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Improvement of emulsifying properties of Brea gum by controlled thermal treatment

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ABSTRACT

Brea gum (BG), which is an exudate from *Cercidium praecox* tree, was purified and subjected to thermal treatment at 110 °C during different times: 24, 48, 72 and 96 h. Treatment effect was evaluated by analyzing the molecular mass distribution of proteins and polysaccharides, by color and spectrophotometric analyses and by an emulsifying test.

BG thermal treated presented changes in the molecular mass of its protein fractions and in its color parameters as a result of non-enzymatic browning reactions. The new molecular species generated were identified by spectrophotometry as Maillard reaction products of the different reaction phases. The modifications in BG structure produced an increase in its surface activity and improved its emulsifying/stabilizing capacity, obtaining corn oil emulsions stable for several months. Finally, the results showed that 24 h of treatment at 110 °C was enough to improve the emulsifying properties of BG.

1. Introduction

Brea gum (BG) is an exudate obtained from the bark of *Cercidium praecox* tree (Ruiz & Pavon) Harms [= *Parkinsonia praecox* (Ruiz & Pavon) Hawkins] (Bertuzzi, Slavutsky, & Armada, 2012) that is widespread in tropical and sub-tropical areas of America, and well-adapted to arid and semi-arid areas (Anderson, Weiping, & Lewis, 1990). Particularly, in Argentina, this tree grows in the semi-arid northwest region. BG consist mainly of acid polysaccharides (83.7%) formed by L-arabinose, D-xylose, glucuronic and 4-*O*-methyl-D-glucuronic acids. It also includes 7.52% of protein as part of its structure and does not contain dextrin or tannins. In a previous work, a characterization of BG molecular components has been carried out and the findings showed that BG has a complex macromolecular structure consisting of several fractions with heterogeneous chemical composition and polydisperse molecular mass distributions (Castel et al., 2016). The major fraction determined in BG structure was a polysaccharide of 2.79×10^3 kDa. Another fraction consisted of proteins in a wide range of molecular mass that in part were forming aggregates. Also, it has been revealed the presence of a polysaccharide–protein complex of 1.92×10^5 kDa which gives high interfacial activity and good emulsifying properties to the gum (Castel, Rubiolo, & Carrara, 2017). Many studies have demonstrated the importance of these polysaccharide–protein complexes and the protein distribution in controlling the emulsifying/stabilizing properties of gums (Al-Assaf, Katayama, Phillips, Sasaki, & Williams, 2003; Al-Assaf, Phillips, Aoki, & Saaki, 2007; Randall, Phillips, & Williams, 1988, 1989).

On the other hand, the thermal treatment applied to polymers produces a variety of effects according to the nature and conformation of the polymer, the temperature, the treatment time, the pH and the water activity. Thermally treated proteins can undergo processes of denaturation and aggregation that modify their conformation at all organization levels, i.e., primary, secondary and tertiary structure which are critical in many mechanisms that regulate its functionality. In low thermal treatments (temperatures up to 100 °C or slightly higher), low energy bonds, such as hydrogen bridges, are broken and the hydrophobic interactions are reinforced (Lupano, 2013). The unfolding of a native protein could cause the exposure of hydrophobic groups to the environment. Then, if the unfolding degree is high and protein-protein interactions are possible, aggregation occurs which can cause decrease in solubility and protein precipitation (Gregory III, 2010). Other consequences of protein thermal treatment can be gelation, the variation in the capacity of water retention and in the emulsifying and foaming properties. Thermal treatments in polysaccharides modify their structure and, as a consequence, their functional properties change, such as their viscosity. Heating can facilitate gum dissolution, such as guar gum, but it can also cause its degradation if a sufficiently high temperature is reached. In addition, reactions in which there is no carbon-

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carbon bonds breakdown may occur, such as, anomerization, aldose-ketose isomerization and dehydration reactions, and also reactions with carbon-carbon breakdown, leading to the formation of compounds such as acetol, acetoin, diacetyl and acids as levulinic, formic, lactic, pyruvic and acetic acids (López & Suárez, 2005). Another possible thermal treatment effect is the nonenzymatic browning that includes caramelisation which involves only sugars, and the Maillard reaction, which occurs between a carbonyl group, usually a reducing sugar, and an amine, generally of an amino acid, peptide or protein.

Gum arabic (GA), which is extensively used as an emulsifier/stabilizer in beverage emulsion for soft drinks, has been subjected to a process called by the authors as "maturation" which consists of heating GA nodules at 110 °C for at least 24 h (Al-Assaf, Phillips, Aoki, & Sasaki, 2007). Authors have performed this in order to emulate the natural process by which the gum matures in its natural setting. As a result of the thermal treatment applied, the molecular structure of GA has been modified by increasing the aggregation of arabinogalactan components with small amounts of protein to form further amounts of arabinogalactan protein (AGP) component. Later, an emulsification test showed that this treatment applied to GA was effective to enhance the performance of the natural gum by increasing dramatically the interfacial surface properties and coverage of the oil droplets in oil-in-water emulsions.

The objective of this work was to evaluate the effect of time of treatment regarding the molecular structure, color and emulsifying properties of BG.

2. Materials and methods

2.1. Materials

BG exudates were collected as nodules from *Cercidium praecox* trees in the area near to the city of Salta (Argentina) and supplied by a native community. Corn oil (Mazola®, Córdoba, Argentina) was purchased from a local supplier. All reagents were analytical grade.

2.2. Brea gum purification

An aqueous solution of BG exudates (15% w/w) was allowed to stand at 25 °C for 24 h to reach complete hydration. Then, the solution was centrifuged and filtered first through Whatman No.1 filter paper to separate undissolved matters, and later through a 0.5 μm fiberglass membrane under vacuum to remove farther impurities. BG purified solution was freeze-dried and grounded, obtaining a powder with 1.14% w/w of fat and 4.23% w/w of ash as determined by standard methods (AOAC, 1995), and 7.52% w/w of protein determined by Kjeldahl method using 6.6 as N protein conversion factor (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006).

2.3. Thermal treatment

BG modification by thermal treatment was carried out according to Al-Assaf et al. (2007) with modifications. Samples of BG purified powder (10 g) were placed in glass beakers in an oven at 110 °C and removed at different times: 24, 48, 72 and 96 h. Thermal treated samples (BGTTs) were named as BG24, BG48, BG72 and BG96 according to the time they were exposed to the thermal treatment.

2.4. Molecular mass distribution

Molecular mass (MM) distributions of BG and treated samples were determined by gel permeation chromatography (GPC) in a Water 2596 module with a set of two columns Ultrahydrogel 500 and 2000 (Waters, Milford, MA, USA). An UV detector operated at 280 nm and a refractive index (RI) detector were used simultaneously. The flow rate was maintained in 0.5 mL/min at 25 °C using 0.05 M NaNO3 as eluent. 100 μL aliquots of BG or BGTTs solutions (25 mg/mL) were injected on to the column.

2.5. Color analysis

Color tristimulus coordinates (L^*, a^*, b^*) of the BG and BGTTs powders were determined with a 508D Minolta colorimeter (Tokyo, Japan) using a 65° illuminant and 10° observer angle. Total difference of color (ΔE^*) was calculated as described in Eq. (1).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^* is the difference of brightness between a BG and each of the BGTTs, Δa^* is the difference of a value between a BG and each of the BGTTs, and Δb^* is the difference of b value between a BG and each of the BGTTs (Verruck, Schwinden Prudêncio, Olivera Müller, Beddin Fritzen-Freire, & Dias de Mello Castanho Amboni, 2015). Before observations, all samples were subjected to compression with 1 kg weight for 5 s and at least five measurements for each sample were performed.

2.6. Spectrophotometric analyses

Solutions at 10% w/w of BG and BGTTs were prepared at 25 °C with Milli-Q ultrapure water and were stirred until complete dissolution. The absorption of the solutions were measured at 284 nm (early Maillard reaction products), 304 nm (Schiff bases) and 420 nm (late Maillard reaction products) (Chawla, Chander, & Sharma, 2009; Jiménez-Castaño, López-Fandiño, Olano, & Villamiel, 2005; Miralles, Martínez-Rodríguez, Santiago, van de Lagemaat, & Heras, 2007; Wang & Ismail, 2012; Zhu, Damodaran, & Lucey, 2008).

2.7. Emulsification test

2.7.1. Emulsion preparation

First, BG and BGTTs powders were dissolved at 10% w/w at 25 °C in Milli-Q ultrapure water with 0.01% w/w sodium azide and were stored at 4 °C overnight to permit a full saturation of the polymer. Then, emulsions were prepared by a two step method:

(a) 10% w/w corn oil was homogenised with 90% w/w gum solution using an Omni mixer homogenizer (Ivan Sorvall, Inc., Norwalk, Conneticut, USA) for 5 min at 5000 rpm.
(b) then Pre-emulsions were treated by sonication using a 20 Khz sonicator (Ultrasound generator, Sonics and Materials VCX-750, Newton, Conneticut, USA) at 75% of amplitude for 2 min with temperature controller setup to stop when sample reaches 30 °C.

2.7.2. Emulsions rheological characterization

A HAAKE RS80- Rheo stress rheometer (Haake Mess - Technik Gmbh, Germany) was employed to carry out the rheological measurements at 25 ± 0.1 °C. A 40 mm diameter cone-plate geometry with a 4 ºcone angle and a gap of 1 mm were used in all measurements. Samples surfaces were covered with a silicone oil thin layer to avoid dehydration. The shear stress (τ) was measured as function of shear rate ($\dot{\gamma}$) ramp-up from 0.1 to 1000 s^{-1}. Flow curves were fitted with the power-law model:

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

where, τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the consistency index and n is the flow behavior index (Mothé & Rao, 1999).

2.7.3. Particle size determination

Emulsions droplet size distributions were determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS90 device (Malvern Instruments. Ltd., Worcestershire, UK) at 25 °C. Emulsion samples were

appropriately diluted with Milli-Q ultrapure water before measure. Z-average diameter and polydispersity index (PdI) of emulsions were automatically calculated by the instrument as mean of ten readings per sample.

2.7.4. Emulsions stability

Emulsions (7 mL) were transferred into glass tubes so as to perform a sample scan from the bottom to the top using a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). Transmittance (T) and backscattering (BS) measurements as function of the sample height were recorded every day for seven days.

Creaming index (CI) was calculated as the percentage of serum layer height (H_S) from the total emulsion height (H_T) (Gu, Decker, & McClements, 2005):

$$CI\ (\%) = \frac{H_S}{H_T}.100 \quad (3)$$

H_S and H_T were measured from backscattering profile in reference mode (Delta Backscattering, ΔBS), which substracts the first curve (t = 0) from the following curves in order to see the changes regarding the initial state.

Delay time (h), time in which emulsion destabilization reaches 5%, was measured as the time when the clarification peak (bottom zone) increased 5% of its height.

2.8. Statistical analysis

All assays were performed at least three times and means and standard deviations were calculated. Results were analyzed statistically by analysis of variance (ANOVA) and differences between means were determined with LSD test at 5% level of significance ($p < 0.05$) (STATGRAPHIC Centurion XV).

3. Results and discussion

3.1. Molecular mass distribution

In order to evaluate the effect of the thermal treatment on BG molecular mass distribution, GPC elution profiles were monitored by RI and UV at 280 nm simultaneously. BG, BG24, BG48, BG72 and BG96 elution profiles are shown in Fig. 1.

BG elution profile monitored by RI showed two peaks (Fig. 1 A), the first peak (labeled peak 1) appeared at elution volume of 34 mL and the second (peak 2) at 46 mL, being similar to the earlier reported profile (Castel et al., 2016). The reported molecular mass of peaks 1 and 2 were 1.92×10^5 and 2.79×10^3 kDa, respectively (Castel et al., 2016). On the other hand, RI profiles of BG thermally treated samples (BGTT) showed no great variation regarding untreated BG profile, however, a small increase in the height of the peaks was observed by increasing treatment time.

The UV profile of BG presented four peaks (Fig. 1 B). Two peaks eluted at early elution volumes, one between 1 and 5 mL and other at 10 mL. These peaks have previously been interpreted as high MM protein aggregates (Castel et al., 2016). Other peaks eluted at 40 mL and 44 mL presented relative higher absorbance than the first ones. Additionally, a shoulder peak was observed at 34 mL coeluting with the RI peak 1 so it could correspond to a polysaccharide-protein complex as interpreted later by Castel et al. (2016). BGTT profiles monitored by UV detector showed great changes achieved as a result of the thermal treatment. First, the peaks observed at early elution volumes (between 1 and 10 mL) in BG profile disappeared in BGTT profiles indicating that the thermal treatment caused the breakdown of protein aggregates. Besides, UV profiles of BGTT samples presented peaks with high absorbance eluted at approximated volumes of 16, 25, 51, 55 and 59 mL that were not observed in untreated BG. These peaks might be resulted either from the disaggregation of the protein aggregates or from the aggregation of very low MM protein fractions that were not observed in untreated BG profile because of the column resolution range (Yu et al., 2017). Castel et al., 2016 observed low molecular mass protein fractions (from 6.5 to 66 kDa) that were not resolved by this set of columns and possibly aggregate during the thermal treatment appearing later as new peaks in the chromatogram. According to Al-Assaf et al. (2007), thermal treatment applied to GA produced the agglomeration of proteinaceous components within the molecularly disperse system of the gum to increasing the amount of AGP emulsifying component. In others words, protein species covalently bind to the carbohydrates chains forming fractions of greater MM, i.e., Maillard reaction would be occurring. The Maillard reaction involves the formation of brown pigments and consists of condensation of a carbonyl group from a reducing sugar (aldehydes or ketones) and a free amino group (terminal α-amino or ε-amino of exposed lysine residues) from amino acids, peptides or proteins. This reaction is considered to be a complex array of non-enzimatic chemical reactions, which produces a large number of products called Maillard Reaction Products (MRP), such as aromatic compounds, intermediates compounds that absorb UV light and browning compounds identified as melanoidins (Wijewickreme & Kitts, 1997). The variety of species resulting from these reactions explain the different functionalities and reactivities exhibited by Maillard products.

3.2. Color analysis

BG thermal treatment caused browning of the samples as shown in Fig. 2. Color coordinates (L^*, a^* and b^*) and the total difference of color (ΔE^*) of BG and BGTTs are shown in Table 1.

Analyzing BGTT samples parameters, it is observed that all L^* were lower than the one of BG and decreased as the treatment time increased, indicating that the powders had less brightness. On the other hand, a^* and b^* parameters of BGTT samples were higher than the respective BG parameters, but no trends was observed with respect to the treatment time. ΔE^* values also point out the difference in color between BG and BGTTs but did not show a relationship between color and time of treatment. Accordingly, as it is observed in Fig. 2, all BGTT samples were darker and with intense brown tones in comparison with untreated BG. Therefore, a darkening of the systems in which BGTTs would be employed could be expected, however, color change is expected to be minimal because BGTTs would be added in small amounts.

Similarly, many authors have observed the decrease of L^* (brightness) values in samples that have been heated, as an indicator of increased darkness (Farroni & Buera, 2012; Morales & van Boekel, 1998; Yu et al., 2017). According to these authors, decrease in brightness is the result of brown pigments formation in the advanced stage of the Maillard reaction. Besides, increases in a^* and b^* parameters have been related to a yellow/brown coloration associated with protein–aldehyde interactions due to various intermediate or final products of Maillard reaction (Liu, Kong, Han, Sun, & Li, 2014; Liu, Tellez-Garay, & Castell-Perez, 2004; Yu et al., 2017). Even though color formation can be due to both sugar caramelisation and Maillard reaction, severe heating intensity ($T > 120\ ^\circ C$) and extreme pH (lower than 3 or higher than 9) are necessary to cause sugar caramelisation (Reyes, Poocharoen, & Wrolstad, 1982). Therefore, color and brown compounds formation is related mainly to Maillard reaction (Hurrell, Finot, & Ford, 1983). On the other hand, Maillard reaction for formation of protein-polysaccharide conjugates can be achieved under wet or controlled dry-heating conditions as in this work (Pirestani, Nasirpour, Keramat, Desobry, & Jasniewski, 2018). The initial stage of this complex series of reactions involves the sugar-amine condensation and the Amadori rearrangement, and no browning occurs at this stage. The second stage involves sugar dehydration and fragmentation, and amino acid degradation via the Strecker reaction. At the end of the second stage there is a beginning of flavor formation. Following this, the formation of heterocyclic nitrogen compounds occurs, and with that, the browning (Monajjemzadeh et al., 2009). In this final stage, the color changes that

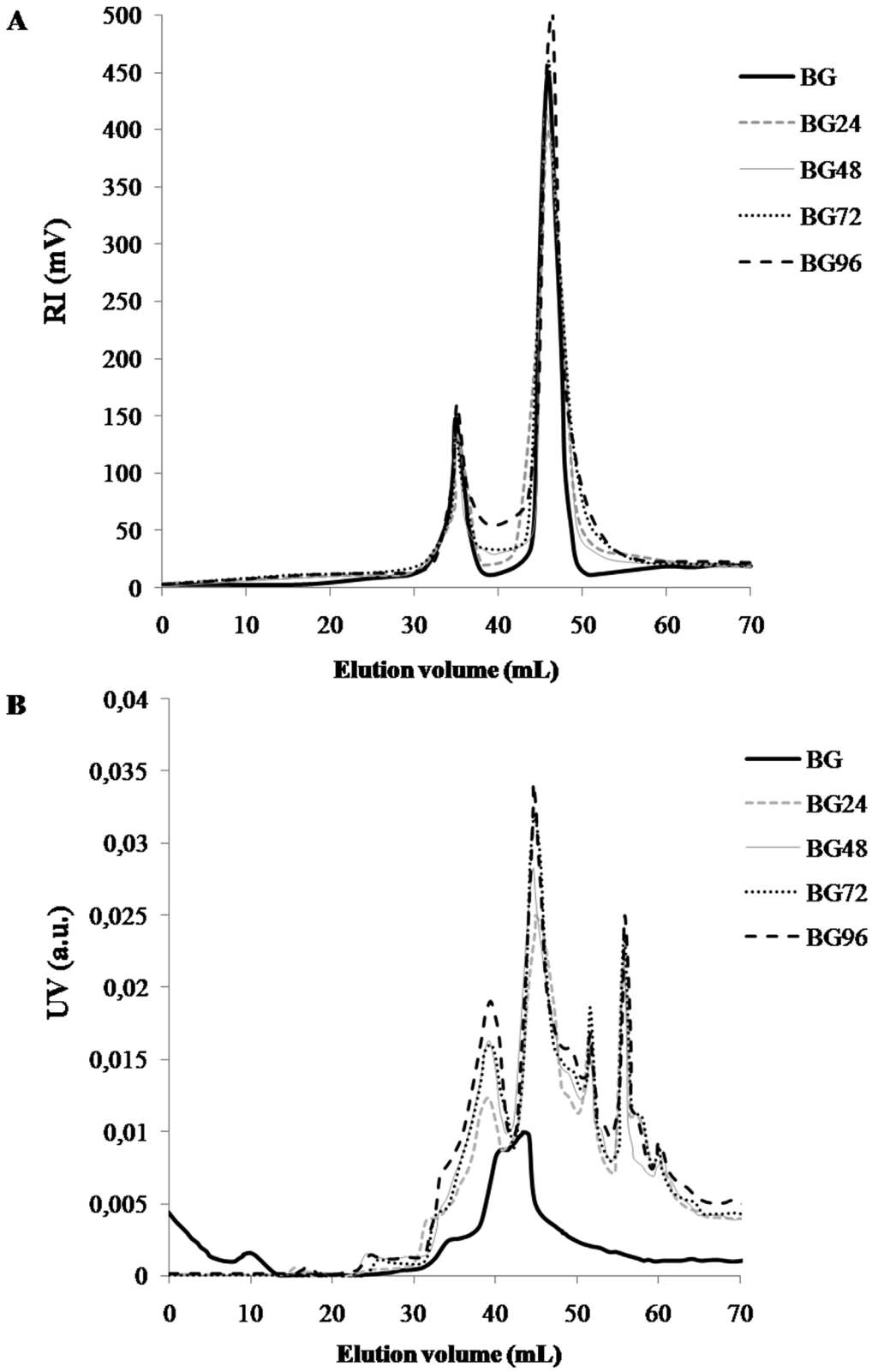

Fig. 1. GPC chromatograms of BG and BGTTs monitored by (A) UV at 280 nm for proteins and (B) refractive index for polysaccharides.

become noticeable are associated with brown pigments formation, which are a complex series of polymers and co-polymers called melanoidins. Hence, brown color development has been widely studied in different model systems (Monti, Borrelli, Ritieni, & Fogliano, 2000; Rizzi, 1997; Tressl, Wondrak, Kruger, & Rewicki, 1998) and has often been used as indicator of the final stage of the reaction (Damjanovic Desic & Birlouez-Aragon, 2011; Leiva, Naranjo, & Malec, 2017; Morales & van Boekel, 1998).

3.3. Maillard products determination

Another way to evaluate the progress of the Maillard reaction is to

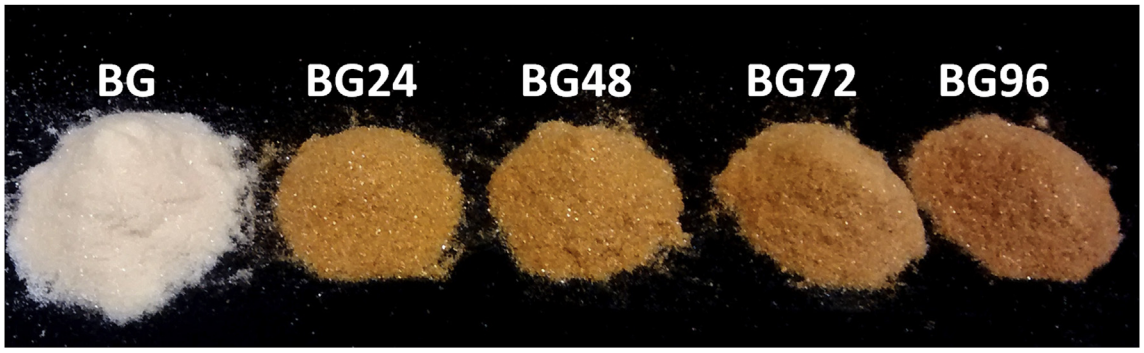

Fig. 2. Photograph of BG and BGTTs powders.

Table 1
Color coordinates and total difference of color values of BG and BGTT powders.

Sample	L^*	a^*	b^*	ΔE^*
BG	85.3 ± 0.0^d	0.7 ± 0.0^a	11.0 ± 0.0^a	0
BG24	56.7 ± 0.3^c	14.1 ± 0.4^c	33.0 ± 0.5^e	41.3 ± 0.7^b
BG48	56.2 ± 0.3^c	13.7 ± 0.2^b	30.1 ± 0.4^d	39.9 ± 0.4^a
BG72	54.7 ± 0.1^b	13.5 ± 0.2^b	27.3 ± 0.1^b	39.8 ± 0.1^a
BG96	53.0 ± 0.7^a	14.1 ± 0.3^c	28.3 ± 0.5^c	41.9 ± 0.9^b

Data expressed as average ± standard deviation (n = 3). Different superscript letters in the same column indicate statistical difference to LSD tests ($p < 0.05$).

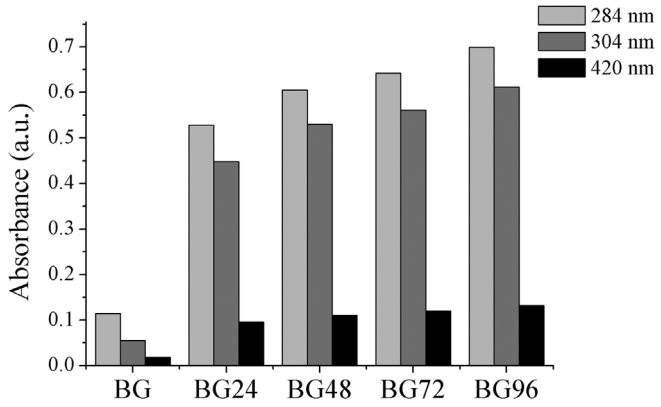

Fig. 3. Absorbance at 284 nm of early intermediates of Maillard reaction, absorbance at 304 nm of Schiff's bases and absorbance at 420 nm of late intermediates of Maillard reaction.

measure the MRPs. The pigments generated have absorbance in the visible spectrum at a particular wavelength. According to the literature, the Schiff bases absorb at 284 nm (Chawla et al., 2009), the Amadori compounds absorb at 304 nm (Wang & Ismail, 2012; Zhu et al., 2008) and the late compounds of the Maillard reaction (browning) are determined by specific absorbance measurements at 420 nm (Jiménez-Castaño et al., 2005; Miralles et al., 2007). Therefore, specific measurements were taken at these wavelengths and results are shown in Fig. 3. Untreated BG showed absorbance at all measured wavelengths indicating the presence of the different MRPs. Then, it can be seen that the absorbances of BGTTs compared to the untreated BG remarkable increased in all wavelengths. Increases of 4.6–6 times the absorbance of the untreated BG could be observed in BGTTs absorbances at 284 nm. This correspond to the formation of uncoloured intermediate products which have been proved to be precursors of Maillard reaction (Lertittikul, Benjakul, & Tanaka, 2007). The absorbances at 304 nm presented the greatest increases (8–11 times) in BGTTs samples regarding untreated BG indicating a great Amadori compounds formation. Furthermore, drastic increases (5–7 times) of absorbance at 420 nm were found in BGTTs samples corresponding to a non-enzymatic browning reaction resulted in colored products. Also, data show that the absorbances at different wavelengths increased with the heating time indicating the progress of the Maillard reaction. These findings are in concurrence with other studies where browning due to heat-induced Maillard reaction is reported (Benjakul, Lertittikul, & Bauer, 2005; Jing & Kitts, 2002; Lan et al., 2010). Moreover, several researchers have reported that browning increased with heating time as it was observed in the present study (Benjakul et al., 2005; Jing & Kitts, 2002; Morales & van Boekel, 1998).

3.4. Emulsifying properties

3.4.1. Emulsions rheology

Fig. 4 shows the curves of shear stress (τ) as function shear rate ($\dot{\gamma}$) of the corn oil emulsions stabilized with BG and BGTTs. In all cases, it can be observed a non-linear relationship between the variables. All BGTT emulsions presented higher shear stress values than that of BG without treatment for the same shear rate, although a tendency with respect to the time of treatment is not distinguished.

On the other hand, the curves were well predicted by power-law model (Eq. (2)), as it is observed in the fitting curves (continuous lines) in Fig. 4 and from the correlation indexes (R^2) in Table 2 which were higher than 0.99. The n values of BGTT emulsions were significantly closer to 1 (0.921–0.945) than the n value of BG emulsion (0.893), indicating that BGTT emulsions conduct were closer to Newtonian behavior. However, there was not a clear trend in n values regarding the treatment time. Besides, consistency index (k) values of all BGTT

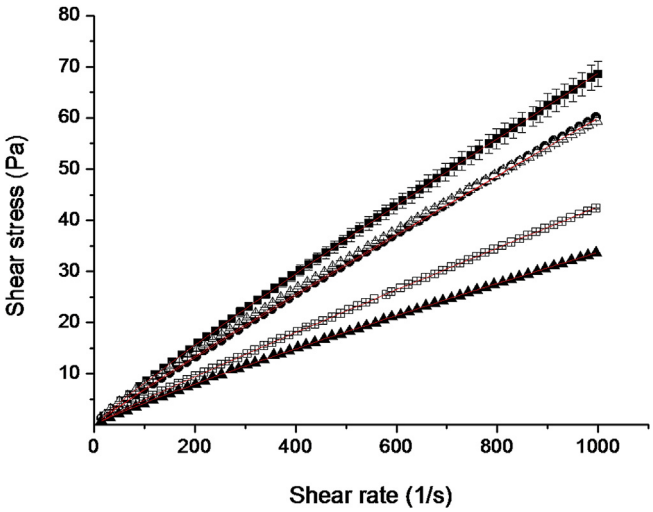

Fig. 4. Shear stress vs shear rate data of corn oil emulsions stabilized with untreated BG (▲), BG24 (●), BG48 (■), BG72 (□) and BG96 (Δ) solutions at 10% w/w. Continuous lines are fits to the power-law rheological model.

Table 2
Effect of thermal treatment time on rheological parameters of BG and BGTTs emulsions.

Emulsion	*k* (Pa s)	*n*	R^2
BG	0.070 ± 0.000[a]	0.893 ± 0.002[a]	0.999
BG24	0.091 ± 0.001[c]	0.940 ± 0.004[b]	0.999
BG48	0.119 ± 0.001[d]	0.921 ± 0.006[b]	0.999
BG72	0.082 ± 0.002[b]	0.932 ± 0.014[b]	0.999
BG96	0.131 ± 0.000[e]	0.945 ± 0.004[b]	0.999

Data expressed as average ± standard deviation (n = 3). Different superscript letters in the same column indicate statistical difference according to LSD tests ($p < 0.05$). *k* is the consistency index, *n* is the flow behavior index and R^2 is the coefficient of determination.

emulsions were higher than *k* value of BG emulsion, indicating higher viscosity of BGTT emulsions. The higher viscosity of BGTT emulsions is possibly due to the fact that, among the remaining polysaccharides in the continuous phase, there are MRPs that increase the viscosity.

3.4.2. Emulsion particles size distribution

Particle size distribution of corn oil emulsions stabilized with 10% w/w BG and 10% w/w BGTTs just after its preparation are presented in Fig. 5 A. It can be seen that all distributions were monomodal. Also, an important decrease in droplet size, hence increase in surface area, can be observed in the emulsions stabilized with BGTTs compared to BG emulsion, so it can be said that the thermal treatment improved the emulsifying capacity of BG. Table 3 shows the Z-average, PdI and mean peak values of corn oil emulsions stabilized with BG and BGTTs. A

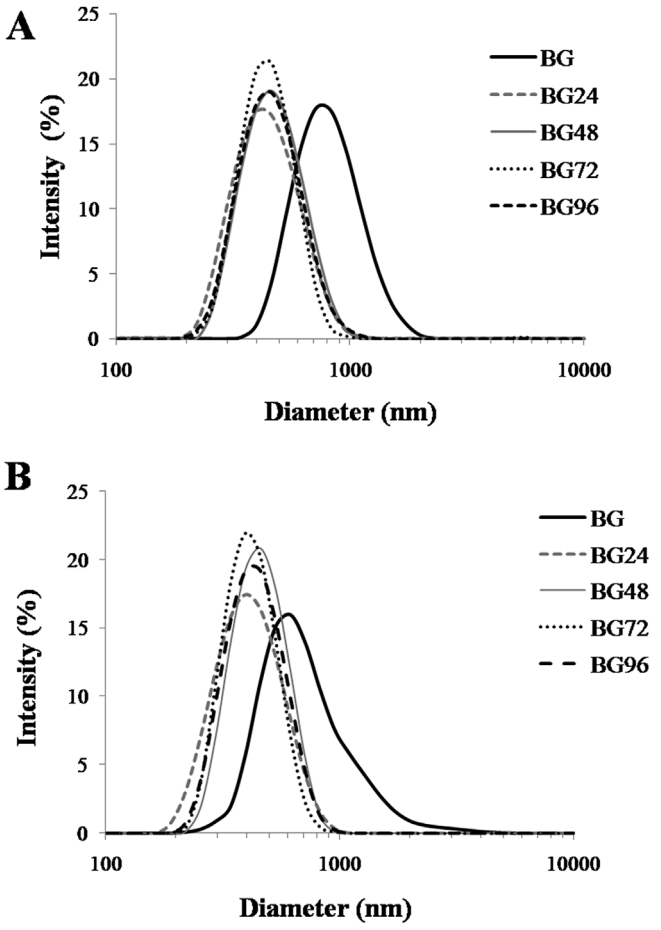

Fig. 5. Particle size distribution of corn oil emulsions stabilized with BG and BGTTs: (A) the day of preparation and (B) after seven days of storage.

significant decrease in Z-average is observed in all emulsions with BGTT compared to the emulsion with untreated BG. When comparing Z-average of BGTT emulsions, there was no clear trend with respect to the treatment time. BG24 emulsion showed the lowest Z-average (0.398 μm) reaching a decrease of 59% in regard to BG emulsion, while BG48 emulsion presented the highest Z-average which was only 9% higher than that of BG24. Also, all BGTT emulsions showed lower polydispersity (PdI) than BG emulsion, but there was no difference on PdI regarding treatment time. Therefore, it could be said that a thermal treatment of 24 h at 110 °C would be enough to improve the emulsifying activity of BG in terms of droplet size and surface area covered. Al-Assaf et al. (2007) reported similar results in emulsions stabilized with thermally treated GA. These authors evaluated different treatment times (24, 54, 72 and 96 h) and observed that 24 and 54 h of thermal treatment produced an increase of AGP component and treatment beyond 54 h produced hydrogel particles which were formed when the molecular mass exceeds 1.45×10^3 kDa. These authors also found that GA samples treated for 54 h were completely soluble and were able to produce emulsion with droplet diameters of 0.88 μm, which correspond to an approximated 39% decrease compared to the emulsions of the starting material. While samples with some gel particles present in their system (72 and 96 h) have less interfacial activity reaching droplet size reductions of only 30%. In contrast with these findings, BGTTs did not show gel particles formation in any of the thermal treatment carried out despite of the high molecular mass of the complexes formed (1.92×10^5 kDa).

On the other hand, decrease in emulsion droplet size was also reported in other oil-in-water emulsion systems stabilized with conjugates of dextran with WPI, bovine serum albumin or 11S globulin *Vicia faba* (Akhtar & Dickinson, 2003; Dickinson & Euston, 1991; Dickinson & Semenova, 1992). MRPs obtained with whey protein isolate (WPI)-maltodextrin were capable of producing emulsion droplets of 0.5–1 μm with either triglyceride oil or orange oil reaching a reduction of 10 times the size obtained with WPI alone (Akhtar & Dickinson, 2007). Nevertheless, it is worth noting that in none of these previous studies have been reached particle sizes as small as those reached in the present study with BGTTs.

3.4.3. Emulsion stability

Corn oil emulsions stability was evaluated by measuring the droplet size of the emulsions by dynamic light scattering after 7 days of storage. Also, stability was analyzed by measuring the Backscattering profile with Turbiscan equipment during the storage time.

Fig. 5 B shows the droplet size distributions of corn oil emulsions stabilized with BG and BGTT after seven days of storage at room temperature. All emulsions preserved the monomodal distribution, and BGTT emulsion presented smaller droplet sizes than the untreated BG emulsion as seen previously in the measurement made on the day of emulsion preparation. As can be seen in Table 3, Z-average of BGTT emulsions increased between 1 and 8% after storage and PdI values were 2–3% higher. Nevertheless, no signs of destabilization were observed in any of the emulsions (Fig. 6). Further, all emulsions prepared with BGTTs remained stable (no phase separation) for more than 10 months (data not shown). High stability of BGTTs emulsions had been foreseen when a decrease in emulsion droplets sizes was observed. Besides, the relatively higher viscosity of BGTTs emulsions, observed previously, would be delaying droplets movement preventing its aggregation therefore increasing emulsion stability. Similarly, emulsions stabilized with GA thermally treated presented no increases in droplet sizes after being subjected to an acceleration test (7 days storage at 60 °C) (Al-Assaf et al., 2007). The enhanced emulsifying properties in terms of droplet size and stability have been attributed to higher interfacial properties of thermal treated GA compared to the starting material as a result of increasing AGP content.

Moreover, WPI-dextran and WPI-maltodextrin conjugates were capable of producing emulsions that maintained droplet size and

Table 3
Z-average diameter, polydispersity index (PdI) and peak mean of corn oil emulsions stabilized with BG and BGTT solutions.

Emulsion	Day 1			Day 7		
	Z-averge (nm)	PdI	Peak mean* (nm)	Z-average (nm)	PdI	Peak mean* (nm)
BG	674 ± 11^{e}	0.20 ± 0.03^{b}	760 ± 48^{c}	755 ± 20^{b}	0.19 ± 0.02^{c}	801 ± 44^{b}
BG24	399 ± 1^{a}	0.09 ± 0.01^{a}	429 ± 3^{a}	430 ± 8^{a}	0.13 ± 0.00^{b}	476 ± 30^{a}
BG48	435 ± 4^{d}	0.08 ± 0.01^{a}	462 ± 5^{b}	442 ± 2^{a}	0.11 ± 0.00^{a}	482 ± 2^{a}
BG72	404 ± 3^{b}	0.08 ± 0.02^{a}	434 ± 10^{a}	431 ± 1^{a}	0.09 ± 0.01^{a}	455 ± 4^{a}
BG96	415 ± 0^{c}	0.07 ± 0.00^{a}	445 ± 3^{a}	438 ± 12^{a}	0.11 ± 0.00^{a}	484 ± 18^{a}

Data expressed as average ± standard deviation (n = 3). Different superscript letters in the same column indicate statistical difference according to LSD tests ($p < 0.05$). *Based on size distribution by intensity.

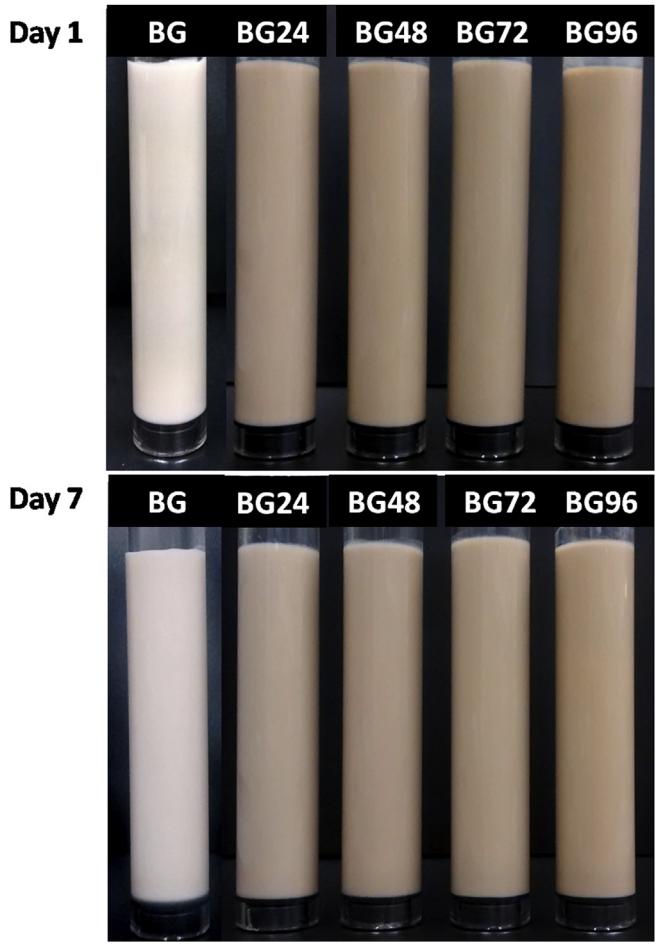

Fig. 6. Photographs of corn oil emulsions stabilized with BG and BGTTs: (A) the day of preparation and (B) after seven days of storage.

presented no noticeable phase separation over a storage period of 2 months (Akhtar & Dickinson, 2007, 2003). Conjugates of dextran with bovine serum albumin and 11S globulin *Vicia faba* also presented droplet size stability after a storage of 3 weeks at 60 °C (Dickinson & Semenova, 1992). Higher stability of emulsion stabilized with MRPs has been proposed to be due a more effective steric stabilization of oil droplets in water than does the adsorbed protein alone (Akhtar & Dickinson, 2007, 2003; Dickinson & Semenova, 1992; Li et al., 2016). This occurs because of a bulky polymeric layer that polysaccharides introduce to the droplet surface which alter the droplet aggregation and the coalescence (Dunlap & Côté, 2005).

Backscattering profiles in reference mode (ΔBS) obtained for BG, BG24, BG48 and BG72 emulsions during seven days of storage are shown in Fig. 7. BG emulsion profile (Fig. 7 A) showed a sharp decrease in ΔBS at the bottom of the sample, which is related to a decrease of droplets concentration at this height and correspond to a clarification process. Moreover, the decrease of ΔBS level along the tube indicates an increase in droplet sizes due to flocculation or coalescence processes. Meanwhile, BGTT emulsion profiles showed no modification during storage which is in agreement with what was observed in Fig. 5. Destabilization parameters for BG emulsion were IC = 1.97% and Delay Time = 26 h, while they were not measurable for the emulsions prepared with the BGTTs. So it is concluded that there were no signs of emulsion destabilization on the samples prepared with BGTTs. These results show that the thermal treatment carried out remarkably improved the emulsifying and stabilizing properties of the BG and that 24 h at 110 °C of treatment is enough to achieve these improvements. In these conditions, has been proved here that Maillard reaction occurs. Therefore, proteins would be binding to the polysaccharides forming protein-polysaccharides complexes responsible for the oil-water interfacial activity (Al-Assaf et al., 2007). Thereby, the enhanced interfacial stabilization of BGTTs might be based on the increased amount of complexes which on the one hand have an amphiphilic character that allow a better adsorption at the oil-water interface and on the other hand improve the steric stabilization of the droplets.

It is now well-recognized that impressive improvements of interfacial functionality can be achieved by Maillard-type conjugates produced via dry-heating of a mixture of proteins and polysaccharides (Akhtar & Dickinson, 2003, 2007; Dunlap & Côté, 2005; Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Guo & Xiong, 2013; Kato, 2002; Kato, Minaki, & Kobayashi, 1993; Li et al., 2016; Yang et al., 2015). However, the complexation and covalent linking of proteins to polysaccharides produced by controlled thermal treatment applied to a natural source of these two biopolymers, as it is the BG, is not yet clearly sighted. Recently, the effect of thermal treatment on the functional properties of cress seed gum (CSG) and xanthan gum has been studied (Naji, Razavi, & Karazhiyan, 2012). Results showed that thermal treatment increased the emulsifying and foaming properties of CSG but has not effect on xanthan gum. Authors attributed the enhanced properties of CSG to an unfolding of the protein moiety caused by the heating, but did not take into account the possible protein-polysaccharide complexation.

4. Conclusions

Thermal treatment applied to BG produced reactions of non-enzymatic browning or Maillard reactions, generating colored compounds of high interfacial/surface activity. This modification improved the emulsifying/stabilizing capacity of BG, obtaining stable corn oil emulsions that present no phase separation for several months. It was concluded that a treatment of 24 h at 110 °C is enough to significantly improve the emulsifying properties of BG, more time did not produced further improvement. The MRPs include protein-polysaccharides complexes responsible for the enhanced surface activity of the treated sample.

Finally, it bears noting that this modification process does not

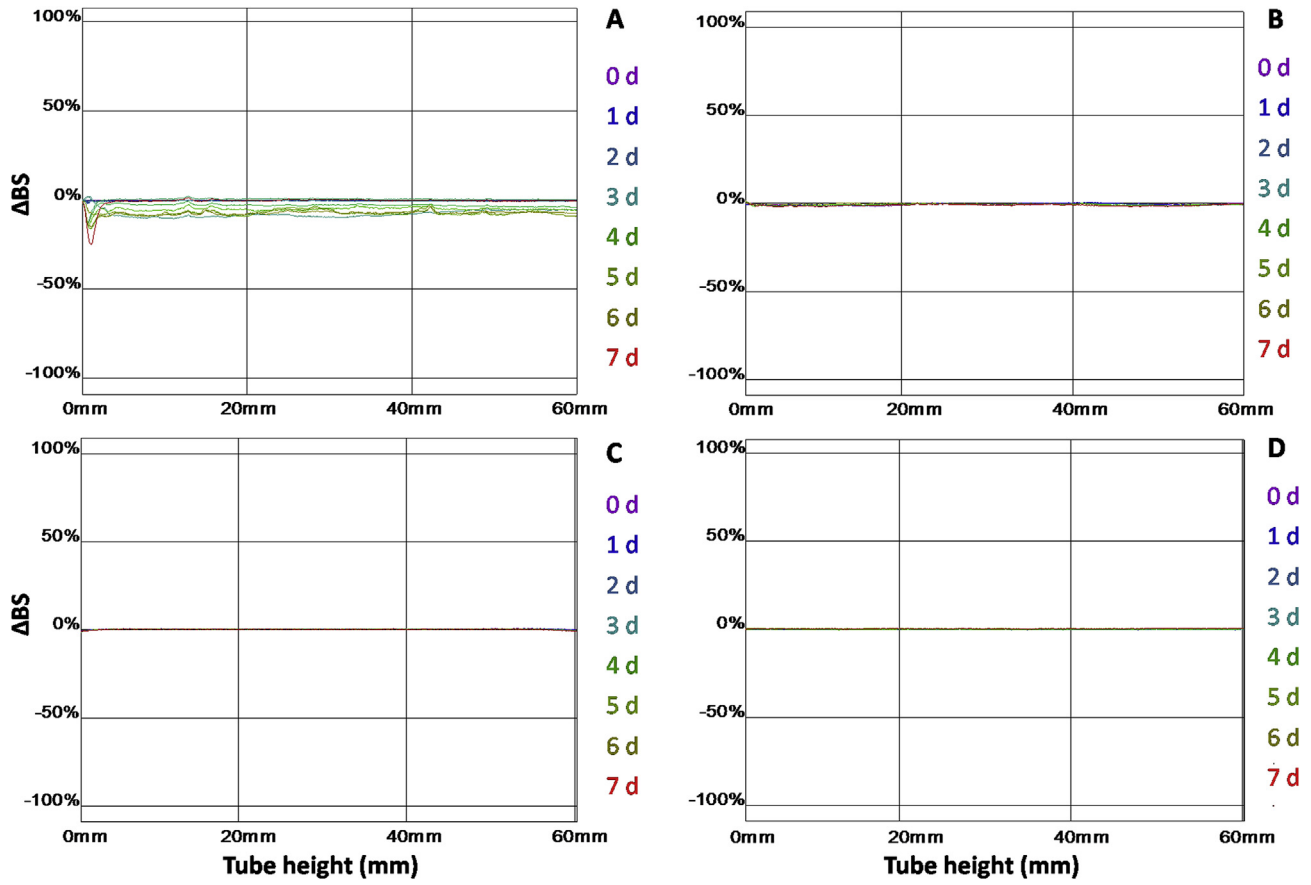

Fig. 7. Delta Backscattering (ΔBS) profiles determined on the day of preparation (0 d) and on the following seven days (1–7 d) of corn oil emulsions stabilized with (A) BG, (B) BG24, (C) BG48 and (D) BG72.

introduce nor remove chemical groups on BG since the effects are achieved by a redistribution of existing components. Furthermore, no chemical agents are involved in the process hence it is completely suitable to be used in food industry.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.foodhyd.2018.07.010.

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