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Impact of pectin or chitosan on bulk, interfacial and antioxidant properties of (+)-catechin and β-lactoglobulin ternary mixtures

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ABSTRACT

The interactions effect between (+)-catechin, β-lactoglobulin and two types of polysaccharides (pectin and chitosan) were studied at pH 4.

Dynamic light scattering (DLS) revealed that pectin formed smaller complexes in the presence of (+)-catechin and β-lg, while chitosan presented a reduced number of populations with higher particle size. Dynamic interfacial data (obtained by a drop tensiometer) revealed that the complexes formed between pectin (+)-catechin and β-lg slowed down the β-lg migration to the oil/water interface; contrarily to the chitosan mixtures where protein migrated faster. The surface dilatational modulus of β-lg was ~45 mN/m and increased to ~60 mN/m when mixing with pectin and (+)-catechin, while the values of the surface dilatational modulus for the chitosan mixed interfacial films decreased to ~35 mN/m. The free (+)-catechin content decreased 12% and 10% when interacting with pectin and chitosan, respectively, as compared to pure (+)-catechin. However, the (+)-catechin antioxidant activity were not affected by the interactions. Complexes formed between polyphenols, proteins and polysaccharides could be used as a good alternative to understand and consequently improve the phytochemicals stability in food matrices.

1. Introduction

Milk proteins, and in particular soluble whey proteins, are commonly used in food foams and emulsions. One of the main proteins of milk whey is β-lactoglobulin (β-lg) (Alomirah & Alli, 2004). This protein has been widely studied and tested as a food ingredient due to its excellent surface-active and colloid-stabilizing characteristics. β-lg is a globular protein stabilized by two disulphide bridges with a molecular weight of 18,350 Da and it possesses a hydrophobic centre (Girard, Turgeon, & Gauthier, 2002). This protein can bind many kinds of endogenous and exogenous agents such as dietary polyphenols (Xiao et al., 2011) as well as polysaccharides (Cooper, Dubin, Kayitmazer, & Turksen, 2005; de Kruif, Weinbreck, & de Vries, 2004; Rodríguez Patino & Pilosof, 2011).

Proteins and polyphenols can interact in solution by the formation of multiple weak interactions (mainly hydrophobic) between amino acid side chains and polyphenol aromatic rings, indicating that it is mainly a surface phenomenon. Sometimes these interactions could be complemented by hydrogen bonding, playing an important role in reinforcing and stabilizing the complexes (Charlton et al., 2002; Kanakis et al., 2011; Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005; Xiao et al., 2011).

Polysaccharides are usually employed to obtain stability in low-pH food products such as acidified milk drinks and yoghurt. Pectin is used in foods as a gelling agent, thickener, texturizing, emulsifier and stabiliser (Kováčová, Synytsya, & Štětina, 2009). The main component common to most pectin's is a backbone chain structure of α-(1 → 4)-linked D-galacturonic acid units interrupted by the insertion of (1 → 2)-linked L-rhamnopyranosyl residues in adjacent or alternate positions. Also, some of the carboxylic groups of galacturonic acid molecules in the pectin chains are methyl esterified. Chitosan is a biocompatible and biodegradable polymer having immense structural possibilities for chemical and mechanical

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modification to generate novel properties and functions in different fields especially in the biomedical field. It is a linear polycationic biopolymer of N-acetyl-D-glucosamine and D-glucosamine residues carrying a positive charge in acidic environments due to the presence of amine groups (Shukla, Mishra, Arotiba, & Mamba, 2013).

Polysaccharides can also interact with polyphenols and these interactions are affected by the polyphenol molecular weight (MW) and the polysaccharide conformational flexibility. Interactions between carbohydrates and polyphenols can be used for microencapsulation which includes the entrapment of the active ingredient in coating material. In that way the active ingredient is isolated (protected) from the environment until its release (Jakobek, 2015). The interaction between polysaccharides and proteins influences the stability and structure of foods. Interfacial protein-polysaccharide complexes can be used to improve the physical stability of oil-in water emulsions (Guzey & McClements, 2007) and foam stability can also be influenced by those interactions (Sperber, Schols, CohenStuart, Norde, & Voragen, 2009).

Little is known about how free polyphenols in the presence of dietary factors may interfere in their release (Argyri, Komaitis, & Kapsokefalou, 2006; Oliveira et al., 2015) and consequently reduce their biological properties. Thus, the main purpose of this work was to analyse bulk and interfacial interactions of ternary mixtures (protein-polyphenol-polysaccharide) as well as the impact on antioxidant properties of polyphenols under low pH conditions.

2. Material and methods

2.1. Materials and reagents list

β-lactoglobulin (≥90%) (+)-catechin (≥99.0%) and high molecular weight (HMW) chitosan was supplied by Sigma–Aldrich® (Sintra, Portugal). Chitosan (Sintra, Portugal) was characterized by a degree of deacetylation >75% and a MW of 624 kDa. Pectin from citrus peel type DF (70% esterification) was provided by CP Kelco (Lille Skensved, Danmark).

The 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), acetic acid, Florisil 60–100 mesh, sodium acetate, sodium carbonate, fluorescein, 2,2'-azo-bis-(2-methyl propionamidine)-dihydrochloride (AAPH) and 6-hydroxy-2,5,7,8-tetramethylbroman-2-carboxylic acid (trolox) were also purchased from Sigma–Aldrich® (Sintra, Portugal). The potassium persulfate was purchased from Merck (Algés, Portugal).

Surface active impurities was removed according to Bahtz et al. (2009). The glassware was properly cleaned to avoid any contamination by any surface-active substance.

2.2. Preparation of the protein-polyphenols-polysaccharide mixtures

The (+)-catechin and β–lactoglobulin powders were dissolved in acetate buffer (pH 4 and ionic strength: 0.1 M) under agitation (400 rpm) at room temperature. Polysaccharides (pectin and HMW chitosan) were dissolved at 70 °C for 30 min. The pH was adjusted with HCl (1 N). The mixed systems were prepared by mixing the appropriate volumes of each solution and stored for 24 h at 4 °C to ensure complexes formation. All samples were prepared in order to reach final concentrations of 0.02% wt for polyphenols, 0.3% wt for protein and 0.38% wt for polysaccharides. These individual concentrations were those found in commercial yoghurt formulations (Oliveira et al., 2015).

One part of the mixed systems was filtered by 3 kDa filters for total antioxidant activity measurements (ABTS, ORAC) and free polyphenol quantification by HPLC-DAD.

2.3. Particle size measurements

Particle size distributions of the samples were obtained by using a Dynamic Laser Light Scattering (DLS) instrument (ZetasizerNano-Zs, Malvern Instruments, Worcestershire, UK) provided with a He–Ne laser beam (633 nm) and a digital correlator, Model ZEN3600. Measurements were carried out at a fixed scattering angle of 173° (Martinez, Farías, & Pilosof, 2010).

2.4. Dynamic interfacial tension

Time-dependent surface pressure (π) and dilatational rheology of the adsorbed β-lg/polyphenol/polysaccharides films, as well as the behaviour of the β-lg alone, were determined at the oil/water (o/w) interface with an automatic drop tensiometer PAT-1 (Sinterface Technologies, Berlin, Germany). A drop (constant volume: 12 μl) of the tested solutions was formed at the tip of a capillary immersed in a cell filled with a purified oil. Measurements were done until the adsorption equilibrium was reached (around 180 min). The surface tension (γ) was accurately (±0.1 mN/m) calculated through the analysis of the droplet profile with the Young-Laplace equation (Labourdenne et al., 1994). The surface pressure (π) is defined as $\pi = \gamma_0 - \gamma$, where γ_0 is the interfacial tension of the clean interface (24 mN/m), in the absence of the emulsifiers, and γ the interfacial tension of the solution at each time (θ). All the experiments were performed at 20 °C and two measurements have been done for each system.

2.5. Interfacial dilatational properties

The interfacial viscoelastic parameters (surface dilatational modulus, E, and its elastic, E_d, and viscous, E_v, components), were measured as a function of the time by applying sinusoidal oscillations in the interfacial area at an amplitude (ΔA/A) of 3% and an angular frequency (ω) of 0.05 Hz. Previously, it was determined these values in order to work in the linear viscoelastic region (data not shown). The sinusoidal perturbation was induced at the interface by injecting and extracting the solution into and from the drop. A Fourier transformation was performed so as to obtain the dilatational parameters of the interfacial films.

The surface dilatational modulus (E) derived from the change in the surface tension (dilatational stress) (equation (1)) resulting from a small change in the surface area (dilatational strain), A (equation (2)), may be described according to equation (3) (Lucassen & Van Den Tempel, 1972):

$$\sigma = \sigma_0 \sin(\omega\theta + \delta) \quad (1)$$

$$A = A_0 \sin(\omega\theta) \quad (2)$$

$$E = \frac{d\sigma}{dA/A} = -\frac{d\pi}{d\ln A} = E_d + iE_v \quad (3)$$

where σ_0 and A_0 are the stress and strain amplitudes, respectively, and δ is the phase angle between stress and strain.

The dilatational modulus is a complex quantity, which is composed of a real and an imaginary part. The real part of the dilatational modulus or storage component is the elasticity of the interfacial film, and the imaginary part or the loss modulus is related to the interfacial viscosity.

2.6. Quantitative analysis of polyphenols by HPLC with DAD detector

For the High-Performance Liquid Chromatography with Diode-Array Detection determinations of free (+)-catechin and β-lg concentrations were carried out with HPLC-DAD (Waters Series 600, Massachussets, USA). A reverse phase Symmetry® C18 column (250 × 4.6 mm i.d. 5 μm particle size and 125 Å pore size) with a guard column containing the same stationary phase (Symmetry® C18) was used.

For (+)-catechin the mobile phase was composed by solvent A: water/methanol/formic acid (92.5:5:2.5 v/v/v) and solvent B: methanol/water (94:6 v/v). The programme began with a linear gradient starting at: 0–30% B in 10 min, 30–50% B in 10 min, 50 to 0% B in 5 min and kept at 0% B during 5 min. The flow rate was 0.75 mL/min, the oven temperature was set as 25 °C and the injection volume was 50 μL. The calibration curve was done with an external standard of (+)-catechin within the range of 6–400 μg/mL.

For β-lactoglobulin quantification, the mobile phase A was composed of water and 1% trifluoroacetic acid (TFA) and mobile phase B included acetonitrile with 1% TFA. The programme began with a linear gradient starting at: 0–50% B in 10 min and from 50 to 0% B in 10 min and kept at 0% B during 5 min. The flow rate was 0.8 mL/min, the oven temperature was set as 30 °C and the injection volume was 50 μL. The calibration curve was performed with β-lg in the range of 0.2–6 mg/mL.

Photodiode array detector (Waters, Mildford, MA, USA) spectra were measured over the wavelength range 200–600 nm in steps of 2 nm. The analysis was monitored at wavelengths of 278 nm for (+)-catechin and 220 nm for β-lactoglobulin. Results were expressed as micrograms/mL.

2.7. Antioxidant activity measurements by ABTS and ORAC

The free radical-scavenging activity was determined by 2,2-azinobis-3-ethylbenzo thiazoline-6-sulphonic acid (ABTS) radical decolourization assay (Giao et al., 2007). The radical cation was produced by reacting ABTS with potassium persulfate. The $ABTS^{+}$ solution was diluted with ultra-pure water to an absorbance of 0.70 ± 0.02 at 734 nm (Shimadzu 1240 UV–visible spectrophotometer) and equilibrated at 23 °C. The absorbance reading was exactly 6 min after initial mixing of 1.0 ml of diluted $ABTS^{+}$ with 10 μL of polyphenol solution. Calibration curve was made with ascorbic acid in the range of 0.02–0.50 mg/mL and all the determinations performed in triplicate were expressed as mg ascorbic acid equivalent/mL.

The Oxygen radical absorbance capacity (ORAC-FL) assay was based on that proposed by Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, and Recio (2011). Briefly, the reaction was carried out at 40 °C in75 mM phosphate buffer (pH 7.4) and the final assay mixture (200 μL) contained fluorescein (70 nM), AAPH (14 mM), and antioxidant [Trolox ($9.98 \times 10^{-4} - 7.99 \times 10^{-3}$ μmol/mL) or sample (at different concentrations)]. The fluorescence was recorded during 137 min (104 cycles). A FLUO star OPTIMA plate reader (BMG Labtech, Offenburg, Germany) with 485 nm excitation and 520 nm emission filters was used. The equipment was controlled by the FLUO star Control software version (1.32 R2) for fluorescence measurement. Black polystyrene 96-wellmicroplates (Nunc, Denmark) were used. AAPH and Trolox solutions were prepared daily and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4). All reaction mixtures were prepared in duplicate and at least three independent runs were performed for each sample. Final ORAC-FL values were expressed as mg of Trolox-equivalents/mL.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows. Normality of data distribution was tested by Kolmogorov–Smirnov method.

Statistical significance values of the groups' means was made by one-way analysis of variance with Dunn's post hoc test to compare groups' means. The statistical analyses performed were considered significant when $P < 0.05$.

3. Results and discussion

3.1. Bulk interactions as assessed by particle size measurements

Dynamic light scattering (DLS) has been used in many studies to characterize the interactions between proteins/polysaccharides in the bulk phase (Kazmierski, Wicker, & Corredig, 2003; Sperber et al., 2009). Fig. 1A shows that β-lg solutions at pH 4 presented a main population with a diameter size of 6.5 nm and a small population at higher sizes. Nevertheless, the volume size distribution indicated that the first one was the only significant population (Fig. 1B). The predominant peak observed probably corresponds to monomers and dimers as reported by Martinez et al. (2010) for β-lg solutions (4%wt, pH 3.5), which showed a bimodal distribution with a predominant lower size peak at 5 nm. The second peak (only observed in the intensity plot) may correspond to octamers of β-lg in the pH range of 3.7–5.2 Gottschalk, Nilsson, Roos, and Halle (2003).

β-lg mixed with (+)-catechin showed that the β-lg population at higher sizes was induced by the polyphenol addition, indicating that this molecule induced the formation of protein aggregates (Fig. 1C). However, once again this second population can be neglected as seen in the volume size distribution (Fig. 1D).

The intensity size distribution of pectin, revealed only one population with a maximum value at 1281 nm (Fig. 1E). The citrus pectin MW is around 145–180 kDa (Morris et al., 2008). According to this MW, the corresponding hydrodynamic diameter obtained from the DLS software should be close to 22 nm. This difference between the estimated and the measured values indicated a strong tendency of pectin to self-associate forming aggregates between 700 and 2000 nm (Lima, Soldi, & Borsali, 2009), which was corroborated in the volume particle size distribution (Fig. 1F).

The mixture of β-lg and pectin showed size distributions with only one peak at 396 nm (Fig. 1G and H). This result could be due to a strong electrostatic interaction between β-lg and pectin. Several studies reported β-lg and pectin complexes formation at pH 4–4.5, value that falls in between the pI of β-lg and the pKa of pectin. The ester groups of pectin can form hydrogen bonds with the hydroxyl, amine, phenyl and carboxylic groups in β-lg (Girard et al., 2002; Jones, Decker, & McClements, 2009; Zimet & Livney, 2009).

Mixtures of pectin and (+)-catechin presented no changes in the particles size diameter when compared with pure pectin (Fig. 1I and J), indicating that, if there are interactions between these molecules, they would not modify the pectin aggregates size. This could be of great interest for the food industry since complexes formed between pectin and polyphenols have been proved to reduce astringency (Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014).

Fig. 1K and L show the results for the ternary mixture of pectin, β-lg and (+)-catechin. As can be seen, they presented no differences with the mixture of pectin and β-lg (Fig. 1G and H). These results indicated that the polyphenol did not interfere in the protein-pectin interactions and probably formed a ternary complex that fell in the same range. Similar results were obtained by Thongkaew, Gibis, Hinrichs, and Weiss (2014).

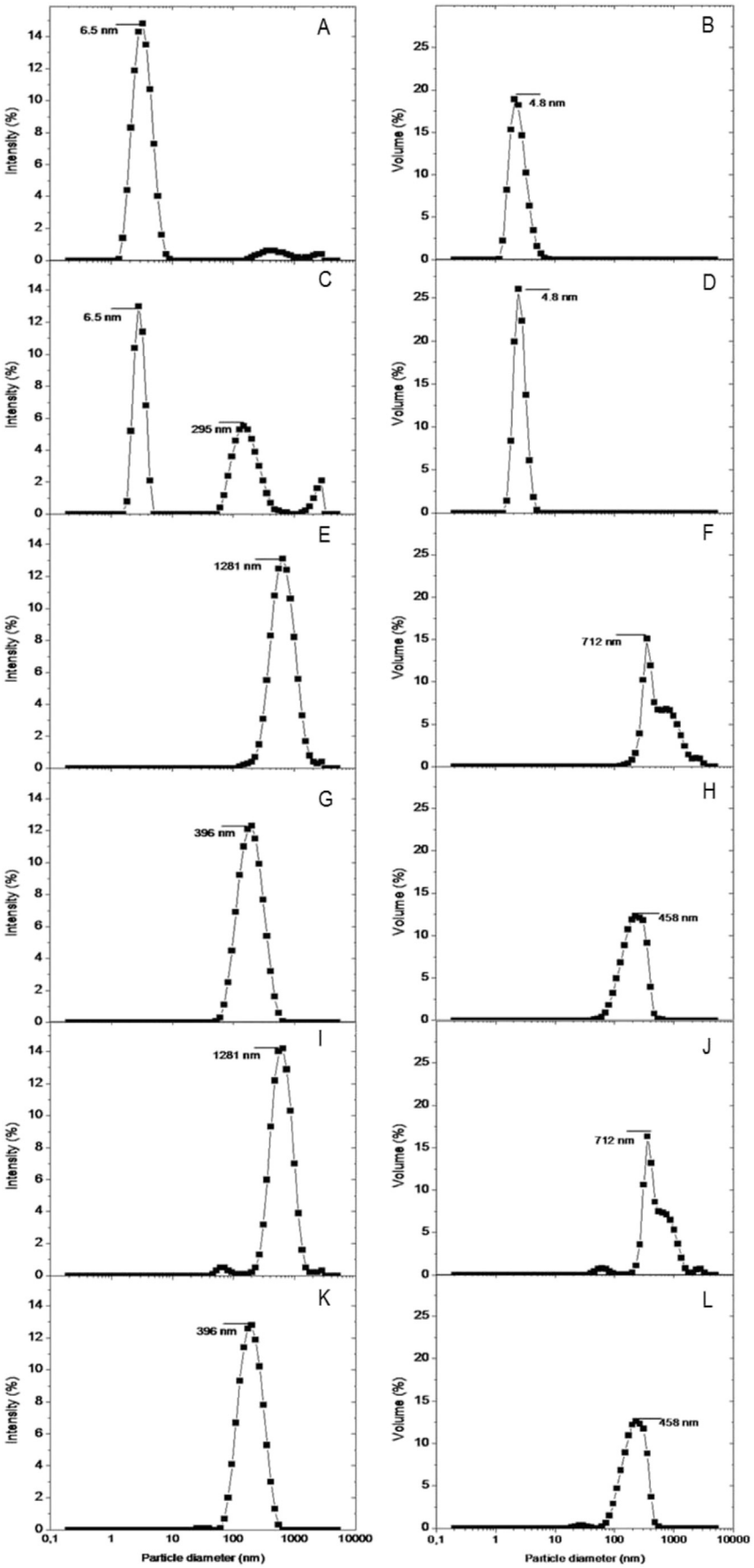

Fig. 1. Intensity and volume particle size distribution of pure β-lg, pectin and their mixtures with catechin at pH 4.0 and 25 °C. A–B: β-lg; C–D: β-lg-catechin; E–F: pectin; G–H: β-lg-pectin; I–J: pectin-catechin; K–L: β-lg-pectin-catechin.

Chitosan presented a multimodal intensity size distribution (Fig. 2E). However, the predominant size population showed a maximum value at 4.8 nm as stated by the volume size distribution (Fig. 2F). According to the DLS software and the MW of chitosan, the size of these particles should be around 47 nm. The existence of several populations reflects a high polydispersity and the tendency to self-associate (Yang et al., 2014; Zoltán, Balogh, Saokham, Jansook, & Loftsson, 2015). Chitosan presented no changes in the particle size distribution when it was mixed with β-lg, at least as could be seen in the volume particle size distribution (Fig. 2G and H). On the other hand, some changes were observed in chitosan and (+)-catechin mixtures (Fig. 2I and J). Interactions between chitosan and tea polyphenols through nanoparticles formation have already been reported by Liang et al. (2011). The polyphenols were entrapped by ionic interaction between the positively charged amine of hydrochloride chitosan and negatively charged carboxyl groups of carboxymethyl chitosan.

The particle size distributions of the ternary mixture chitosan-(+)-catechin-protein (Fig. 2K and L) showed similar results to the mixture of the protein with the polysaccharide.

As explained by Jakobek (2015), the mechanisms of polyphenol/polysaccharide associations would be similar as for proteins, where the adsorption is mediated by hydrogen bonds and hydrophobic interactions. However, by comparison to polyphenol/protein interactions, much less is known regarding the interaction between polyphenols and polysaccharides. Nevertheless, the polymeric character of most polysaccharides and their versatile physicochemical properties would anticipate interactions driven by similar factors governing flavonoid/protein interactions (Bordenave, Hamaker, & Ferruzzi, 2014).

3.2. Interactions at the o/w interface

In order to corroborate that the complexes are indeed formed and to assess how these particles behave of at the o/w interface, interfacial measurements were carried out. Surface pressure (π) time evolution of β-lg and its mixtures with (+)-catechin and pectin or chitosan at the o/w interface are shown in Figs. 3 and 4 (A, C and E). The polysaccharides (pectin and chitosan), as well as the (+)-catechin presented no interfacial activity (data not shown). Thus, it can be deduced that the increment of π with time observed in these figures is due to the protein adsorption.

The β-lg and (+)-catechin mixture presented a similar trend but with slightly higher π values as compared to pure β-lg solution (Fig. 3A). On the other hand, a slower migration of β-lg to the o/w interface was observed in the presence of pectin (Fig. 3C) or in the ternary mixture (Fig. 3E), since lower π values were reached at the beginning of the adsorption step. This may be related to the β-lg–pectin interactions observed previously (Fig. 1G).

On the contrary, the interactions of β-lg with chitosan (Fig. 4C) and chitosan-(+)-catechin (Fig. 4E) increased the surface activity, which may be attributed to the presence of the polysaccharide in the subphase. According to Qun and Ajun (2006), the variation of surface tension values depends on the molecular weight of chitosan, although it could also influence the conformation adopted by the chitosan in the aqueous medium according to the values of pH, ionic strength and degree of acetylation as different molecular interaction forces may be promoted (van der Waals, hydrogen-bonding, hydrophobic and electrostatic interactions). It could also affect the way the protein components dissociate from the complexes formed at the o/w interface (K.G. Zinoviadou, Scholten, Moschakis, & Biliaderis, 2012) that also depend on the experimental conditions of the aqueous medium.

It is very important to analyse the interfacial dilatational parameters as they are involved in the stability of dispersed systems, such as oil-in-water emulsions (Dickinson, 2009). As a general trend, it was observed that the E values increased with time (Figs. 3 and 4B, D and F) indicating that interactions between the adsorbed molecules did occur (Pizones Ruiz-Henestrosa, Martinez, Carrera Sánchez, Rodríguez Patino, & Pilosof, 2014).

At long adsorption times (~180 min), β-lg E values increased when other components are present, but with the exception of the systems with chitosan. The highest increment of E was observed in the ternary mixture β-lg-pectin-(+)-catechin (Fig. 3F), which indicated a more elastic interfacial film formation. As it was deduced from the DLS results (Fig. 1), pectin formed complexes with β-lg (Fig. 1G and H) with a higher particle size and similar results were obtained for the ternary mixture (Fig. 1K and L). This better interfacial behaviour (highest E values) in the presence of (+)-catechin (Fig. 3F) implicate a very important result as this mixture could be interesting to form and stabilize oil-in-water emulsions. Similar results were obtained by Wan, Wang, Wang, Yuan, and Yang (2014) when analysing a soy protein isolate and resveratrol mixture.

On the contrary, less structured interfacial films were formed in the presence of chitosan (Fig. 4D and F), since the E values decreased as compared to those of pure β-lg. So, the rheological properties were worsened in the presence of chitosan, which could be possibly due to the interactions between protein and chitosan. Thus, reducing protein flexibility and avoiding the protein conformational changes necessary to form an elastic film.

These results showed that the interactions in the bulk of different components lead to systems which have a direct effect on the composition and structure of the o/w interface (Zinoviadou, Scholten, Moschakis, & Biliaderis, 2014).

3.3. Quantification of free (+)-catechin and β-lg

To determine the percentage of (+)-catechin and β-lg bound or being part of complexes with polysaccharides, the free amount of these compounds was tested by HPLC-DAD. Table 1 shows the results for (+)-catechin. As can be seen, free (+)-catechin concentration decreased 12% in ternary mixtures with pectin and almost 10% in ternary mixtures with chitosan (Table 1). The remaining mixtures presented variations between 2 and 5% (Table 1). These results are different from those found by other authors since green tea polyphenols were retained by proteins up to a 90% (Rodríguez, von Staszewski, & Pilosof, 2015). Differences could be attributed to the amount of different compounds present in the extract, different methodologies used to determine the polyphenol concentration (Folin Ciocalteu's reagent) and different conditions of pH (pH = 6).

β-lg interacts with both (+)-catechin and polysaccharides to form complexes, thus free protein would be reduced. The free β-lg content decreased 24, 23 and 78% for the mixtures with (+)-catechin, pectin and chitosan, respectively (Table 2). In the ternary mixtures, the reduction of free β-lg was 44 and 85% for pectin and chitosan, respectively (Table 2). These could be the result of a stronger interaction between the protein and chitosan and even more in the presence of polyphenol than for system where pectin was present. Although mixtures with chitosan showed volume size distributions similar to those of pure β-lg, the intensity size distributions indicated that larger complexes were formed (Fig. 2). Interactions between chitosan and β-lg would be strong enough to maintain the complexes even during HPLC determinations. Chitosan molecules can adopt a spread conformation in solution because of the electrostatic repulsion force existing between the amine groups along the molecular chain. The carboxyl groups on the surface of the protein molecule may form hydrogen bond with amine groups maintaining a compact three-dimensional structure without spreading and keeping an inner hydrophobic core (Guzey

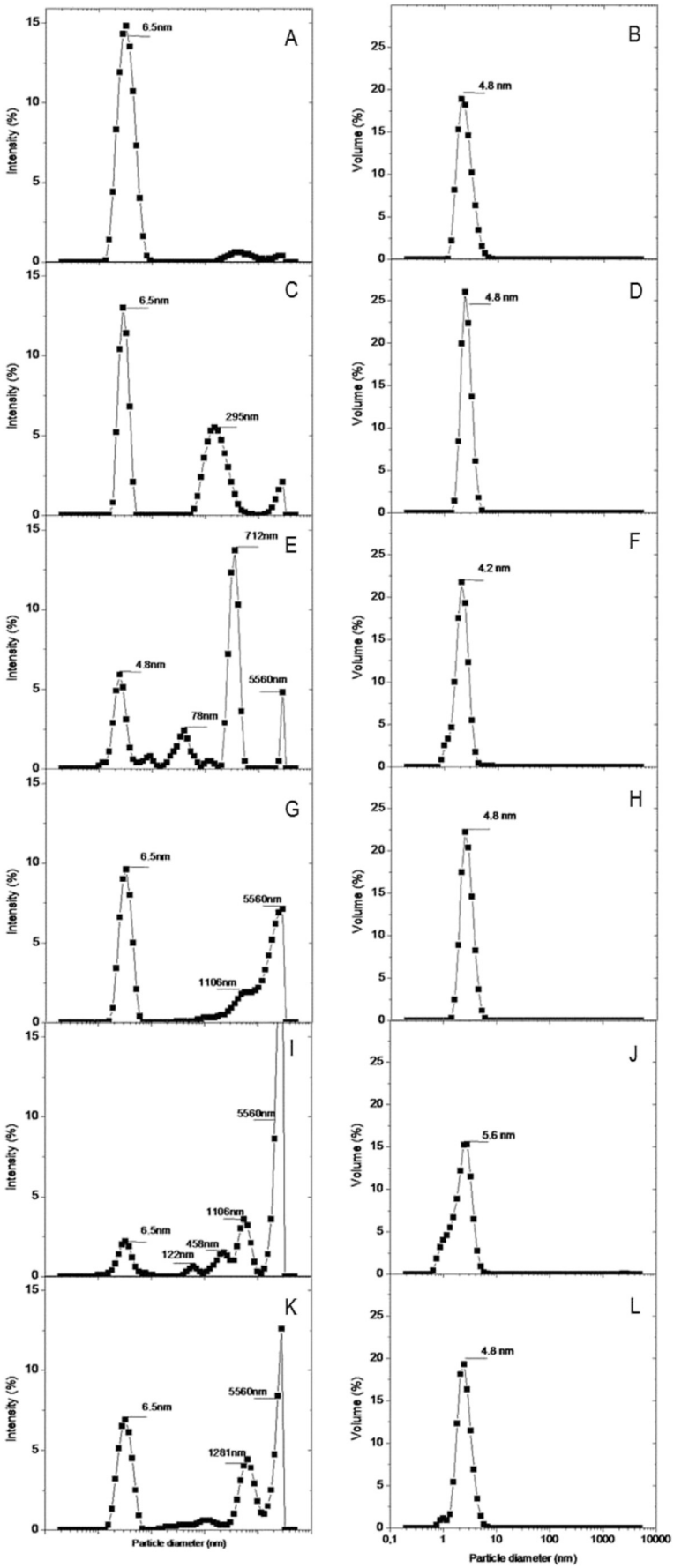

Fig. 2. Intensity and volume particle size distribution of pure β-lg, chitosan and their mixtures with catechin at pH 4.0 and 25 °C. A–B: β-lg; C–D: β-lg-catechin; E–F: chitosan; G–H: β-lg-chitosan; I–J: chitosan-catechin; K–L: β-lg-chitosan-catechin.

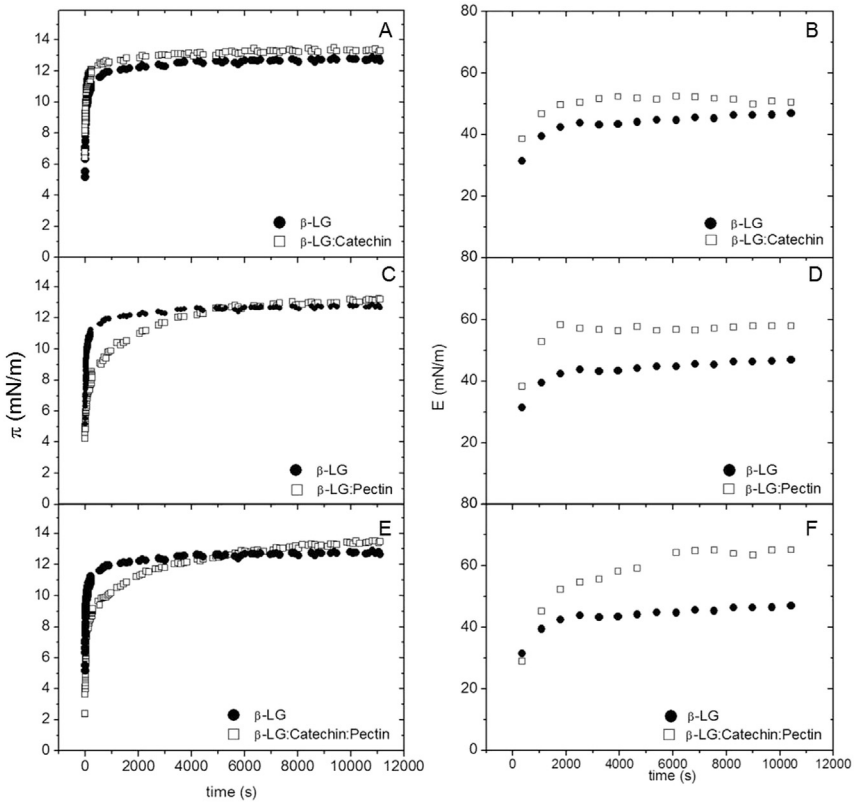

Fig. 3. Surface pressure time evolution and dilatational modulus for pure β-lg and its mixtures with pectin and catechin at pH 4.0 and 25 °C.

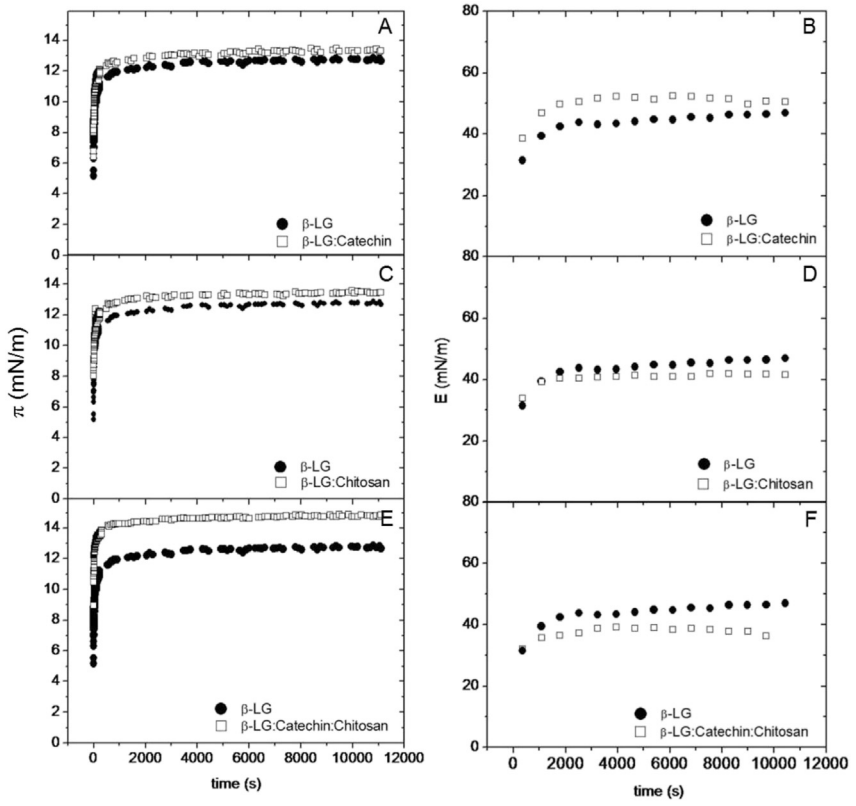

Fig. 4. Surface pressure time evolution and dilatational modulus for pure β-lg and its mixtures with chitosan and catechin at pH 4.0 and 25 °C.

Table 1
Antioxidant activity (ABTS and ORAC) and free (+)-catechin concentration in the presence of β-LG and polysaccharides (pectin and chitosan).

Sample	ABTS (mg ascorbic acid/mL)	ORAC (mg Trolox/mL)	Free (+)-catechin (μg/mL)
(+)-Catechin	0.267 ± 0.001[a]	0.78 ± 0.04[a]	181.57 ± 1.49[b]
(+)-Catechin + β-LG	0.275 ± 0.002[a]	0.81 ± 0.02[a]	185.12 ± 0.23[a]
(+)-Catechin + Pectin	0.280 ± 0.002[a]	0.82 ± 0.03[a]	185.49 ± 0.53[a]
(+)-Catechin + Chitosan	0.261 ± 0.002[a]	0.81 ± 0.02[a]	176.79 ± 0.74[c]
(+)-Catechin + β-LG + Pectin	0.256 ± 0.002[b]	0.79 ± 0.05[a]	163.41 ± 0.21[e]
(+)-Catechin + β-LG + Chitosan	0.255 ± 0.001[b]	0.79 ± 0.09[a]	168.04 ± 0.19[d]

[a] Different letters represent significant differences ($P < 0.001$) between free polyphenol and interactions with polysaccharides and β-LG.

Table 2
Free β-lactoglobulin concentration in the presence of (+)-catechin and polysaccharides (pectin and chitosan).

Sample	Free β-lg (mg/mL)	% Loss
β-LG	4410 ± 0.540[a]	–
(+)-Catechin + β-LG	3347 ± 0.190[c]	24
β-LG + Pectin	3398 ± 0.463[b]	23
β-LG + Chitosan	0.968 ± 0.133[e]	78
(+)-Catechin + β-LG + Pectin	2448 ± 0.474[d]	44
(+)-Catechin + β-LG + Chitosan	0.672 ± 0.105[f]	85

[a] Different letters represent significant differences ($P < 0.001$) between free protein and in interactions with polysaccharides and β-LG.
% Loss represented the content of protein that did not appear in the elution time of pure β-lg.

& McClements, 2006).

3.4. Antioxidant activity of (+)-catechin

The antioxidant capacity of (+)-catechin and its mixtures with the biopolymers was determined by the methods of ABTS and ORAC. The antioxidant capacity of (+)-catechin determined by ABTS revealed a slight but significant decrease for both ternary mixtures (Table 1). However, ORAC determinations presented no significant ($P > 0.05$) variations in the antioxidant capacity. Although proteins or polysaccharides interact and form complexes with (+)-catechin as stated by HPLC, the complexes would still retain some antioxidant activity because not the whole polyphenol molecule is compromised in the interaction. Most of them have neighbouring hydroxyl groups on two aromatic rings, which are responsible for the antioxidant activity, and it is likely that, even if the hydroxyl groups on the second ring are still free to allow the molecule to act as an antioxidant (Von Staszewski, Pilosof, & Jagus, 2011).

4. Conclusions

This work demonstrated that (+)-catechin interacted with β-lg and the polysaccharides to form complexes. These interactions impacted on the interfacial properties of β-lg at the oil/water interface, which consequently would have an effect on the respective emulsions. The (+)-catechin mixtures with each biopolymer or ternary formulations did not affect the antioxidant properties of the polyphenol. Thus, complexes formation of polyphenols with proteins and polysaccharides could be used as a good alternative to improve the phytochemicals performance in food products. Food components forming complexes with bioactive compounds may protect them from degradation during gastrointestinal digestion and becoming more available for absorption *in vivo*.

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