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PHYSICO-CHEMICAL AND BROMATOLOGICAL CHARACTERISTICS OF ARENCA AND RHEOLOGICAL PROPERTIES OF OIL-IN-WATER EMULSIONS CONTAINING ISOLATED PROTEIN

Características físico-químicas e bromatológicas de arenca e propriedades reológicas de emulsão óleo em água que contenham proteína isolada

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ABSTRACT

The design, formulation and development of a new product or the improvement of a traditional product are dependent on the knowledge of the physicochemical, bromatological and rheological characteristics of that product. An important aspect of the study of food is complex dispersions such as emulsions. For preparation and formulation of emulsions, surfactants like protein are used to constitute a molecular barrier that helps emulsions to form and stabilizes dispersions. The aim of this work was to standardize an oil in water (O/W) food emulsion with Arencas (*Triportheus magdalenae*) isolated protein. For this procedure, a physicochemical and bromatological characterization of fish muscle has been done, in which a protein percentage of 17.85±0.12 has been achieved. This has allowed for the recovery of 72-90% of isolated protein to be used in food products such as salad dressing, mayonnaise, spreads, dressings and other products. Stable emulsions with adequate rheological and microstructural characteristics were prepared using 40% w/w palm oil and different concentrates of isolated protein from Arenca, between 2.5 and 3.5% w/w. Therefore, we have obtained an oil in water (O/W) food emulsion with isolated proteins from Arenca that presented non-Newtonian fluid type pseudoplasticity and homogeneous distribution of droplets.

Index terms: Isolated protein; rheology; microstructure.

RESUMO

A concepção, formulação e o desenvolvimento de um novo produto ou a melhoria de um produto tradicional, dependem do conhecimento das características físico-químicas, bromatológicas e reológicas de seus componentes. Um aspecto importante na investigação de alimentos é a dispersão de complexos, tais como as emulsões. No entanto, para elaboração e formulação de emulsões, são utilizadas proteínas como surfactantes para constituir uma barreira molecular que estabiliza as dispersões e ajuda na sua formação. Neste trabalho, objetivou-se padronizar uma emulsão óleo em água (O/A) com proteína isolada de Arenca (*Triportheus magdalenae*). Para esse procedimento, foi realizada a caracterização físico-química e bromatológica do músculo do peixe, no qual se obteve uma porcentagem de proteína de 17,85±0,12. Isso permitiu uma recuperação 72-90% de proteína isolada para ser utilizada em produtos alimentares como molho para salada, maionese, cremes de passar no pão, molhos e outros produtos. Emulsões estáveis com características reológicas e microestruturais adequadas foram preparadas, utilizando óleo de palma 40% m/m em concentrações entre 2,5 e 3,5% m/m do isolado de proteína a partir de Arenca. A obtenção de emulsão óleo em água (O/A) com proteínas isoladas de Arenca apresentou comportamento não-Newtoniano tipo fluido pseudoplástico e distribuição homogênea de gotículas.

Termos para indexação: Proteína isolada; reología; microstrutura.

INTRODUCTION

Emulsions consist of droplets of one liquid dispersed in another immiscible liquid. They are in a metastable dispersion; external shear energy is used to rupture large droplets into smaller ones during emulsification (Mason, 1999). Emulsions can be classified according to the distribution of the oil and aqueous phases. A system in which oil droplets are dispersed in an aqueous phase is called an oil-in-water or O/W emulsion, while a system which consists of water droplets dispersed in an oil phase is called a water-in-oil or W/O (Muñoz; Alfaro; Zapata, 2007). These colloidal dispersions are unstable (Dickinson; Stainsby, 1988) and tend to change in their properties over time. Several of the most important physical mechanisms responsible for the instability of emulsions are gravitational separations, like creaming and sedimentation, flocculation, coalescence and phase inversion (Taylor, 1995; Tcholakova et al., 2002; Wilde et al., 2004).

The behavior of oil-in-water emulsions is defined by three parts of the system: the fat or oil that is in the interior of the emulsion droplets; the interfacial material between this lipid material and the aqueous phase; and

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the aqueous phase itself. Each of these phases can be chemically complex. The lipid may be partly or wholly crystalline and it may be subject to chemical changes such as lipolysis or oxidation. The interfacial material can be composed of proteins or small emulsifiers such as monoglycerides, esters phospholipids, or mixtures of these components. The aqueous phase may contain ions, which can destabilize emulsions, or macromolecules such as polysaccharides, which may have stabilizing or destabilizing effects (Dalgleish, 2006). Proteins tend to reduce the interfacial tension because of their amphiphilic nature and, for this reason, proteins can be used as functional ingredients in the formation and stabilization of food emulsions and foams, as well as improve the stability and produce desirable physicochemical properties in oil-in-water emulsions (McClements, 1999; 2004) Stability is the most important factor to be considered in emulsion technology; an emulsion is stable when there is no change in the size distribution or the spatial arrangement of droplets over the experimental time-scale (Gallegos; Franco, 1999). Emulsifiers have two main functions: providing colloidal stability to the droplet for a long time, by forming an electrically charged layer at its interface with the continuous phase and lowering the interfacial tension and thereby making droplet formation less energy intensive; and stabilization of the emulsion by restricting the mobility of the drops of the disperse phase, due to an increase in viscosity and sometimes viscoelasticity of the continuous phase (Muñoz; Alfaro; Zapata, 2007). In food emulsions, egg yolk is the most widely used emulsifier; however much effort has been devoted for decades to replace egg yolk as an emulsifier in order to avoid the presence of cholesterol from the yolk or the development of salmonella in yolk-containing food products. These efforts have led to the use of different kinds of mixtures of macromolecular and low-molecular weight emulsifiers, or proteins of different systems like crayfish and vegetable (Romero; Cordobés; Puppo, 2008) as emulsifiers and the evaluation of model mayonnaise stabilized by processed egg products with different cholesterol contents (Moros; Franco; Gallegos, 2002). Fish protein has been characterized because of its low fat content and high protein content, which includes essential amino acids (Vollmer; Schenker; Sturm, 1999). The protein content of fish is 12-23% on a wet basis, with 70-80% being globulins, 10-20% being albumins and 2-4% being keratin and collagen (Badui, 2006).

Rheology and emulsion stability are interrelated such that emulsion rheology may not be understood without considering the structural parameters of the emulsion, like the rheology of the continuous phase, the nature of the particles, and the inter particle interaction (Gallegos; Franco, 1999; Gallegos et al., 2002). The aim of this work was to standardize an oil in water (O/W) food emulsion with isolated protein of Arenca (*Triportheus magdalenae*). To do this, we used different factors and analyzing the rheological and microstructural characteristics. due to parameters of homogenization, as well as the composition of the mixture, determine the physicochemical properties of the emulsion (Sunder; Scherze; Muschiolik, 2001) and most of the properties of emulsions depend on the emulsion microstructure, the emulsifiers used and the viscosity on the continuous phase. The microstructure is mainly a function of droplet size and droplet size distribution. Droplet size has an essential importance because it has great influence on physical, microbiological, rheological and optical characteristics, bioavailability or dose response, taste and other properties (Schubert; Engel, 2004).

MATERIALS AND METHODS

Material

Arenca (*Triportheus magdalenae*) (average weight 95 g - 120 g) were obtained at Cienega of Marialabaja in the Bolivar department, packed in plastic bags and transported with ice in a ratio of 1:1 w/w to the laboratory of food technology at Cartagena University. The fish were kept on ice during preparation for analysis and until subjected to analysis.

Physicochemical and bromatological analysis

The analysis determining pH, moisture, ether extract and crude protein of Arenca (*Triportheus magdalenae*) was done in triplicate using the procedures described for the Association of Official Analytical Chemist (Association Of Official Analytical Chemists-AOAC, 1998). The values were expressed as a percentage of samples (wet weight basis) ± S.D. Crude protein (Nx6.25) was determined according to the kjeldahl method and moisture content was determined by oven drying at 105 °C to a constant weight. Ash content was determined by heating in a muffle in the furnace at 550 °C to constant weight. Ether extract was determined by the soxhlet extraction system and pH was measured after the fish muscle was homogenised with 10 volumes of deionised water using a pH meter HANNA HI 9126.

Isolation and characterization of protein

Isolated protein may be produced by extracting with acidified water (pH adjusted to the isoelectric

point of the proteins), which removes soluble materials and some minor proteins, as well as odour and flavour substances. Aqueous extraction is following by centrifugation to separate solubilised protein (supernatant) from insoluble matter (Yada, 2004). If the native structure is required for the functionality of protein, the protein solution was kept to prevent denaturalization. Isolated fish protein was prepared from Arenca (*Triportheus magdalenae*) by alkaline extraction of the soluble protein and isoelectric precipitation. The fish were homogenized using a high speed blender with acetic acid 0.01M (1:9) adjusting to pH 3, measured with a pH meter HANNA HI 9126. The dissolution was centrifuged (model JOHAN B3ay11) at 4000rpm/10minutes (Ruiz-Marquez et al., 2010). The supernatant was then kept at 4 ºC. The moisture content, ether extract, ash and protein were determined from Arencas (*Triportheus magdalenae*) protein isolated according to the Association of Official Analytical Chemist (AOAC, 1998).

Formulation and standardization of emulsions

Different oil-in-water emulsions were prepared using palm oil, proteins isolated from Arenca (*Triportheus magdalenae*) and deionised water to analyse the influences of isolated protein concentration and speed emulsification. A previous study reported that an increase in the agitation speed produced only a slight decrease in the mean droplet diameter and that emulsification time also reduced the droplet diameter (Gallegos et al., 2002). A 3^2 complete factorial design was used to investigate the relationship between the different treatments applied. To determine a comparison of levels of the factors in that process performance will be better, show in Table 1.

Table 1: Experimental design of the emulsions.

Sample codes	Protein concentration *	Homogenization speed (rpm)
1	2.5	16800
2	3.5	12400
3	2.5	12400
4	3.5	16800
5	3.0	12400
6	3.0	16800

*Expressed in weight per weight percentage (w/w) relative to 100% of the emulsion.

The agitation for the premixes of the different emulsions were always performed at 60 rpm for 5 minutes. Then, emulsions were prepared by adding the oil to water-protein solution to pH 4±0.02 using a homogenizer, rotor stator system, Ultra Turrax T25, equipped with a dispersion element S25N-10G ST, at 12400 to 16800 rpm for 15 minutes for all emulsions. Finally, emulsions were stored at 4 ºC and placed at 25 ºC for 30 minutes before taking any measurements.

Rheological and microstructural evaluations

Tests were performed on viscous emulsions made with a Brookfield DV-E viscometer strain (Brookfield Engineering Laboratories, Massachusetts, USA). Readings between 0 and 100 scale units were taken (spindle nº3) at rotational speeds between 5 to 100 rpm. Scale values were read after 90 seconds under shear. For each emulsion sample, the measurements were an average of three replicates. The temperature (25 ºC) of the emulsion was maintained during the measurements. The microstructure of emulsions was measured using a standard optical microscope Leica D500 Germany. Photographs were taken from typical fields to compare the variability of the different emulsions.

RESULTS AND DISCUSSION

Physicochemical and bromatological characterization of Arenca (Triportheus magdalenae)

The post mortem pH of Arenca (*Triportheus magdalenae*) muscle was 6.53±0.13. It has been reported that the pH of fish muscle is generally in the range of 6.2-6.6, depending on a variety of factors such as species, fishing ground, feeding of the fish, storage, temperature and buffing capacity of the meat (Chaijan, 2011).

The chemical composition of Arenca (*Triportheus magdalenae*) is shown in Table 2. These results are comparable to other fish species. The most important and predominant component, after high moisture content, was protein content at 17.85±0.12% which is an intermediate value in comparison with other species. This value can be attributed to the presence of enzymes. The protein content in the muscle tends to be ±18% (wet weight) depending on the species and variety, the state of nutrition, and the reproductive animal cycle, as well as the part of the organism (Badui, 2006; Chaijan, 2011; Sinoski, 1994; Spinelly; Dassow 1982).

Fish composition shows an inversely proportional relationship between fat and moisture. The ethereal extract percentage of fish is low and depends on different

parameters according to the species such as age, body area, sexual cycle, feeding, environmental conditions, harvest season and storage ability (Suzuki, 1987). The moisture content of analyzed fish samples ranged from 66.24% to 72.34% with a mean value of 68.74±2.70. In literature, the reported levels of moisture content in previous studies were similar to our samples and pelagic fish, such as arenques and sardines, have high fat content in optimal conditions (Vaclavik, 2002). In this assay present a value of 12.46±3.2.

Obtaining and characterizing isolated protein

Proteins were solubilized at pH 3.45±0.05, presented an increment in viscosity of the mixture of fish and acetic acid until desired pH was reached. After centrifugation, four distinct phases were observed: the first phase contained lipids; the second had a high protein content; and the last two phases contained mush, skin, debris, spine and muscles. To make the emulsion, the second phase, which contained the isolated protein, was used. This corroborated that fish protein isolates can be defined as a semi-liquid product obtained from the whole fish or parts thereof. This state is achieved by proteolytic enzymes contained in the same fish. This pH prevents the decomposition of the product (Figueroa; Sánchez, 1997). The chemical composition of isolated Arenca (*Triportheus magdalenae*) protein is shown in Table 3. A moisture content of 92.36±2.58% was determined using a range of fish isolated (Figueroa; Sanchez, 1997). Since a drying process was not done in the extraction process, a protein content of 13.44±5.86% was obtained, which is a 70-90% recovery rate, with percentages of fat and ash of 0.057±3.37% and 0.16±1.16%, respectively.

Standardization of emulsions

The emulsions were prepared using different concentrations of protein isolated from Arenca (*Triportheus magdalenae*) and different levels of emulsification velocity using 40% w/w oil and 60% w/w deionized water. Six formulations were used that are shown in Table 1. The premixes were made with an agitation speed of 60 rpm for 5 minutes. Then, they were prepared by adding the oil to the water-protein solution to a pH of 4±0.02 using a homogenizer to reduce particle size and entanglement for emulsion stability. The emulsions obtained after the homogenization process were stable and kept their physical characteristics until rheological and microstructural analysis.

Rheological and microstructural properties

Rheological and microstructural characterizations were performed on the emulsions after 48 hours processing and stabilization at 25 °C. Viscosity, protein concentration and different processing homogenization rates were studied. Figure 1 show that the apparent viscosity decreased with increasing shear rate applied for all samples; this behavior is defined as a characteristic of a Non-Newtonian fluid type Pseudoplastic (Macosko, 1994). Pseudoplastic behavior in the dispersions with proteins is due to the gradual orientation of the molecules in the direction of flow in order to reduce the frictional resistance and deformation of proteins by hydration in the direction of flow. Hydrogen bonds and some weak bonds are broken,

Table 2: Chemical composition of Arenca (*Triportheus magdalenae*).

	Moisture	Protein* (Nx6.25)	Fat*	Ash*
Arenca (*Triportheus magdalenae*)	68.74±2.70	17.85±0.12	12.46±3.2	1.20±0.10
Sardina[a] (*Sardinops melanosticta*)	72	23.1	2.9	--
Sardina[b,c] (*Sardinella aurita*)	72.53	20.6	3.999	1.53

*The percentages are expressed on a wet weight basis. [a] Suzuki, 1987. [b] Cabello et al. 1995. [c] Delgado-Bottini, Vall-Puig and Gonzales-Cantillo 2001.

Table 3: Chemical composition of Arenca (*Triportheus magdalenae*) isolated protein.

	Moisture	Protein* (Nx6,25)	Fat*	Ash*
Arenca (*Triportheus magdalenae)* protein isolated	92.36 ± 2.58	13.43±5.86	0.053±3.37	0.16±1.15
Fish isolated[a]	77.2	16.7	1.3	4.8

*The percentages are expressed on wet weight basis. [a]Figueroa and Sanchez, 1997.

which leads to the dissociation of aggregates of protein networks (Fenema, 2000). As the shear rate increased, the particle-particle interaction was disturbed and eventually disrupted, which resulted in a reduction in flocs size and resulted in a decrease in viscosity. At higher shear rates, the viscosity reached a constant value because all flocs and large particles were completely disrupted. Therefore, only individual and small particles remained in the system (McClements, 1999).

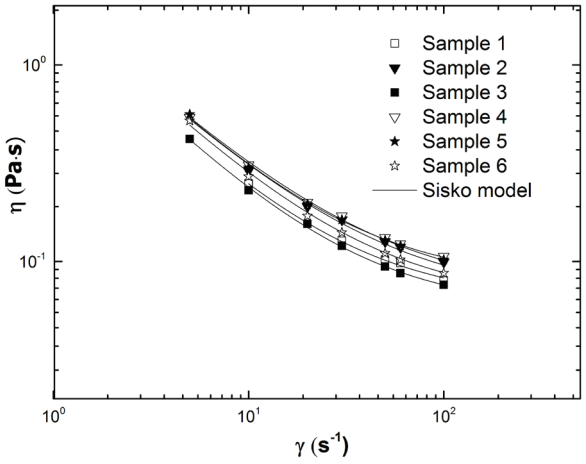

Figure 1: Viscous flow curves of different O/W emulsions sampled at room temperature (25 ºC). The solid lines represent adjusting to Sisko model.

Due to the emulsions' behavior, different models can be used to adjust the experimental data (viscosity versus shear rate). In this case, data was adjusted to a Sisko model. The Sisko model is giving by the Equation 1:

$$\eta = \eta_{\infty} + K_s \gamma^{n_s - 1} \quad (1)$$

Where η is the apparent viscosity (Pa s), η_{∞} is the index of performance, K_s is the consistency coefficient, η_s is the flow behavior index and γ is the shear rate (s^{-1}). This expression is used to describing the flow behavior of most emulsions and suspensions in the practical shear rate range of 0.1 to 1000 s^{-1}.

Many actual flows for structured liquids take place at shear rates at which the viscosity is just coming out of the power-law region of the flow curve and flattening off towards η_{∞}. This situation is easily dealt with by simply adding a Newtonian contribution to the power-law description of the viscosity. The quality of fit for the Sisko model to the experimental results can be checked by comparison, shown in Figure 1, which corresponds the fitting equation with the actual viscosity data. The infinite shear rate Newtonian viscosity and the consistency index of the Sisko model, which represents the differences between the viscosity and the infinite shear rate viscosity, increased exponentially with concentration of protein isolated from Arenca (*Triportheus magdalenae*). The tendency to reach the infinite-shear rate Newtonian viscosity was observed. The experimental data obtained by modeling are shown in Table 4, and showed a very close fit to experimental data, as shown by the high correlation coefficients ($0.99574 < R^2 < 0.99934$). At very low flow rates of deformations, the viscosity is not constant and is called apparent viscosity. At some points, the apparent viscosity begins to decrease and, generally, the curve enters a logarithmic phase indicating Sisko model behavior.

Table 4: Sisko model parameters for the different sample codes at 25 ºC.

Sample codes	[a]	k_s [b]	[c]	R^2
1	0.063	1.77	0.019	0.999
2	0.076	2.24	0.010	0.997
3	0.056	1.73	0.022	0.998
4	0.084	2.25	0.009	0.999
5	0.075	2.18	0.013	0.996
6	0.065	2.14	0.017	0.995

[a] Standard deviation for η_{∞} (index of performance) is always lower than 10^{-3}. k_s [b] Standard deviation for, (consistency coefficient) is always lower tan 10^{-2}. [c] Standard deviation for η_s (flow behavior index) is always lower tan 10^{-3}.

This decrease in viscosity with shear rates is called shear thinning. One can observe the start of flattening as shear rates increase and, if subjected to high cutting speed, there will be a second region of constant viscosity. This behavior was observed due to the low speed handling of the formation since sometime the behavior typical positing along the axis of the deformation speed is too low. This situation sometime arises when both behavior in both high and low speeds are hard to see and only then is a part of the behavior of the fluid (Barnes, 2000). For all samples, when the velocity of emulsification and protein concentration increased, this led to an increase in the performance index, the consistency coefficient, and a decrease in the flow behavior index, showing an increase in

the apparent viscosity and an increase in pseudoplasticity, which can be explained by the decreases in droplet size and polydispersity.

The fluid behaviors are directly related to changes in the microstructure, which result from phase deformation and different types of breakup. Rheological measurements can probe the microstructure and contribute directly to stress through interface tension. The formation of interfacial tension gradients and the reduction in interfacial tension of the system are important factors during homogenization, due to the fact that they facilitate the further disruption of emulsion droplets (i.e. less energy is required to break up a droplet) and, consequently, lead to emulsions with lower droplet size distributions and higher interfacial area between the oil and water phases (Floury et al., 2000; McClements, 1999). The interfacial area available for the interaction between droplets is highest when this average is lowest. For this reason, an increase in the viscosity and their viscoelastic properties is observed in the case of an emulsion stabilized with protein, as protein chains are adsorbed at the interface of different droplets and the continuous medium interacts to form an internal network antiparticle to provide propertis of high viscosity and viscoelasticity (Demetriades; Coupland; McClements, 1997; Hunt; Dlagleish, 1994; Sanchez et al, 2000). The results of the microscopic characterization of emulsions stabilized from different experimental conditions using a rotor stator device are shown in Figure 2. The microstructure showed significant differences in distribution and droplet size according to the protein concentration and velocity of emulsification. Polydispersed emulsions were observed with a droplet surrounded by a continuous aqueous phase. As the protein concentration increased, a better size distribution of droplets was observed, due to the fact that proteins play an important role in the stability of emulsions.

Some proteins are dissolved in the aqueous phase and the proteins that act as an emulsifier are in the surrounding surface of the fat globules, which leads to a greater diffusion of proteins. Molecules solubilized in the continuous phase of the interface facilitates create an interfacial surface (Franco; Berjano; Gallegos, 1997). In the samples with the same amount of protein [2.5% (a, d) 3.0% (b, f) and 3.5% (c, e)] but different homogenization speeds, it was observed that emulsion at higher speed of homogenization (d, f, e) has a lower droplet size and better distribution. The sample with a low protein concentration and treatment, Figure 2a (12400 rpm and 2.5% of protein) showed that a low viscosity leads to low resistance of the fluids against the flow, a large droplet size and the worst

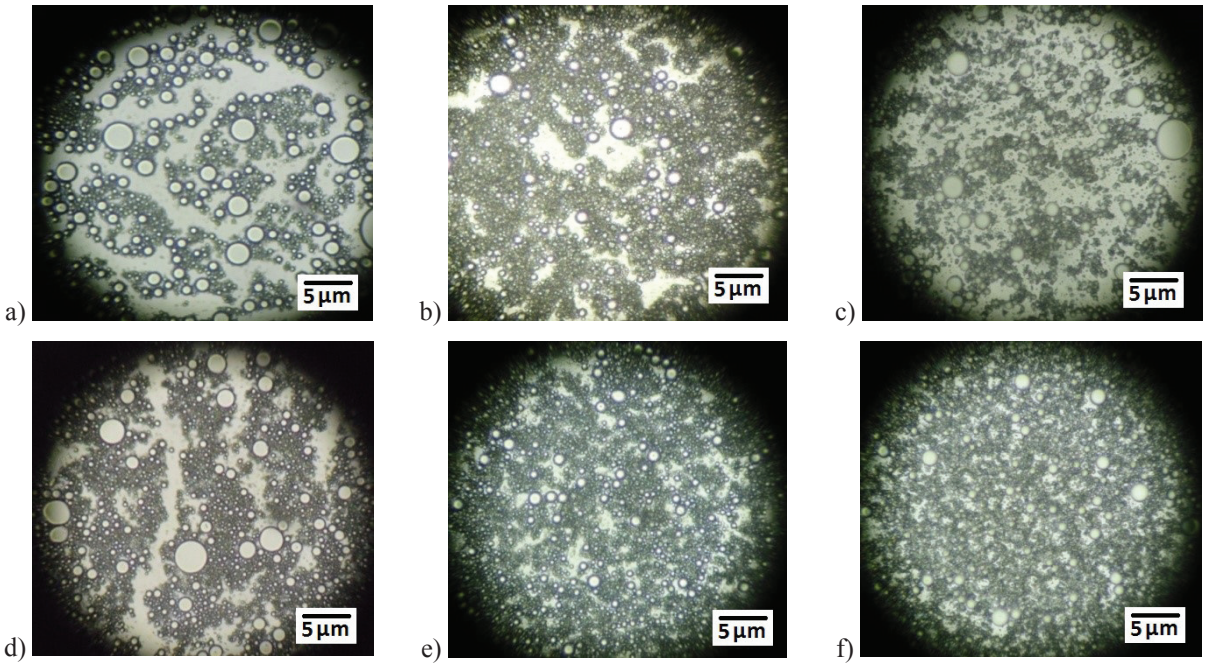

Figure 2: Micrograph of homogenized emulsions at 12400 rpm (a, b, c), and 16800 rpm (d, e, f) at isolated protein concentrations of 2.5% wt (a, d), 3.0% wt (b, e) and 3.5% wt (c, f).

particle size distribution. In contrast, the emulsion with a higher concentration and speed, Figure 2f (3.5% of protein and 16800 rpm) showed better characteristics, with a highest viscosity that led to high resistance of the fluid against the flow, a smaller droplet size and better distribution representing better stability. We concluded that a smaller droplet size and higher viscosity in emulsions stabilized with Arenca (*Triportheus magdalenae*) protein gave evidence for a direct relationship between particle size distribution and pseudoplastic parameters. However, reaching an excessive emulsifier state is undesirable, as the reduction in droplet size may induce lower emulsion stability (Barnes, 2000).

CONCLUSIONS

The fish species Arenca (*Triportheus magdalenae*) can be considered an important source of protein. In this study, a quantity of 13.46±5.86% of protein was obtained, which was a recovery rate of 70-90% in whole fish. This protein was used to develop and formulate oil in water food emulsions that were stable and contained a higher protein concentration, up to 3.5%, without the use of another stabilizer or preservative.

The rheological behaviors of the emulsions were pseudoplastic and fitted perfectly to a Sisko model with a high correlation coefficient ($R^2 > 0.995$). When protein concentration was increased and greater speed in homogenization processing was used, the stability and microstructure of the emulsion changed, resulting in a droplet diameter reduction. Processing speed refers to the speed of emulsification during processing and leads to smaller droplets. Sample 4 shows that at higher protein content and velocity of emulsion, a better droplet size distribution, the highest viscosity and better stability are obtained.

Finally, the information gained here about protein isolated from fish could be useful for the formulation and development of new industrial products that use emulsions such as salad dressings, mayonnaise, spreads, dressings and other products.

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