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Food emulsion type oil in water prepared with high-protein from shrimp (*Penaeus vannamei*) heads flour – SHF

Emulsiones alimentarias del tipo aceite en agua preparadas con harina con alto contenido proteico a partir de cabezas de camarón (*Penaeus vannamei*)

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ABSTRACT

The use of flour from shrimp (*Penaeus vannamei*) heads with a high content of protein (SHF) to stabilize food emulsions type oil in water (o/w) is an alternative to take advantage of the by-products of the shrimp industry. The aim of this work was to prepare food emulsion type oil in water (o/w) using the SHF due to the high percentage in proteins; for this procedure a physicochemical and bromatological characterization of flour of shrimps (*Penaeus vannamei*) heads has been done, in which a percentage of protein 51 %, moisture of 11,82 %, fat 8,52 % and 22,23 % of ash has been obtained. The base emulsions may be used in food products such as salad dressing, mayonnaise, spreads, dressings and other products. The different emulsions with adequate rheological and microstructural characteristics were prepared using different concentrations of palm oil (20, 30 and 40%w/w) and different concentrate of SHF (0,5, 1 and 2 % w/w). Therefore, we have obtained a food emulsion stable type oil in water (O/W) with 2 % w/w of SHF, which presented a behavior non-Newtonian fluid type shear-thinning and homogeneous distribution of droplets.

Keywords: shrimps *(Penaeus vannamei)* heads flour – SHF, emulsion, rheology, shear-thinning.

RESUMEN

El uso de harinas obtenidas a partir de las cabezas de camarones *(Penaeus vannamei)* - SHF con un altos contenido en proteína para la estabilización de emulsiones alimentarias del tipo de aceite en agua (O/W) son una alternativa para poder aprovechar estos residuos de la industria del camarón. El objetivo de este trabajo fue preparar emulsiones alimentarias aceite en agua (O/W), utilizando la SHF, debido a su alto porcentaje de proteínas. Se realizó la caracterización fisicoquímica y bromatological de la harina SHF, obteniendo unos porcentajes del 51 % en proteína, 11,82 % de humedad, 8,52 % de grasa y 22,23 % de ceniza. Las emulsiones logradas pueden ser utilizadas en productos alimenticios tales como ensaladas, mayonesa, productos para untar, aderezos, entre otros. Se prepararon emulsiones bases con diferentes concentraciones de aceite de palma (20, 30 y 40 % w/w) y de SHF (0,5, 1 y 2 % w/w). A las diferentes emulsiones que presentaron estabilidad se les realizaron las caracterizaciones reológicas y microestructural. Por lo tanto, se obtuvieron emulsiones estables del tipo aceite en agua (O/W) con 2 % w/w de SHF que presenta un comportamiento no newtoniano del tipo reofluidificante y la distribución homogénea de gotitas.

Palabras clave: Harina de cabezas de camarón *(Penaeus vannamei)*- SHF, emulsión, reología, reofluidificante.

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Introduction

Shrimps are a highly nutritious food; its caloric value is about 4,5 KJ/g; contains 75-80 % of water, 18-20 % of proteins and about 1 % of fat. Their exoskeleton is formed about 17-42,2 % of protein, 17-32 % of chitin, 17-4 % of pigments and 41-46 % of ash, so it has a high percentage of calcium, magnesium and phosphorous (Soro, 2007).

The most important by-product material in shrimp processing industries is head waste, which accounts for approximately

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35-45 % of the whole shrimp weight (Meyer, 1986). Shrimp head waste is a rich source of chitin (11 % dry weight basis) (Sánchez-Camargo, *et al*, 2011), protein (50 % to 65 % dry weight basis), nutrients (Shahidi & Synowiecki, 1991) and enzyme (Heu, *et al*, 2003). Therefore, making use of by-products has drawn considerable attention in recent years.

Proteins are macromolecule containing carbon, nitrogen, oxygen and sulphur, some found phosphorus, iron, zinc and copper (Robinson, 1991). Besides their nutritional function, proteins play an important role in the sensory attributes of foods; the functional properties had been related to the physical and chemical properties that affect the behavior of proteins in food systems during processing, storage, preparation and consumption (Hill, *et al*, 1998). The functional properties of proteins are a consequence of their physicochemical properties such as size, molecular mass, distribution charge, hydrophobicity, hydrophilicity, flexibility, rigidity and molecular structure (Damodaran, 1997).

An emulsion is a thermodynamically unstable dispersion of two immiscible liquids, usually non-polar and polar nature, in which one forms small droplets (0,1 to 100 microns) is called dispersed or internal phase and the other, phase continues or external. In practice, it must contain a third component, an emulsifier, amphiphilic substance that facilitates the formation of the emulsion by decreasing the interfacial tension between the non-polar phase (oil) and the polar (aqueous) and provides at least some physical stability for a while, which may be shorter or longer, depending on the composition, processing characteristics and external conditions during aging (Muñoz *et al*, 2007). To maintain the physical stability of an emulsion, a suitable emulsifier is imposing used to lie in the interface, forming a protective barrier that hinders coalescence. Stability of the final emulsion is conditioned by its structural and rheological properties, which depend on the way the emulsion is processed. The processing variables affect the structural parameters (i.e., distribution of droplet sizes, particle interactions, microstructure of the continuous phase) leading to important differences in the rheological behavior and stability of emulsions (Sánchez, *et al.*, 2000).

An emulsifier is amphiphilic molecule. It has a low or high molecular weight and tends to migrate rapidly adsorbed on the oil-water interface, promoting the formation of droplets with a lower power consumption, and the formation of the emulsion and reduce the interfacial tension. Emulsifiers also facilitate the formation of emulsions, providing the interface from a certain physical stability, but may be for a short period of time (Muñoz *et al.*, 2007).

However, interactions between the deformable interfaces of droplets play an important role in the emulsion rheology. The most basic interactions are that of excluded volume; the second basic interaction results from the work done against the stress to create an additional droplet surface area when two droplets deform as they are forced together; and finally, the surfactant typically provide a short range repulsion thus preventing droplet coalescence (Mason, 1999).

In the field of food emulsions macromolecular proteins as emulsifiers are predominating, due they are present in significant amounts in edible natural raw materials. While the best known of proteins for use as emulsifiers raw materials are animal origin i.e., eggs and milk, vegetable sources are being used increasingly, such as soybean, peas, lupins or wheat gluten (Bengoechea, *et al.*, 2006, 2008; Ruiz-Márquez *et al.*, 2010).

Proteins to exert their function remain a mechanism emulsifier in three stages. The first is the convective transport from the continuous phase to the interface; the second one is the adsorption at the interface and, the third is a reorganization of its structure at the interface which is called the surface denaturation (Hill *et al.*, 1998).

The aim of this work was stabilized food emulsions type oil in water using shrimps (*Penaeus vannamei*) heads flour - SHF due to the high percentage in proteins and evaluate rheology and microscopically the stabilized emulsions.

Materials and methods

Shrimps *(Penaeus vannamei)* heads (SH) were provided by Oceanos S.A (Cartagena, Colombia). Xanthan gum was kindly provided by Tecnas S.A. (Medellín, Colombia). Other ingredients used in food emulsions were purchased from a local supermarket. All other chemical reagents were of analytical grade.

Preparation of shrimps (Penaeus vannamei) heads flour - SHF

Shrimps (*Penaeus vannamei*) heads (SH) were transported using a relation of ice or 1:1 and washed with water for 10 minutes. After that, it was heated for 10 minutes and dried in a conventional oven at 80 ºC for 4 hours. The dried SH were grinded in a disc mill IKA MF 10 and sieve to obtaining a better particle size distribution of shrimps *(Penaeus vannamei)* heads flour (SHF). The content of moisture, ether extract, ash and protein, had been determinate from SHF, according to the Association of Official Analytical Chemist (AOAC, 1998).

Formulation and standardization of Emulsions

Different oil-in-water emulsion using palm oil, SHF, xanthan gum and deionized water were prepared, analyzing the influences of the blend of high-protein flour concentration with the gum and the amount of oil. The speed and time of emulsification were constant in the process. A completed factorial design 32 have been done in comparison of levels of the factors in that process performance will be better. The variables studied were amount of flour in percentage of 0,5, 1,0 and 2,0 % w/w and amount of oil in percentage of 20, 30 and 40 % w/w show in table 1. The xanthan gum (XG) was used as an auxiliary agent for their emulsifiers characteristics on a percentage of 0,2 % w/w for all samples. Then, the emulsions were prepared mixing the flour and xanthan gum with water for 5 minutes using a magnetic stirring and the oil was added to water-flour-gum solution using a homogenizer, rotor stator system Ultra Turrax IKA T10, equipped with a dispersion element S25N-10G ST, working at 29 900 RPM for 15 minutes for all emulsions. Finally, the emulsion stabilized were stored at 4 ºC and were placed at 25 ºC for 30 minutes before taking the measurement at 24 hours after preparation.

Rheological and Microstructural characterization

The test was performed viscous emulsions made with a Brookfield RVF viscometer strain (Brookfield Engineering Laboratories, Massachusetts, USA). Readings between 0 and 100 scale units were taken (spindle nº 3) at rotational speeds between 1 to 100 rpm. Scale values were read after 60 second 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 emulsion was measured using a standard optical microscope Leica D500 Germany. Photographs were taken from typical fields to compare the changed ability of standardized emulsion.

Table 1. Experimental design of the emulsions

Sample Codes	SHF concentration*	Oil concentration*
1	0,5	20
2	2,0	20
3	1,0	20
4	0,5	30
5	2,0	30
6	1,0	30
7	0,5	40
8	2,0	40
9	1,0	40

*Expressed in weight per weight percentage (w/w) in basis to 100% of emulsion.
Source: Authors

Results and discussion

Proximate composition of shrimps (Penaeus vannamei) heads flour - SHF

Table 2 shows the chemical composition (% dry weight) of SHF. A flour with a high concentration of protein was obtained. The crude protein content obtained was 51 % ± 1,0 using a factor of 6,25, the moisture content was 11,82 % ± 1,2, lipids of 8,52 % ± 1,0 and 22,23 % ± 1,1 of ash. The amount of proteins shows an important and inversely proportional value compared with lipids, so the amount of protein obtained showed that the SHF has a high content of this component. However, these results were compared with values reported in the literature. The other flours present a contents of protein of 39,9 ± 1,82, 19,3 ± 1,02, moisture, 12,5 ± 1,34 of lipids and 12,3 ± 1,07 of ash (Oliveira, *et al.*, 2007); the proximate chemical composition of the redspotted shrimp residue value was 49 ± 1 of protein (Sánchez-Camargo *et al.*, 2011). Ibrahim *et al.* (1999) reported values of protein of the shrimp waste of *Penaeus spp* 47,75-47,43 %, and 49,47 % of *Penaeus monodom* (Nargis, *et al.*, 2006); investigations report an amount of protein in flours at 50,27 % - 47,5 % (Cruz-Suarez *et al.*, 2000).

Table 2. Proximate chemical composition of shrimps (*Penaeus vannamei*) heads flour – SHF

Components %	SHF
Total protein (N x 6,25)a,b	51% ±1
Moisture content	11,82%±0,9
Fat content	8,52% ±1
Lipids content	22,23±0,5

a Values on a dry weight basis (d.w.).
b Mean of three determinations ± standard deviation.
Source: Authors

Standardization of emulsions

The emulsions were prepared using different concentrations of SHF (51 % ± 1,0 of protein) and different levels of oils and deionized water. Nine (9) formulations were obtained that shown in Table 1 by developing an experimental design. The emulsions with sample code 2, 5 and 8 obtained after completed the homogenization process was stable and keeping their physical characteristic until rheological and microstructural analysis in 24 and 48 hours, these emulsions contain a percentage of 2 % w/w of SHF and a percentage of 20, 30 and 40 % w/w of oil, respectively. The xanthan gum (XG) was used as auxiliary agent in all samples, with the aim to produce a better entanglement between the components to SHF (Gamonpilas, C. *et al.*, 2011); XG has been associated with the ability to produce a large increase in the viscosity of a liquid by adding a very small quantity which helps to prevent oil separation in emulsions (Papalamprou, *et al.*, 2005). The emulsions formulated at levels of 0,5 and 1 % w/w of SHF, respectively, are not stabilized in the process of emulsification. The emulsions were destabilized through a sedimentation phenomenon which is detected when the density of the disperse phase is greater than of the medium. This is the result of gravity, when the density of the droplets and the medium are not equal (Robins, 2000; Tadros, 2009). SHF can be used to stabilize emulsion type oil in water using percentage higher than or equal to 2 %w/w. The obtained emulsions after the homogenization process were stable and kept their physical characteristics until rheological and microstructural analysis.

Rheological and microstructural properties

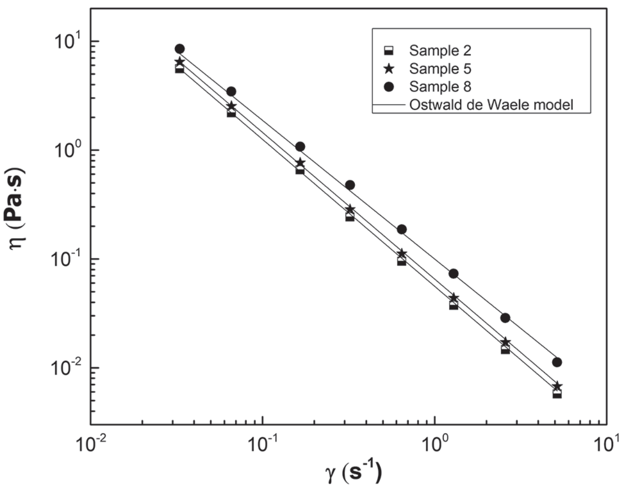


Figure 1. Viscous flow curves of different O/W emulsions samples at room temperature (25 °C). The solid lines represent adjusting to Ostwald de Waele model**.**
Source: Authors

The variations of viscosity of emulsions stabilized in functions of SHF and oils amount were studied 24 and 48 hours after their preparations. A diminution of apparent viscosity with increasing shear rate applied to all samples was observed, characteristic of this behaviour is defined as Non-Newtonian fluid type Shear-thinning (Macosko, 1994). Comparing the viscosity behavior of the emulsions in relation to the amount of protein, a similar behavior can be observed in the emulsions with different amounts of oil for samples code 2, 5 and 8, considering that these emulsions were stabilized with the same percentage of shrimps (*Penaeus vannamei*) heads flour (SHF). Some proteins are dissolved in the aqueous phase and the proteins act as an emulsifier like fish proteins (Quintana *et al.*, 2015) and vegetable protein (Ramirez - Brewer *et al.*, 2016).

The viscosity of proteins dispersions varies in relation of the apparent diameter and the polipectics molecules (Poveda & Elena, 2001). This diameter depends on the intrinsic characteristics of proteins molecules like: mass, size, volume, structure, ease of deformation, and other factors like pH. Likewise, the viscosity of proteins dispersion can depend on protein-dissolvent interactions, what influence in swelling, solubility and specific hydrodynamic sphere around the molecule. Finally, protein-protein interactions that determine the size of the aggregates at elevated protein concentrations (Poveda & Elena, 2001).

The shear-thinning behaviors can be explain for the progressive orientations of molecules in displacement direction, the deformations of the hydration sphere around the proteins in the sliding direction and the breaking of hydrogen bonds and other weak links, causing the dissociation of protein aggregates or network (Poveda & Elena, 2001). Due to the emulsions behaviour, different models can be used to adjust the experimental data (viscosity versus shear rate). In this case was adjusted to Power law or Ostwald de Waele model in terms of apparent viscosity; the model is given by the Expression (1):

$$\eta = k\dot{\gamma}^{(n-1)} \tag{1}$$

Where η is the apparent viscosity (Pa s), k is the consistency coefficient, n is the flow behaviour index and $\dot{\gamma}$ is the shear rate (s^{-1}). In Figure 1, a lineal decrease of viscosity call the potential drop represented by the Ostwald de Waele model can be observed. It was determined that all results are rheograms viscous flow model in accordance with the Ostwald de Waele, the setting values are presented in Table 3 with a coarse adjustment of $R^2 > 0{,}985$. The rheological behaviour of emulsions (dilute, moderately concentrated, and highly concentrated) is strongly influenced by the droplet size (Pal, 1997, 2011).

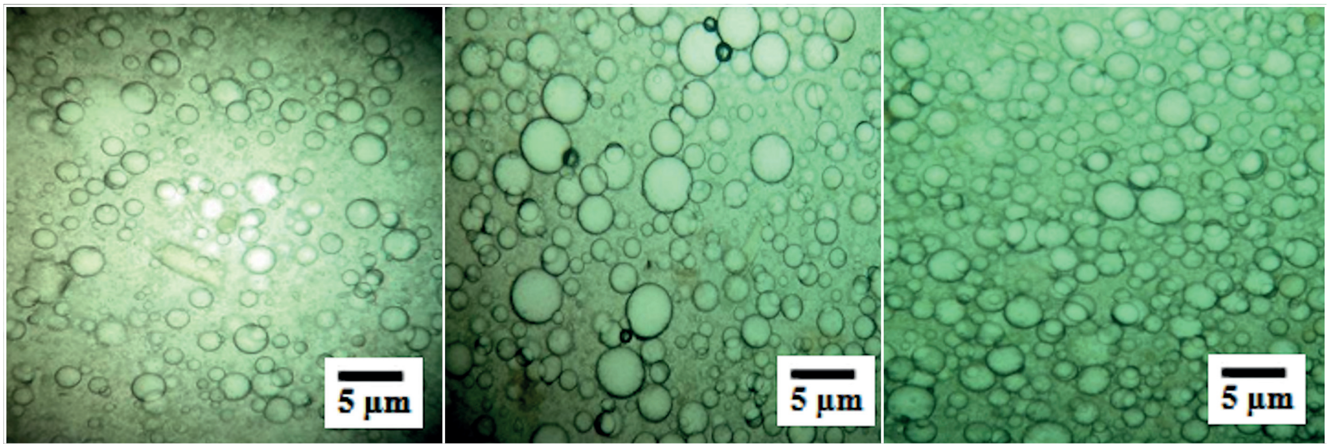
As the shear rate increased, the particle–particle interaction was deformed and eventually disrupted, which resulted in the size reduction of the flocs and resulted in decreasing of viscosity. At higher shear rate, the viscosity reached a constant value because all flocs and large particles were completely disrupted (McClements, 2004), so a smaller droplet size and higher viscosity in emulsions stabilized with proteins gave evidence for a direct relationship between particle size distribution and shear-thinning parameters (Quintana *et al.*, 2015).

Table 3. Ostwald de Waele model parameters for the different sample codes at 25 °C

Sample codes	$K (Pa.S^n)^a$	n^b	R^2
No. 2	0,056	0,651	0,999
No. 5	0,065	0,653	0,999
No. 8	0,100	0,726	0,985

[a] Standard deviation for (consistency coefficient) is always lower tan 10^{-2}
[b] Standard deviation for n (flux behaviour) is always lower than 10^{-3}.
Source: Authors

The results of the microscopic characterizations shown in Figure 2, as can be observed, that the droplet size distributions of the stabilized emulsions present an increase in the rheological parameters in relation to the oil concentration, this is accompanied by a decrease in mean droplet diameter. A qualitative analysis of stabilized emulsions was observed; the sample code 8 with the highest amount of oils showed better characteristics, with a highest viscosity that led to high resistance of the fluid against the flow, a similar droplet size and better distribution representing better stability, considering that emulsions had the same percentage of SHF. Partal *et al*. (1997), Guerrero *et al*. (1998) and Ramirez - Brewer *et al*. (2016) reported that the increase in rheological functions of emulsions with oil concentration is accompanied by a decrease in mean droplet diameter.



Sample code 2	Sample code 5	Sample code 8

Figure 2. Micrograph of stabilized emulsions.
Source: Authors

The emulsions contain the least amount of oil, sample code 2, (20 % w/w) showing a decrease in the apparent viscosity and a decrease in shear-thinning, which can be explained by the decrease in droplet size and polydispersity. Polydispersed emulsions were observed with a droplet surrounded by a continuous aqueous phase. This smaller droplet size is observed compared with the sample code 5 (30 % w/w). Sample code 5 presented an intermediate distribution compared with the other samples.

Many proteins are surface-active molecules that can be used as emulsifiers because of their ability to facilitate the formation and improve the stability and produce desirable physicochemical properties in oil-in water emulsions (Norde, 2003; Quintana *et al.*, 2015). Proteins adsorb to the surfaces of freshly formed oil droplets created by the homogenisation of oil–water–protein mixtures, where they facilitate further droplet disruption by lowering the interfacial tension and retard droplet coalescence by forming protective membranes around the droplets (Walstra, 2003). We concluded that the higher the concentration of oil and the amount of protein emulsions presented best results of stability could be obtained.

Conclusions

The shrimp *(Penaeus vannamei)* heads are considered as by-products; They have a great source of protein and present an important feature as raw material for stabilization emulsions, due to the high-proteins shown in bromatological characterization (51 % w/w).

The high-proteins present in the shrimp *(Penaeus vannamei)* heads can stabilize emulsions using concentrations higher than or equal to 2 % w/w of the total weight of the emulsion, regardless of the concentrations of oil, with the use of small amount adjuvant.

The rheological study of emulsion type oil in water (o/w) stabilized with flour obtained from shrimps *(Penaeus vannamei)* heads flour – SHF exhibit a non-Newtonian fluid behaviour in all emulsion stabilized, adjusting well to the Oswald de Waele model.

Microscopic evaluation showed that regardless of the concentration of oil in the emulsions, these were stable, but in the concentration of 40 % had a higher oil distribution and smaller droplets therefrom. The results concluded that the higher concentration of oil droplet distribution is best achieved.

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