996; German, 1999; Hu, 2003). One of the main reasons is the production and availability of many emulsion-type

food articles. There are different scientific studies on the oxidation of bulk oils and food emulsions (Franlel, 1994; Chaiyasit, 2007). There are various factors that influence the oxidation process, such as the size and concentration of oil droplets, the thickness and content of the interfacial region, and the electrical charge of the droplets (Coupland, 1996; Hu, 2003). The type of emulsifier and emulsified oil used are very important, too (Finkel, 1991; Mei, 1999).

Whereas most studies are on synthetic surfactants (Brij, Tween), it is the natural proteins that are recommended as emulsifiers for the food industry. Regarding oxidation mechanisms, unsaturated vegetable oils (e.g. walnut, canola, olive) are of primary interest.

The rheological properties of food emulsions have been studied by many food scientists (Sherman, 1968; Race, 1991; Shoemaker, 1992). Knowledge of these properties is important for a variety of reasons: the shelf life of many food emulsions depends on the rheological characteristics, and also the sensory descriptors of food emulsions are related to their rheological properties (spreadability, smoothness, pourability, hardness). The major factors influencing emulsion rheology are disperse phase volume fraction, droplet size and droplet-size distribution, rheology of component phases, and colloidal interactions (Dickinson and Golding, 1997a; Dickinson, 2000).

Olive oil has been widely used for centuries, due to its widely known health benefits (Alonso et al., 2006). It is possible to incorporate olive oil into

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oil-in-water emulsions, but its unsaturated lipids are susceptible to oxidation. These oxidation processes and rheological properties can cause alteration in the characteristics of food emulsions, such as taste, texture, and shelf life.

The objective of the present work was to investigate the rheological properties and oxidation processes of oil-in-water emulsions with olive oil and protein emulsifiers.

Materials and Methods

Materials

Virgin olive oil was obtained from the local market. Its characteristics were as follows: acidity (% oleic acid) – 0.27; peroxide value (meq O₂/kg of oil) – 1.2; K₂₃₂ [where K=absorbance/C (g/100 mL oil)] - 1.6. 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 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 3.6 and 4.0.

Oxidative stability evaluation

Experimental design

Olive oil sample and model O/W emulsions (200 mL) were stored in screw-capped glass containers (200 mL) at 20°C prior to analysis. Oil was separated from the O/W emulsions by repeated freeze-thaw cycles, followed by centrifugation (Jacobsen et al., 1999). Containers were sampled every four days. Two containers of each sample were independently analyzed in each sampling and each parameter was measured twice. The results are expressed as mean values.

Analytical determinations

The progress of lipid oxidation was assessed by measuring free acidity, peroxide value (PV), and specific extinction coefficient K₂₃₂. These quality indicators were determined according to European

Union standard methods (Annexes II and IX in European Community Regulation EEC/2568/91).

Free acidity, given as % oleic acid, was determined by titration of an oil solution dissolved in ethanol/ether mixture with ethanolic solution of potassium hydroxide.

Peroxide value, given in milliequivalents of active oxygen per kilogram of oil (mequiv/kg), was determined as follows: a mixture of oil and chloroform/acetic acid 3:2 (v/v) was left to react in darkness with saturated potassium iodine solution; the free iodine was then titrated with a sodium thiosulfate solution.

The extinction coefficient K₂₃₂ was calculated from the absorption of a 0.2% solution of the oil in iso-octan at 232 nm, using a UV spectrophotometer.

Rheological studies

Emulsion viscosity was measured at 20±0.1°C over a shear rate-range of 13.5 s⁻¹ to 364.5 s⁻¹ using a rotational viscometer RV-2. The shear data were then analyzed according to the power-law equation $\tau = K \cdot \dot{\gamma}^n$ 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 $\dot{\gamma}$ (s⁻¹) were characteristic of shear-thinning behavior.

Emulsion stability studies

Emulsion stability was monitored as the emulsions were placed in transparent test tubes and stored at room temperature for a period of 30 days. Three different layers were observed and measured: a lower droplet-depleted “serum” layer, an intermediate “emulsion” layer, and a droplet-rich “creamed” upper layer.

Statistical analysis

All experiments were performed on paired 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 analysis of fatty acid content shows (%): palmitic – 10.41; palmitoleic – 0.37; stearic – 2.89; oleic – 73.22; linoleic – 13.11. The olive oil is the richest source of monounsaturated fatty acid oleic acid and there is strong evidence that this fatty acid may affect the risk of breast cancer (García-Segovia et al., 2006). Olive oil contains more oleic acid and less linoleic and linolenic acids and this renders olive oil more resistant to oxidation.

Figure 1 and 2 shows the dynamics of the oxidation process of olive oil and O/W emulsions during the storage period through two basic indicators – acidity and peroxide value. As for the olive oil, the values of these indicators were within

quality limits (acidity $\leq 1\%$; PV ≤ 20) during the storage period.

The oxidative processes were more intensive in olive oil dispersed in O/W emulsions.

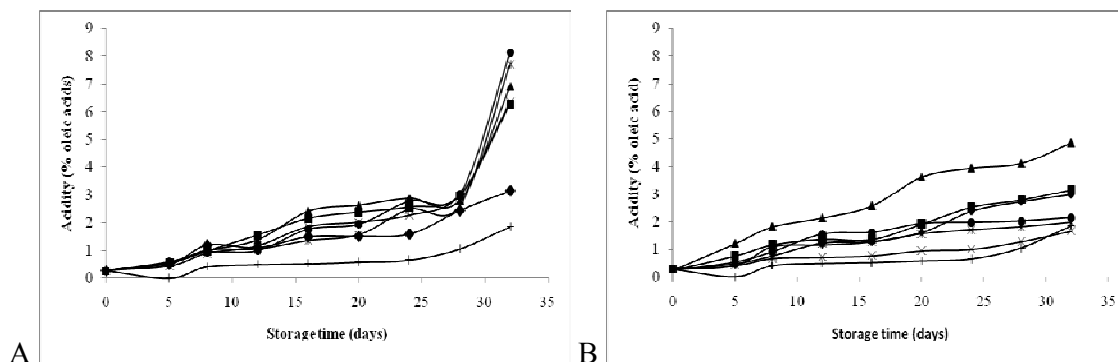


Figure 1. Changes in acidity over storage period at 20 °C in O/W emulsions with virgin olive oil and emulsifiers WPI (A) and SI (B): Virgin olive oil (\square); emulsions with 30 % v/v dispersive phase and emulsifier (% w/v) 4 ($-\diamond-$); 6 ($-\blacksquare-$); 8 ($-\blacktriangle-$); emulsions with 70 % v/v dispersive phase and emulsifier (% w/v) 4 ($-\square-$); 6 ($-\blacksquare-$); 8 ($-\bullet-$).

The differences in lipid oxidation were demonstrated in the emulsion samples. The influence of the emulsifier type and concentration, as well as the dispersive phase volume, was evaluated.

Generally, a more intensive oxidation process was measured in the emulsions with 30% olive oil than in the emulsions with 70% oil. An exception to this tendency was the emulsions with 4% emulsifier. In most studied emulsions, the increase of emulsion concentration delayed oil oxidation. This tendency was measured in the emulsions with WPI, as well as in the emulsions SI. It can be noticed that the emulsions with SI were more oxidatively stable than the same emulsions with WPI. The results show that several factors influenced the oxidation stability of the studied emulsions: (a) the increase of emulsifier concentration resulted in smaller size of droplets and greater interface region and opportunities for oxidation processes; (b) the increase of the oil

phase resulted in closely packed droplets and delay in oxidation; (c) various emulsifiers adsorbed on the interface in different manner, and acted as barrier to the diffusion of lipid oxidation initiators.

Other authors who studied the oxidation processes in O/W emulsions reported similar findings (Coupland and McClements, 1996; Hu et al., 2003; Paraskevopoulou et al., 2007).

Comparative studies on the specific extinction coefficient values (K_{232}) of olive oil and olive oil O/W emulsions are shown at Table 1. The measurement of the K_{232} coefficient was used to determine the level of conjugated dienes present in olive oil. The oxidation products of oils display characteristic spectra in the ultraviolet region and at 232 nm, and the measurement of absorbance at 232 nm is an indication of oxidative stability. The results confirmed the measured tendency described above. The limit of K_{232} (2.5) was exceeded before 12 storage days in the emulsions with WPI, whereas the emulsions with SI were more stable.

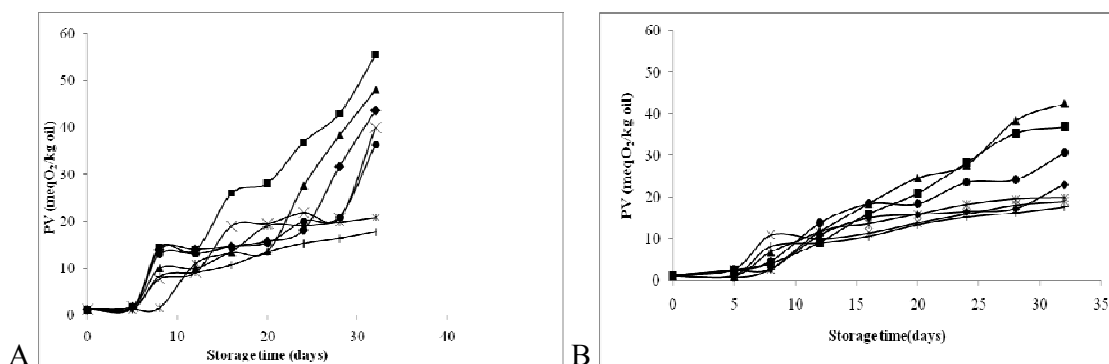


Figure 2. Changes in peroxide values over storage period at 20 °C in O/W emulsions with virgin olive oil and emulsifiers WPI (A) and SI (B): Virgin olive oil (\square); emulsions with 30 % v/v dispersive phase and emulsifier (% w/v) 4 ($-\diamond-$); 6 ($-\blacksquare-$); 8 ($-\blacktriangle-$); emulsions with 70 % v/v dispersive phase and emulsifier (% w/v) 4 ($-\square-$); 6 ($-\square-$); 8 ($-\bullet-$).

Table 1. Changes in specific extinction coefficient values (K_{232}) over storage time at 20°C in virgin olive oil and model O/W emulsions with 30 and 70 % v/v dispersed phase and 4 %, 6 %, 8 % w/v emulsifiers (WPI and SI).

Dispersed phase	Emulsifier	K_{232} values				
		Day				
		0	12	16	20	24
Virgin olive oil		1.6±0.07	2.16±0.01	2.24±0.02	2.97±0.02	3.12±0.08
Emulsions with whey protein isolate						
Oil	Whey protein isolate					
30%	4%	1.6a	2.82b	2.54b	3.95c	4.28d
	6%	1.6a	3.03b	3.06b	4.11c	4.78d
	8%	1.6a	2.93b	3.09b	3.46c	6.28d
70%	4%	1.6a	2.64b	2.83b	2.94b	3.41c
	6%	1.6a	2.66b	3.23c	3.49c	4.01d
	8%	1.6a	2.68b	2.69b	3.06c	3.23c
Emulsions with soy isolate						
Oil	Soy isolate					
30%	4%	1.6a	1.64a	1.78a	2.3b	2.84c
	6%	1.6a	1.89b	1.95b	2.83c	3.66d
	8%	1.6a	2.03b	2.14b	3.28c	4.11d
70%	4%	1.6a	1.97b	2.19b	2.68c	3.02c
	6%	1.6a	1.59a	1.78a	2.51b	3.19c
	8%	1.6a	2.09b	2.23b	3.11c	3.78d

Values are the mean of duplicate samples. Within a column values for which the superscript is identical are not significantly different ($P < 0.05$).

Rheological properties

The rheological properties of the model oil-in-water emulsions with SI and WPI were examined. The influence of several factors on these properties was analyzed.

As can be seen in Table 2, the apparent viscosity (η) of all investigated model emulsions increased with the increasing disperse phase volume fraction and emulsifier concentration. The influence of the disperse phase volume fraction was more significant. Generally, the viscosity of a nonfloculated

emulsion increases with increasing disperse phase volume fraction, because the presence of droplets increases the energy dissipation associated with fluid flow (MacClements, 2005). The emulsions exhibited a shear-thinning behavior that may be caused in part by the presence of aggregated droplets. It was shown that this increase in viscosity was slower in the emulsions with lower emulsifier concentration. Similar tendency has been shown by other authors (Roesch and Corredig, 2002).

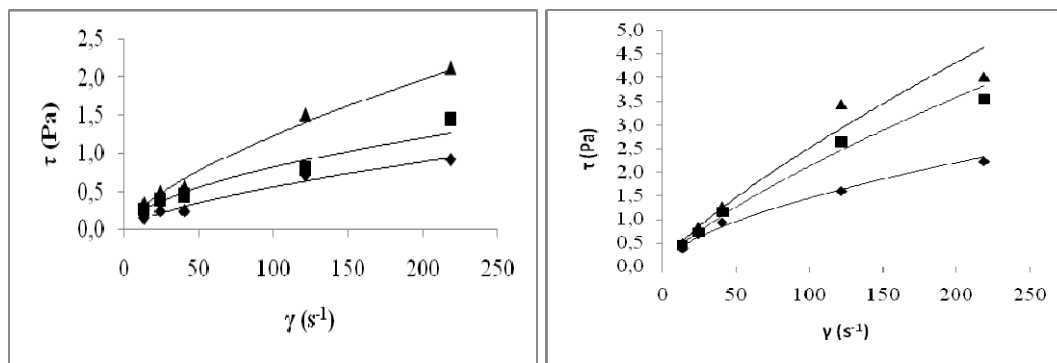
Table 2. Shear viscosity of the emulsions as a function of emulsifier concentration and oil phase volume.

Dispersed phase	Emulsifier	Apparent viscosity η (mPa.s) at γ (s^{-1}):				
		13.5	24.3	40.5	121.5	218.7
Whey protein isolate 30%	4%	11.2	10.2	6.1	5.9	4.2
		20.2	16.3	11.9	6.6	6.6
		25.4	20.1	13.7	12.4	9.7
	70%	654.3	334.7	219.4	187.2	165.2
		1342.6	1002.2	834.5	625.2	405.3
		1633.2	1543.2	1021.6	812.5	512.1
Soy protein isolate 30%	4%	28.3	28.1	23.1	13.2	10.2
		33.4	29.4	28.6	21.7	16.2
		36.2	34.5	31.4	28.3	18.4
	70	835.1	541.9	483.6	297.2	206.8
		2133.5	1834.3	1261.2	621.6	542.6
		2997.6	2162.2	1831.2	1201.3	805.3

The rheological characteristics can be illustrated with the flow curves (shear stress versus shear rate) shown in Figure 3 and Figure 4. Viscosity decreased with increasing shear stress (pseudoplasticity). This tendency is exhibited more strongly in the emulsions with higher emulsifier concentration and dispersed phase volume fraction. The non-Newtonian behavior is more pronounced, and the model emulsions exhibit rheological behavior – plasticity. They have some yield stress values and these values are higher in the O/W emulsions with SI. The reason is that these

emulsions contain three-dimensional networks of aggregated droplets. The oil droplets are packed so closely together that there is strong resistance to flow. The samples with lower emulsifier concentration and dispersed phase volume exhibited pseudoplastic behaviour.

Table 3 represents the consistency coefficient (K) and flow behavior index (n) of the evaluated O/W emulsions. The rheological properties of the samples with the protein emulsifiers were in close agreement with the Herschel-Bulkley model ($r^2=0.97-0.99$).



A.

B.

Fig.3. Flow curves of food emulsions with 30 % olive oil and emulsifiers WPI (A) and SI (B) with concentration 4 % (-♦-); 6 % (-■-) and 8 % (-▲-).

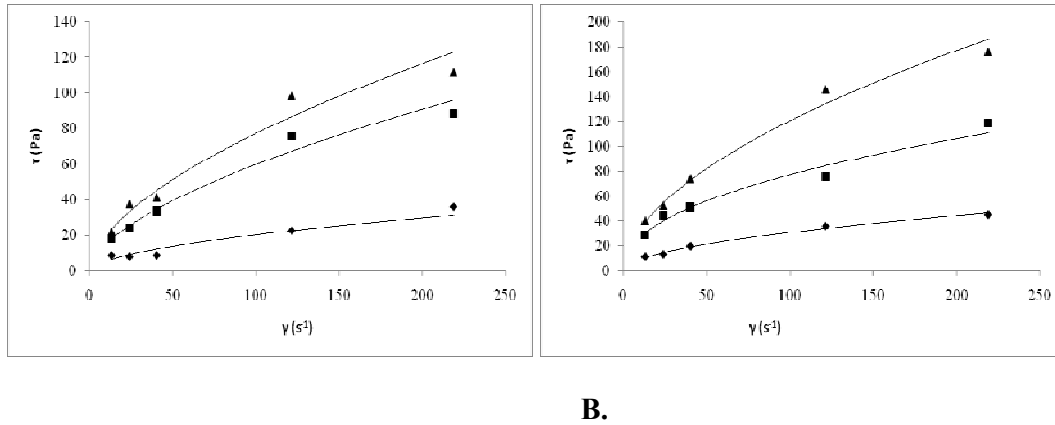


Figure 4. Flow curves of food emulsions with 70 % olive oil and emulsifiers WPI (A) and SI (B) with concentration 4 % (-♦-); 6 % (-■-) and 8 % (-▲-).

Table 3. Rheological characteristics of O/W emulsions with WPI and SI – consistency coefficient (K), and flow behavior index (n).

Emulsions with:		Rheological characteristics:		
Dispersed phase:	Emulsifier:	K (Pa.s)	n	r ²
Whey protein isolate (WPI)				
30%	4%	0.03a	0.66a	0.971
	6%	0.06b	0.56b	0.973
	8%	0.05b	0.68a	0.982
70%	4%	1.55c	0.56b	0.980
	6%	3.66d	0.61b	0.983
	8%	5.03e	0.59b	0.973
Soy protein isolate (SI)				
30%	4%	0.09b	0.60a	0.972
	6%	0.07b	0.75c	0.990
	8%	0.07b	0.79c	0.984
70%	4%	2.69f	0.53b	0.982
	6%	9.17j	0.46d	0.971
	8%	9.43j	0.55b	0.991

Values shown are the means of duplicate samples. Within a column, values for which the superscript is identical are not significantly different at the $P < 0.05$ level.

The comparison between the rheological characteristics of the evaluated emulsions with both protein emulsifiers showed that the emulsions with SI are more viscous. This is due to different emulsifying and stabilizing properties of the protein emulsifiers. They could be able to form emulsions with different droplet size and distribution, as well as an interfacial droplet region with different composition and structure.

The viscosity of the model emulsions was measured at various stages of storage. The structure of emulsions was evaluated as stable. The viscosity values increased slowly during the storage period.

This is probably because of destruction processes like coalescence and creaming.

Furthermore, data on Emulsion Stability (ES) was collected and analyzed. The most stable emulsions during the first 10 days were the samples with both emulsifiers and 70% oil phase. Droplet creaming during storage was retarded by the extremely high viscosity of model O/W emulsions at low shear stresses. The emulsions with soy isolate exhibited stability during the entire investigated period. Creaming processes were observed in the O/W emulsions with lower oil concentration. An 11-29% intermediate, not separated “emulsion” layer was observed in

emulsions with whey protein isolate, and a 28-86% layer was observed in emulsions with soy isolate. Emulsion stability was dependent on emulsifier concentration and disperse phase volume. Creaming behavior correlated well with the viscosity of the emulsion systems. The emulsions with higher viscosity showed better emulsion stability against creaming. This should be attributed to the formation of a droplet network. The interdroplet interactions within this network delayed creaming.

Conclusion

The high content of unsaturated fatty acids – oleic $C_{18:1}$ (73.22%) and linoleic $C_{18:2}$ (13.11%) in olive oil was confirmed. It can be concluded that the lipid oxidation of emulsions with olive oil occurred more rapidly than the oxidation of bulk oil. Emulsions with 70% dispersive phase volume were more oxidatively stable than emulsions with 30% olive oil. Regarding the emulsifier type, emulsions with SI exhibited better oxidative stability than emulsions with WPI.

The rheological characteristics of the investigated emulsions showed a dependency on the type and concentration of the used emulsifiers, as well as on the volume of the dispersive phase. The viscosity increased with the increasing volume of the dispersed phase and emulsifier concentration. The emulsions exhibited Newtonian or pseudoplastic behaviour at lower oil and emulsifier concentrations, and non-ideal plastic behaviour at higher concentrations.

Regarding emulsion stability, measurements showed that the emulsions at lower oil concentration were less stable. The emulsions with higher oil volume and soy isolate exhibited good emulsion stability over the entire storage period.

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