



Encapsulated betalains (*Opuntia ficus-indica*) as natural colorants. Case study: Gummy candies



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ARTICLE INFO

Keywords:

Betain
Gelatin
Rheological properties
Optical microscopy
Gummy candy

ABSTRACT

Gummy candies were selected as model food system to incorporate betalain-rich capsules (BC) obtained by ionic gelation with calcium alginate from a betalain-rich extract of *Opuntia-ficus-indica* fruit. Ten formulations were obtained by a factorial design (2 × 5) with two types of gelatin (GA and GB), and five betalain-rich capsules/gelatin (BC/G) ratios (from 70/30 to 30/70). The rheological and morphological properties of all formulations were studied, finding that the viscoelastic, gel strength, and stress relaxation properties were dependent on the G type content in the ratio BC/G. Optical micrographs also showed that G type, bloom, and BC/G ratio had a marked influence on the gummy candies structure. These data suggested that the G addition in higher concentrations allows to develop a more rigid gel. The betanin stability in the food system was evaluated during storage at 4 °C for 30 days and there was no significant variation in total colour parameters for all samples (ΔE^* values less than four). Thus, the stability of the betalain colour in the gummy candy was confirmed, exhibiting this food product a vivid red-purple colour, such representing a promising application for these natural pigments in food industry.

1. Introduction

In modern food industry, natural pigments have gained the attention of both researchers and consumers due to their safety as well as their nutraceutical properties and biological activity. Most of these natural pigments are health-promoting ingredients because they have showed preventive effects against several diseases such as, cancer, atherosclerosis, arthritis, cataracts, neuro-degenerative and cardiovascular diseases, among others (Carocho, Morales, & Ferreira, 2018).

Betalains, commonly extracted from cactus pear fruits (*Opuntia* spp.), are water-soluble pigments that are capable to confer red-purple (betacyanins), or yellow-orange colours (betaxanthins) to different foods. They also to exhibit health benefits as antioxidants, anti-cancer, anti-lipidemic and antimicrobial activities (Gengatharan, Dykes, & Choo, 2015; Stintzing et al., 2005). The colour stability and antioxidant activity of these pigments are restricted because of their rapid degradation in the presence of oxygen, light, pH, water activity, or temperature (Tsai, Sheu, Wu, & Sun, 2010; Celli & Brooks, 2017;

Betancourt, Cejudo-Bastante, Heredia, & Hurtado, 2017). However, the stability of these bioactive compounds could be markedly improved with the encapsulation. Although, spray drying is the most commonly used technique to protect these pigments, the equipment required is expensive. In addition, the amount of encapsulated betalains will be dependent on the process conditions, such as, inlet and outlet air temperatures, feed flow rate, atomisation speed or pressure, and feed to carrier ratio, among others (Gandía-Herrero, Cabanes, Escribano, García-Carmona, & Jiménez-Atiénzar, 2013; Otálora, Carriazo, Iturriaga, Nazareno, & Osorio, 2015; Otálora, Carriazo, Iturriaga, Osorio, & Nazareno, 2016; Robert, Torres, García, Vergara, & Sáenz, 2015; Vergara, Saavedra, Sáenz, García, & Robert, 2014). In contrast, ionic gelation through extrusion dripping is a simple, fast, efficient, environment friendly and low-cost encapsulation technique for these bioactive compounds that does not require specialised equipment, high temperature, or organic solvents (Gorbunova, Bannikova, Evtsev, Evdokimov, & Kasapis, 2018; Otálora et al., 2016).

New trends in the food industry are focused on the development of

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fortification foods; replacing artificial colorants by natural pigments, which exhibit specific functionalities and health benefits (Crupi, Dipalmo, Clodoveo, Toci, & Coletta, 2018). In this regard, gummy candies, came up as an appropriate food system for delivery of functional pigments, and they are visually attractive for a great number of consumers, from children to elderlies. In addition, gummy candies are a system suitable to the palatability and stability of pigment during storage in contrast with instability of pigments in an emulsified medium (e.g. yogurt). Before food preparation, producers should assess the stability of the ingredients and additives including colourants. This issue can be solved if, instead of lyophilised fruit pulp, encapsulated betalains are used. In this case, pigment should be released just before preparation. However, it is necessary to evaluate the effect of alginate calcium incorporation on texture and rheological properties of gummy candies, that are of relevant importance for gel-like products (Cappa, Lucisano, & Mariotti, 2013; Marfil, Anhe, & Telis, 2012); and the colour, as an important indicator of food quality and acceptability (Cappa, Lavelli, & Mariotti, 2015).

Applications of betalains in confectionery are quite limited in literature; for example, pigments in red pitaya fruit puree (*Hylocereus polyrhizus*) were used in the gummy production with gelatin and high-methoxyl pectin (Hani, Romli, & Ahmad, 2015). Beetroot - saffron powders, pitaya juice, and beetroot pomace have been assayed as natural pigment in candy formulations (Chranioti, Nikoloudaki, & Tzia, 2015; Coria-Cayupán & Nazareno, 2015; Kumar, Kushwaha, Goyal, Tanwar, & Kaur, 2018; Rodríguez-Sánchez, Cruz y Victoria, & Barragán-Huerta, 2017). Additionally, the betanin-nanoliposomes have been used in the manufacturing of gummy candies (Amjadi, Ghorbani, Hamishehkar, & Roufegarinejad, 2018). Up to now, there are scarce reports concerning the development of a food system as gummies that includes betanin-capsules as the natural colouring additive. Thus, the aim of this work was to assess the incorporation of betalains obtained from ionic gelation-capsules (as natural colouring additive) to prepare gummy candies and evaluate their effect on the texture properties and colorimetric stability of this food model.

2. Material and methods

2.1. Materials

Opuntia ficus-indica fruits (purple pulp) corresponding to clone number 1279 were collected from the cactus collection belonging to the Faculty of Agronomy and Agroindustries (National University of Santiago del Estero) of the province of Santiago del Estero located in the Argentinean region of the dry Chaco (27 450 S, 64 1800 W, 170 m over sea level). Sodium alginate (SA) (Emulgel CP 3792) was provided by Saporiti (Buenos Aires, Argentina) and used as received without further purification. Calcium chloride dihydrated ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was purchased from Cicarelli Laboratories (Buenos Aires, Argentina). Type-A gelatin (GA, from pork skin, bloom 300, isoelectric point 7–9) was purchased to Sigma-Aldrich (Milwaukee, WI, USA), and type-B gelatin (GB, from bovine bones, bloom 285, isoelectric point 4.2–6.5) to Gelco (Medellín, Antioquia, Colombia).

2.2. Preparation of betalain-rich extract (BE)

Cactus fruit pulp was crushed in a homogeniser and the seeds were removed by filtration. The homogenised cactus fruit pulp was lyophilised in a freeze-dryer Labconco Freezone 4.0 (Kansas City, MO, USA) until reach a final moisture value between 1.9 and 2.3 g/100 g (wet base). Then, the sample was kept in hermetic flasks with light protection and stored in a freezer at $-18\text{ }^\circ\text{C}$ until be used. To obtain betalain-rich extract (BE), lyophilised cactus pulp was macerated with a phosphate buffer (pH 5.5) using a 1:2 g/mL pulp/buffer ratio for ionic gelation. The betalain composition of this extract was analysed by HPLC-MS/MS in Otálora et al. (2015), who reported the presence as major

components of betacyanins (betanin and isobetanin), and betaxanthin (indicaxanthin).

2.3. Betalain-rich encapsulates by ionic gelation

The BE was combined with SA (15 g/L) at pH 5.5 in a ratio of 1:0.015 g/g, respectively, using a homogeniser (Phillips, Buenos Aires, Argentina) for 2 min at low speed. The mixture was slowly added (drop-wise by using a 5 mL pipette with a 1.43 ± 0.03 mm internal diameter tip) on a 0.15 mol/L calcium chloride solution, following the procedure reported by Otálora et al. (2016). The hydrogel beads were kept in the calcium chloride solution for 1 min (hardening time) and washed with distilled water. Subsequently, they were filtered and washed again with distilled water. Hydrogel beads dehydration was performed by air-drying at $30 \pm 1\text{ }^\circ\text{C}$ for 24 h in a forced-air circulating oven (Termo Dalvo SRL, Model TDC 30, Buenos Aires, Argentina). In the manufacturing of the gummy candies, the betalains encapsulated are disintegrated to guarantee an increase in the bioactive compounds content in the matrix.

2.4. Preparation of betalain-rich additives

To manufacture the gummy candies, encapsulated betalains were previously released to guarantee the total incorporation of the bioactive compounds in the matrix. The dried betalain-rich capsules (BC) (300 mg) were disintegrated in water (pH 5.0; food-grade citric acid) (500 mL) at $20\text{ }^\circ\text{C}$, by using an orbital shaker (Orbit Environ Shaker, Lab Instruments, Melrose Park, IL, USA) at 180 rpm.

2.5. Gummy candies preparation

To prepare of gummy candies, gelatin powder (G) was mixed with water (pH 5.0) at $60\text{ }^\circ\text{C}$ until complete dissolution. After that, the solution was cooled down until $30\text{ }^\circ\text{C}$ to add the BC solution in different BC/G ratios (g/g) (70/30, 60/40, 50/50, 40/60, and 30/70), for both G types. Each formulation was poured into cactus pear fruit-shape metal templates and cooled at room temperature to form the gels to be immediately analysed.

2.6. Characterisation of food system

2.6.1. Viscoelastic properties

Viscoelastic properties of gummy candies were measured by using a rotational rheometer (Malvern Bohlin-Instruments CVOR, Malvern, UK). The temperature was maintained at $25\text{ }^\circ\text{C}$ by a Peltier element in the lower plate. The measurements were carried out using a cone/plate geometry (40 mm diameter and angle 2°). Fresh gels were prepared according the procedure explained in section 2.5, and after gelation the samples were carefully cut into disk-shaped slices by using a scalpel. Disk samples were put on the lower plate of the rheometer for the measurements. The strain sweep test was used to ensure that the measures were in the linear viscoelastic region (LVR). The evolution of the storage modulus (G') was determined by time sweep experiments at shear oscillatory frequency of 1 Hz, and strain amplitude of 1% during 21 min.

2.6.2. Gel strength and stress relaxation

Gel strength was determined according to a previously described method (Holzwarth, Korhummel, Siekmann, Carle, & Kammerer, 2013). The strength of each sample was measured by using a Model TA-TX-Plus Texture Analyser (Stable Micro Systems, Godalming, UK) with a spherical probe (P/0.25S) of $\frac{1}{4}$ -inch diameter. Subsequent penetration of the gels at room temperature ($20\text{ }^\circ\text{C}$) up to the depth of 20 mm was performed at a constant velocity of 0.5 mm/s. The maximum force (Pa) was recorded as the breaking point of the sample. For stress relaxation, gummy candy samples were compressed by spherical probe until the

deformation reached 30% at a deformation velocity of 0.5 mm/s and kept under compression for 3 s at 20 °C.

2.6.3. Optical microscopy

Gummy candies samples were cut into blocks of 2 mm thick using a razor blade. The internal structures of each sample were exposed by cutting the gummy in halves. The samples were mounted on a slide. Reflected light microscopy images of samples were recorded with Nikon E600 upright microscope (Nikon Instruments, Melville, NY, USA) equipped with a bright 100 W halogen illumination and a black and white Lumera Infinity 3-1UC camera with 1.4 Mpx for image recording. Objective lens was Nikon 10×/0.25 Ph1 and working distance 200 mm. Illumination was set manually while the gain of the camera was adjusted automatically. Images were recorded using Infinity Capture version 5.0.4 program.

2.7. Betalain stability in the food system

Gummy candy formulations were wrapped in polyethylene film to minimize water and stored for 30 days at 4 °C in the dark. For each sample the colour parameters were determined at initial time (zero time) and after 30 days of storage. The CIELAB parameters (L^* , a^* , b^*) of encapsulates were measured by using a Mini Scan EZ HunterLab (Hunter Associated Laboratory, INC, Reston, Virginia, USA). Total colour change (ΔE^*) was calculated as follows (Eq. (1)):

$$\Delta E^* = [(L_i^* - L_o^*)^2 + (a_i^* - a_o^*)^2 + (b_i^* - b_o^*)^2]^{0.5} \quad (1)$$

where L_o^* , a_o^* , and b_o^* are the values of the samples at zero time and L_i^* , a_i^* , and b_i^* the measured values of the sample after 30 days of storage (Obón, Castellar, Alacid, & Fernández-López, 2009).

2.8. Statistical analysis

Data of the gummy candy characterisation were reported as the mean \pm standard deviation for determinations performed ($n = 3$). The Kruskal-Wallis test was used to identify differences among the means with the statistical package InfoStat/P version 1.1 computing program (Di Rienzo et al., 2012). Differences at probability level $p \leq 0.05$ were considered significant.

3. Results and discussion

3.1. Characterisation of model food system

Commercially available gelatins for the food industry (type A and type B) were used in this study. Type A (produced via an acid process) and type B (produced via an alkaline process) exhibit different physicochemical properties (Ali, Kishimura, & Benjakul, 2018) that influence the gelation behaviour of gels. The effects of the ratio BC/G on gummy candy rheology are shown in Fig. 1. Storage modulus (G') is a quantitative representation of the elasticity or solid-like behaviour of viscoelastic materials. In gels, an increase in G' is related to a higher effect of physical crosslinks or molecular interactions such producing harder materials. Comparing the results presented in Fig. 1, the G' values for gummy candies containing GA were higher than the corresponding GB samples. This is likely because the gelatin powder was dissolved at pH 5.0, that is below the pI (pH 7–9) of GA. At this condition, the aggregation and electrostatic forces for GA, that influence the ability of chains to form junction zones, are much higher than for GB whose pI (pH 4.2–6.5) is different. Fig. 1 also showed that the G' value increased significantly over time for samples with higher proportion of G (30/70 and 40/60 BC/G ratio); in contrast, the gummy candies with low G proportion (70/30 BC/G ratio) showed a rapid stabilisation and a decrease of G' . These results suggest that the gel hardness changes are directly related to the G content. The inter-chain associations that lead

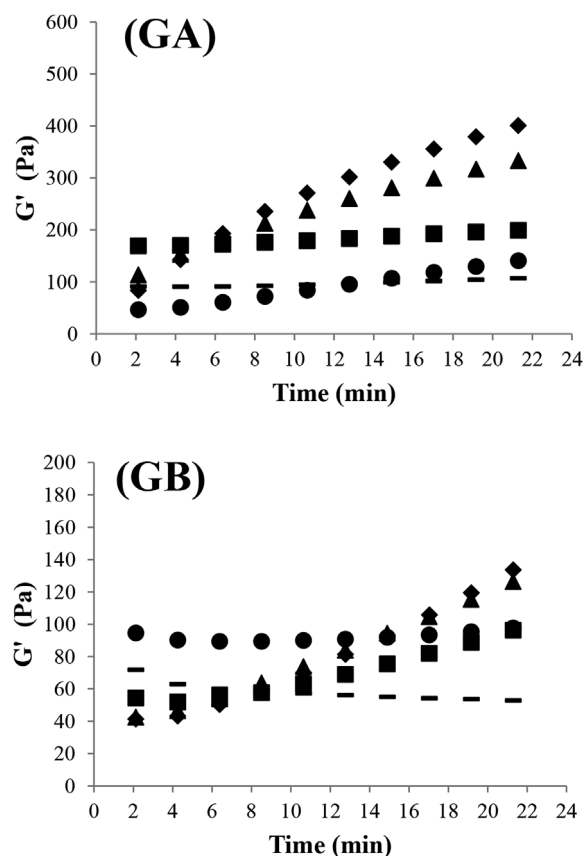


Fig. 1. Effect of gelatin type (GA or GB) and BC/G (betalain-rich capsules/gelatin) ratios on storage elastic modulus (G') of gummy candies at 25 °C. BC/G ratio: 30/70 (◆), 40/60 (▲), 50/50 (■), 60/40 (●), and 70/30 (▼).

the formation of cross-links or junction zones separated by flexible strands, are stabilised by non-covalent interactions (Ziegler & Foegeding, 1990), such leading a cross-linking with a fast increase in the G' value. In other way, the G' value remains constant or slightly decreased with the BC content, because of the linearisation of elastic modulus after the beginning of gelation; this could be explained by the presence of alginate in BC, that acts as a barrier to restrict the triple-helix forming ability of the gelatin molecules. Hence, a less rigid network is obtained with the consequent cross-linking density decrease (Semenova & Dickinson, 2010). The combination of alginate present in BC with G, could allow a phase separation either by polymer molar mass or ionic strength (Tolstoguzov, 2003), or by the interaction between carboxyl groups of SA in BC with the amino groups of the gelatin molecules (Saarai, Kasparkova, Sedlacek, & Saha, 2013; Zhao, Li, Carvajal, & Harris, 2009). If G concentration is high, the gummy candies formulation can gel, but if the BC proportion is increased to intensify the colour, a viscous heterogeneous solution is formed; thus, the ratio BC/G is the key parameter to avoid the hydrogels breakdown.

The influence of BC/G ratio on the gel strength of gummy candies is shown in Table 1. In general, the gel strength was higher in gummy candies with major proportion of G, being these values higher for samples containing GA in comparison with those having GB. These results are in agreement with those reported by Pang, Deeth, Sopade, Sharma, and Bansal (2014) who studied the effect of type B gelatin concentration on the textural properties of gelatin gels, and with Morales, Martinez, and Pilosof (2016) who studied the interaction between gelatin and casein glycomacropeptide and its influence on the textural properties of those mixed gels. The gel strength of gels is related to the amino acid composition and content, the molecular weight distribution, the ratio of α -chain, and the amount of β -component of the gelatin (Gómez-Guillén et al., 2002).

Table 1

Effect of gelatin type and BC/G ratios on the gel strength (g) and stress relaxation (%) of gummy candies and total colour change (ΔE^*) in BC/GA and BC/GB model foods with different ratios (after stored at 4 °C during 30 days).

Ratio BC/G	Property					
	Gel strength (Pa)		Stress relaxation (%)		ΔE^*	
	GA	GB	GA	GB	GA	GB
70/30	9 ± 1 ^a	8 ± 1 ^a	561 ± 65 ^a	268 ± 10 ^b	3.7 ± 0.3 ^a	3.9 ± 0.4 ^a
60/40	16 ± 2 ^b	14 ± 2 ^b	91 ± 7 ^c	47 ± 2 ^d	3.4 ± 0.2 ^a	3.3 ± 0.3 ^a
50/50	16 ± 1 ^b	16 ± 2 ^{b,d}	71 ± 15 ^c	44 ± 6 ^d	2.7 ± 0.2 ^b	2.9 ± 0.2 ^b
40/60	24 ± 2 ^c	18 ± 1 ^d	31 ± 9 ^e	33 ± 10 ^e	1.8 ± 0.1 ^c	2.1 ± 0.2 ^d
30/70	27 ± 4 ^c	23 ± 2 ^{c,e}	14 ± 2 ^f	20.7 ± 0.5 ^g	1.3 ± 0.1 ^c	1.3 ± 0.1 ^c

All data are the mean of measurements ($n = 3$) ± standard deviation. Different letters in the same row and column (a-g) for each property, indicate a statistical difference ($p < 0.05$) among samples with different gelatin type.

The effect of BC/G ratio on the stress relaxation is also shown in Table 1. The variation of stress relaxation with the BC/G ratio was opposite to the behaviour of gel strength, obtaining lower values of stress relaxation when the proportion of G was increased. These data suggested that a greater proportion of G in the relation BC/G allowed the formation of gels with higher junction zones, that increased as the gelation proceeds (Tau, & Gunasekaran., 2016). Matsukawa et al. (2014) reported similar results during the measurement of stress relaxation and spatial distribution in agar gels under large deformation.

The micrographs of the surface and internal structures of gels formed only with GA and GB are shown in Fig. 2. The gels formulated with GA showed a uniform network with the presence of strands, in contrast with GB samples that showed a fine-grained and homogeneous structure, exhibited strands forming a network microstructure. The uniform structure of GA samples is related to the higher bloom strength (300 bloom), compared with the coarser gel structure of GB gels, which exhibited a lower bloom strength (285 bloom). These results were

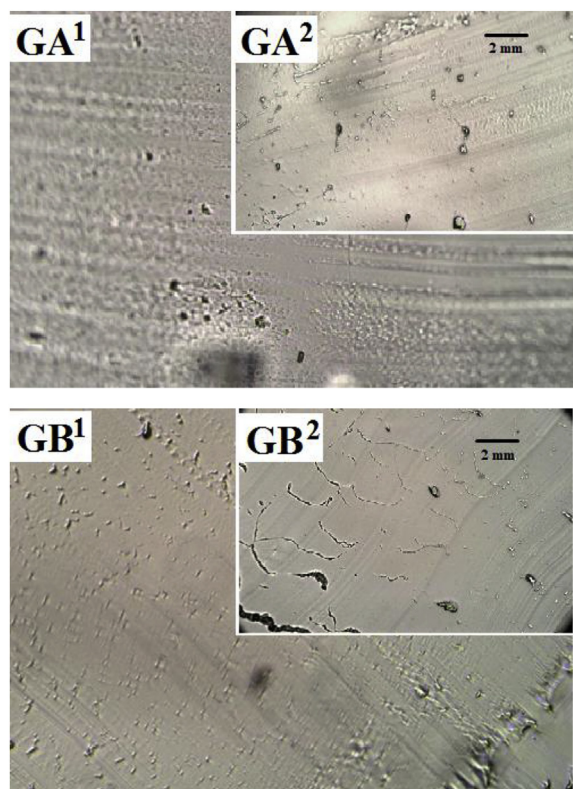


Fig. 2. Representative optical micrographs images of gummy candies obtained with only gelatin type A (GA) and type B (GB),⁽¹⁾ surface and ⁽²⁾ internal structures at 10X.

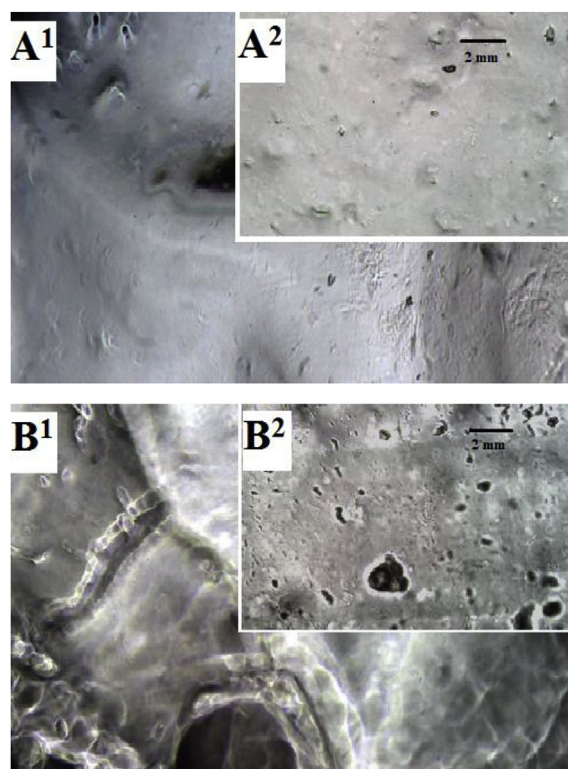
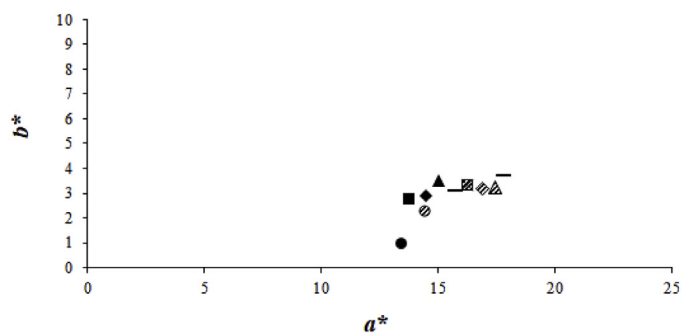
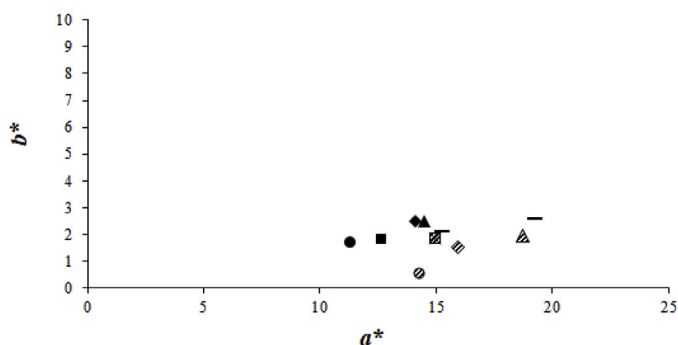


Fig. 3. Representative optical micrographs images of gummy candies obtained with gelatin type B (GB) at 70/30 (A) and 30/70 (B) BC/G ratios, ⁽¹⁾ surface and ⁽²⁾ internal structures at 10X.

similar to those reported by Benjakul, Oungbho, Visessanguan, Thiansilakul, and Roytrakul (2009). Therefore, GB was chosen for the subsequent analysis of optical microscopy.

The microstructures of two gummy candies formulated with GB are compared in Fig. 3. When the amount of disintegrate capsule (BC) was higher (BC/GB 70/30), the surface structure was looser and homogeneous without the presence of strands (Fig. 3A); in contrast, when the ratio BC/GB was 30/70, the microstructure was dense, rough with small voids and with the presence of strands and micelles (Fig. 3B). These results suggest that the G molecules overlap each other by effect of hydrogen bonding, hence, the degree of heterogeneity of the microstructure in the gels increases with the G content. Similar results were obtained by Saarai et al. (2013), in hydrogels prepared with different ratios of SA and G. Regarding internal structure, the gummy candies formulated at 70/30 BC/GB ratio were very smooth feeble, in contrast to the sample made with 30/70 BC/GB ratio, whose internal structure was rough, uneven, and compact (Fig. 3B). These results showed that the increased of G contributed to the formation of a compact structure,

BC/GA**BC/GB**

because the free volume space reduction within the gel. This is likely due to intermolecular attractive forces among protonated amine (NH_3^+) groups of the protein and carboxylate (CO_2^-) groups of the alginate of BC.

3.2. Betalain stability in food system

The colour parameters (L^* , a^* and b^*) for gummy candies formulated with GA and GB during the storage are shown in Fig. 4. Both samples, BC/GA and BC/GB were located in the first quadrant of a^* , b^* diagram, showing an increase in the a^* values as the proportion of BC was higher. Also, it is shown that after 30 days, the sample colour changed showing a decrease in the a^* values, such indicating that samples are less red than those in zero time. These changes were more significant in the BC/GB samples. The L^* and b^* values of the gummy were constant as a function of time storage as BC proportion was higher in the BC/G ratio. Total colour change (ΔE) (Table 1) between GA and GB was clearly depending on the relation gelatin in the matrix and was due to the decrease in a^* value, where ΔE^* values were higher in samples with lower proportion of G suggesting a protective role of this hydrocolloid on the colour stability of pigment. The data of Table 1 shows that ΔE^* value in gummy candies were less than 4, value that is considered undistinguishable by human eye (Witzel, Burnham, & Onley, 1973), after storage at 4 °C during 30 days. The results showed that colour of samples with lower proportion of BC is more stable during the storage at 4 °C.

4. Conclusions

A novel model food system (gummy candy) was prepared by mixing gelatin type B with a natural colouring additive obtained from betalain-rich capsules. These gummy candies showed appropriate gelling and morphological properties to be used in confectionery industry. The food system sample stored at 4 °C during 30 days, did not significantly

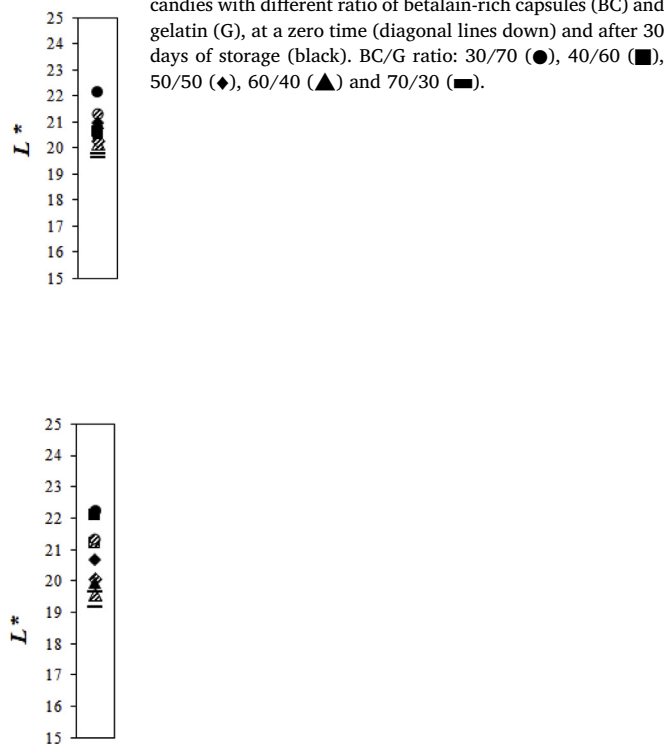


Fig. 4. Colour parameter (L^* , a^* , and b^*) values of gummy candies with different ratio of betalain-rich capsules (BC) and gelatin (G), at a zero time (diagonal lines down) and after 30 days of storage (black). BC/G ratio: 30/70 (●), 40/60 (■), 50/50 (◆), 60/40 (▲) and 70/30 (■).

change its colour ($\Delta E^* > 4$). Although encapsulated betalains have a potential to be successfully used as colouring additives in the food industry, further research is still necessary to evaluate the bioaccessibility of the bioactive compounds in order to justify the real applications as a system with biofunctional properties.

Note

The authors declare that there is no any conflict of interests.

Funding

Authors greatly acknowledge the financial support provided by COLCIENCIAS, Colombia (contract RC. No. 707-2013), CICYT-UNSE (Project 23/A213), and MINCYT (CO 13/02), Argentina. M.C.O. acknowledges CONICET for her doctoral fellowship.

Acknowledgments

Authors are grateful with Dr. Martha Quicazán from ICTA (Instituto de Ciencia y Tecnología de Alimentos), Universidad Nacional de Colombia, for kindly lend us the texturometer, and Dr. Jose Carriazo from Department of Chemistry, Universidad Nacional de Colombia, for his help on the experimental design for optical microscopy.

Abbreviations

GA	Type A gelatin
GB	type B gelatin
BE	betalain-rich extract
G	gelatin powder
BC	betalain-rich capsule
BC/G	betalain-rich capsules/gelatin
G'	storage elastic modulus

SA sodium alginate
 ΔE^* total colour change

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