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Influence of green tea polyphenols on the colloidal stability and gelation of WPC

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ABSTRACT

Green tea extracts are being widely used in food products due to their health-promoting properties. Polyphenols can interact with food proteins leading to the formation of soluble or insoluble complexes; therefore they could alter functional properties of proteins. The objective of the present work was to study the colloidal stability and gelation characteristics of a whey protein concentrate (WPC) in the presence of green tea polyphenols. Mixtures of WPC35 (8 and 30% w/v) and green tea polyphenols (0.25–1% w/v) were prepared at pH 4.5 and 6.0. The size of particles formed was analyzed by light scattering, while gelation was characterized by means of dynamic rheometry and texture analysis of gels. At pH 6.0, the particles were smaller and had a higher net charge than at pH 4.5, which accounted for by a less precipitation of the system at pH 6.0. The G′ parameters of gels upon cooling at 35 °C increased with increasing polyphenols concentration at both pH values. However, the relative viscoelasticity decreased. The texture analysis indicated that the addition of polyphenols improved the firmness and adhesiveness of the gels at pH 6.0, while no significant differences were seen at pH 4.5. The results obtained in this work indicate that pH-dependent interaction between green tea polyphenols and WPC induces the formation of aggregates that modifies the viscoelastic and texture properties of the gels.

1. Introduction

Functional foods can be regarded as foods with a health benefit beyond satisfying traditional nutritional requirements (Sanders, 1998). Moreover, there is an increasing demand by consumers of new functional food products and the easiest way for developing these products, is the incorporation of functional ingredients to already known food stuff.

Polyphenols are major constituents of plants and are consumed by humans in food and beverages such as wine, tea, fruits and chocolate. Especially, green tea polyphenols have received increased attention since many epidemiological studies suggest an association between the prevention of diseases and the consumption of polyphenol-rich beverages or foods (Scalbert, Manach, Morand, & Rémésy, 2005).

Polyphenols have a significant affinity for proteins that leads to formation of soluble complexes, which can grow in size and even form sediments. Many authors have developed models to explain protein–polyphenol complexes formation and precipitation (Charlton et al., 2002; Jöbstl, O'Connell, Fairclough, & Williamson, 2004; Lin, Chen, Cheng, & Chen, 2004; Poncet-Legrand et al., 2006; Richard, Lefeuvre, Descendit, Quideau, & Monti, 2006; Siebert, Troukhanova, & Lynn, 1996). Most of these models propose that protein–polyphenol complexes are formed by multiple weak interactions (mainly hydrophobic) between aminoacids side chains and polyphenol aromatic rings, indicating that the association of polyphenols with proteins is principally a surface phenomenon. Sometimes these interactions could be complemented by hydrogen bonding, which would play an important role in reinforcing and stabilizing the complexes. Additionally, each polyphenol is able to bind more than one protein, acting as a linker between two proteins. Thus, in the last few years, polyphenol–protein interaction has been studied by several techniques like spectroscopy, chromatography, flow nephelometry and electron microscopy among others (Charlton et al., 2002; Jöbstl et al., 2004; Lin et al., 2004; Poncet-Legrand et al., 2006; Richard et al., 2006; Siebert et al., 1996). However, there are little experimental

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data relating to the effects of polyphenol–protein interactions on protein functional properties (e.g. foaming, gelation, emulsification). Indeed, most studies have been limited to the interaction of a particular protein or a narrow range of polyphenols. Further research needs to be done over a range of proteins and polyphenols in order to elucidate the effects of polyphenols on protein functional properties that may influence the polyphenol application in food processing.

Whey protein (WP) products are important food ingredients because of their desirable functional properties, such as gelation, foaming and emulsification. The major protein in whey is β-lactoglobulin (β-lg; 50%) and is its primary gelling agent that dominates the thermal behaviour of the total WP system (Relkin, 1996). Native β-lg has two disulfide bonds and one free thiol group, which is buried within the protein structure. Upon heating, β-lg undergoes complex changes. Above 60 °C the protein monomer is partially unfolded and non-polar groups, and the buried thiol group, are exposed (Iametti, De Gregori, Vecchio, & Bonomi, 1996). Subsequent protein aggregation can take place depending mainly on protein concentration and pH (Verheul, Roefs, & de Kruif, 1998). At neutral pH thiol/disulfide exchange reactions, leading to the formation of disulfide bonds, are mainly involved. At lower pH the contribution of thiol groups in the formation of aggregates is lower and large aggregates formed are mainly physical aggregates (Hoffmann & van Mil, 1999).

The effect of pH on β-lactoglobulin gels has been previously studied using viscoelastic measurements (Stading & Hermansson, 1990), large deformation properties (Stading & Hermansson, 1991) and microscopy (Langton & Hermansson, 1992). Texture depends on mechanical properties, which in turn depend largely on the structure of the gel network. Mechanical properties can be divided into small deformation properties and large deformation properties. Small deformation properties are measured using non-destructive methods, whereas testing by large deformation includes fracture properties of the material. Large deformation properties are usually performed by compression or tension (Aguilera & Stanley, 1999).

The objective of this study was to investigate the effects of polyphenol–protein interactions on the colloidal stability and gelation of a whey protein concentrate.

2. Materials and methods

2.1. Materials

Whey protein concentrate with 35% protein content (WPC35) was kindly supplied by Milkaut S.A. (Santa Fé, Argentina). Its composition was: lactose 48.8%; protein 38.3%; ash 7.5%; moisture 3.2% and fat 2.2%.

Green tea extract powder (Sunphenon® 90MD) from Taiyo International, Inc. (Minneapolis, Minnesota) contained >95% total polyphenols, >75% total catechins, >45% Epigallocatechin gallate (EGCG) and <6.0% caffeine.

2.2. Preparation of WPC-polyphenols mixtures

Samples were prepared by dissolving WPC35 (8% w/v) and green tea polyphenols (0.25–1% w/v) in Milli-Q ultrapure water at room temperature and stirring for 30 min pH (4.5 or 6.0) was adjusted with 0.5 N HCl or 0.5 N NaOH.

2.3. Precipitation kinetics

In order to determine the precipitation kinetics, the mixtures were placed in 5 mL graduated test tubes and stored at 20 °C for 8 days. The volume of upper phase resulting from precipitation was visually determined over time. Each sample was repeated in duplicate.

2.4. Amount of precipitated polyphenol

To determine the percentage of polyphenols precipitated by WPC35 after 24 h of storage at 20 °C, an aliquot from the top of each test tube was taken and the polyphenols in the upper phase (UP) were determined using the Folin–Ciocalteau method as described by Singleton and Rossi (1965). Briefly, a calibration standard curve of gallic acid was prepared and the results were expressed as mg gallic acid equivalents/L. The samples were properly diluted in order to obtain an absorbance in the range of the prepared calibration curve. Then, the Folin–Ciocalteau reagent and the saturated Na_2CO_3 solution were added sequentially. After standing for 2 h at 25 °C, the absorbance was measured at 765 nm using a UV–Vis spectrophotometer, Metrolab 330 (Metrolab S.A., Buenos Aires, Argentina). Also, solutions of WPC35 (8% w/v) alone were measured in order to discount any interference in the absorbance. The percentage of polyphenol precipitated was calculated as:

$$\textit{Polyphenol precipitated}(\%) = \frac{\textit{Total polyphenol} - \textit{polyphenol in UP}}{\textit{Total polyphenol}} \times 100 \qquad (1)$$

2.5. Electrophoresis

After 24 h of storage at 20 °C, an aliquot from the top of each test tube was taken and subjected to SDS-PAGE in order to evaluate the protein pattern of precipitation. The polyacrylamide gel electrophoresis (PAGE) was carried out with a Mini-Protean II dual slab cell system (Bio-Rad Laboratories, Hercules, CA) according to the procedure of Laemmli (1970). Each sample was diluted in a proportion 1:4 with the sample buffer (pH 6.8, 0.5 M Tris–HCl, with glycerol, SDS and β-mercaptoethanol). The resolving and stacking gels contained 12.5% and 4% acrylamide, respectively. All samples were loaded with a total volume of 20 μL and the running buffer contained Tris-Base 0.025 M, glycine 1.4% w/v and SDS 0.1% w/v at pH 8.3. Proteins were stained with Coomassie brilliant blue solution (0.1%) and destained with a mixture 1:1 of methanol–glacial acetic acid (20%).

2.6. Particle size and ζ-potential measurements

Particle size distribution was determined after 24 h of storage in the same samples of previous assay (*2.3*) by light scattering using a Mastersizer 2000 with a Hydro 2000MU as dispersion unit, from Malvern Instruments (Worcestershire, United Kingdom). Samples were gently agitated before poured into the beaker containing acetate buffer 0.01 M (pH 4.5) or phosphate buffer 0.01 M (pH 6.0). The pump speed was settled at 1800 rpm. Based on protein characteristics, the refractive index (RI) used for dispersed particles was 1.354, while the absorption parameter was zero. Particle size is reported as the volume–surface mean diameter or Sauter diameter ($D_{32} = \sum n_i d_i^3 / \sum n_i d_i^2$) and the equivalent volume–mean diameter or De Brouckere diameter ($D_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of particles of diameter d_i (Allen, 1992). The particles sizes are reported as the average and standard deviation of ten readings made on a sample.

The zeta potential ζ was measured in a Zetasizer Nano-Zs (Malvern Instruments, Worcestershire, United Kingdom) using the laser Doppler velocimetry (LDV) technique, (measurement range

from 5 nm to 10 μm). In this technique, a voltage was applied across a pair of electrodes placed at both ends of a cell containing the particle dispersion. Charged particles are attracted to the oppositely charged electrode, and their velocity was measured and expressed per unit field strength as the electrophoretic mobility μe. Then, the zeta potential ζ was calculated by the Dispersion Technology Software provided by Malvern according to Henry's equation:

$$\zeta = \frac{3\eta}{2\varepsilon F(\kappa a)}\mu e$$

where ζ is the zeta potential of the sample, μe is the electrophoretic mobility, ε is the dielectric constant of water, F(κa) is the function of the dimensionless parameter κa, which was determined to be 1.5 according to Smoluchowski approximation that is usually used when the radius of particle is much larger than the Debye length of the electric double layer (Russel, Saville, & Schowalter, 1989). Samples were diluted 1:100 in acetate buffer 0.01 M (pH 4.5) or in phosphate buffer 0.01 M (pH 6.0) before loading them in the cell and temperature was set at 25 °C.

2.7. Dynamics of gelation and viscoelasticity

Samples were prepared by dissolving WPC35 (30% w/v) and green tea polyphenols (0.25–1% w/v) in Milli-Q ultrapure water at room temperature and stirring for 30 min pH (4.5 or 6.0) was adjusted with 0.5 N HCl or 0.5 N NaOH.

Dynamic oscillation measurements were performed in a MCR300 controlled stress rheometer from Paar Physica (Graz, Austria). Samples were poured onto the bottom plate of a parallel plate measuring system, with a gap of 1 mm. The temperature of the bottom plate was controlled with a Peltier system (Viscotherm VT2, Paar Physica), and liquid silicone was applied to the exposed surfaces of the sample to prevent evaporation. During gelation experiments, the frequency was 1 Hz and the strain was kept at 0.01%, a value found to be in the linear viscoelastic region in preliminary experimentation. The samples were heated from 25 °C to 90 °C at a rate of 10 °C/min and kept at 90 °C for 15 min, which was sufficient time to allow G′ to equilibrate, and then cooled to 35 °C at 25 °C/min. At this point the values of G′, G″ and tanδ were recorded. The temperature at which the storage and loss modulus (G′ and G″) crossed over was taken as the gel point (T_{gel}). The frequency dependence of gels was measured at 25 °C with a constant strain of 1% at a frequency range of 0.01–5 Hz. The strain used (1%) is also within the linear viscoelastic region of the gel formed. Dynamic oscillation values quoted are the means of three measurements on different samples.

2.8. Texture properties of gels

The texture profile analysis (TPA) is an imitative test that provides standardized values of food texture by deformation of the product via a pivotal motion (resembling the human jaw). A two-bite cycle is employ and the stress developed in the food sample is measured as the sample is compressed (Friedman, Whitney, & Szczesniak, 1963). The values for texture attributes were obtained by mathematical functions from the resulting force–time curve (Rosenthal, 1999). This particular test was chosen because preliminary experiments demonstrate the existence of attributes (i.e. stickiness) that could be well described by the parameters of the TPA test (i.e. adhesiveness). Additionally, TPA has successfully been used to analyze WPC gels before (Jara, Pérez, & Pilosof, 2010; Spahn, Baeza, Santiago, & Pilosof, 2008).

The gels for texture analysis were prepared into glass cylinders (13 mm diameter × 55 mm height) containing 4 mL of the same samples of previous assay (2.6) by heating for 20 min at 90 ± 1 °C in a dry bath. The TPA was performed at 25 °C in a Texture Analyzer model TAXT2i from Stable Microsystems (Godalming, United Kingdom) using a cylindrical probe (P/36R 36 mm diameter). Cylindrical specimens of the gels (13 mm diameter × 20 mm height) were compressed to 20% at a compression rate of 0.5 mm/s. The following parameters were quantified and are defined by Bourne (1982) as: hardness (the peak force of the first compression of the product), springiness (how well a product physically springs back after it has been deformed during the first compression), adhesiveness (the negative force area for the first cycle, representing the work necessary to pull the compressing probe away from the sample), and cohesiveness (the strength of the internal bonds making up the food). The experiments were performed in triplicate.

3. Results and discussion

3.1. Colloidal stability of WPC – polyphenol mixtures

Fig. 1 shows the phase separation of WPC–polyphenol complexes in mixtures of WPC (8% w/v) and different polyphenol concentrations (0.25–1% w/v). The behaviour of single WPC is also included for comparison. At pH 4.5, a very fast phase separation was

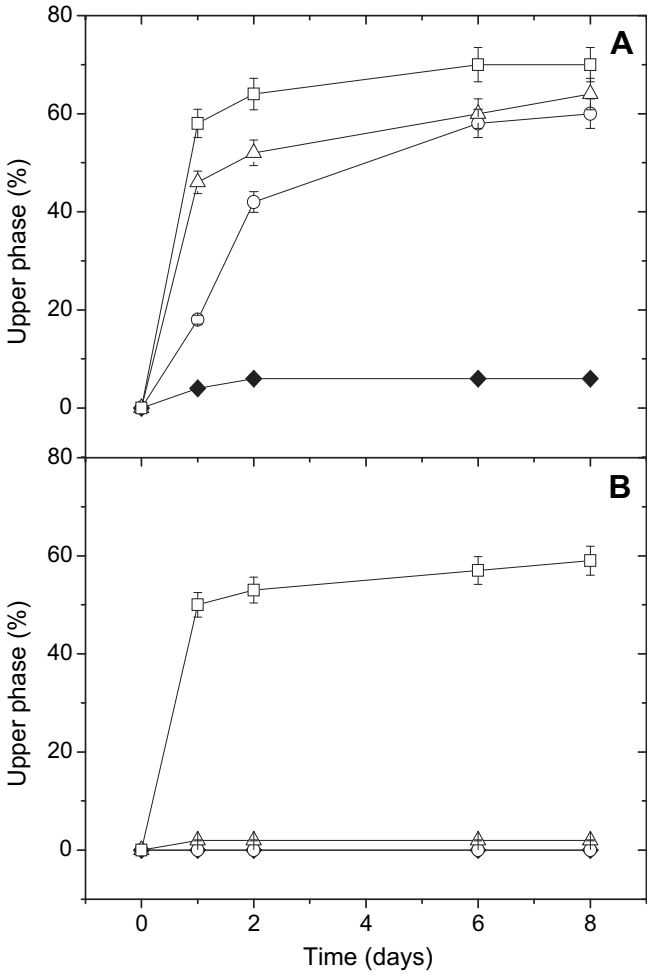

Fig. 1. Phase separation over time of WPC35 (8% w/v) (◆) and WPC35 (8% w/v)-polyphenol mixed systems at (A) pH 4.5 and (B) pH 6.0. Polyphenol concentrations are (○) 0.25%, (△) 0.5% and (□) 1% w/v.

observed in the first two days of storage for every polyphenol concentration (Fig. 1A), while at pH 6.0 this was only observed in the presence of 1% of polyphenols (Fig. 1B). At pH 4.5, close to the isoelectric point of the main whey proteins (β-lactoglobulin, α-lactalbumin and BSA), the net charge of the complexes is minimal and the interactions between them were promoted by polyphenol bridging (Charlton et al., 2002). As a result these bigger particles of reduced solubility enhanced flocculation.

The amount of polyphenols that remains in the upper phases of WPC35-polyphenols mixtures after 24 h of storage was determined as an estimation of the extent of precipitation of polyphenols by whey protein (Table 1). Additionally, the proteins in the upper phases were also analyzed by SDS-PAGE. The protein profile in Fig. 2 corresponding to single WPC (0% w/v polyphenol) showed two main bands corresponding to β-lg (18.3 kDa) and α-la (14.2 kDa). Also the band corresponding to aggregated caseinomacropeptide (CMP) could be identified (Nakano, Ikawa, & Ozimek, 2007) at a molecular weight close to 31 kDa. The band denominated B I of molecular weight of approximately 36 kDa and the bands of molecular weight above 90 kDa, denominated B II, could correspond to immunoglobulin heavy chains (Kinghorn, Norris, Paterson, & Otter, 1995; Pessela et al., 2006).

At pH 4.5, the intensity of bands corresponding to β-lactoglobulin, α-lactalbumin, B I and B II decreased gradually with increasing concentrations of polyphenols (Fig. 2A), while at pH 6.0 the intensity of these bands did not change until 1% of polyphenol was added (Fig. 2B). The same trend was observed for the precipitation of polyphenols since at pH 4.5 a gradual increase from 36 ± 1 to 83 ± 5 could be observed, while at pH 6.0 only with 1% w/v of polyphenols a high percentage of precipitated polyphenols (70 ± 2) was noticed (Table 1). Thus, both pH and polyphenol concentration play an important role in the colloidal stability of the mixture. It appears the existence of a pH-dependent threshold concentration of polyphenols over which the complexes became insoluble and precipitated. Moreover, no specificity of polyphenols for some of the protein fractions could be observed.

The other main factors affecting the solubility of the systems are the size of the aggregates formed, the chemical nature of both the protein and the polyphenol and the solvent composition (Naczk, Oickle, Pink, & Shahidi, 1996; Poncet-Legrand et al., 2006; Prigent et al., 2009). However, it is worth to notice that binding and precipitation are related but not identical, since the factors that mainly affect these processes may be different. For example, Charlton et al. (2002) showed that binding affinities between salivary proteins and polyphenols at different pH values were found to be identical, but the amount of visible precipitate, as well as the particle size measured by light scattering, was pH dependant. Thus, the study of the size and charge of the soluble primary complexes formed between polyphenols and WPC would be of help in order to explain the subsequent flocculation and precipitation of the system.

3.2. Protein–polyphenol complexes characterization

Light scattering techniques have been used for studying the complexation of proteins and polyphenols over a wide range of conditions (Charlton et al., 2002; Jöbstl et al., 2004; Lin et al., 2004; Poncet-Legrand et al., 2006; Siebert et al., 1996). This method gives quantitative and qualitative information about the size of complexes formed, as well as on the rate of particle formation. Thus, the size and charge of the complexes formed upon 24 h of storage of WPC (8% w/v)-polyphenol (0.25–1% w/v) mixtures were analyzed by light scattering.

At pH 4.5, the D_{32} value was higher than 0.35 μm from the beginning because at a pH close to the pI of whey proteins, association is promoted (Fig. 3A). The addition of increasing concentrations of polyphenols (0.25 and 0.5% w/v) produced a slight increase of $D_{32;}$ however, the D_{43} value, which is an index of particles flocculation/aggregation, was in every case higher than 1 μm and explains the rapid phase separation observed in Fig. 1A. In particular, with 1% of polyphenols the aggregates formed are much bigger (D_{32} higher than 0.5 μm and D_{43} of almost 4 μm), which led to a faster precipitation (Figs. 1A and 2A).

At pH 6.0, single WPC showed a D_{32} value of 0.31 μm almost constant over time (Fig. 3C). In the presence of polyphenols (0.25% and 0.5% w/v) this value increased slightly over time, but it was always lower than 0.35 μm. These particles of small size will stay soluble for long periods of time as can be seen in Fig. 1B. On the contrary, the presence of 1% of polyphenols promoted the formation of larger aggregates that continued to grow in time, reaching a D_{32} of almost 0.4 μm (Fig. 3C). Fig. 3D shows that the aggregates formation is more important in the presence of 1% (w/v) polyphenols and, for long storage times, particles of 1 μm could be obtained, which would precipitate as shown in Fig. 1B.

Table 1
Polyphenols precipitated (Mean % ± SD) by WPC35 (8% w/v) after 24 h incubation.

Polyphenols concentration	pH 4.5	pH 6.0
0.25% w/v	36 ± 1	5 ± 1
0.5% w/v	51 ± 3	11 ± 1
1% w/v	83 ± 5	70 ± 2

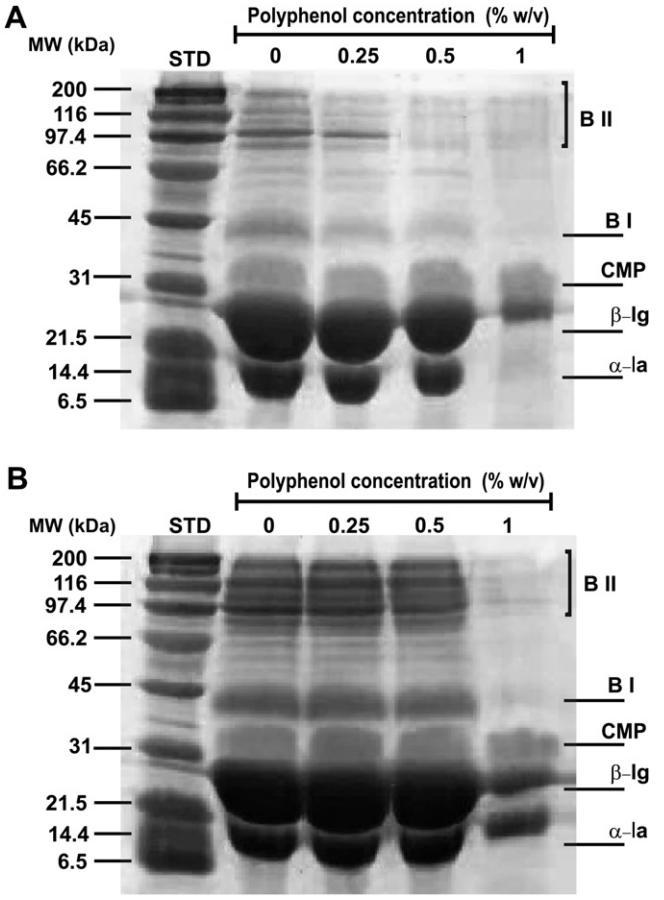

Fig. 2. SDS-PAGE patterns of WPC35 (8% w/v)-polyphenol mixed system upper phases after 24 h at (A) pH 4.5 and (B) pH 6.0.

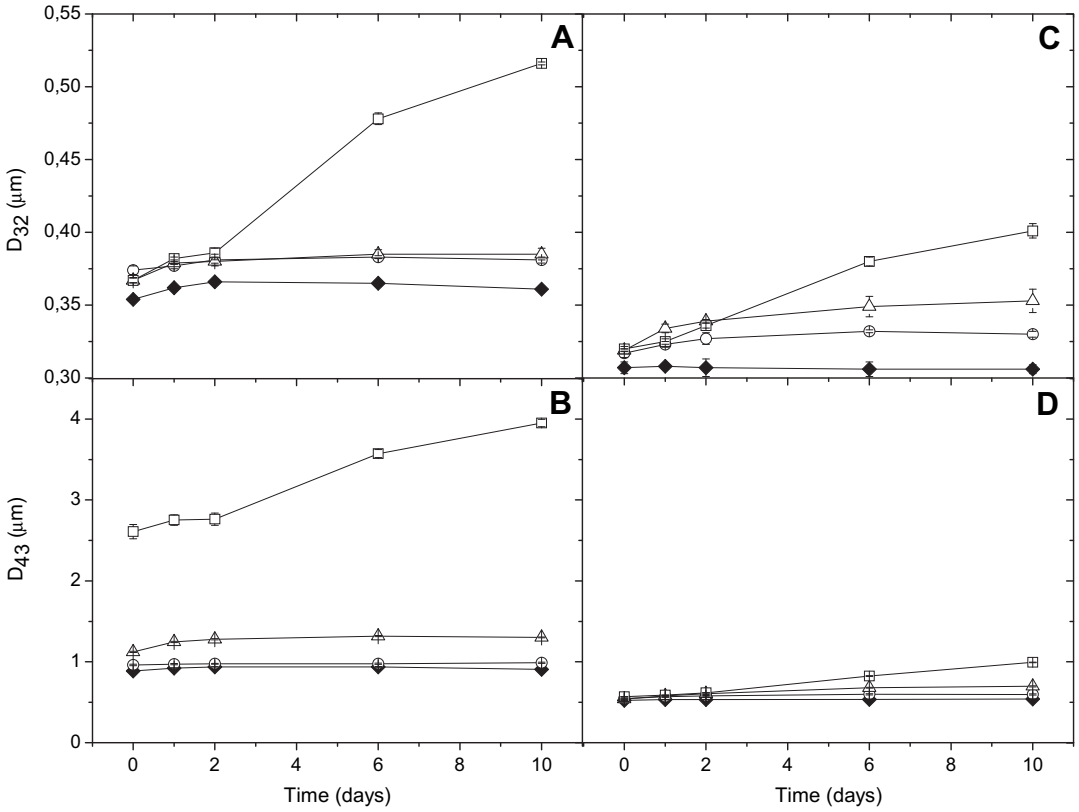

Fig. 3. Mean surface (D_{32}) and volume (D_{43}) diameters of WPC35 (8% w/v) (◆) and WPC35 (8% w/v)-polyphenol mixed systems at (A–B) pH 4.5 and (C–D) pH 6.0. Polyphenol concentrations are (○) 0.25%, (△) 0.5% and (□) 1% w/v.

The zeta potential measurements indicated that WPC at pH 4.5 had a ζ potential of −0.37 mV (Fig. 4) that is very close to its isoelectric state. This value was practically not modified by the addition of polyphenols. The low ζ potential also explains the low stability of these systems at this pH, where particles tend to flocculate and precipitate. At pH 6.0 the WPC had a ζ potential of −34.9 mV which further decreased by the addition of polyphenols (−50 mV average) (Fig. 4). At pH 6.0, close to the pKa, the protonated phenolic groups of polyphenols could be deprotonated and the generated oxygen center imparts a high negative charge density, which further decreases the ζ-potential value of the complexes. This high net charge of particles plays an important role in preventing flocculation (Fig. 1B); however at 1% (w/v) polyphenols, a phase separation was observed in spite of a strongly negative ζ potential. This indicates that other factors than the net charge, are affecting the flocs formation, as the relative concentration of polyphenol to WPC. In fact at 1% (w/v) of polyphenols a strong precipitation of WPC-polyphenols occurs irrespective of the ζ potential of particles as shown in Figs. 1 and 2, which is correlated to the formation of flocs or aggregates of 1–3.5 μm (Fig. 3B and D).

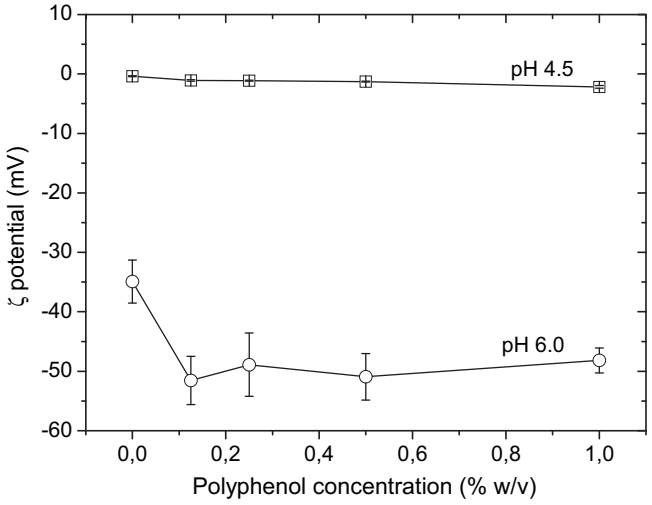

Fig. 4. Zeta potential of WPC35 (8% w/v)-polyphenol mixed systems as a function of polyphenol concentration at pH 4.5 and 6.0 and 25 °C.

It can be concluded that the precipitation of aggregates occurs in a three step process: in the first stage and at low polyphenol concentration, polyphenols associate with the proteins to form small soluble complexes. In a second stage and with increasing polyphenol concentration, each polyphenol-coated protein starts to cross-link with other soluble complexes, where the cooperative weak intermolecular bridging interactions are also carried out by the polyphenols. This leads to the formation of bigger particles or flocs with less solubility. Finally, the spontaneous aggregation of these insoluble flocs, leads to phase separation (Charlton et al., 2002; Jöbstl et al., 2004; Richard et al., 2006; Siebert et al., 1996).

3.3. Gelation of WPC–polyphenol complexes

3.3.1. Gelation dynamics

Storage modulus (G′) and loss modulus (G″) were recorded after 24 h of storage to reflect the structure development during heating WPC–polyphenol mixtures at pH 4.5 and 6.0. For these experiments 30% (w/v) of WPC was used because a minimum

concentration of protein (10% w/v) was required to gel WPC. The polyphenol concentrations tested were 0.25–1% (w/v).

The gel points (T_{gel}) were determined at the time/temperature where the elastic component rapidly started to rise and crossed the viscous component. T_{gel} for WPC with and without polyphenols at pH 4.5 and 6.0 are given in Table 2A and B, respectively. It can be seen that T_{gel} for WPC at pH 4.5 was 86.2 °C, while at pH 6.0 was four degrees higher (90.0 °C) because aggregation is favoured close to the protein isoelectric point. Pérez, Wargon, and Pilosof (2006) found that 12% dispersions of WPC (with 78% protein content) at neutral pH have a T_{gel} of 82 °C. This difference could be due to the different composition of the material, i.e. WPC35 was higher in lactose (48.8%) than WPC80 (5.2%). The thermal formation of a gel structure by whey proteins can be effectively affected by various sugars including lactose (Bordenave-Juchereau, Almeida, Piot, & Sannier, 2005; Garrett, Stairs, & Annett, 1988).

Taking into account that polyphenols can promote binding between different protein molecules leading to aggregation, a decrease of the T_{gel} is expected for mixed systems. Interestingly, this was only observed for samples at pH 6.0, where the increment of polyphenol concentration led to a gradual decrease in gelation temperature. At pH 4.5 no changes in T_{gel} were observed because a maximum aggregation is already achieved in WPC at this pH.

Fig. 5 shows the time evolution of G′ for all the mixtures. At pH 4.5 the addition of polyphenols significantly modified G′ evolution (Fig. 5A), but did not affect G′ evolution at pH 6.0 (Fig. 5B). At pH 4.5, except at 0.5% (w/v) polyphenols, the G′ evolution was lowered by polyphenols. Nevertheless at the end of heating period and upon cooling all systems showed G′ values of the same order.

Table 2A and B also shows the elastic modulus and viscous modulus of gels after cooling down to 35 °C, as affected by polyphenol content. G′ resulted in every case higher than G″, indicating the preponderant elastic nature typical of protein gels. Additionally, both G′ and G″ showed higher values at pH 4.5 than at pH 6.0. The structure of whey protein gels is mainly determined by β-lactoglobulin and depends on the pH (Capitani, Pérez, Bertoldo Pacheco, & Pilosof, 2007). The decrease of reactivity of SH groups of β-lg occurs close to the pI. Therefore, non-covalent interactions are mainly involved in the structure of these gels. At neutral pH intermolecular disulfide bonds are favoured (Schokker, Singh, & Creamer, 2000).

The gelation parameters (G′, G″ and tanδ) upon cooling at 35 °C gradually increased with the increment of polyphenol concentration. Similar results were found by Wu, Clifford, and Howell (2007) for egg albumen gels in the presence of 1 and 2% (w/v) of instant green tea.

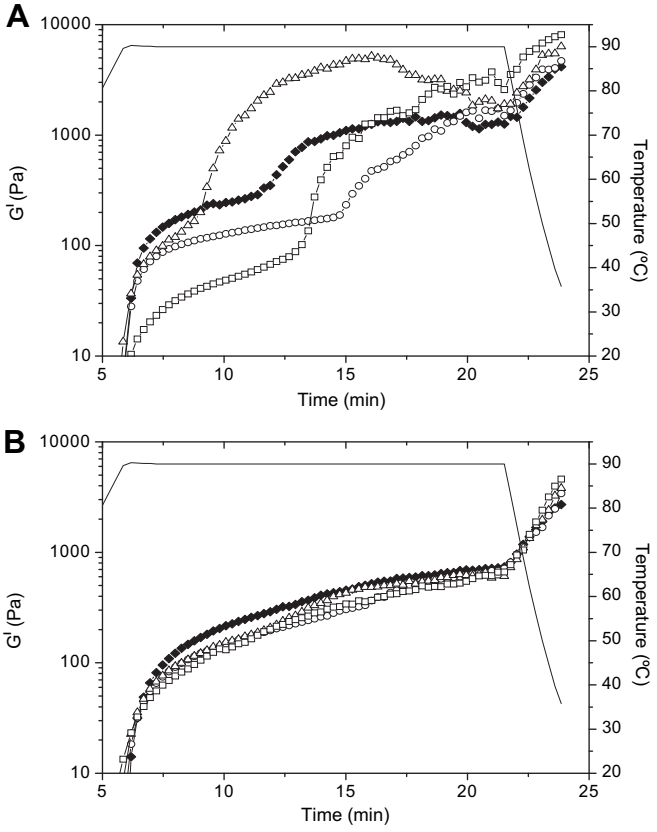

Fig. 5. G′ time evolution of WPC35 (30% w/v) (◆) and WPC35 (30% w/v)-polyphenol mixed systems at (A) pH 4.5 and (B) pH 6.0. Polyphenol concentrations are (○) 0.25%, (△) 0.5% and (□) 1% w/v.

Table 2
Gelation parameters and frequency dependence of WPC35 (30% w/v) as affected by increasing concentration of green tea polyphenols. Storage modulus (G′), loss modulus (G″) and damping factor (tanδ) upon cooling at 35 °C and 1 Hz are reported. Frequency dependence (n) was measured at 25 °C and the correlation coefficient (R^2) arises of the calculation of n by linear regression: $\log G' = n\log f + K$, where f is the oscillation frequency and K is a constant.

Polyphenol	T_{gel} (°C)	G′(Pa)	G″(Pa)	Tanδ	n	R^2
A: pH 4.5						
0%	86.2 ± 0.3	4160 ± 40	1009 ± 67	0.24 ± 0.02	0.1413	0.9971
0.25%	86.2 ± 0.1	4685 ± 89	1199 ± 15	0.26 ± 0.02	0.1617	0.9892
0.5%	86.2 ± 0.2	6327 ± 252	1919 ± 86	0.30 ± 0.01	0.2181	0.9970
1%	86.2 ± 0.2	8110 ± 437	2738 ± 75	0.34 ± 0.01	0.1944	0.9989
B: pH 6.0						
0%	90.0 ± 0.4	2710 ± 471	575 ± 16	0.21 ± 0.03	0.1539	0.9790
0.25%	87.9 ± 0.2	3417 ± 415	790 ± 31	0.23 ± 0.01	0.1536	0.9942
0.5%	86.2 ± 0.1	3800 ± 59	879 ± 38	0.23 ± 0.02	0.1641	0.9971
1%	86.2 ± 0.1	4605 ± 177	1297 ± 19	0.28 ± 0.01	0.2320	0.9927

Values are mean ± 3 RSD.
T_{gel} is the gelation temperature.

Tanδ is considered to represent the relative viscoelasticity within the gel network (Aguilera, Xiong, & Kinsella, 1993). Thus, the gels formed in the presence of polyphenols have a less relative viscoelasticity, because they exhibited higher tanδ values.

As described by Stading and Hermansson (1990), the frequency dependence of G′ can also provide information about gel structure. The degree of frequency dependence can be expressed by the constant n:

$$\log\ G' = \mathrm{n}\log\ f + \mathrm{K}$$

where G′ is the storage modulus, f is the oscillation frequency and K is a constant. The n-value is regarded as an indication of the viscoelastic nature of the gels: n is zero for purely elastic gels and becomes higher with increasing relative contribution from the viscous component (less elastic). The n-values obtained for the WPC without polyphenols were the lowest for both pH (Table 2), which is expected since they also have the lowest tanδ, corroborating their predominantly elastic nature. It can be noted that these n-values gradually increased with the increment of polyphenol concentration, reflecting a change of mechanical properties that results in more aggregated gels with less elastic nature (Table 2).

3.3.2. Gel texture

Texture properties of the gels are shown in Fig. 6. Hardness and adhesiveness of WPC gels at pH 4.5 were not significantly affected by the addition of polyphenols (Fig. 6A and B). However, both

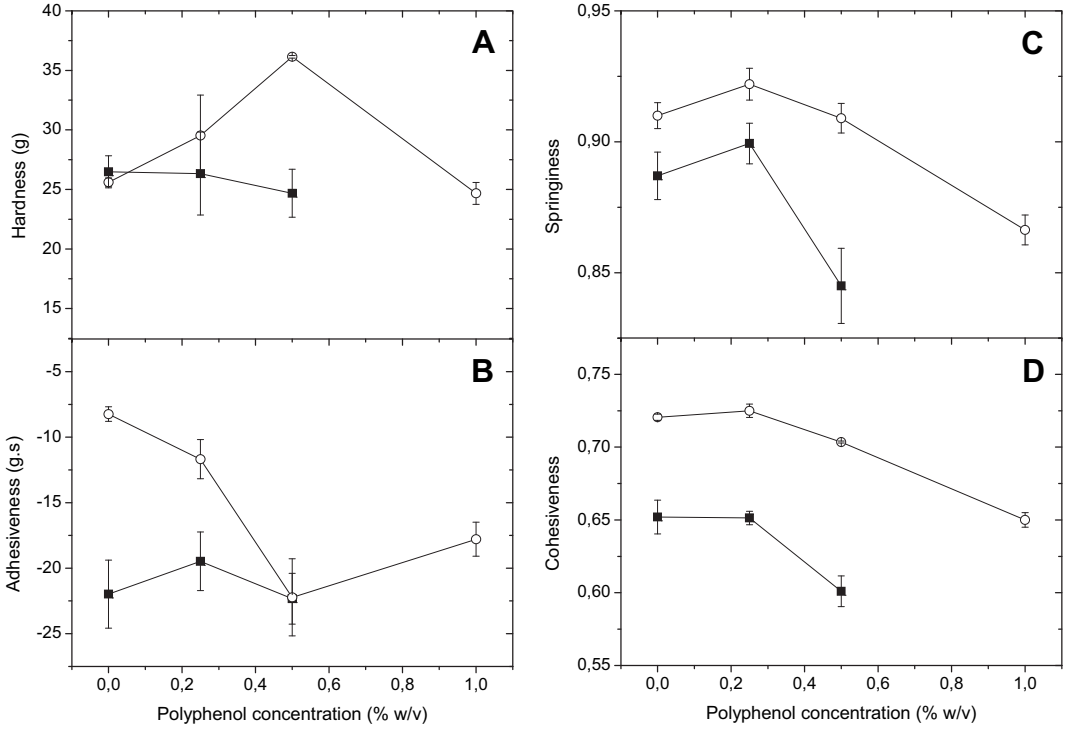

Fig. 6. Textural parameters obtained by TPA of WPC35 (30% w/v) gels with increasing concentrations of polyphenols (0.25–1% w/v) at pH (■) 4.5 and (○) 6.0.

springiness and cohesiveness of these gels presented significantly lower values with 0.5% (w/v) of polyphenols (Fig. 6C and D). This indicated that more than 0.25% (w/v) of polyphenols at pH 4.5 negatively affected the quality of the gel. Additionally, gels formed at this pH resulted pasty, which made difficult to take them out of the test tubes. Similar results were obtained by Mleko, Li-Chan, and Pickus (1997), who evaluated WPI gels at pH 4.0 and 5.0. pH plays a main role on gelling ability of WPC because within the isoelectric range of whey proteins, the lack of repulsive forces leads to the formation of particulate gels. Moreover, when polyphenols were added at 1% w/v self-supporting gels could not be obtained, which yields to samples inappropriate for texture measurements. Particulate gel structures are formed when conditions allow proteins to associate rapidly into large and disordered aggregates (Foegeding, Bowland, & Hardin, 1995). The presence of polyphenols, which decreases solubility by increasing protein intermolecular interactions, could have a similar effect on the microstructure of the gel. This indicates that polyphenols added at pH close to pI further promoted protein aggregation and particulate gel formation upon heating.

At pH 6.0 the texture profile analysis indicated higher hardness and adhesiveness for gels with 0.25 (30 ± 3 g) and 0.5% w/v (36 ± 0.1 g) of polyphenols as compared with WPC alone (26 ± 0.4 g) (Fig. 6A and B). However, when polyphenol concentration increased to 1% w/v, hardness decreased (25 ± 0.9 g) up the value of WPC alone. Adhesiveness also decreased at this polyphenol concentration (−18 ± 1 g s) as compared with 0.5% w/v of polyphenols (−22 ± 3 g s) but maintained a higher level than that with 0.25% (−12 ± 1 g s). Springiness and cohesiveness did not present significant differences with 0.25 and 0.5% (w/v) of polyphenols as compared with WPC alone (Fig. 6C and D), but showed an important decrease when polyphenol concentration was 1% (w/v). Similar behaviour was found by Balange and Benjakul (2009) when studying the gel properties of mackerel surimi added with phenolic compounds at pH 6.8. They observed that breaking force and deformation of gels increased as the phenolic compounds were added up to a particular level of 0.5% w/v. When this concentration was exceeded a noticeable decrease of textural parameters was recorded. They concluded that the gel strength is maximum when an optimal concentration of 0.5% w/v polyphenols was achieved. These authors associated this behaviour with the self-aggregation of phenolic compounds at high concentration levels, which leads to a loss in protein cross-linking capability. However, based on the present results (Table 1) it could be noticed that polyphenols continued to stack to proteins rather than self-aggregated. Thus, a better explanation could be that whey proteins, in the presence of high polyphenol concentration, would be totally coated by polyphenols and they would be unable to interact between them and form a gel. The aggregation promoted by polyphenols would be beneficial up to a threshold point over which it would start to be an obstacle for the generation of stronger interactions between the forming gel proteins.

4. Conclusions

The colloidal stability of WPC–polyphenol mixtures depends on pH and on polyphenol concentration. There is a critical polyphenol concentration over which phase separation occurs being this concentration pH-dependent. At pH 4.5, close to isoelectric point of whey proteins, the presence of polyphenols has a more negative effect on the colloidal stability of mixtures because of the formation of big aggregates that precipitate rapidly. In addition, the viscoelastic character of WPC gels strongly decreased.

At pH 6.0, polyphenol concentrations of 0.25 and 0.5% (w/v) can be added without producing precipitation or affecting negatively the viscoelasticity and texture of WPC gels.

In conclusion, green tea polyphenols can be viewed as modifiers of protein clusters size distributions in protein dispersions and determinant of gelation behaviour in thermally induced WPC gels. If the right polyphenol concentration is

chosen, the desired health-promoting effects of the functional ingredient can be achieved without negatively affecting the functional properties of WPC.

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