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Ultrasound impact on whey protein concentrate-pectin complexes and in the O/W emulsions with low oil soybean content stabilization

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ABSTRACT

Consumers' preference for products with reduced levels of fat increased in the last years. Proteins and polysaccharides have an important role due to their functional and interaction properties because, when combined in ratios and pH of higher potential for electrostatic interactions they may act as emulsifiers or stabilizers. This study evaluated the ultrasound impact on the electrostatic interaction between pectin (PEC) and whey protein concentrate (WPC) at different WPC:PEC ratios (1:1 to 5:1), and its effect on the emulsification and stability of emulsions formulated with WPC:PEC blends (1:1, 4:1) at low soybean oil contents (5 to 15%). Zeta potential analysis showed greater interactions between biopolymers at pH 3.5, which was proven in FTIR spectra. Rheology and turbidimetry showed that the ultrasound reduced the suspension viscosity and the size of the biopolymer complexes. Suspensions were Newtonian, whereas the emulsions showed shear-thinning behavior with slight increase in apparent viscosity as a function of oil content, and remained stable for seven days, with small droplets ($< 8\,\mu m$) stabilized and entrapped in a pectin network evidenced by confocal laser microscopy. Sonication was successfully applied to emulsion stabilization, improving the functional properties of WPC:PEC blends and enabling their application as low-fat systems, providing healthier products to consumers.

1. Introduction

Fats have many important functions in the human organism, but the most important fat characteristic is its contribution to the sensorial appeal of foods, being determinant for their texture. Nevertheless, fat consumption in significant amounts has been related to obesity increase and some cancer types, while ingestion of saturated fatty acids is associated to the increase of blood cholesterol and coronary diseases. Fat mimetics may offer a safe, effective way of maintaining foods' palatability with controlled amounts of fat and/or energy [1–3].

Mixed systems based on colloidal particles of natural biopolymers such as protein and polysaccharides have been more and more used in food processing for application as encapsulation systems, delivery of active ingredients, or to modulate food sensory properties. In particular, these blends may be used as fat mimetics in food formulations [4]. Most of these applications involves an emulsification step during processing [5,6].

Emulsions are thermodynamically unstable systems and tend to destabilization with time, resulting in two separated liquid phases [7]. Formulation of emulsions that remain stable for considerable periods (weeks to years) needs the addition of emulsifiers and/or stabilizers and use of mechanical forces (homogenization process) to reduce the droplet sizes [8]. Proteins act as emulsifiers due to their amphiphilic nature, reducing the interfacial tension at the oil-water interface and producing a film coating the oil droplets, preventing their aggregation [8]. Concentrated (WPC) or isolated (WPI) whey proteins are valuable ingredients due to their suitable functional properties, including high water solubility, viscosity increase, gelation ability, emulsification, aeration, color, texture, and flavor improvement, besides contributing to increase the nutritional value of formulations [9].

Polysaccharides act as stabilizers by gelation or viscosity increasing of the continuous aqueous phase [10]. Pectins are hydrocolloids par excellence due to their hydrophilic character and ability of binding great amounts of water, producing viscous solutions and being used as thickening, texturizing, emulsifying or stabilizing agent [11]. Pectin methoxylation degree determines its gelation level, being the stronger the gelation the higher its methoxylation degree [12,13].

High intensity ultrasound application aiming to change biopolymers' functional properties, such as viscosity and gelation ability has been studied [14–16]. Sonication is one of the methods that allow preparation of emulsions with reduced size of droplets [17].

Complexation between proteins and polysaccharides may be used to

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create emulsions highly viscous or similar to gels, but with oil content quite lower than in conventional emulsions. This complexation is induced by electrostatic interactions between the biopolymers at a pH in which they present opposite charges [18].

Many studies have reported the impact of protein-polysaccharide ratio, pH, temperature, and other parameters on complexes and emulsions, but few works have investigated the influence of sonication on these systems. In this context, this study evaluated the effects of ultrasound application and different biopolymer ratio in the complexation by electrostatic interaction between whey protein concentrate and high methoxyl pectin, as well as on the stability and rheology of low oil content emulsions for application as possible fat mimetics.

2. Material and methods

2.1. Materials

The materials used were high methoxyl pectin (PEC) (Dupont™ Danisco®, Cotia, Brazil), whey protein concentrated (WPC) (Alibra Ingredientes®, Campinas, Brazil) and commercial soybean oil (Liza®). McIlvaine buffer solution (disodium phosphate – citric acid) was prepared according Morita & Assumpção [19] and used for preparing the biopolymer stock solutions.

2.2. Stock solution preparation

Stock solutions of whey protein concentrated and high methoxyl pectin, both in the concentration of 20 g/kg, were prepared in McIlvaine buffer solution at pH 3.5 ± 0.05, stirred for 3 h and left to rest for 12 h at 25 °C to guarantee complete hydration. Sodium azide (0.3 g/kg) was added to the solutions to prevent the microbial growth.

2.3. Protein-polysaccharide blends (mixed systems)

Mixed systems were prepared by mixing the appropriated mass of stock solutions, with a total biopolymer quantity fixed at 2.0% (w/w), to result the desired concentrations of each biopolymer. Protein:polysaccharide blends (WPC:PEC) at ratios 1:1, 2:1, 3:1, 4:1, and 5:1 were prepared with and without ultrasound application and transferred to test tubes. All the samples remained at rest for 24 h to allow interaction between the biopolymers. For the sonicated samples, the stock solutions were taken to an ultrasound device (Sonic Ruptor 4000, Omni International, EUA) equipped with a 12.7 mm diameter probe tip and sonicated continuously for 5 minutes at a frequency of 20 kHz and power of 320 W. To avoid sample overheating during sonication, a water circulation cooling system was used.

2.4. Emulsion preparation

Oil in water emulsions (O/W) were prepared according to Kaltsa et al. [20] with some modifications. The biopolymer amount was fixed at 2.0% (w/w) in the aqueous phase and WPC:PEC ratios were of 1:1 and 4:1. Soybean oil (5, 10 and 15% based on the solution total mass) was dispersed in WPC solution with Ultra turrax (T-25, IKA, Germany) for 4 min at 15000 rpm. After that, the desired pectin solution amount was added and the emulsion was agitated during 4 minutes at the same rotational frequency. The emulsions were then sonicated (20 kHz, 120 W) for one minute with the probe immersed 1 cm below the emulsion surface, transferred to test tubes, and left at rest for 48 h.

2.5. Zeta potential measurements

Pure PEC and WPC solutions at 2 g/kg were investigated in relation to the surface charge according to Marfil [21] in the pH range 3 to 7 (± 0.05) adjusted with HCl 1 N and NaOH 1 N at 25 °C using a Zeta Plus Analyzer (Brookhaven Instruments Corporation, USA). Solutions were prepared with and without application of ultrasound.

2.6. Turbidimetry

Protein:polysaccharide complexes formation was investigated by turbidimetry at pH 3.5 ± 0.05 at different WPC:PEC ratios (1:1; 2:1; 3:1; 4:1 e 5:1), with and without ultrasound application. The biopolymer amount was fixed at 0.5 g/kg and the pH was adjusted using NaOH 1 N or HCl 1 N. Measurements were made in spectrophotometer (SP-22, Biospectro, Brazil), at 590 nm [21–23], using deionized water as reference.

2.7. Rheological measurements

Rheological assays were made in an AR-2000EX rotational rheometer (TA Instruments, Delaware, USA), using geometry of serrated parallel plates (∅40 mm) according to Albano et al. [24] with some modifications. For the protein-polysaccharide blends the gap was 300 μm and for the emulsions the gap was 400 μm. After introduction in the rheometer, the samples were left to rest for 2 min to reach the measurement temperature. The rheological behavior of emulsions was evaluated in function of the system composition, at 25 °C, and following heating–cooling ramps. Flow curves (shear stress versus shear rate) were determined following downward (100 a 0.1 s^{-1}) and upward shear rate ramps (0.1 a 100 s^{-1}) at 25 °C. The Newton (Eq. (1)) and Power Law (Eq. 2) models were adjusted to the flow curves allowing calculation of rheological parameters in function of the system composition.

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

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

in which τ (Pa) is the shear stress, μ (Pa·s) the viscosity, $\dot{\gamma}$ (s^{-1}) the shear rate, K the consistency index (Pa·s^n), and n the flow behavior index.

Heating-cooling assays were carried out with emulsions prepared with WPC:PEC ratios of 1:1 and 4:1 and 15% oil, at shear rate of 50 s^{-1} and three temperature ramps on the following sequence: from 25 to 72 °C, from 72 to 5 °C and from 5 to 25 °C at 2 °C/min, with 30 s of rest between each ramp.

2.8. Infrared spectrum (FTIR-ATR)

FTIR analysis was made in liofilized samples (Liotop – L101 for about 96 h) of the pure materials (WPC and pectin) and for the mixed systems with WPC:PEC ratio 1:1 and 4:1, with and without ultrasound application. Spectra were obtained at the interval 4000–650 cm^{-1} with resolution of 4 cm^{-1}, with 32 scans using a spectrophotometer Perkin-Elmer FTIR (Spectrum One) connected to a total reflectance attenuator (FTIR-ATR), with the help of the software Origin Pro 8.0 for data evaluation.

2.9. Optical microscopy

Emulsion aliquots were placed on glass slides and covered with coverslips. The microstructure was evaluated in optical microscope (Olympus CX31) with a coupled video camera (Olympus, SC30), with objective lens of 40× (bar 20 μm).

2.10. Confocal laser scanning microscopy (CLSM)

Confocal microscopy was made for the emulsions according to Lamprecht et al. [25] with some modifications, using the fluorescent dyes FITC (fluorescein isothiocyanate) and Rhodamine B. The dyes were dissolved in DMSO (dimethyl sulphoxide) at 1 mg/mL and added in the amount of 40 μl for each 2.0 g of biopolymer in solution. The

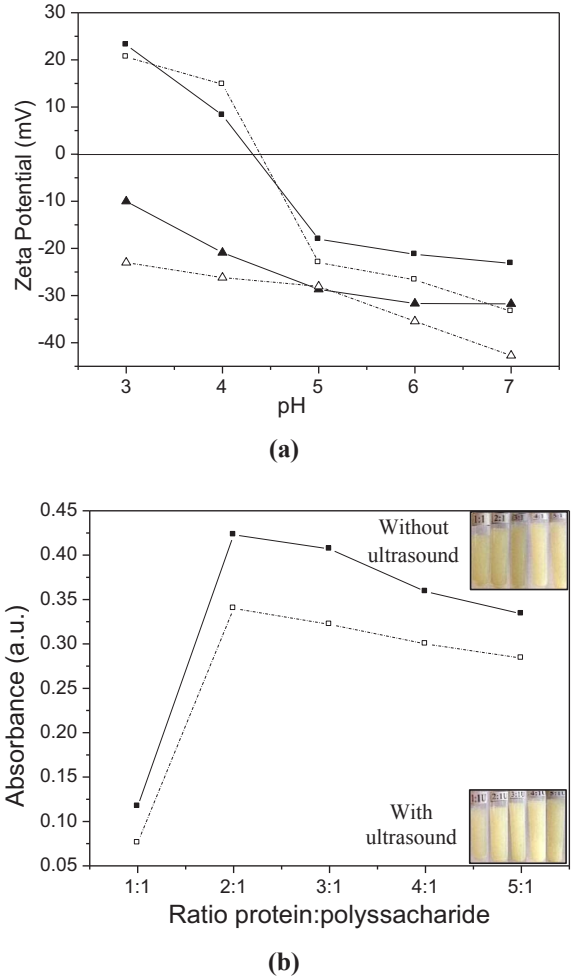

Fig. 1. (a) Zeta potential of pure solutions of WPC (squares) and PEC (triangles) at different pH. (b) Effect of the ratio WPC:PEC at pH 3.5 on the absorbance at 590 nm. With (open symbols) and without ultrasound application (closed symbols).

observations were made on a confocal laser scanning microscope (ZEISS LSM 710) with objective lens of 40× and the images were analyzed on Zen 2010 software. The laser was adjusted for the green/red fluorescence mode, that produced two excitation wavelength, 488/543 nm, respectively. Green and red fluorescent images were obtained from two separated channels and then superimposed.

2.11. Droplet size distribution analysis

The average and size distribution of emulsion droplets was determined by laser diffraction (Laser Scattering Spectrometer Mastersizer 2000S, Malvern Instruments Ltd., U.K). To avoid multiple dispersion effect, the emulsions were diluted in deionized water.

3. Results and discussion

3.1. WPC-pectin complex formation

3.1.1. Zeta potential and turbidimetry

Surface charge of WPC and PEC showed similar behavior with and without ultrasound application (Fig. 1). Pectin showed negative values throughout the pH range (3−7), and its electronegativity increased with pH increase, in a range of −10.02 mV to −31.75 mV without ultrasound and −22.99 mV to −42.71 mV with ultrasound, a characteristic of anionic polysaccharides (Fig. 1a). Souza et al. [26] evaluated zeta potential of pure solutions of high methoxyl pectin in the pH range 3–7 and found negative values in the whole range of pH studied, with the electronegativity increasing from −21 mV (pH 3) to −50.3 mV (pH 7). The negative liquid charge of the pure high methoxyl pectin solutions is consequence of the non-protonated carboxyl groups (COO–) for solutions with pH above of its pKa ≈ 2.9[27].

Zeta potential of WPC varied from 23.18 mV (pH 3), 8.26 mV (pH 4) to −23.18 mV (pH 7) without ultrasound, and from 20.61 mV (pH 3), 14.82 mV (pH 4) to −33.34 mV (pH 7) with ultrasound. These values are consistent with those found by Nogueira [28] and Souza et al. [26]. The isoelectric point was found at pH 4.5, which is in the range of 4.5 to 5.22 previously reported for whey proteins [26,28–31].

Taking into account the profile showed by WPC and PEC, a restricted range of interaction between pH 3.0 and 4.0 was identified and the pH 3.5 was adopted for the following assays.

Concerning turbidimetric analyses, WPC showed absorbance values of 0.047 a.u and 0.09 a.u, while pectin showed 0.003 a.u and 0.008 a.u, both with and without sonication, respectively. Similar values were found by Freitas et al. [32] on the evaluation of the high methoxyl pectin absorbance in a wide range of pH (3 −7).

Samples with different WPC:PEC ratios showed similar behavior (Fig. 1b), however, systems without ultrasound application presented higher absorbance in relation to those treated with ultrasound, indicating that sonication reduced the size of formed complexes, generating a better homogenization and, consequently, a less turbid solution (Fig. 1b). Madadlou et al. [33] observed that sonication (35 and 135 kHz) resulted in the decrease of turbidity and casein particle diameters in any pH value between 6.35 and 11.4. Martini et al. [34] investigated the ultrasound effect in whey proteins and found a decrease of about 90% in the turbidity when ultrasound was applied.

The experimental data indicated a drastic increase in absorbance when the WPC:PEC ratio was changed from 1:1 to 2:1, indicating that the increase of the protein proportion leaded to higher electrostatic interaction between the biopolymers and caused more intense complexation. Further increase in protein proportion (WPC:PEC ratios of 3:1, 4:1 and 5:1) showed a trend of stabilization in the absorbance values. According to Hosseini et al. [35] at higher protein:polysaccharide relations, samples are less turbid without sedimentation, indicating that the colloidal dispersions contain small complexes with higher stability. The results indicated that ultrasound favored the dispersion stability due to a reduction in the complex sizes and did not affected the interaction potential between the biopolymers.

At pH 3.5 the protein concentration increase provided a charge balance between the ionized biopolymers, with the formation of stable coacervates as a result of interactions between the opposite charges. Jones et al. [36] evaluated the stability of particles of β-lactoglobulin (β-Lg) complexed with high methoxyl pectin and observed a turbidity increase when the solution pH decreased, indicating a great number of formed complexes with high stability. According to Albano and Telis [37] these systems did not show visual phase separation, indicating biopolymers' miscibility, which occur more in diluted solutions with low concentration of macromolecules. There are a few cases – for example, blends of whey albumin and pectin [38], in which a genuine and complete miscibility in non-diluted dispersions of two biopolymers has been observed [10].

On the other hand, the high turbidity of the blends indicated high charge density and strong interaction among the polyelectrolytes, although macroscopic phase separation did not occur [39]. In the sonicated samples a less turbid system was observed, indicating higher homogeneity and smaller complex sizes. A significant reduction in the size of protein aggregates upon ultrasound treatment has been observed in whey proteins [14,16]. Madadlou et al. [33] observed that sonication resulted in a decrease in casein solution turbidity and in the re-assembled micelles diameter at any studied pH value, the magnitude of decrease being greater for samples treated with higher ultrasound frequency. On the other hand, Hosseini et al. [35] observed, by isothermal titration calorimetry, that sonication decreased the interaction strength

between b-lactoglobulin and alginate, which was attributed to the lower negative charge density of sonicated alginate when compared to the intact polysaccharide, leading to lower complexation. Nevertheless, in the present work the ultrasound treated pectin showed a slightly lower zeta-potential than the non-sonicated one (Fig. 1a), reinforcing the hypothesis of particle size reduction due to sonication. The lower complex sizes and consequent higher stability may be highly favorable for emulsions stabilized by electrostatic interaction between oppositely charged biopolymers with reduced droplet sizes obtained by ultrasound application.

Albano and Telis [37] observed by optical microscopy that the system WPC:PEC exhibited small scattered soluble complexes that increased slightly with the increase in WPC proportion in the solution. Freitas et al. [32] evaluated the effect of soy protein:high methoxyl pectin ratio at pH 3.0 and 3.5 and observed, by turbidimetry and optical microscopy, that complexes formed in pH 3.5 were smaller, more soluble and stable, which turns their use appropriated for food systems.

Although the obtained soluble complexes without ultrasound were small, the sonication was able to further reduce their size. After sonication, many small WPC particles are formed as a result of bigger particle disruption and their surface acquire a smooth aspect [16]. This observation explains the low observed viscosity and its decrease with ultrasound application, which is discussed during evaluation of the rheological behavior.

3.1.2. Infrared spectroscopy (FTIR-ATR)

Pure WPC spectrum showed the expected characteristic chemical groups (Fig. 2): the band 3285 cm^{-1} is referent to the stretching of the N—H group; the stretching C—H (CH_2 and CH_3 groups saturated) is indicated at 2932 cm^{-1}; at 1632 cm^{-1} there is the stretching C═O (amide I); and, finally, 1536 cm^{-1} corresponds to the stretching C═O (amide II).

Chen et al. [40] determined the FTIR spectrum of whey protein isolated (WPI), which revealed the presence of characteristic functional groups at 1645, 1535, 1456, and 1043 cm^{-1} corresponding, respectively, to the stretching C═O and N—H, C—H and C—N, basically the same chemical groups found in the whey protein concentrated (WPC). Lee et al. [41] evaluated the region of amide I group to determine structural changes in the secondary structure of the protein adsorbed in the emulsion interface, and the amide group was in the band from 1700 to 1600 cm^{-1}.

For pure pectin, the chemical groups present in the bands from 3425 cm^{-1} to 2930 cm^{-1} are referent to hydroxyl groups (O—H); at 1720 cm^{-1} and 1580 cm^{-1} there is the stretching C═O of the carboxyl acid I and carboxyl acid II, respectively, and at 1020 cm^{-1} the saccharide structure (stretching C—O—C of the ether group).

Fellah et al. [42] when evaluating pectin methyl-esterification found almost the same chemical groups. Previous studies about pectin suggested that the esterified group shows bands in the range of 1350–1450 cm^{-1}: 1380 cm^{-1} corresponding to the CH_3 symmetrical stretching and 1440 cm^{-1} corresponding to the asymmetric stretching [43]. The same stretches were found in the bands 1449 cm^{-1} for the symmetric stretching and in 1356 cm^{-1} for the asymmetric stretching.

Samples WPC:PEC 1:1 without (Fig. 2a) and with sonication (Fig. 2b) showed intermediate spectra to both pure biopolymers, with a protein wide band and the ether group band of the polysaccharides being more prominent. The blend WPC:PEC 4:1 revealed the protein characteristic bands, with the ether group less accentuated in comparison to WPC:PEC 1:1, indicating that there is excess of protein charges and greater interaction of the amino groups of proteins with the carboxylic acid groups of polysaccharides. Polysaccharides such as pectin have free carboxylic groups, which confer negative charge to the molecules, while proteins such as WPC have positive charge due to the amino group presence. During the electrostatic interaction, the carboxylic groups of the polysaccharides interact with the amino groups of the proteins to form complexes that contain amine [44]. Thus, it is possible to conclude that the complexation between pectin and whey protein concentrate effectively occurred.

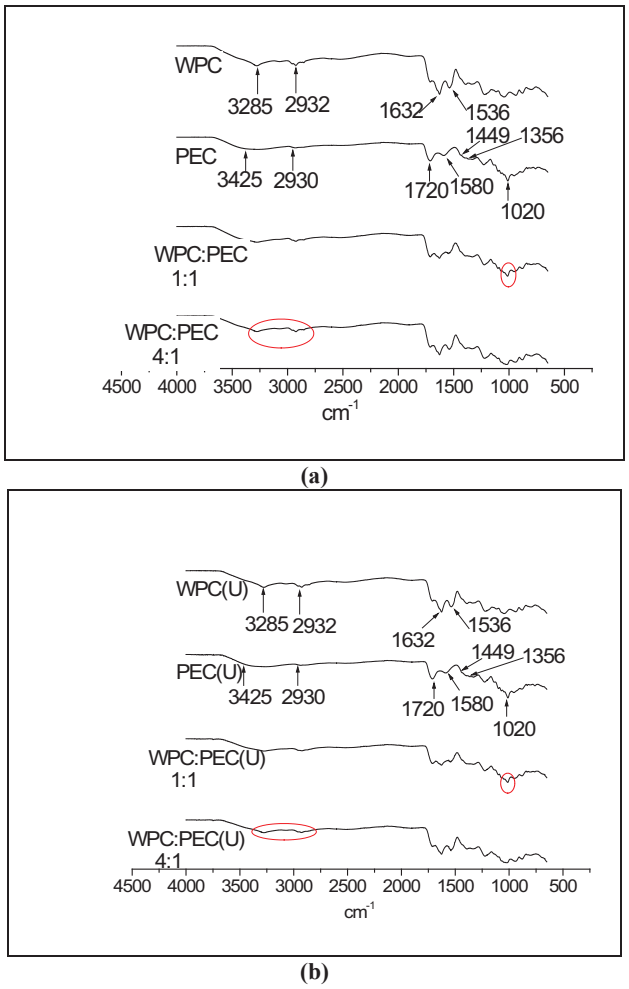

Fig. 2. FTIR spectra of the pure WPC and PEC and their blends at ratios 1:1 and 4:1 without ultrasound application (a) and with ultrasound (U) application (b).

Regarding sonicated samples (Fig.2b), the protein bands were slightly less accentuated in WPC:PEC 4:1, indicating that ultrasound application did not changed the infrared spectrum, since the functional groups remained the same and there were not relevant structural differences either in pure biopolymers neither in their blends. Therefore, FTIR analysis confirmed the complexation and the predominant biopolymer in each formulation, evidencing that ultrasound did not interfere in the electrostatic interactions between WPC and PEC, but only affected the complex sizes.

3.1.3. Rheological behavior

The flow curves of WPC-Pectin suspensions were evaluated following downward and upward shear rate ramps in order to detect possible thixotropic behavior in the samples, which was not observed as the downward and upward flow curves were practically superimposed [24]. In addition, the analysis of shear stress versus shear rate dependence indicated linear behavior and the Newton's model showed good adjustment to data, with high coefficients of determination ($R^2 > 0.98$). The calculated values of viscosity are presented in Table 1 and the absence of thixotropy is reinforced by the similar values of viscosity obtained for ramp 1 (downward) and ramp 2 (upward).

The systems showed low viscosity values that were reduced with the increase in the protein proportion, with or without ultrasound application, although ultrasound application potentiated the viscosity reduction in comparison to the non-sonicated samples (Table 1). The

Table 1
Viscosity values (μ (mPa.s)) at 25 °C of the complexes at different WPC:PEC ratios without and with ultrasound application and proportions among the components of the mixed system (WPC, PEC and water).

WPC:PEC	Without Ultrasound		With Ultrasound		Proportions of the components	
	Ramp 1 μ	Ramp 2 μ	Ramp 1 μ	Ramp 2 μ	PEC:H_2O	WPC:H_2O
1:1	10.4	10.3	5.5	5.5	1:98	1:98.0
2:1	4.3	4.3	3.1	3.1	1:147	1:73.5
3:1	2.5	2.5	1.9	1.9	1:196	1:65.3
4:1	1.9	1.8	1.5	1.4	1:245	1:61.3
5:1	1.6	1.5	1.4	1.4	1:294	1:58.8

viscosity decrease with protein content increase is justified by the water increase in relation to the pectin amount, which was not enough to gel the continuous aqueous phase and to increase or to maintain the suspension viscosity, as suggested by the ratio PEC:H_2O indicated in Table 1. Perrechil and Cunha [45] evaluated the rheological behavior of the cream and serum phases of the emulsions with sodium caseinate and jatai gum and verified lower viscosity in the cream phase, which was attributed to the chemical composition of the separated phases, because the cream phase showed higher water content.

In the present work, there are evidences that the WPC:Pectin complexes have been broken by ultrasound application, having their viscosity reduced. Viscosities changes were also observed in chocolate mousse prepared with ultrasound treatment [46]. This effect is similar to the high-pressure homogenization: both of the processes reduce the complex sizes by action of the turbulence, cavitation and shear forces [47]. Different results have been reported regarding the effects of ultrasound on the rheological behavior of protein suspensions. Some researchers related that sonication did not cause significant change in viscosity of whey protein [9,48], whereas Zisu et al. [49] reported a strong correlation between the decrease in protein particle size caused by sonication and the reduced suspension viscosity. Non-Newtonian behavior has been observed in more concentrated (10% w/w) protein suspensions. Arzeni et al. [14] evaluated the ultrasound effect on suspensions of WPC, soy protein isolate, and egg white powder and fitted the Power law to the respective flow curves. These authors verified a decrease in the consistency index after protein sonication, suggesting that the consequent reduction in particle size caused a decrease in the apparent viscosity. Jambrak et al. [50] described a great increase in the consistency index of soy protein isolate concentrated suspensions (10% w/w) after ultrasound treatment.

3.2. O/W emulsions stabilized by WPC-pectin complexes

The emulsions presented themselves clear and homogeneous, without phase separation, independently of WPC:PEC ratio and oil content, indicating to be stable systems with low oil content, what is of great interest and relevance for the food industry regarding fat application and substitution, enabling development of healthier and less caloric formulated foods.

The results indicate that the ultrasound treatment conditions and biopolymer ratios used were suitable to produce stable systems, allowing protein adsorption at the oil/water interface, followed by stabilization promoted by pectin adsorption building a bilayer on the droplets formed through sonication. The increase in the thickness of adsorbed polymer layer and the probable zeta potential increase tend to stabilize the emulsion, inhibiting the droplet aggregation by means of improved electro-steric repulsions [51].

The stability of the studied emulsions may be discussed in function of the different ratios between the compounds presented in the formulations (Table 2). In WPC:PEC 1:1 systems, the WPC:oil ratio is 1.6 smaller than in WPC:PEC 4:1 system; in contrast, the PEC:oil and

Table 2
Proportions among the different components (PEC, WPC, oil and H_2O) of the emulsions, values of consistency index (K [mPa.s^n]) and of flow behavior index (n) at 25 °C with different soybean oil contents at WPC:PEC ratios 1:1 and 4:1.

Oil (%)	WPC:Oil	PEC:Oil	PEC:H_2O	Ramp 1		Ramp 2	
				K	n	K	n
			WPC:PEC 1:1				
5	1:5.3	1:5.3	1:98	12.2	0.944	12.6	0.936
10	1:11.1	1:11.1	1:98	15.8	0.928	17.4	0.923
15	1:17.6	1:17.6	1:98	25.6	0.917	26.5	0.947
			WPC:PEC 4:1				
5	1:3.3	1:13.2	1:245	3.1	0.935	2.9	0.944
10	1:6.9	1:27.8	1:245	2.7	0.964	3.0	0.946
15	1:11.0	1:44.1	1:245	4.2	0.947	3.9	0.965

PEC:H_2O ratios are 2.5 times bigger. In the case of WPC:PEC 1:1 this was favorable, because despite the available protein quantity in relation to the oil content (WPC:Oil) was low for the oil/water interface overlay, the pectin quantity was enough to compensate and to stabilize the system due to the higher PEC:H_2O ratio, which favored the viscosity increase of the continuous phase and stabilized the emulsions. Pectin is used mainly as a stabilizing agent due to its ability to increase the solution viscosity. However, it also can be used as an emulsifying substance because of its tensoactive properties. In emulsions produced only with pectin, an overlap of stabilizing and emulsifying effects will occur [52].

In contrast, in the emulsions WPC:PEC 4:1, the contrary occurred, since the quantities of water (low ratio PEC:H_2O) and oil (low ratio PEC:oil) in relation to the pectin quantity were high, hindering the formation of a polysaccharide network in the continuous phase and reducing the oil droplet stabilization, in addition to cause viscosity reduction. The absence of phase separation may be attributed to higher protein availability in relation to oil (higher ratio WPC:oil), favoring a higher overlay of the oil/water interface and compensating the smaller quantity of pectin and principally the sonication that promoted higher homogenization and reduction in droplet sizes, stabilizing the emulsions. In addition, increasing oil content increased the volumetric fraction of the dispersed phase and facilitated interactions between biopolymers in the continuous phase, improving the system stability. Schmidt et al. [52] concluded that the protein content and the pectin esterification degree were the main parameters influencing the emulsion characteristics, as well as the polysaccharide concentration used directly influenced the occurrence of phase separation or kinetically stable systems [18,53].

3.2.1. Optical and confocal laser scanning microscopy

Fig. 3 shows the optical micrographics of WPC:PEC systems 1:1 and 4:1 two days after the preparation. The systems are unpackaged with small dispersed droplets in the continuous phase, characteristic of stable emulsions. Ferreira et al. [54] evaluated different methods of homogenization in emulsions and found that ultrasound caused the greatest reduction in droplet size and a considerable decrease in their creaming index, improving emulsion stability. A positive effect of ultrasound on droplet size reduction and low creaming index was also found by Albano et al. [55] in emulsions prepared with soy protein isolate and sodium alginate. As expected, the droplet population increased considerably with the oil content increase, but with permanence of small droplets. According to Kaltsa et al. [20] the ultrasound can generate very stable emulsions that require little surfactant, besides producing emulsions with small droplet sizes. All the emulsions remained stable after 7 days of preparation.

Laser confocal micrographics showed that oil droplets were coated by the protein (in green) and covered and entrapped by the pectin (in red), what formed a network in the continuous phase (Fig. 4),

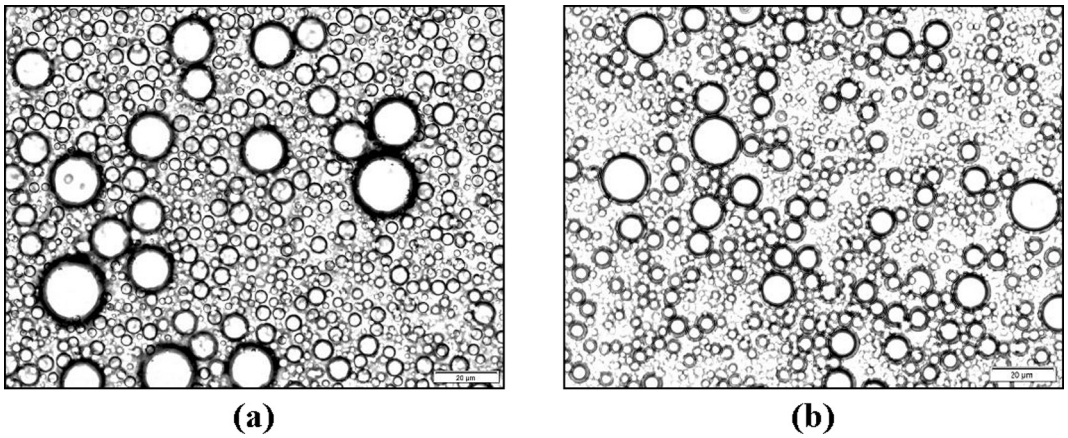

Fig. 3. Optical micrographics of emulsions composed of 15% soybean oil, at ratios WPC:PEC 1:1 (a) and 4:1 (b) after two days of storage. Scale bar 20 μm.

indicating that the protein is present at the interface and the pectin is (mainly) present in the continuous phase, stabilizing the system. In systems WPC:PEC ratio 4:1 (Fig. 4b) there was a higher quantity of droplets covered by protein (in green) and a less dense continuous phase (in red), confirming the previous discussion on the effects of different ratios among the compounds in solution.

Perrechil and Cunha [53] evaluated the confocal laser micrographics of emulsions and found that the protein remained around the oil droplets, while the excess amount was homogeneously distributed in the continuous phase. The fluorescence signals confirmed the presence of a protein layer adsorbed on the emulsion droplets and, when pectin was used as a second layer, the fluorescence was detected too, indicating the presence of regions covered by high methoxyl pectin [56].

Based on the micrographics, it can be concluded that the sonication and the bilayer formation between WPC and pectin was highly efficient to prevent droplets flocculation, providing emulsion stabilization. Emulsions stabilized by bilayers show a narrow droplet size distribution, indicating that the additional layer of high methoxyl pectin helps to prevent droplet flocculation, possibly due to steric effects [56].

3.2.2. Rheological properties

Based on the comparison between downward and upward shear rate ramps, as discussed in item 3.1.3, the flow curves corresponding to the emulsions showed absence of thixotropy. Nevertheless, non-Newtonian behavior was observed and fitting of Power Law model (Eq. 2) to the data resulted in high coefficients of determination ($R^2 > 0.999$). The rheological parameters indicated shear-thinning behavior ($n \geq 0.92$) with consistency index values (K) below 26.5 mPa.s^n (Table 2). For the emulsions with WPC:PEC ratio 1:1 there was a slight decrease in the flow behavior index and an increase in the consistency index caused by increasing oil content (Table 2), which is explained by the increasing volumetric fraction of the emulsion dispersed phase. However, the behavior indexes of WPC:PEC 4:1 systems were a little higher than that of WPC:PEC 1:1 samples and the consistency indexes decreased. This behavior is justified by the ratio among the different components present in the emulsions (Table 2), since the lower PEC:H_2O ratio rendered less viscous emulsions, leading the systems to approach the Newtonian behavior. The observed tendency to Newtonian behavior reveals the emulsion stability against shear, which can be attributed to an adequate balance between the stabilizing biopolymers, to the droplet size distribution, structure development, and lower resistance to flow.

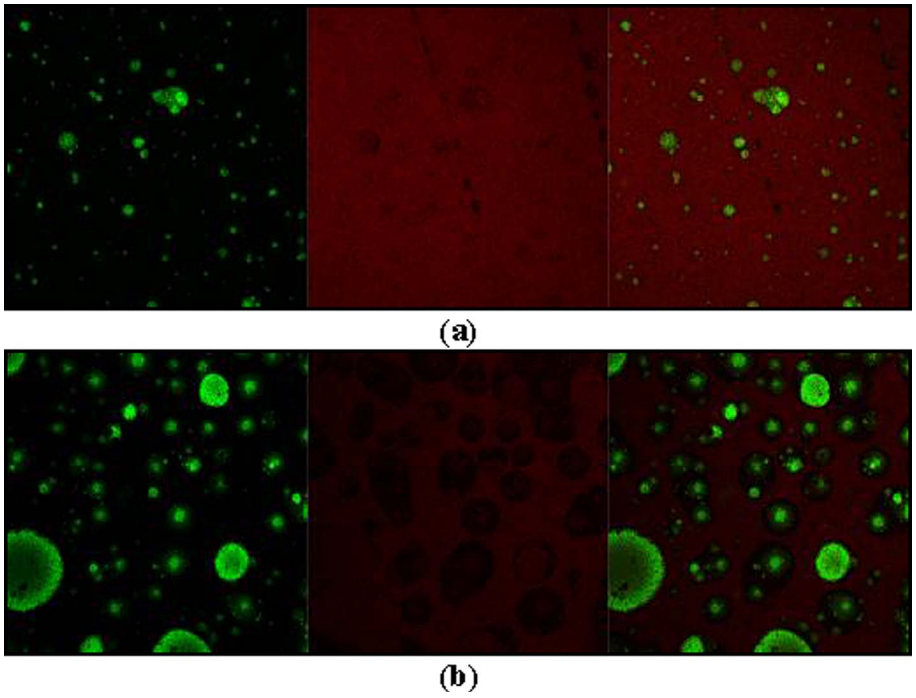

Fig. 4. Laser confocal micrographics of emulsions with 15% soybean oil at ratios WPC:PEC 1:1 (a) and 4:1 (b). Green images: PEC stained using Rhodamine B; red images: WPC stained using FITC. The images were obtained from two separated channels and then were superimposed. Scale bar 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Non-Newtonian behavior was also found by Simo et al. [18] in emulsions prepared with pectins, however they found that emulsions with lower pectin concentration showed lower resistance to flow. Piazza et al. [57], when evaluating the interfacial rheology of films formed by soy proteins and high methoxyl pectin, found a stabilizing and structuring effect of pectin, which was attributed to the formation of protein-polysaccharide aggregates at the oil/water interface. The effect of polysaccharides in the rheological properties of O/W emulsions was investigated by Perrechil and Cunha [53], who observed that emulsions with low polysaccharide concentration showed behavior close to Newtonian (n=0.92), whereas emulsions with increasing κ-carrageenan concentration presented shear-thinning behavior.

The system formulation and homogenization process affects the rheological behavior of emulsions. In the present case, the pectin concentration and sonication parameters resulted in relatively low apparent viscosities (9.4, 11.3, 17.4 mPa.s to the ratio 1:1 and 2.2, 2.3 and 3.2 mPa.s to the ratio 4:1, for 5, 10, and 15% oil content, respectively, calculated at $\dot{\gamma} = 100\,s^{-1}$), although being enough to prevent destabilization. Kaltsa et al. [20] evaluated different ultrasonic treatments in emulsions and polysaccharide solutions, and found that the flow curves of all formulations exhibited shear-thinning behavior over the shear rate tested. However, some polysaccharide based systems presented viscosity reduction when sonication time and intensity was increased, with their rheological parameters indicating a tendency to Newtonian behavior. Perrechil e Cunha [45] found that emulsions stabilized by biopolymers and homogenized at high pressures (10–60 MPa) were stable, showed Newtonian behavior and low viscosity, with this behavior being attributed to the reduction in the droplet size caused by high-pressure homogenization, which also led to a decrease in the creaming velocity and, consequently, an increase in the emulsions stability.

Similarly, it is important to highlight that the electrostatic interaction between high-methoxyl pectin and whey proteins combined to ultrasound application allowed the achievement of highly stable emulsions with low oil content in the present work.

Heating-cooling ramps caused changes in rheological behavior of the emulsions when subjected to higher temperatures (72 °C), which could be correlated to the pure biopolymer behavior. This effect showed that the biopolymer present at higher amount in the formulation had a predominant role in the emulsions' rheological behavior. In WPC:PEC 1:1 emulsions with 15% oil (Fig. 5a), the first heating ramp showed a viscosity increase at temperatures above 40 °C with prominent peaks at 50 and 65 °C. This increase in viscosity is probably due to gelation of pectin, which, when evaluated pure (Fig. 5c) also showed a viscosity increase at 50 °C. The high methoxylation pectin and pH 3.5 may have favored the formation of this gel. According to Kuhn et al. [58] the mechanism of gel formation for pectins depends directly on the esterification degree, as in the case of high methoxylation degree, aggregation and gel formation can be induced at low pH and in the presence of soluble solids. At low pH values (between 3 and 4) the electrostatic repulsion between pectin chains is suppressed, allowing mainly hydrophobic interactions and hydrogen bonds [58].

In the second ramp (cooling), the viscosity was lower at higher temperatures compared to the first ramp (heating), and increased with the temperature decreasing. Pectin suspensions heated above the pectin gelation temperature and followed by cooling form gels due to chain–chain interactions, especially hydrophobic interactions and hydrogen bonding [59].

Finally, the third ramp showed that, when returning to room temperature, the emulsion practically recovered its initial characteristics, presenting intermediate values of viscosity in relation to the first two ramps. When the pH of a pectin suspension is adjusted to 2.8–3.5 in the presence of a certain concentration of soluble solids, cooling the suspension gives rise to a gel, which retains its characteristics, even when reheated at temperatures close to 100 °C [59].

The influence of pectin on the rheological behavior of the emulsions is evidenced by analyzing a pure pectin solution (60 g/kg) at the same emulsion testing conditions (Fig. 5c). The ramps showed that pectin gelled with heating, peaking at 50 °C, but on the second and third ramps the viscosity increased with decreasing temperature and, upon cooling, returned to its initial characteristic, proving the reversible gel behavior.

These emulsion characteristics make their application highly favorable as fat mimetics in formulating sauces or desserts. For instance, for sauces or soups that are consumed hot immediately after preparation, the viscosity is potentiated, giving a better sensorial appeal in the tasting of hot foods. In the same way, it is favorable for desserts consumed refrigerated or at room temperature, such as puddings, pasteurized dairy drinks, as it maintains their initial characteristics at low temperatures even when subjected to thermal treatments, such as HTST (high-temperature, short-time) pasteurization at 72 °C [60], followed by cooling for storage (5–18 °C) and consumption at room temperature (25 °C).

The samples WPC:PEC ratio 4:1 showed a similar behavior, however with a bigger dispersion in the experimental data, possibly because there is a lower pectin concentration in this emulsion. It was observed only one viscosity peak at 65 °C in the heating ramp (Fig. 5b), which is near to the protein gelling temperature [58].

In the WPC:PEC 4:1 ratio, where there is protein predominance, the viscosity of the first ramp (heating) up to 40 °C was lower than the other ramps and above 40 °C the viscosity was potentiated, but on ramp 2 (cooling) and ramp 3 (heating up to 25 °C) the emulsion maintained the viscosity reached after heating, indicating the formation of irreversible gels, characteristic behavior of whey proteins. Under specific conditions, whey proteins form non-reversible gels, which entrap large amounts of water and non-protein compounds, resulting in excellent functional properties that assist in the formation of low fat products [3].

This behavior was also observed when the protein was evaluated pure (60 g/kg) (Fig. 5d). After the first heating ramp, gelation took place, providing a viscosity increase, potentiated between 60 and 70 °C; the high viscosity remained along the cooling ramp, characterizing the irreversible gel property expected for this protein.

It is important to highlight that both of the effects (pectin and protein) occurred during the emulsions heating-cooling process, because the emulsions stabilization is due to the joint action of the biopolymers. Li and Zhong [61], when evaluating the aggregation and gelling properties of preheated whey protein and pectin mixtures at different pHs, it was concluded that hydrogen bonds and hydrophobic attraction are partially responsible for the formation of gels and they emphasize that to control these properties it is necessary to control the mass ratio between the biopolymers.

3.2.3. Droplet size distribution

Droplet size distribution confirmed the images obtained by optical and confocal laser microscopy (Figs. 3 and 4) and the effect of ultrasound, showing small droplet sizes, which are characteristic of stable emulsions, and indicated by the low values of average droplet diameters ($D_{3,2}$): 2.14, 2.02, and 2.6 μm, respectively for emulsion with 5, 10, and 15% oil contents at WPC:PEC 1:1 ratio, and 1.88, 2.38 and 2.94 μm for 5, 10 and 15% oil contents, respectively, at WPC:PEC 4:1.

The droplet size distributions are shown in Fig. 6. Emulsions with WPC:PEC ratio 1:1 (Fig. 6a) showed bimodal distribution, mainly at 15% of oil with droplet size peaks at 1.8 μm and 8 μm. At 5% oil content the bimodal distribution was attenuated with a peak at 1.8 μm and small shoulder at 5 μm. Emulsion with 10% oil content showed monomodal size distribution.

The bimodal behavior of the systems 1:1 may be attributed to the biopolymers concentration used in the emulsion formulation. Simo et al. [18] verified that the addition of low levels of pectin leaded to bimodal distribution and concluded that a pectin critical concentration must be exceeded before a strong network of gel be formed in the emulsions. In contrast, Kaltsa et al. [62] evaluated the effect of the WPI proportion with Tween 20 in the droplet size distribution and concluded that an excess of WPI also caused a bimodal distribution.

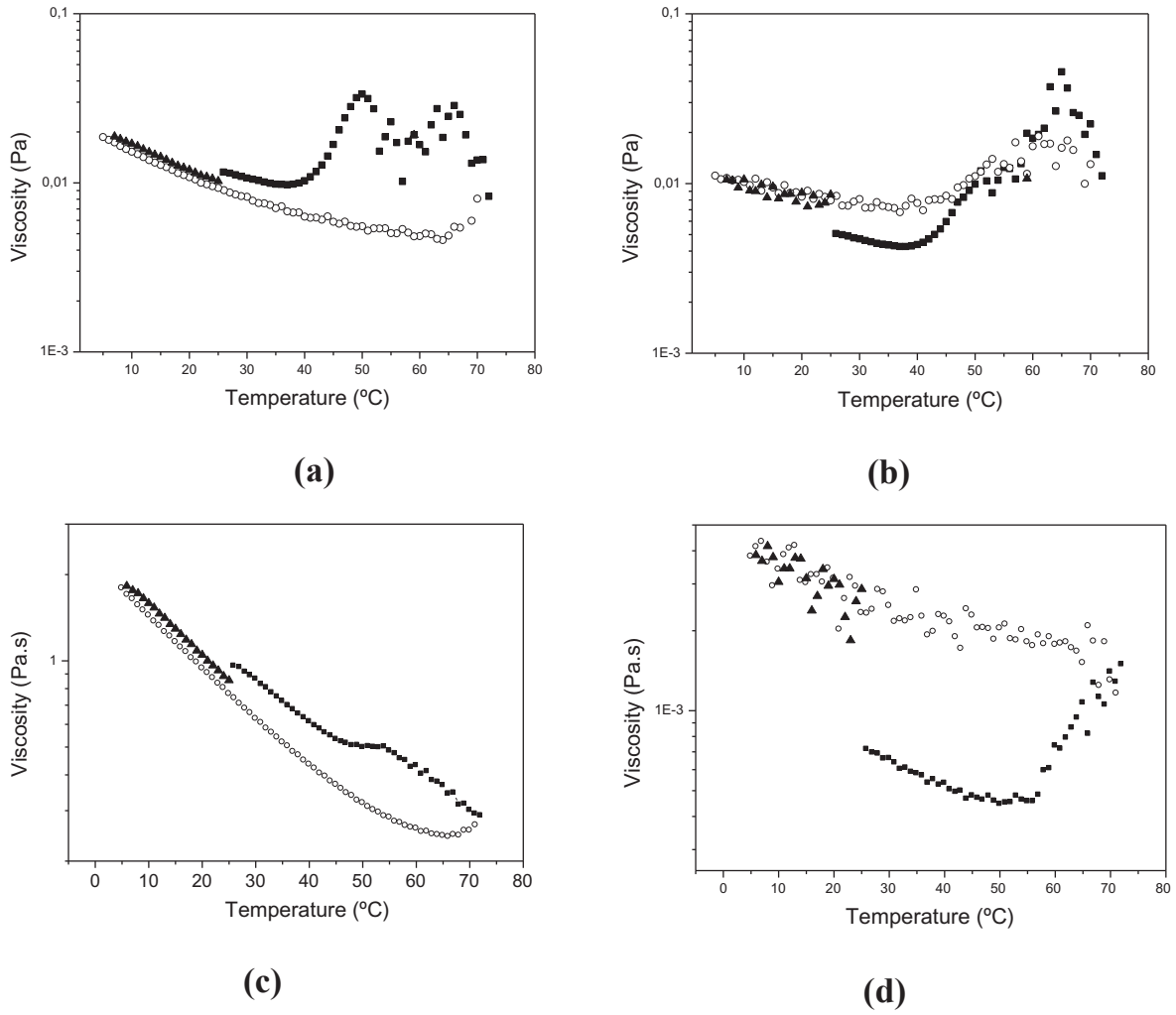

Fig. 5. Heating-cooling ramps: ramp 1 from 25 to 72 °C (■); ramp 2 from 72 to 5 °C (○); and ramp 3 from 5 to 25 °C (▲), of emulsions at ratios 1:1 (a) and 4:1 (b) with 15% soybean oil and of pure (c) pectin and (d) WPC.

Regardless the biopolymer used in the emulsions, the balance of their ratio is paramount for a narrower distribution of droplet sizes.

In the WPC:PEC ratio 4:1 which presented higher concentration of WPC, the size distribution was monomodal with predominance of small droplets. Possibly, the higher amount of WPC allowed an improved protein coating on the oil droplets/water interface. Perrechil and Cunha [53] observed that the increase of sodium caseinate concentration up to a certain limit resulted in the reduction of the oil droplet diameters. The availability of higher amounts of protein in the system assumes increasing importance with the increase in oil content: small droplet sizes at increasing volumes of the dispersed phase implies in higher interfacial area to be stabilized.

On the other hand, the average droplet size increased with increasing oil content, with droplet size peaks at 2.2, 2.8, and 5.04 μm for 5, 10, and 15% oil content due to the increased volume of dispersed phase (Fig. 6b). Similar results were reported by Piriyaprasarth et al. [63] when they analyzed the droplet size of pectin-stabilized emulsions, using different concentrations of rice bran oil. In parallel, the droplet size increase may also have been favored by the bilayer formation, as observed by Mohmmadi et al. [64] that nano-encapsulated of olive leaf phenolic compounds through WPC–pectin complexes and found that droplet sizes of W/O/W emulsions stabilized by WPC:pectin were higher than the droplet sizes in systems stabilized only by WPC. These authors concluded that the simultaneous use of two biopolymers increased the thickness of the interfacial layer around the droplets and, consequently, their size.

4. Conclusion

WPC:PEC complexes showed higher electrostatic interaction at pH 3.5 without separation of macroscopic phases. Turbidimetry indicated higher complexation with increasing protein content, but lower absorbance values in sonicated samples, indicating that the ultrasound application leaded to smaller complex particle sizes, independently of the biopolymer ratio used. This behavior was confirmed by the lower viscosity of samples treated with ultrasound. The complexation between WPC and pectin was demonstrated by FTIR analysis. The effect of ultrasonic treatment was highly favorable to emulsion stabilization, as the emulsions containing 5, 10, and 15% oil remained stable in the course of 7 days, showing narrow droplet size distributions and shear thinning behavior ($n \geq 0.92$) with low apparent viscosities values, however, the values obtained were enough to prevent destabilization. The heating-cooling rheological assays showed that the emulsions were able to recover their rheological characteristics after being subjected to thermal treatment, allowing their use as fat mimetics in different foods products and enabling formulation of foods with lower fat contents.

Acknowledgments

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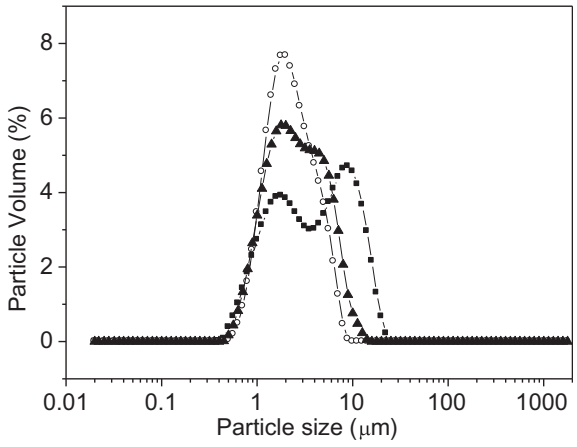
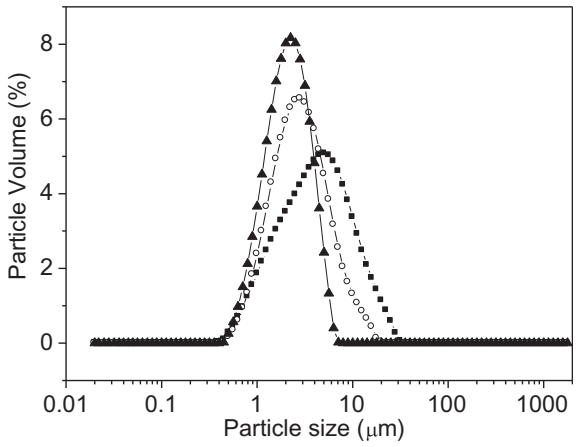


(a)



(b)

Fig. 6. Droplet size distribution in O/W emulsions at ratios WPC:PEC (a) 1:1 and (b) 4:1 with 5% (▲), 10% (○), and 15% (■) soybean oil.

References

[1] Y. Mao, D.J. Mcclements, Fabrication of viscous and paste-like materials by controlled heteroaggregation of oppositely charged lipid droplets, Food Chem. 134 (2012) 872–879.

[2] J.C. Ospina-E, A. Sierra-C, O. Ochoa, J.A. Pérez-Álvarez, J. Fernández-López, Substitution of saturated fat in processed meat products: a review, CRC Crit. Rev. Food Sci. 52 (2012) 113–122.

[3] M.V.S. Pinheiro, A.L.B. Penna, Substitutos de gordura: tipos e aplicações em produtos lácteos, Alimentos e Nutrição 15 (2004) 175–186.

[4] I. Capron, S. Costeux, M. Djabourov, Water in water emulsions: phase separation and rheology of biopolymer solutions, Rheol. Acta 40 (2001) 441–456.

[5] O.G. Jones, D.J. Mcclements, Recent progress in biopolymer nanoparticle and microparticle formation by heat-treating electrostatic protein-polysaccharide complexes, Adv. Colloid Interface 167 (2011) 49–62.

[6] J.M. Aguilera, Why food microstructure? J. Food Eng. 67 (2005) 3–11.

[7] E. Dickinson, An Introduction to Food Hydrocolloids, University Press, Oxford, 1992.

[8] D.J. McClements, Food Emulsions: Principles, Practice, and Techniques, CRC Press, Washington, 2005.

[9] G. Kresic, V. Lelas, A.R. Jambrak, Z. Herceg, S.R. Brnic, Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins, J. Food Eng. 87 (2008) 64–73.

[10] E. Dickinson, Hydrocolloids at interfaces and the influence on the properties of dispersed systems, Food Hydrocolloid 17 (2003) 25–39.

[11] J. Bowers, Food Theory and Applications, Macmillan Publishing Company, New York, 1992.

[12] H.D. Belitz, W. Grosch, Química de los alimentos, second ed., Acribia, Zaragoza, 1988.

[13] S.H. Cristensen, Pectins, in: M. Glicksman (Ed.), Food Hydrocolloids, CRC Press, Boca Raton, 1986, pp. 205–230.

[14] C. Arzeni, K. Martínez, P. Zema, A. Arias, O.E. Pérez, A.M.R. Pilosof, Comparative study of high intensity ultrasound effects on food proteins functionality, J. Food Eng. 108 (2012) 463–472.

[15] N.A. Camino, O.E. Pérez, A.M.R. Pilosof, Molecular and functional modification of hydroxypropylmethylcellulose by high-intensity ultrasound, Food Hydrocolloid 23 (2009) 1089–1095.

[16] L. Gordon, A.M.R. Pilosof, Application of high-intensity ultrasounds to control the size of whey proteins particles, Food Biophys. 5 (2010) 203–210.

[17] J. Chandrapala, C. Oliver, S. Kentish, M. Ashokkumar, Ultrasonics in food processing, Ultrason. Sonochem. 19 (2012) 975–983.

[18] O.K. Simo, Y. Mao, T. Tokle, E.A. Decker, D.J. McClements, Novel strategies for fabricating reduced fat foods: heteroaggregation of lipid droplets with polysaccharides, Food Res. Int. 48 (2012) 337–345.

[19] T. Morita, R.M.V. Assumpção, Manual de Soluções, reagentes e solventes, Blucher, São Paulo, 2007.

[20] O. Kaltsa, C. Michon, S. Yanniotis, I. Mandala, Ultrasonic energy input influence on the production of sub-micron o/w emulsions containing whey protein and common stabilizers, Ultrason. Sonochem. 20 (2013) 881–891.

[21] P.H.M. Marfil, V.R.N. Telis, Microencapsulação de óleo de palma por coacervação complexa em matrizes de gelatina/goma arábica e gelatina/alginato. (Doctoral thesis) IBILCE/UNESP, São José do Rio Preto, 2014; p. 135.

[22] Y.A. Antonov, O.M. Zubova, Phase state of aqueous gelatin–polysaccharide (1) polysaccharide (2) systems, Int. J. Biol. 29 (2001) 67–71.

[23] A.S. Prata, C.R.F. Grosso, Estudo dos parâmetros físico-químicos envolvidos na formação de microcápsulas produzidas por coacervação complexa. (Doctoral thesis) UNICAMP: Campinas, 2006; p. 267.

[24] K.M. Albano, C.M.L. Franco, V.R.N. Telis, Rheological behavior of Peruvian carrot starch gels as affected by temperature and concentration, Food Hydrocolloid 40 (2014) 30–43.

[25] A. Lamprecht, U.F. Schäfer, C.M. Lehr, Influences of process parameters on preparation of microparticle used as a carrier system for Ù – 3 unsaturated fatty acid ethyl esters used in supplementary nutrition, J. Microencapsul. 18 (2000) 347–357.

[26] F.N. Souza, C.G.M.S. Cordeiro, M.C.E. Ribeiro, K.S. Chaves, M.L. Gigante, C.R.F. Grosso, Production and characterization of microparticles containing pectin and whey proteins, Food Res. Int. 49 (2012) 560–566.

[27] M.C. Ralet, V. Dronnet, H.C. Buchholt, J.F. Thibault, Enzymatically and chemically de-esterified lime pectins: characterisation, polyelectrolyte behaviour and calcium binding properties, Carbohyd. Res. 336 (2001) 117–125.

[28] G.F. Nogueira, C.R.F. Grosso, Produção e caracterização de micropartículas com multicamadas obtidas por gelificação iônica associada à interação eletrostática. (Masters dissertation). UNICAMP: Campinas, 2013; p. 173.

[29] O.G. Jones, D.J. McClements, Biopolymer nanoparticles from heat-treated electrostatic protein–polysaccharide complexes: factors affecting particle characteristics, J. Food Sci. 75 (2010) N36–N43.

[30] M.H. Ly, M. Aguedo, S. Goudot, M.E. Le, P. Cayot, J.A. Teixeira, T.M. Le, J.M. Belin, Y. Wache, Interactions between bacterial surfaces and milk proteins, impact on food emulsions stability, Food Hydrocolloid 22 (2008) 742–751.

[31] P. Walkenstrom, A.M. Hermansoon, Mixed gels of gelatin and whey proteins, formed by combining temperature and high pressure, Food Hydrocolloid 11 (1997) 457–470.

[32] M.L.F. Freitas, K.M. Albano, V.R.N. Telis, Characterization of biopolymers and soy protein isolate-high-methoxyl pectin complex, Polímeros (2016) In press.

[33] A. Madadlou, M.E. Mousavi, Z. Emam-Djomeh, M. Ehsani, D. Sheehan, Comparison of pH-dependent sonodisruption of re-assembled casein micelles by 35 and 130 kHz ultrasounds, J. Food Eng. 95 (2009) 505–509.

[34] S. Martini, R. Potter, M.K. Walsh, Optimizing the use of power ultrasound to decrease turbidity in whey protein suspensions, Food Res. Int. 43 (2010) 2444–2451.

[35] S.M.H. Hosseini, Z.E. Djomeh, S.H. Razavi, A.A. Moosavi-Movahedi, A.A. Saboury, M.S. Atri, P.V. Meeren, β–Lactoglobulin-sodium alginate interaction as affected by polysaccharide depolymerization using high intensity ultrasound, Food Hydrocolloid 32 (2013) 235–244.

[36] O. Jones, E.A. Decker, D.J. Mcclements, Thermal analysis of b-lactoglobulin complexes with pectins or carrageenan for production of stable biopolymer particles, Food Hydrocolloid 24 (2010) 239–248.

[37] K.M. Albano, V.R.N. Telis, Caracterização reológica da interação eletrostática entre concentrado proteico de soro e pectina em função da aplicação de ultrassom, XXXVII Congresso Brasileiro de Sistemas Particulados. Anais do XXXVII Congresso Brasileiro de Sistemas Particulados, vol. 2, Editora Edgard Blücher, São Paulo, 2015, pp. 1378–1385.

[38] M.G. Semenova, V.S. Bolotina, A.P. Dmitrochenko, A.L. Leontiev, V.I. Polyakov, E.E. Braudo, V.B. Tolstoguzov, The factors affecting the compatibility of serum albumin and pectinate in aqueous medium, Carbohyd. Polym. 15 (1991) 367–385.

[39] C. Thies, How to Make Microcapsules: Lecture and Laboratory Manual, St. Louis, Missouri, 1995.

[40] B. Chen, H. Li, Y. Ding, H. Suo, Formation and microstructural characterization of whey protein isolate/beet pectin coacervations by laccase catalyzed cross-linking, LWT – Food Sci. Technol. 47 (2012) 31–38.

[41] S.H. Lee, T. Lefevre, M. Subirade, P. Paquin, Effects of ultra-high-pressure homogenization on the properties and structure of interfacial protein layer in whey protein-stabilized emulsion, Food Chem. 113 (2009) 191–195.

[42] A. Fellah, P. Anjukandi, M.R. Waterland, M.A.K. Willians, Determining the degree of methylesterification of pectin by ATR/FT-IR: methodology optimization and comparison with theoretical calculations, Carbohyd. Polym. 78 (2009) 847–853.

[43] A. Synytsya, J. Copikova, P. Matejka, V. Machovic, Fourier transform Raman and infrared spectroscopy of pectins, Carbohyd. Polym. 54 (2003) 97–106.

[44] M. Saravanan, K.P. Rao, Pectin-gelatin and alginate-gelatin complex coacervation for controlled drug delivery: influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules, Carbohyd. Poly. 80 (2012) 808–816.
[45] F.A. Perrechil, R.L. Cunha, Oil-in-water emulsions stabilized by sodium caseinate: influence of pH, high-pressure homogenization and locust bean gum addition, J. Food Eng. 97 (2010) 441–448.
[46] D. Pingret, A.-S. Fabiano-Tixier, E. Petitcolas, J.-P. Canselier, F. Chemat, First investigation on ultrasound-assisted preparation of food products: sensory and physicochemical characteristics, J. Food Sci. 76 (2011) C287–C292.
[47] S. Le Hénaff, Microparticules de complexes de protéines de lactosérum et de xanthane comme substitut de matière grasse. (Mémoire de maîtrise) Université Laval: Québec, 1996.
[48] A.R. Jambrak, T.J. Mason, V. Lelas, G. Kresic, Ultrasonic effect on physicochemical and functional properties of a-lactalbumin, LWT – Food Sci. Technol. 43 (2010) 254–262.
[49] B. Zisu, R. Bhaskaracharya, S. Kentish, M. Ashokkumar, Ultrasonic processing of dairy systems in large scale reactors, Ultrason. Sonochem. 17 (2010) 1075–1081.
[50] A.R. Jambrak, V. Lelas, T.J. Mason, G. Krešic, M. Badanjak, Physical properties of ultrasound treated soy proteins, J. Food Eng. 93 (2009) 386–393.
[51] M. Evans, I. Ratcliffe, P.A. Williams, Emulsion stabilisation using polysaccharide–protein complexes, Curr. Opin. Colloid Interface 18 (2013) 272–282.
[52] U.S. Schmidt, K. Schmidt, T. Kurz, H.-U. Endreß, H.P. Schuchmann, Pectins of different origin and their performance in forming and stabilizing oil-in-water-emulsions, Food Hydrocolloid 46 (2015) 59–66.
[53] F.A. Perrechil, R.L. Cunha, Stabilization of multilayered emulsions by sodium caseinate and – carrageenan, Food Hydrocolloid 30 (2013) 606–613.
[54] S. Ferreira, C.R. Malacrida, V.R.N. Telis, Influence of emulsification methods and use of colloidal silicon dioxide on the microencapsulation by spray drying of turmeric oleoresin in gelatin-starch matrices, Can. J. Chem. Eng. 94 (2016) 2210–2218.
[55] K.M. Albano, C.P. Oliveira, V.R.N. Telis, Efeito da aplicação de ultrassom na obtenção de emulsões o/w preparadas com complexos isolado proteico de soja:alginato de sódio, In: annals: XI Brazilian Meeting on Chemistry of Food and Beverages V Simpósio de Engenharia e Ciência de Alimentos, 2016, pp. 664–670.
[56] N. Xiang, Y. Lyu, G. Narsimhan, Characterization of fish oil in water emulsion produced by layer deposition of soy β-conglycinin and high methoxyl pectin, Food Hydrocolloid. 52 (2016) 678–689.
[57] L. Piazza, N. Durr-Auster, J. Gigli, E.J. Windhab, P. Fischer, Interfacial rheology of soy proteins – high methoxyl pectin films, Food Hydrocolloid 23 (2009) 2125–2131.
[58] K.R. Kuhn, C.S.F. Picone, R.L. Cunha, Food Gels, in: V.R.N. Telis (Ed.), Biopolymer Engineering in Food Processing, CRC Press, New York, 2012, pp. 111–144.
[59] E.P. Ribeiro, E. Seravalli, Química de Alimentos, Blucher, Mauá, 2007.
[60] P.X. Qi, D. Ren, Y. Xiao, P.M. Tomasula, Effect of homogenization and pasteurization on the structure and stability of whey protein in milk, J. Dairy Sci. 98 (2015) 2884–2897.
[61] K. Li, Q. Zhong, Aggregation and gelation properties of preheated whey protein and pectin mixtures at pH 1.0-4.0, Food Hydrocolloid 60 (2016) 11–20.
[62] O. Kaltsa, P. Paximada, I. Mandala, E. Scholten, Physical characteristics of submicron emulsions upon partial displacement of whey protein by a small molecular weight surfactant and pectin addition, Food Res. Int. 66 (2014) 401–408.
[63] S. Piriyaprasarth, M. Juttulapa, P. Sriamornsak, Stability of rice bran oil-in-water emulsions stabilized by pectin-zeincomplexes: effect of composition and order of mixing, Food Hydrocolloid 61 (2016) 589–598.
[64] A. Mohammadi, S.M. Jafari, E. Assadpour, A.F. Esfanjani, Nano-encapsulation of olive leaf phenolic compounds through WPC–pectin complexes and evaluating their release rate, Int. J. Biol. Macromol. 82 (2016) 816–822.