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ORIGINAL ARTICLE

Stability and rheological behavior of coconut oil-in-water emulsions formed by biopolymers

Eliana da Silva Gulão[1,2], Clitor Junior Fernandes de Souza[1,3], Angélica Ribeiro da Costa[4], Maria Helena Miguez da Rocha-Leão[2] and Edwin Elard Garcia-Rojas[1,4]*

[1]Programa de Pós-graduação em Ciência e Tecnologia de Alimentos – PPGCTA, Universidade Federal Rural de Rio de Janeiro – UFRRJ, Seropédica, RJ, Brasil

[2]Departamento de Ciência de Alimentos, Instituto de Química, Universidade Federal do Rio de Janeiro – UFRJ, Rio de Janeiro, RJ, Brasil

[3]Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Universidade Federal da Grande Dourados – UFGD, Dourados, MS, Brasil

[4]Laboratório de Engenharia e Tecnologia Agroindustrial – LETA, Universidade Federal Fluminense – UFF, Volta Redonda, RJ, Brasil

edwinr@id.uff.br

Abstract

Proteins are frequently used as emulsifiers and stabilizers. In this work, two proteins with different isoelectric points were used: lactoferrin and ovalbumin. Solutions containing different proteins ratios, with different pH values, were stored for 7 days at 25 °C to analyze the system stability. Systems containing 3% w/v lactoferrin remained stable at all pH values studied, while systems containing 1% w/v ovalbumin remained stable only at a high pH value (8.0). Emulsions containing a mixture of proteins remained stable at a pH between the isoelectric points of the two proteins, which was attributed to an electrostatic bond because of the opposite charges of proteins at this pH. During the analysis of rheological properties, it was possible to observe a non-Newtonian behavior of the emulsions, using the models of Carreau and Cross to describe the pseudoplastic behavior of suspensions. This study provides important information for the use of functional ingredients.

Keywords: *emulsion stability, oil-in-water emulsion, polymers, emulsifiers.*

1. Introduction

Proteins are widely used as emulsifiers in foods. For this reason, the proteins have been widely studied to better understand the mechanisms involved in the stabilization of emulsion systems. The thick layer formed around the droplets as a consequence of protein adsorption prevents coalescence[1-4]. However, emulsions emulsified and/or stabilized with proteins are highly sensitive to stresses such as pH, ionic strength, and temperature[5-7].

Lactoferrin (Lf) is a glycoprotein obtained from milk, with a molecular weight of 80 kDa and an isoelectric point (pI) close to 8.0; its main feature is the ability of each molecule to bind to two iron ions[8]. For be located in several tissues is considered a multifunctional protein participating in different physiological processes, as: regulation of iron absorption in the gut, immune response, antioxidant property, anticancer and anti-inflammatory properties and protection to microbial infection[9]. Studies of emulsion stability using lactoferrin have shown that the protein has a great stabilizer capacity[10-12]. On the other hand, ovalbumin (OVA), the most abundant egg protein, is widely used in the food industry due to its ability to form gels with other polymers. It has a molecular weight of 42 kDa, is negatively charged at a neutral pH, and has an isoelectric point of approximately 4.8[13]. In strong electrostatic repulsion conditions (pH far from its pI and at a low ionic strength), denatured ovalbumin forms linear, semi-flexible aggregates, while their level of branching considerably increased when the electrostatic repulsions are screened[14]. Recently, other researchers have studied the emulsifying properties of ovalbumin associated with gum arabic. In the first study, Niu et al.[15] studied the stability of emulsion w/o by complexes formed between egg albumin and arabic gum under stress conditions as changes in pH, ionic strength and heating, observing stability in acid pH conditions, in the absence of salt ions and upon heating, while in a second study, Niu et al.[16] studied the stability of emulsion w/o using the same complex, showing improved physical and oxidative stability in the ratio 1: 2 and, through the rheology, noted that this proportion provide systems more viscous with higher storage modulus.

Emulsions have been very suitable for encapsulating lipophilic components. These systems can be produced from natural ingredients with high nutritional quality using relatively simple processing operations, such as mixing and homogenization[2,17].

The physico-chemical properties of the formulation may influence the obtaining process, the type and stability of the system, as well as the phase behavior of the dispersion. The main phenomena involved in the O/W emulsion instability process

are flocculation and coalescence. In flocculation occurs the reversible aggregation of droplets with maintenance of the interfacial film forming a two-dimensional network, whereas coalescence can be induced by thinning and rupturing the film between droplets, when two or more droplets of the dispersed phase approach each other with enough energy to melt and form a larger droplet[4-6].

Studies involving emulsions have increased due to the possible application of functional ingredients to the food, such as vegetable oils. Coconut oil is a vegetable oil obtained from fresh or mature coconut fruits by mechanical or natural processes, with or without the use of heat in the absence of chemical refining[18]. Its consumption is increasing, not only because of its pleasant taste but also because of its potential health benefits[19].

The aim of this work was to study the stability and rheological properties of model oil-in-water emulsions prepared with lactoferrin and ovalbumin as emulsifiers.

2. Materials and Methods

2.1 Materials

Dehydrated lactoferrin (Bioferrin® 2000, 95% w/w putity) was obtained from Glanbia Nutritionals (Fitchburg, WI, USA) and ovalbumin (A5503, 98% w/w purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Unrefined coconut oil of the brand Copra (Brazil) was obtained from the local market. This study used analytical reagents and ultrapure water (Master P&D, Gehaka, Brazil) with a conductivity of 0.05 μS/cm.

2.2 Protein solution preparation

Lf (3%, 2.25%, 1.5% and 0.75% w/v) and OVA (3%, 2.25%, 1.5% and 0.75% w/v) concentrations were prepared by previously solubilizing the proteins without pH adjustment in ultrapure water and agitating them with the aid of a magnetic stirrer (NT101, Novatecnica, Brazil) for approximately 3 hours. The solutions containing the mixture of the two proteins (3% Lf; 2.25% Lf-0.75% w/v OVA, 1.5% Lf-1.5% w/v OVA, 0.75% Lf-2.25% w/v OVA; 3% w/v OVA) were adjusted with HCl 0.5 mol/L or NaCl 0.25 mol/L to different pH values (2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0) and shaken with the aid of a magnetic stirrer.

2.3 Emulsion preparation

Emulsions were prepared by mixing 10% v/v of coconut oil with 90% v/v of the aqueous protein solution, with different ratios of protein and pH values as mentioned at section 2.2. The coconut oil was subjected to a 25 °C at a thermostatic bath with a precision of 0.05 °C (MPC-108A, Huber, Offenburg, Germany) to obtain it in liquid form. The solutions were homogenized using an Ultra-Turrax T 10 Basic (Gehaka, Brazil) at 25 °C for 2 minutes at 13.000 rpm and were subsequently subjected to ultrasound for 3 minutes at 25 °C (Ultrasonic Processor, Hielscher, Germany) at a frequency of 30 kHz (100% amplitude and 0.5 cycles per minute).

2.4 Properties and stability of emulsions

2.4.1 Zeta-potential and droplet size measurements

The Zeta-potential and droplet size distribution were obtained by dynamic light scattering (DLS) measurements on emulsions stored in quiescent conditions after 1 and 7 days an incubator (TE-424, Tecnal, São Paulo, Brazil) at 25 °C. The emulsions were diluted with deionized water to a pre-set pH value at a ratio of 1:200 v/v and were placed in capillary tubes, which were placed inside the equipment (Nano-ZS, Malvern Instruments, Malvern, UK). The samples were equilibrated for 1 minute in the instrument. Intensity-weighted z-average diameter was obtained from droplet size distribution.

2.4.2 Influence of temperature on the stability of emulsions

Emulsions that demonstrated stability after the zeta potential and z-average diameter experiments were subjected to temperature variations (30 to 90 °C with a range of 10 °C) in a thermostatic bath with a precision of 0.05 °C (MPC-108A, Huber, Offenburg, Germany) and were maintained at each temperature for 20 minutes for further evaluation of the droplet diameter. Following the methodology proposed by Bengoechea et al.[20], emulsions were diluted with deionized water (1:200 v/v) with a pre-adjusted pH for analysis.

2.4.3 Rheological properties

The rheological measurements of the emulsions were performed with a controlled stress rheometer (Thermo Scientific, Mars III Haake, Karlsruhe, Germany) using a cone-plate sensor (diameter 60 mm, angle 1°, gap 0.052 mm). The temperature of the test was precisely controlled by a Peltier system on board, and a protection cover (solvent trap) was used to prevent the evaporation of water during analysis. All analyses were performed in duplicate, and the average time before the tests was 5 min. Steady shear tests were performed using a ramp shear rate from 0 to 500 s^{-1} at a fixed temperature of 25 °C. The models of Newton (1), Power Law (2), Herschel-Bukley (3), Cross (4) and Carreau (5) were tested to describe the flow behavior.

$$\tau = \mu(\dot{\gamma}) \quad (1)$$

$$\tau = K\left(\dot{\gamma}\right)^n \quad (2)$$

$$\tau = \tau_0 + K\left(\dot{\gamma}\right)^n \quad (3)$$

$$\eta = \eta_\infty + \left(\frac{\eta_0 - \eta_\infty}{1 + \left(\lambda\dot{\gamma}\right)^n}\right) \quad (4)$$

$$\eta = \eta_\infty + \left(\frac{\eta_0 - \eta_\infty}{\left(1 + \left(\lambda\dot{\gamma}\right)^2\right)^{\frac{N}{2}}}\right) \quad (5)$$

In these models, τ_0, τ, λ, $\dot{\gamma}$, μ, η, η_0, η_∞, K, n, and N represent the yield stress (Pa), shear stress (Pa), relaxation time (s), shear rate (s^{-1}), viscosity (Pa·s), apparent viscosity (Pa·s), zero-shear rate of viscosity (Pa·s), infinite-shear rate of viscosity (Pa·s), consistency coefficient (Pa·s^n), flow behavior index and potency index, respectively.

2.4.4 Optical microscopy

Emulsions containing 10% v/v of coconut oil were observed through an optical microscope (Eclipse E-200, Nikon, Japan) amplified 60x with coupled camera (Evolution VF, MediaCybernetics, USA). In order to compare, the five different formulations were evaluated in pH values in which they presented stability and instability, according to the zeta potential and z-average diameter results. The emulsions were diluted with deionized water with a pre-set pH value at a ratio of 1:200 v/v.

3. Results and Discussion

3.1 Properties and stability of emulsions

3.1.1 Influence of pH and ratio of biopolymers

Initially, we evaluated the ζ-potential of each protein and mixtures containing 3% w/v Lf and 3% w/v OVA for comparison with their behavior in the emulsions, as seen in Figure 1A. It can be observed that Lf presented change in electric charge in the individual protein solution, occurring dislocation of negative charge in the pH 11.8 (-31.1) to positive at pH 1.8 (+16.45) with zero load point (pI) close to 8.0, as also reported in the literature[9]. OVA showed a similar behavior, suffering displacement of negative charge in the pH 11.7 (-26.3) to positive charge at pH 1.2 (+24.3) with zero load point near pH 5.0, in agreement with the data reported by Croguennec et al.[14]. The mixtures containing 3% Lf-3%OVA w/v showed negative charge (-20.96) at pH 11.7 to the positive charge (+20.2) in pH 1.18, with zero load point near pH 6.5, being among those of the original polymers. At this pH, Lf is positively charged and OVA negatively charged, which indicates the possible electrostatic bonding between proteins.

Figure 1B shows the results of the ζ-potential of emulsions with different protein proportions after the seventh day of their formation. Emulsions containing 3% Lf w/v showed behavior similar to that of aqueous proteins (Figure 1), having a positive charge from pH 1.7 to pH 8.0 and a point of zero charge near the pI of the protein. Emulsions containing 3% OVA w/v also showed behavior similar to that of the aqueous solution of the original polymer, with a zero load point near the pI protein (between 4.5 and 5.0). The behavior of emulsions containing mixtures of both proteins, varied according to the relative amount of Lf and OVA used in the system.

It can be observed that all the emulsions showed a positive charge at low pH and a negative charge at higher pH values, with a zero load point between 5.0 and 6.2. However, when the concentration of Lf was higher than that of OVA (2.25-0.75% w/v Lf-OVA), the zero-charge point (6.2) was close to pI of Lf. Conversely, when the concentration of OVA was higher than that of Lf (2.25-0.75% w/v OVA-Lf), the zero-charge point (5.0) was near to pI of OVA.

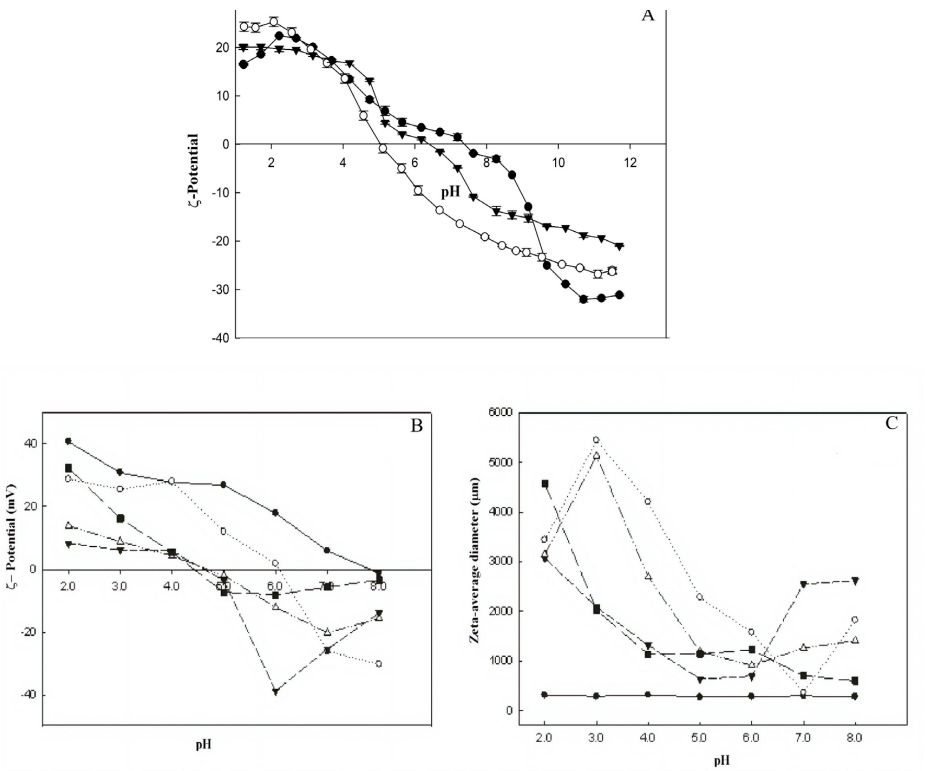

Figure 1. (A) Effect of pH on the zeta potential of the solutions containing 3% w/v Lf (●), 3% w/v OVA (○) and mixtures containing 3-3% w/v Lf:OVA (▼); Influence of pH on emulsions containing 10% v/v coconut oil at the seventh day after its formation on the zeta potential (B) and intensity-weighted z-average diameter at the seventh day after their formation (C), when: (●) 3% w/v Lf; (○) 2.25-0.75% w/v Lf:OVA; (▼) 1.5-1.5% w/v Lf:OVA; (Δ) 2.25-0.75% w/v OVA:Lf; (■) 3% w/v OVA.

Similar behavior was observed in emulsions containing lactoferrin and β-lactoglobulin[21,22]. The composition of the proteins also exerted a significant influence on the stability to aggregation of the droplets as a function of pH. In Figure 1C, it can be observed that the emulsions containing 3% w/v Lf remained stable from pH 2.0 to 8.0, with no aggregation of droplets (z-average diameter < 310 nm). The high stability of these emulsions can be attributed to a combination of electrostatic and steric repulsion between the droplets. Presumably, Lf molecules are oriented at the oil-water interface so that the hydrophilic groups of protein point to the aqueous phase and provide a strong steric repulsion[6,21,22].

Visually, it can be observed that in emulsions formed with 3% w/v Lf at all the pH values, no phase separation was evidenced (Figure 2-I). For emulsions containing 3% w/w OVA, the stability to droplet aggregation was noticeably influenced by pH (Figure 2-II). It can be observed that at pH values from 2.0 to 6.0, a strong aggregation of droplets (z-average diameter > 1200 nm) occurred, but at higher pH values (pH 7.0 to 8.0) the emulsions presented lower zeta-average diameter (z-average diameter < 702 nm). The same occurred in the emulsions containing 2.25-0.75% Lf-OVA w/v (Figure 2-III), with less droplet aggregation at higher pH (6.0 to 8.0). This fact can be explained by those emulsions having a greater electrostatic repulsion instead of steric repulsion between the oil droplets and ovalbumin molecules, leading to emulsion stability. Consequently, as particles tend to aggregate when the pH is below or near the pI of protein, an electrostatic repulsion is not strong enough to overcome the attraction forces between the molecules[23].

All emulsions containing mixtures of Lf and OVA showed an aggregation of molecules at low pH values, with the most stable being between pH 5.0 and 7.0, which are intermediate values between the isoelectric points of the proteins. These results suggest that Lf and OVA are capable of forming an interfacial complex that improves the stability of the emulsion aggregation. Based on the study by Tokle et al.[21], who observed the stability of emulsions formed by lactoglobulin and lactoferrin, it can be suggested that the same model may be used for emulsions formed between Lf and OVA, assuming that the complex can be formed in different ways that lead to a variety of interfacial structures: Lf adsorbs the surface of the droplets, and then OVA adsorbs at the top, forming a multilayer; OVA adsorbs the surface of the droplets, and then Lf adsorbs at the top, forming a multilayer; Lf and OVA in solution form a complex, which is then adsorbed to the surfaces of droplets, forming a mixture layer; Lf and OVA are adsorbed to the surfaces of

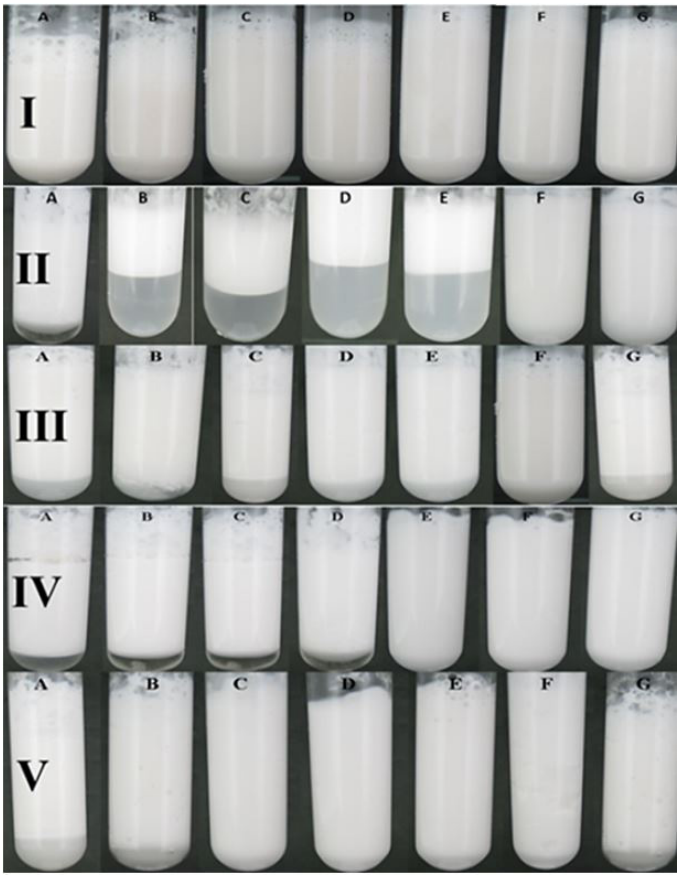

Figure 2. Visual appearances of o/w emulsions containing 10% v/v coconut oil and solutions at the seventh days of quiescent storage after their formation, when: (I) 3% w/v Lf; (II) 3% w/v OVA; (III) 2.25-0.75% w/v OVA:Lf; (IV) 2.25-0.75% w/v Lf:OVA; (V) 1.5-1.5% w/v Lf:OVA, when (A) pH 2.0; (B) 3.0; (C) pH 4.0; (D) pH 5.0; (E) pH 6.0; (F) pH 7.0; and (G) pH 8.0.

droplets, forming a mixture layer that may be a monolayer or a multilayer. In addition, among emulsions containing mixtures of proteins, those containing 2.25-0.75% OVA-Lf w/v (Figure 2-IV) at pH 7.0 showed the smallest droplet diameter (361 nm) and maintained a fully stable condition during the 7 days of storage. Although the emulsions containing only OVA were stable to gravitational separation (or creaming/or phase separation) at a pH values ranging from 7.0 to 8.0, when OVA (1.5% w/v) was mixed with Lf (1.5% w/v), the resultant emulsions showed no phase separation (or creaming/or gravitational separation) in the pH range 5.0-6.0 (Figure 2-V). This fact would be associated with lower z-average values (< 700 nm) (Figure 1C).

3.1.2 Influence of temperature

Initially, all the samples containing only albumin (OVA 3% w/v) were adjusted to pH 8.0; the samples containing only lactoferrin (Lf 3% w/v) were adjusted to pH 7.0; and all the emulsions containing the protein mixtures were adjusted to pH 6.0, being the pH at which the emulsions showed higher stability at 25 °C. Emulsions containing 3% w/v Lf showed larger-diameter droplets, indicating an initiation of aggregation close to 60 °C, which is indicated as the first denaturation temperature of Lf[24]. Figure 3-I shows the droplet diameter of emulsions containing 3% w/v Lf (pH 7.0), 3% w/v OVA (pH 8.0) and mixtures with different proportions Lf:OVA and a pH between the isoelectric points of the proteins (6.0). Upon reaching temperatures above 80 °C, aggregation of the molecules occurred, and an emulsion with a gel aspect appeared. Similar results were observed by Tokle et al.[21], indicating that although at this pH (far from its pI), a strong electrostatic repulsion occurs, hydrophobic bonds between protein and lipid droplets overcome the electrostatic repulsion of micelles, leading to approach and subsequent aggregation. According to Croguennec et al.[14], when the denaturation temperature is reached, an exposure of nonpolar amino acid occurs, resulting in hydrophobic interactions.

In turn, emulsions containing 3% w/v OVA did not show aggregation at all temperatures studied, with larger-diameter droplets just above 80 °C but no visually observed aggregation (Figure 3 II-E). Samples containing mixtures of proteins (Figures 3II-B, 3 II-C and 3 II-D) presented behavior that varied with the concentration of each biopolymer, but all showed instability and presented an aggregation of droplets between 60 and 70 °C. At these temperatures, it can be observed that the droplet diameter was higher in emulsions containing a higher concentration of lactoferrin (2.25-0.75% w/v Lf:OVA > 6.000 nm) and lower when the concentration of OVA was higher (2.25-0.75% w/v OVA:Lf, <4.000 nm), due to the greater instability of Lf at high temperatures. We can also observe that the emulsions containing mixtures showed higher droplet diameters at lower temperatures than did emulsions containing each protein isolate separately. Similar results were observed by Tokle et al.[21] when studying the formation of emulsions from interactions between lactoferrin and β-lactoglobulin. It can be suggested that a possible electrostatic interaction between the molecules and OVA/LF, with the pH is being between the pI of the two proteins, caused changes in the physical layers of the proteins involving the oil droplets. The emulsion gelation occurs when the interfacial aggregates begin to overlap, and hardening occurs mainly due to the rearrangement of the network-like molecules on a scale of different lengths[25]. It is the result of chemical interactions (electrostatic and hydrophobic) between molecules of a single protein or from the combined interactions between protein molecules. The destabilization of the native protein tertiary structure increases their interactions at a level that ultimately causes the formation of a stable network[25,26].

3.1.3 Rheological properties

The flow behavior of the emulsions prepared at pH where they exhibited the highest stability and the apparent viscosity vs. shear rate curves are shown in Figure 4.

The apparent viscosity of all the studied emulsions decreased with the increasing shear rate and exhibited non-Newtonian behavior. The Carreau and Cross models, which describe the pseudoplastic behavior of suspensions, were used to model the flow curves of the emulsions evaluated. These models are useful for predicting apparent viscosity over a wide shear rate range[27], in contrast to the power law model that was also used in this work. The parameters obtained by these models are shown in Table 1. Many dispersed systems behave like a non-Newtonian fluid that presents three distinct regions during the flow: (i) at low shear rate, the apparent viscosity tends to reach a Newtonian

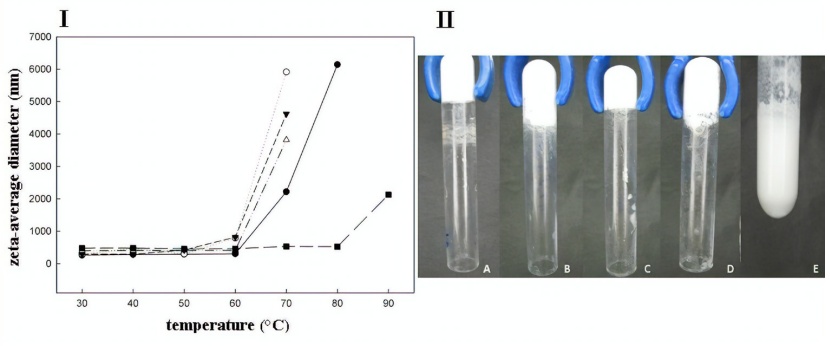

Figure 3. (I) Intensity-weighted z-average diameter for o/w emulsions containing 10% v/v of coconut oil subjected to thermal treatments at different temperatures. (●) 3% w/v Lf, pH 7.0; (○) 2.25-0.75% w/v Lf:OVA, pH 6.0; (▼) 1.5-1.5% w/v Lf-OVA, pH 6.0; (Δ) 2.25-0.75% w/v OVA:Lf, pH 6.0; (■) 3% w/v OVA, pH 8.0; (II) Photographs at bulk scale of o/w emulsions subjected to heating. (A) 3% w/v Lf, 80 °C; (B) 2.25-0.75% w/v Lf:OVA, 70 °C; (C) 1.5-1.5% w/v Lf-OVA, 70 °C; (D) 2.25-0.75% w/v OVA:Lf, 70 °C; and (E): 3% w/v OVA, 90 °C.

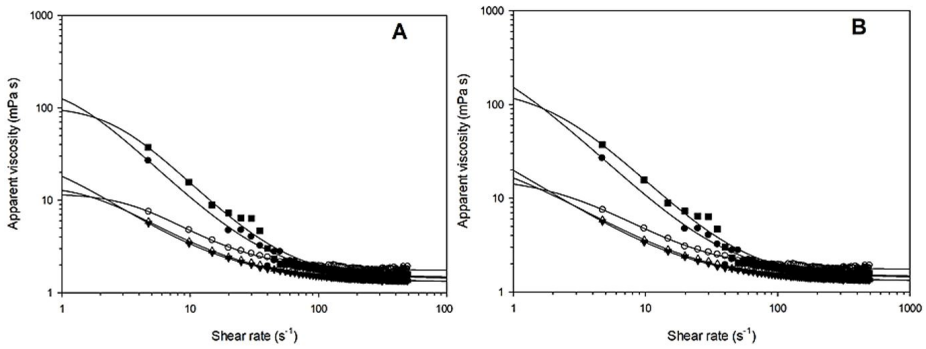

Figure 4. Apparent viscosity as a function of the shear rate of the o/w emulsions containing 10% v/v coconut oil, when: (●) 3% w/v Lf, pH 7.0; (○) 2.25-0.75% w/v Lf:OVA, pH 6.0; (▼) 1.5-1.5% w/v Lf:OVA, pH 6.0; (Δ) 2.25-0.75% w/v OVA-Lf, pH 6.0; (■) 3% w/v OVA, pH 8.0. Continuous lines represent the models fitted Carreau (A) and Cross (B) to the data.

Table 1. Adjustment parameters of flow curves of Carreau (Equation 5), Cross (Equation 4), and power law (Equation 2), models for the studied emulsions.

Sample	Carreau					Cross					Power Law		
	η_0	λ	N	η_∞	R^2	η_0	λ	n	η_∞	R^2	K	n	R^2
3% LF	0.365	0.873	0.683	0.0015	0.997	0.4510	1.6472	1.3757	0.0015	0.997	0.0886	0.1839	0.893
2.25%LF-0.75OVA	0.0219	0.2625	0.5917	0.0017	0.995	0.0170	0.3123	1.2556	0.0017	0.995	0.0085	0.7113	0.746
1.5% LF-1.5% OVA	0.0583	1.3629	0.5016	0.0013	0.999	0.1254	5.5669	1.0142	0.0013	0.999	0.0061	0.7253	0.752
2.25%OVA-0.75%LF	0.0278	0.5678	0.5057	0.0013	0.999	0.0369	1.3349	1.0407	0.0013	0.999	0.0066	0.7122	0.770
3% OVA	0.2059	0.3870	0.7175	0.0014	0.994	0.1544	0.4763	1.4700	0.0014	0.994	0.1555	0.0397	0.971

λ = relaxation time (s); η = apparent viscosity (Pa·s), η_0 = zero-shear of viscosity (Pa·s); η_∞ = infinite-shear rate of viscosity (Pa·s); K = consistency coefficient (Pa·s^n); n = flow behavior index; N = potency index; R^2 = correlation coefficient.

plateau where the viscosity is independent of the shear rate (zero shear viscosity (η_0)[28]; (ii) at intermediate shear rates, pseudoplastic behavior is observed, with apparent viscosity decreasing with increasing shear rate; and (iii) again, the fluid behaves as Newtonian at high shear rate (infinite shear viscosity, η_∞). Although not presenting the Newtonian flow regime at low shear rates, the models that best fit the data were Carreau and Cross, justified by the higher determination coefficient values $R^2 > 0.99$. Due to the limitations of the equipment, only the obtained values of shear rate rate above 4.0 s^{-1} are presented; therefore, it was not possible to observe the Newtonian flow regime at low shear rates. Figure 4 shows the fit of these plotted models (Carreau and Cross) against the measured data.

The parameters analysis (Table 1) reveals that only the proteins used had influence on the Newtonian viscosity at low shear rates. The emulsions formulated with only Lf and OVA presented higher values of η_0. Higher values of η_0 suggest that stronger interactions occurred between proteins and oil droplets compared to Lf-OVA complexes[29]. It is also known that the protein surface coating (mg/m^2) of Lf-stabilized emulsions, prepared at pH 7.0, is greater than emulsions stabilized with β-lactoglobulin, due to their higher molar mass[22]. When it comes to the pseudoplastic region, where the Power Law was maintained, the viscosity characteristic slope was also different for emulsions containing only the protein and those with the Lf-OVA complexes, and the N parameter (Carreau model) is related to this region's slope.

The emulsions formed by the complexes presented lower values of N and this behavior is related to the increase of the pseudoplastic character, reinforcing the interactions and the formation of an entanglement between the proteins in the emulsion. This pseudoplastic region may be associated with droplet flocculation, which increases the apparent dispersed phase volume and increases the formation of non-spherical aggregates[30]. In fact, the emulsions formed by the complexes showed greater droplet aggregation as shown in section 3.1.1, but did not account for stability of these emulsions. The time constant (λ) is related to the instability of the emulsions against creaming. Thus, emulsions exhibiting longer times showed greater stability due to strong droplet-droplet interactions[30].

The emulsion containing 1.5-1.5% w/v Lf:OVA presented the highest λ, and among the emulsions formed by the two proteins, also obtained a smaller z-average diameter, as can be seen in Figure 1C. It is possible to affirm that the emulsions containing only protein (Lf or OVA) presented greater resistance to the flow ($> \eta_0$), but the formulations containing the two proteins presented a greater stability, requiring a higher shear stress to occur the break-up of the structure. For high shear rates, all emulsions presented infinite shear viscosity (η_∞).

3.1.4 Optical microscopy

Order to better observe the interaction effect between Lf and OVA on the stability of emulsions, images of all formulations at the pH values were obtained on the optical microscope, where they were more stable or unstable, according to the results of zeta potential and droplet diameter, when: 3% w/v Lf, pH 7.0; 3% w/v Lf, pH 3.0; 3% w/v OVA, pH

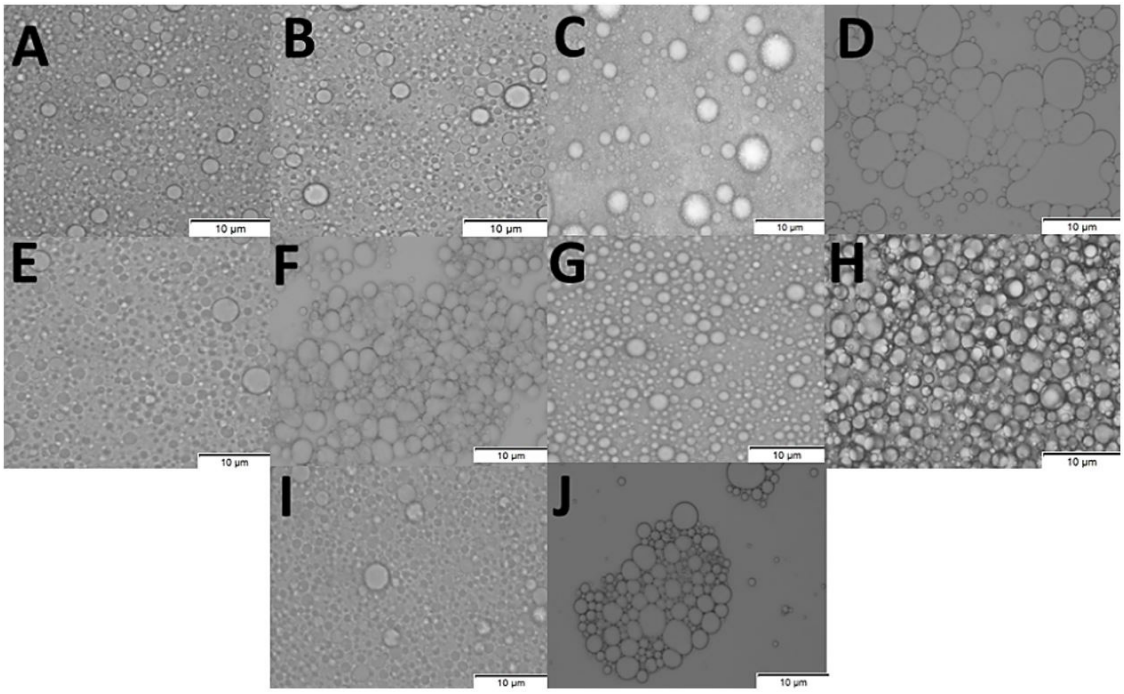

Figure 5. Photomicrographs of o/w emulsions containing 10% v/v coconut oil at the seventh day of quiescent storage after their formation. (A) 3% w/v Lf, pH 7.0; (B) 3% w/v Lf, pH 3.0; (C) 3% w/v OVA, pH 8.0; (D) 3% w/v OVA, pH 3.0; (E) 1.5% w/v Lf:OVA, pH 6.0; (F) 1.5% w/v Lf:OVA, pH 3.0; (G) 2.25-0.75% w/v Lf:OVA, pH 6.0; (H) 2.25-0.75% w/v Lf:OVA, pH 3.0; (I) 2.25-0.75% w/v OVA-Lf, pH 6.0; (J) 2.25-0.75% w/v OVA-Lf, pH 3.0.

8.0; 3% w/v OVA, pH 3.0; 1.5% w/v Lf:OVA, pH 6.0; 1.5% w/v Lf:OVA, pH 3.0; 2.25-0.75% w/v Lf:OVA, pH 6.0; 2.25-0.75% w/v Lf:OVA, pH 3.0; 2.25-0.75% w/v OVA-Lf, pH 6.0; 2.25-0.75% w/v OVA-Lf, pH 3.0. The results can be seen at Figure 5. It is noted that emulsions containing 3% w/v Lf were stable at pHs 7.0 and 3.0 (Figures 5A and 5B), which corroborated with previous results (Figure 1C), since the emulsions containing only Lf presented constant diameter of particle, from pH 2.0 to pH 8.0. The emulsions containing mixtures of Lf and OVA were more stable at pH values between the pI of the proteins, confirming previous results (Figures 1B and 1C).

It is noticed that emulsions at pH 6.0 containing ratios of 1.5% w/v Lf:OVA, (Figure 5E), 2.25-0.75% w/v Lf:OVA (Figure 5G) and 2.25-0.75% w/v OVA-Lf (Figure 5I), were stable. However, when the pH of same systems was adjusted to pH values below pI of proteins (3.0), the approximation of droplets was observed, causing aggregation and possibly flocculation, as there is no evidence of micelles disruption (Figures 5F, 5H and 5J). In this pH value, Lf and OVA presented positive charges as seen on zeta-potential results (Figure 1A), therefore, there is no electrostatic interaction and consequently no formation of electrostatic complexes that would allow the repulsion between droplets.

Emulsions containing 3% w/v OVA were stable at pH 8.0 (Figure 5C) and unstable at pH 3.0 (Figure 5D). At pH 3.0, the emulsion coalescence could be observed, with the approaching and melting of droplets forming large aggregates. A similar phenomenon was observed when studying the stability of double w/o/w emulsions containing only WPI as emulsifier[31]. The authors attributed the emulsions coalescence to the formation of a thin polymer layer at the interface of the droplet, since the electrostatic complexes formed by the interaction between polymers can promote the formation of more resistant layers at the interface of the oil droplets, facilitating repulsion between droplets and supporting the stability of the system.

4. Conclusions

This study demonstrated that the physicochemical properties of emulsions can be modulated by varying the ratio between two globular proteins with different isoelectric points: lactoferrin and ovalbumin. At pH values between isoelectric points, the proteins have opposing charges and can form electrostatic complexes. These complexes can alter the stability of the oil droplets relative to pH and temperature. Although ovalbumin is able to stabilize the emulsions only at a restricted pH, the addiction of lactoferrin to the system provided stability to the emulsions in a higher pH range. This approach may be useful for designing emulsion-based systems for use in functional foods and beverages.

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