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Functional Jelly Beans Based On Hydrocolloids And Citrus Cremogenates

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***Abstract*— Jelly beans were obtained from sugar, gelling agents, and citric cremogenates as source of bioactive compounds. To the best of our knowledge, the blends of modified starch and low methoxyl pectin (LMP) as well as the effect of citric cremogenates on the formulation of confectionery gels and their functional properties have hardly been reported. This work was focused on: i) obtaining cremogenates from different citrus fruits and characterizing them physicochemically; ii) formulating jelly beans based on acetylated starch, LMP, and cremogenate; iii) assessing the quality attributes and global acceptability. Furthermore, the changes on rheological behaviour due to cremogenate addition and calcium-induced gelation in composite model systems were studied.**

Viscoelasticity studies confirmed the type-gel structure of the formulated systems. Likewise, rheological results correlated with TPA profiles of jelly beans. General acceptability scores were 6.84 for formulations containing orange cremogenate and 5.72 for grapefruit ones. Composite developed systems have a protective effect of ascorbic acid exhibiting a slight reduction during the drying step especially in the case of samples containing grapefruit cremogenate.

The functionalization of the matrix with calcium, involved in the chemical gelation mechanism of pectin, can incorporate this mineral in the final product, being this relevant from a nutritional point of view.

Keywords*— Jelly beans, orange and grapefruit cremogenates, nutritional contribution, hydrocolloids blend systems, rheological properties, sensorial acceptability.

I. INTRODUCTION

Nowadays, consumer attitudes and behaviors' have moved towards healthy eating and food safety. Customers prefer gummy jelly candies due to their unique texture and chewability; they are based on a gelling agent (gelatin) as well as sweeteners, acidulants, coloring and flavorings additives. Thus, the nutritional imbalance of these products places them in a food category socially perceived as unhealthy.

The blend of different hydrocolloids offers an interesting alternative to develop new matrices with different textures. Starch–hydrocolloid combinations have been extensively used in processed food matrices. Hydrocolloid addition may modify the gel-like character of starch pastes, depending on the starch type as well as on the polymer type and concentration [1]. In this sense, pectin is frequently used in systems containing fruits. Different polysaccharides with strong gelling and water-binding properties are industrially used due to their influence on the food matrix stability; and particularly these polymers are successfully applied for developing products containing fruits [2].

Low methoxyl pectin (LMP) has the ability to associate ions due to a high content of negative charges and can gel in the presence of divalent cations (usually calcium) by ionotropic gelation [3,4]. Sharma, Naresh, Dhuldhoya, and Merchant [5] pointed out that the products formulated using a combination of pectin and starch, are the so-called *"jelly beans"*. Compared with jelly gummies products the texture is long and viscous. Meanwhile, the changes in consistency can be achieved modifying the pectin/starch ratio or by the type of the pectin or starch used. Additionally, Shi and BeMiller [6] also suggested that ion-charged gums could act as cross-linking agents in starch-gum systems.

An innovative aspect of this research is the use of a natural product (cremogenate), as a source of minerals, vitamins, bioactive compounds, and fiber. According to the Argentinean normative [7], citric cremogenate is defined as the crushed fruit with their skin or shell, conserving their essential oils and compounds related to the flavor of the fruit. It should present the proportions of juice, pulp and peel corresponding to those of the fruit. As regard, the obtaining of cremogenates from other vegetables sources such as granadilla (*Passiflora ligularis*) has been reported as well as their characterization [8].

In order to make progress on the healthy confectionery the identification of ingredients naturally rich in bioactive compounds that could be exploited in the jelly beans and establishing their health effects inside the food matrix is an important scientific inquiry that could take potential societal benefit.

To the best of our knowledge, the blends of modified starch and LMP as well as the effect of citric cremogenates on the formulation of confectionery gels

and their functional properties have hardly been reported.

This work was focused on: i) obtaining cremogenates from different citrus fruits and characterizing them physicochemically; ii) formulating jelly beans based on acetylated starch, low methoxyl pectin, and cremogenate; iii) assessing the quality attributes and global acceptability. Furthermore, the changes on rheological behavior due to cremogenate addition and calcium-induced gelation in composite model systems were studied.

II. MATERIAL AND METHODS

A. Cremogenate preparation and characterization

Cremogenate preparation

The cremogenates were obtained from different healthy and ripe citrus fruits selected according to size and appearance, orange Valencia (*Citrus sinensis*) and grapefruit (*Citrus paradisi*) pink variety, which were purchased from the local market.

The fruits were washed with chlorinated water (250 mg Cl_2/L) and they were sliced without discarding any constituent. The cremogenates were obtained by processing of whole fruit with extraction equipment Centrifugal Juicer (Philip Juicer, Argentine). For all assays the cremogenates were obtained from 2 kg of fruit per sample.

Physicochemical analysis of cremogenates

Total soluble solids (TSS) expressed in ºBrix determined by using AOAC official method [9] by placing a drop of filtered cremogenate into a digital refractometer (Atago, USA). Measurements were performed in triplicate.

Titratable acidity (TA) was measured using AOAC method [9]. Color was determined by a Minolta colorimeter CR 300 Series (Osaka, Japan) calibrated with a standard (Y = 93.2, x = 0.3133, y = 0.3192). Samples were analyzed in triplicates, recording six measurements for each sample.

In order to estimate the cremogenate yield, samples of fruit were cut, weighed and, their corresponding cremogenates were obtained. Cremogenate yield was defined as g of cremogenate/100g of fruit based on the weight of fresh whole fruit.

For cremogenate ash determination, porcelain crucibles were dried at 105°C overnight to remove water. The crucibles were placed in a vacuum desiccator to reach room temperature and the weights were recorded. Approximately 10 g of sample was poured into the porcelain crucibles. Ash content was determined in duplicates in a muffle furnace at 550°C according to the method described by Marshall [10].

To quantify the ascorbic acid (AA) content of cremogenates HPLC analysis was performed. Samples of cremogenates (about 10 g) were centrifuged twice at 12.000 rpm for 15 min (Centrifuge 5415 D, USA) in order to remove the suspended particles. Then the samples were diluted (1:5) with mobile phase and afterwards, the samples were filtered with Millipore membrane with pore size 0.45 µm (Millipore Corporation, France). The HPLC system used was a Waters equipment (Selangor, Malaysia), model R-414. Solute elution was analyzed by using a UV detector and RI detector. The liquid chromatographic method used for the determination of ascorbic acid (AA) consisted of an isocratic elution procedure with UV-Visible detection at 245 nm. Separations were carried out on a 5 mm RP C18 column of 150 mm-4.6 mm (WAT 045905, Ireland). The employed mobile phase was a 93:7 mixture of HPO_3 0.5 g/100mL and acetonitrile [11]. Chromatographic peak in the samples of cremogenates and jelly beans were identified by comparing the retention time with that of reference standard.

B. Jelly beans formulation

Jelly beans were made from synthesized acetylated starch, (substitution degree of 0,084±0,002) (Arcor, Tucumán, Argentine), and from low methoxyl pectin solution (LMP) Genu Pectin 104 (CP Kelco, Denmark) and sugar (Ledesma, Jujuy, Argentine). In order to prepare the hydrocolloid solution, 8 g of pectin (LMP) was slowly dispersed in 100 mL of hot distilled water (80°C) under constant stirring for 1 h. In preliminary tests the concentrations of each ingredient were optimized, being these for 100 g of suspension: 13 g acetylated starch, 35 g sugar, and 32 g LMP solution prepared at 8 g/100g.

LMP solution was incorporated and the system was gelatinized during 20 min at 90°C in a thermostatic bath. Then, samples were cooled at room temperature, and 20 g of cremogenate were added. In order to provoke the calcium-induced gelation, the suspensions were molded using cylindrical acrylic molds (1 cm height and 2.6 cm diameter) pulverized with calcium lactate gluconate solution of 10 g/100mL (food grade, Jungbunzlauer, Germany). Calcium lactate gluconate (CLG) solution was used due to displays high bioavailability, neutral taste and is highly soluble among the calcium salts commonly used for mineral enrichment. Finally, the samples were submitted to the drying process at 30ºC during 29 h, being this condition optimized through preliminary tests. Fig. 1 shows a representative scheme of the steps involved in the obtaining process of jelly beans.

C. Study of the interaction between the ingredients of jelly beans formulation using composite model systems

In order to study the individual and combined effects of the ingredients, rheological analysis using different model systems were performed. Blends of AS, sugar and pectin, in the concentrations previously described, with and without addition of cremogenates were analyzed (Table 1).

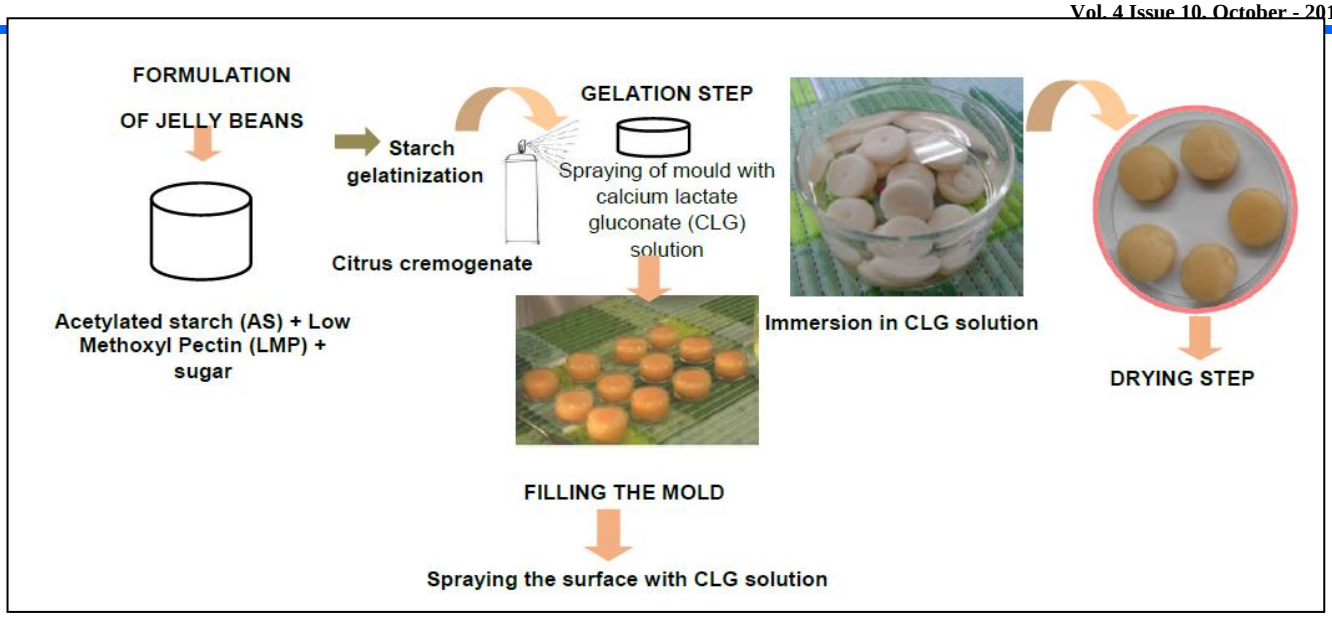

Fig. 1. *Representative scheme of the steps involved in the obtaining process of jelly beans.*

Furthermore, the effect of addition of gelling agent (calcium) on the rheological properties of the system was studied.

The linear viscoelastic properties of the samples with and without cremogenate were studied with controlled stress rheometer RheoStress 600 ThermoHaake (Haake, Germany) using rough plate-plate geometry (3 cm diameter). A gap of 1 mm was employed. Before measurements were taken, samples remained between the plates during 3 min for equilibration. Then tests were conducted at 20°C.

TABLE I. COMPOSITE MODEL SYSTEMS PREPARED TO STUDY THE INTERACTION BETWEEN INGREDIENTS OF JELLY BEANS FORMULATIONS

Model systems	AS	Sugar	LMP	Cremogenate	Water	Ca^{+2}
MS0	X	X	X			
MS1	X	X	X		X	
MS2-O	X	X	X	X		
MS2-G	X	X	X	X		
JB-C	X	X	X		X	X
JB-O	X	X	X	X		X
JB-G	X	X	X	X		X

Nomenclature used: MS0: model system corresponding to the blend of hydrocolloids and sugar gelatinized; MS1: model system corresponding to the blend of hydrocolloids and sugar gelatinized with water addition to a final concentration of 20% w/w; MS2 model system corresponding to the blend of hydrocolloids and sugar gelatinized with cremogenate (O: orange and G: grapefruit) addition to achieve a final concentration of 20% w/w; JB-C: jelly bean control (without cremogenate but with calcium solution addition to induce the gelation), JB-O: jelly bean containing orange cremogenate, JB-G: jelly bean containing grapefruit cremogenate.
AS: acetylated starch 13% (w/w), sugar: 35% (w/w), water: 20% (w/w), cremogenate: 20% (w/w), LMP: low metoxyl pectin: 32% (w/w), Ca^{+2}: calcium lactate gluconate: 10% (w/v).

Dynamic assays were performed to characterize the viscoelastic behavior of the formulations. In the first step, a stress sweep (0.001–100 Pa) was made to determine the linear viscoelasticity range at constant frequency (1 Hz). Then, frequency sweeps (0.01–100 Hz) were performed at constant stress. Dynamic rheological parameters recorded were: storage modulus (G'), loss modulus (G''), tangent of the phase angle (tan δ = G''/G') and complex shear stress (G*). All rheological assays were performed at least by triplicate.

D. Physicochemical characterization and quality attributes of jelly beans

Humidity content and water activity

Water activity (a_w) of the samples before and after the drying process was measured at 25°C with a Water Activity Meter Aqualab series 3 (Decagon Devices Inc., Washington, USA).

Humidity content was gravimetrically quantified in a vacuum oven (DZF-6030A, ICSA, Argentine) at 500 mbar and 60°C, until reaching constant weight. The results were expressed as percentage g of water/100g sample. In both cases the average of three determinations, was informed.

Color

Surface color before and after the drying process was measured using a Chroma Meter CR 400 (Konica Minolta Sensing, Japan) as was previously described. Besides, color differences (ΔE) were calculated considering the chromaticity parameters of samples before drying process (a_0*, b_0* and L_0*):

$$\Delta E = \sqrt{((a* - a_0*)^2 + (b* - b_0*)^2 + (L* - L_0*)^2)} \quad (1)$$

The average of at least five determinations of each formulation tested, performed in two independent experimental batches, was informed.

Texture

Jelly beans texture profile analyses (TPA) were performed in a TAX T2i Texture Analyzer (Stable Micro Systems Ltd, Godalming, Surrey, UK). Samples were

compressed to 30% between flat plates employing a 75 mm diameter aluminum probe (P75). The test consisted of two successive compression cycles with a rest period in between; thus, it attempts to simulate the chewing process. The measurement conditions were as follows: speed pre and post-test to 5 mm s^{-1}, test speed 1 mm s^{-1} and time between cycles 5 s. Data were processed with the Texture Expert software. Hardness, springiness, cohesiveness, gumminess and adhesiveness were quantified [12]. The average of six determinations, performed in two independent experimental batches, was informed.

Sensory evaluation

A sensory panel was performed to evaluate general acceptability of jelly beans. Sensory tests were carried out with a 50-member panel, conformed of frequent and non frequent consumers of this kind of product.

Panelists group consisted of 33 female and 17 male aged between 25 to 55 years old. Samples were randomly coded with three digit numbers and placed in trays; they were presented to the evaluators randomly arranged and coded with three-digit numbers. Drinking water was provided as neutralizing agent for rinsing the mouth between samples. The evaluators were instructed to examine first, the attributes of a sample, and then just the other. The attributes analyzed were overall acceptability, color, texture, and flavor according to a box-scale (1-9) anchored in the following steps: 'dislike extremely', 'indifferent' and 'like extremely'. For each one of these attributes, the average response of panelists was reported.

E. Nutritional contribution

To determine the calcium content, samples of cremogenates and jelly beans were poured into porcelain crucibles and ashes were obtained in a muffle at 550°C as was described previously. Ashes were dissolved in HCl 2 mol/L and measured by atomic absorption spectrometry, with $LaCl_3$ (as interference suppressor), by using an atomic absorption spectrophotometer AAnalyst 100 (Perkin Elmer, Japan), air-acetylene flame, 0.7 nm slit, and 422.7 nm wavelength. Duplicates were run with each set of samples. Mean values of calcium content (expressed as g Ca^{+2}/100g ash) were reported.

To perform the calibration curve, standard solutions of Ca^{+2} (5-50 mg/L) were prepared from a 100 mg/L stock solution of $CaCl_2$, prepared with ultrapure water (Milli-Q plus). Determinations were made from the ashes of the samples obtained after 29 h of drying, as was previously described. The ashes were dissolved with HNO_3 (1: 1) 10 g/100mL, bringing to final volume of 100 ml with Milli-Q water. The determinations were performed in triplicate.

For Ascorbic acid (AA) quantification samples were previously frozen at 80°C, in order to prevent the vitamin degradation. Afterwards, thawed and crushed samples (about 0.5 g) were mixed with 2.5 ml of 5 g/100mL aqueous solution of metaphosphoric acid (Sigma Aldrich, USA) and were centrifuged during 15 min at 12.000 rpm (Centrifuge 5415D, USA). The supernatant was separated and filtered on 0.45 µm Millipore membranes (Millipore Corporation, France) and finally injected into the HPLC equipment. The same procedure than that performed for cremogenate characterization was used.

F. Statistical analysis

The InfoStat Software (Version 2008) (InfoStat Group, Argentine) was used. Analysis of variance (ANOVA) and comparison of means with the Fisher's least significant difference (LSD) test were conducted, at a significance level p = 0.05.

III. Results and Discussion

A. Cremogenate obtaining and characterization

Total soluble solids (TSS) and titratable acidity (TA) are the most frequently chemical parameters tested in both fruits and derived juices and are considered as critical indicators of sensory quality [13].

TABLE 2. PHYSICOCHEMICAL PROPERTIES OF GRAPEFRUIT AND ORANGE CREMOGENATES

		Orange cremogenate	Grapefruit cremogenate
pH		4.2 (0.4)[a]	3.3 (0.1)[b]
Titratable acidity % (w/v citric acid)		0.6 (0.04)[a]	2.7 (0.01)[b]
Total soluble solids (ºBrix)		10	9
Ash content (%)		0.46 (0.06)[a]	0.62 (0.003)[b]
Ascorbic acid content (mg/100ml)		98.9 (1)[a]	72.2 (0.9)[b]
Citric acid content (mg/100ml)		1.27 (0.06)[a]	4.85 (0.071)[b]
Calcium content (mg/100g)		50.3 (0.05)[a]	51.8 (0.1)[b]
Yield (% based on fresh whole fruit weight)		31.47[a]	38.85[b]
Color parameter	L*	54.82 (1.1)[a]	46.76 (0.44)[a]
	a*	-0.46 (0.19)[a]	7.47 (0.26)[b]
	b*	27.23 (1.78)[a]	13.24 (0.35)[b]
	C*	27.24 (1.78)[a]	15.2 (0.41)[b]
	h	90.97 (0.39)[a]	60.58 (0.59)[b]

Informed values correspond to the average obtained ones and standard deviations are indicated in brackets. Different letters within the same row indicate significant differences ($p<0.05$).

The grapefruit and orange cremogenates showed TA values of 2.7 and 0.6%, respectively (Table 2). Schvab, Ferreyra, Gerard, and Davies [14] reported similar citric acid content for samples of orange juice. Likewise, citric acid corresponds to 80-95% of total acids in these fruits.

Ascorbic acid content of orange and grapefruit cremogenates were 72.2 and 98.9 mg AA/ 100mL, respectively. These results suggest that selecting fruits are a rich source of a functional component such as ascorbic acid, with the added asset of providing characteristic fruit components, such as color and flavor.

Total soluble solids of fruit juices consist mainly of sugars (80-85%), being sucrose, glucose and fructose

the most abundant in proportions 2: 1: 1 [15]. Citric acid and other acids and their salts, nitrogenous compounds and other soluble, such as water-soluble vitamins constitute the remaining solid composition [15]. Thus, the ºBrix of citrus juices indicates all existing soluble solids therein; not only the sugars present.

Concerning to the cremogenates surface color, the high values of the b* parameter in the orange cremogenate is attributed to the high carotenoids content in this kind of derivative, being this parameter statistically ($p<0.05$) higher than the corresponding to grapefruit cremogenate (Table 2). In the last case, positive values of a* and b* parameters were obtained because of the yellowish-red characteristic given by the presence of pigments such as lycopene in addition to carotenoids [16].

B. Ingredients interaction and gelling mechanism in the jelly beans

The proposed model systems (Table 1) allowed to analyze the individual and combined effects of the different ingredients in order to evaluate their contribution to the structural matrix development.

Acetylated starch was completely gelatinized even in the presence of pectin and sugar. However, the rheological behavior of MS0 corresponded to that of a concentrated viscous solution with G" practically coinciding with G' across the analyzed frequency range (Fig. 2a). Meanwhile, water addition (MS1) caused the typical dilution effect on the observed behavior (viscous solution), approaching G' values to G" ones at frequencies higher than 10 Hz (Fig. 2a).

From stress sweeps was determined the linear viscoelastic range extended up to 100 Pa in which the viscoelastic moduli was independent of the oscillation stress for both formulations, showing the strength of the developed structure. In this regard, Steffe [17] established that strong gels may remain in the linear viscoelastic region over greater strains than weak gels.

The mechanical spectrum obtained from the frequency sweep was used to determine the difference between entanglement networks. The addition of citrus cremogenates led to systems exhibiting typical gel behaviors (Fig. 2b). The differences observed in the rheological behavior of both systems (MS2-O and MS2-G), containing different types of cremogenates, could be associated to their different composition, especially pectin, citric acid, pH and micronutrients. According to Agudelo, Varela, Sanz, and Fiszman [3] these results could be attributed to the greater concentration of solids in the formulation and also to the increase in the pectin content due to the fruit. Additionally, Fraeye, Duvetter, Doungla, Van Loey, and Hendrickx [18] explained that the pectin present in fruits and vegetables is responsible to provides cell-cell adhesion and gives the tissues mechanical strength due to crosslinking in the cell wall. Citrus peel is a rich source of bioactive molecules and its albedo layer is a good source of pectin [19]. Moreover, the addition of grapefruit cremogenate increased the G' values (Fig. 2b). This trend could be attributed to the higher pectin content of grapefruit compared to orange considering that the flavedo and albedo of citrus fruits contain the greatest amounts of pectin [20]. Likewise, this result draws the attention taking into account the citric acid content of the cremogenate, since it has been reported that it is capable of hydrolyzing the starch [21].

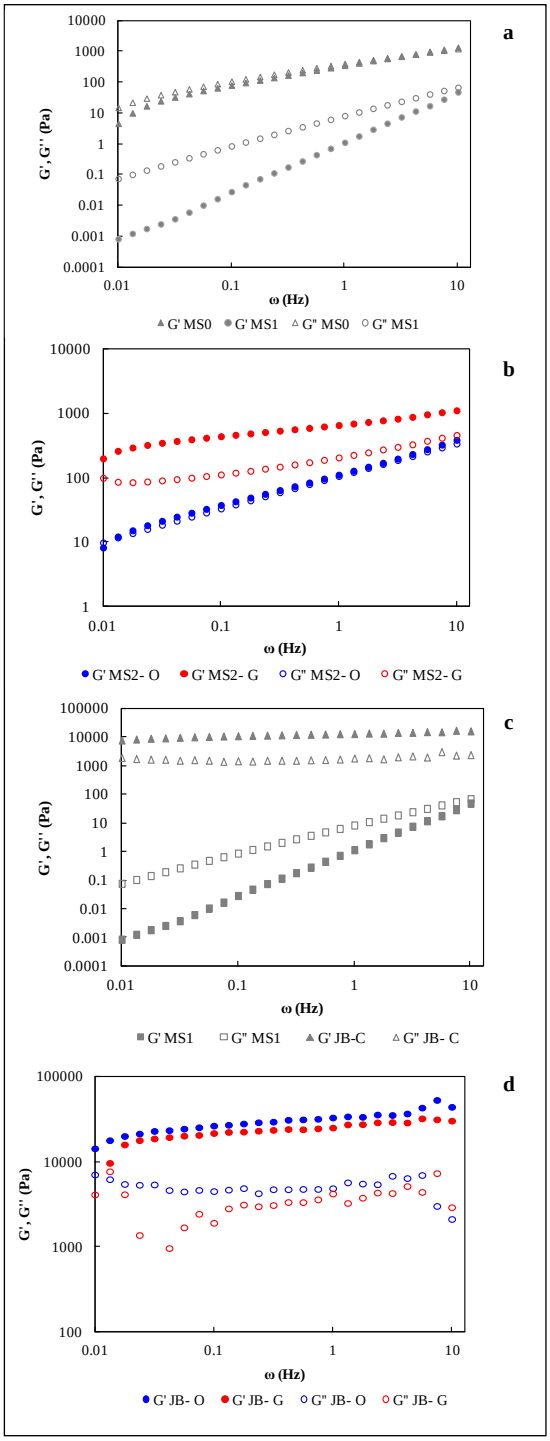

Fig. 2. *Mechanical spectra of different model blend systems: a) MS0 and MS1; b) MS2-O and MS2-G; c) MS1 and JB-C and d) JB-O and JB-G. Full symbols corresponds to G` curve and empty symbols to G" one. Nomenclature used is described in Table 1.*

As expected, a considerable difference in structure was observed between samples with and without

calcium addition (MS1 and JB-C, Fig. 2c); this behavior was explained considering the low G' value. In the model systems without cremogenate (JB-C), where the gelation was induced with calcium lactate gluconate, the samples showed a gel behavior, with G' significantly ($p<0.05$) greater than G''. The G' and G'' modulus were frequency independent for the frequency sweep window studied (0.01-10 Hz). Low methoxyl pectin gelation is due to the formation of intermolecular junction zones between homogalacturonic smooth regions of different chains; the structure of such a junction zone is generally ascribed to the so called "egg box" binding process. The ionic interaction between Ca^{+2} and free carboxylic groups of pectin molecules supplements the hydrogen bonding in acid-induced gelation thereby strengthening the gel network. As it is well known, the ability of pectin solutions to aggregate or form gels is determined by two main chemical parameters: the number of successive negative cavities required to form a junction zone, and the relative amount of calcium to pectin present in the system. Initial strong association of two polymers into a dimer is followed by the formation of weak inter-dimer aggregation, mainly governed by electrostatic interactions [1,22,23,24]. In this sense, the tan δ values reflected increased viscoelasticity when calcium was present. Likewise, a synergic effect between pectic, calcium and sugar has been reported [25].

The mechanical spectra for the systems containing orange (JB-O) and grapefruit (JB-G) cremogenates are presented in Fig. 2d, showing a significantly increased in the values of both moduli compared to systems without calcium. Although the addition of pectin and calcium to systems containing fruit improved the structure of the prepared gels, it would be rash to assume that interactions as complex as those at cellular level could be established. Carbonell, Costell, and Duran [26] reported that the effects of adding pectin and solids on certain rheological properties of strawberry and peach jams depended on the fruit content and its interaction with other formulation-related factors (pectin and sugar concentration).

The mechanical spectra of the blend systems were mathematically fitted to the Power law model [17]:

$$G' = a\,\omega^{b} \qquad (2)$$

$$G'' = c\,\omega^{d} \qquad (3)$$

where ω is the frequency expressed in Hz and *a*, *b*, *c* and *d* are the fitting parameters. The proposed equations fitted satisfactorily the variation with frequency of both: G' (r^2> 0.9452) and G" (r^2>0.8224) moduli. The values of the fitting parameters are shown in Table 3.

Parameters *a* and *c* values reflect the tendencies previously described. Thus, for MS1 the corresponding values were lower to MS0 indicating the dilution effect. When cremogenate was added, *a* and *c* parameters increased while b and d decreased, evidencing the gel-type behaviors (MS2-O and MS2-G). As can be expected calcium addition led to the development of a strong gel structure correlating with the higher *a* and *c* parameters values and the lower b and d ones (Table 3). According to Khondkar, Tester, Hudson, Karkalas, and Morrow [27] the *b*-value is related to the strength and nature of the gel, being *b*= 0 for a covalent gel, whereas for a physical gel *b*>0. Besides, these authors stressed that b values closer to 0.1, like those of JB-C, JB-O and JB-G, indicates that considerable crosslinking had taken place.

C. Physico-chemical characterization and quality attributes of the obtained jelly beans

Fig. 3a shows the jelly beans obtained before and after the drying process. This step was necessary in order to reduce the humidity content and to reach a final water activity that allows the product safety and stability preservation.

After drying process, during 29 h at 30°C, the samples aw experienced a 20% reduction reaching values for both formulations of 0.734 ± 0.004 slightly higher than those of commercial products (average 0.71) and similar to that informed by Delgado and Bañón [28].

TABLE 3. VISCOESLASTIC BEHAVIOR OF COMPOSITE MODEL SYSTEMS AND JELLY BEANS FORMULATIONS: PARAMETERS OF THE MATHEMATICAL MODELS

	Storage modulus G' (Pa) $G' = a\,\omega^{b}$			**Loss modulus G" (Pa) $G'' = c\,\omega^{d}$**		
	Correlation coefficient (r^2)	**Parameter a (Pa s^b)**	**Parameter b**	**Correlation coefficient (r^2)**	**Parameter c (Pa s^d)**	**Parameter d**
MS0	0.9997	347.4 (0.7) ^{c}	0.598 (0.001) ^{b}	0.9994	390.2 (0.2) ^{d}	0.51 (0.005) ^{b}
MS1	0.9996	1.1 (0.2) ^{a}	1.6 (0.1) ^{c}	0.9995	8.2 (0.1) ^{a}	0.93 (0.01) ^{c}
MS2- O	0.9999	110.35 (2.5) ^{b}	0.54 (0.02) ^{b}	0.9992	106.2 (0.49) ^{b}	0.50 (0.005) ^{b}
MS2- G	0.9953	678.2 (2.9) ^{d}	0.232 (0.007) ^{a}	0.9924	213.9 (2.5) ^{c}	0.33 (0.03) ^{a}
JB- C	0.9882	13100 (28.3) ^{e}	0.0917 (0.004) ^{d}	0.8224	4088 (186.9) ^{f}	0.12 (0.03) ^{d}
JB- O	0.9288	34300 (100) ^{g}	0.129 (0.001) ^{e}	0.9040	584.3 (0.5) ^{e}	0.12 (0.002) ^{d,e}
JB- G	0.9452	26300 (382) ^{f}	0.10 (0.01) ^{d,e}	0.9452	3746 (88) ^{f}	0.19 (0.03) ^{e}

Informed values correspond to the average obtained ones and standard deviations are indicated in brackets. Different letters within the same column indicate significant differences ($p<0.05$). Nomenclature used is described in Table 1.

Bearing in mind, is important to consider that a low water activity gives a microbiological safety that ensures stability and self-preservation of the food product, based on preventing the development of chemical reactions responsible for the deterioration and microorganisms that are potential threats to consumer health.

To achieve stabilization of the jelly beans texture the drying step was carried out. The drying effect was also reflected in the humidity content of jelly beans experienced a reduction of 62% regardless the cremogenate used, resulting in a product with higher solids concentration. The drying kinetics of the developed product containing grapefruit cremogenate is shown in Fig. 3b.

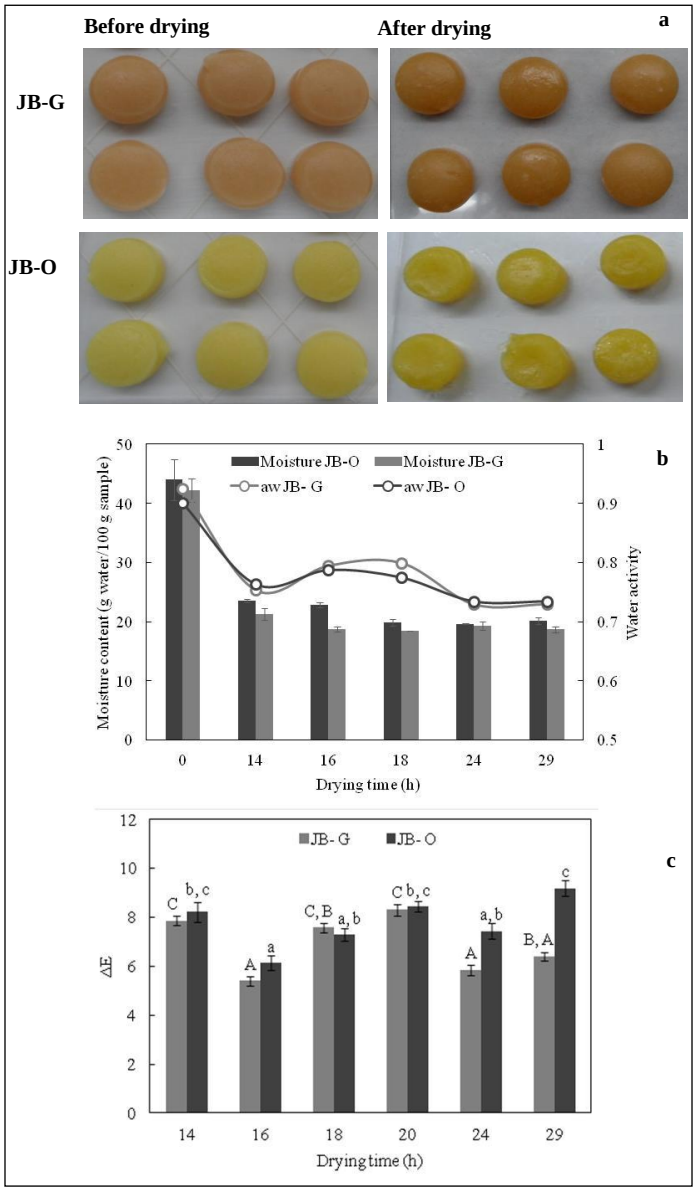

Fig. 3. *a) Photograph of the jelly beans obtained before and after the drying process. JB-G: jelly beans with grapefruit cremogenate and JB-O: jelly beans with orange cremogenate; b) drying kinetics and c) color differences (ΔE) as a function of drying time of developed jelly beans containing grapefruit (JB-G) or orange (JB-O) cremogenates.*

Likewise, after drying step the developed jelly beans presented a volume reduction of 50%, this observation was independent of the cremogenate used. No significant ($p<0.05$) variations were detected after 20 h drying; this behavior was also informed by other authors [28].

As expected, jelly beans presented the typical fruit cremogenate color included in the formulation, which was intensified after drying (Fig. 3a and c). This visual observation is reflected in the increased values of color differences (ΔE), being the changes in b* parameter the largest contributor to this difference. Since values of a* parameter were closer to zero, Chroma follows a similar trend than b* chromaticity parameter. Overall, after 20 h drying no significant difference ($p>0.05$) in the product color differences were observed. Drying step significantly ($p<0.05$) affected the luminosity of samples containing both orange and grapefruit cremogenate, reducing it by about 9%.

The texture properties used to monitor changes in jelly beans are shown in Fig.4a. With regard to the parameters of texture, TPA profiles of jelly beans containing orange or grapefruit cremogenates before and after drying are shown in Fig. 4a. The differences observed in the mechanical profiles of both systems containing different types of cremogenates, could be associated to their different composition, especially pectin, citric acid, pH and micronutrients. According to Agudelo, Varela, Sanz, and Fiszman [1; 3] these results could be attributed to the greater concentration of solids in the formulation and also to the increase in the pectin content due to the fruit. Both hydrocolloids contribute to jelly beans matrix development. Moreover in calcium presence, LMP gelation is due to the formation of intermolecular junction zones between homogalacturonic smooth regions of different chains; the structure of such a junction zone is generally ascribed to the so called *"egg box"* binding process [3, 22].

The hardness was the most affected parameter, since as can be expected drying process led to an increase in the product solid content (Fig.4b). A similar trend was reported by Delgado and Bañón [28] working on jelly gummies. TPA can be used as a quality control tool to monitor the drying time and to estimate the required time for structural stabilization, which was 24 h in orange and grapefruit formulations.

The combined actions of gelling agents (AS and LMP in presence of calcium) on the colloidal system containing sugar, water and other components resulting from cremogenates, constitute a typical firm and chewy structure of gelled candies. Similar results were informed by Delgado and Bañón [28] and Burey, Bhandari, Rutgers, Halley, and Torley [29].

To the best of our knowledge, scarce information on TPA in jelly beans is available in the literature and are dependent on testing geometries [29]. Besides, data comparisons are difficult since in this type of confectionery products, intended mainly for children, the form is an attribute subject to design.

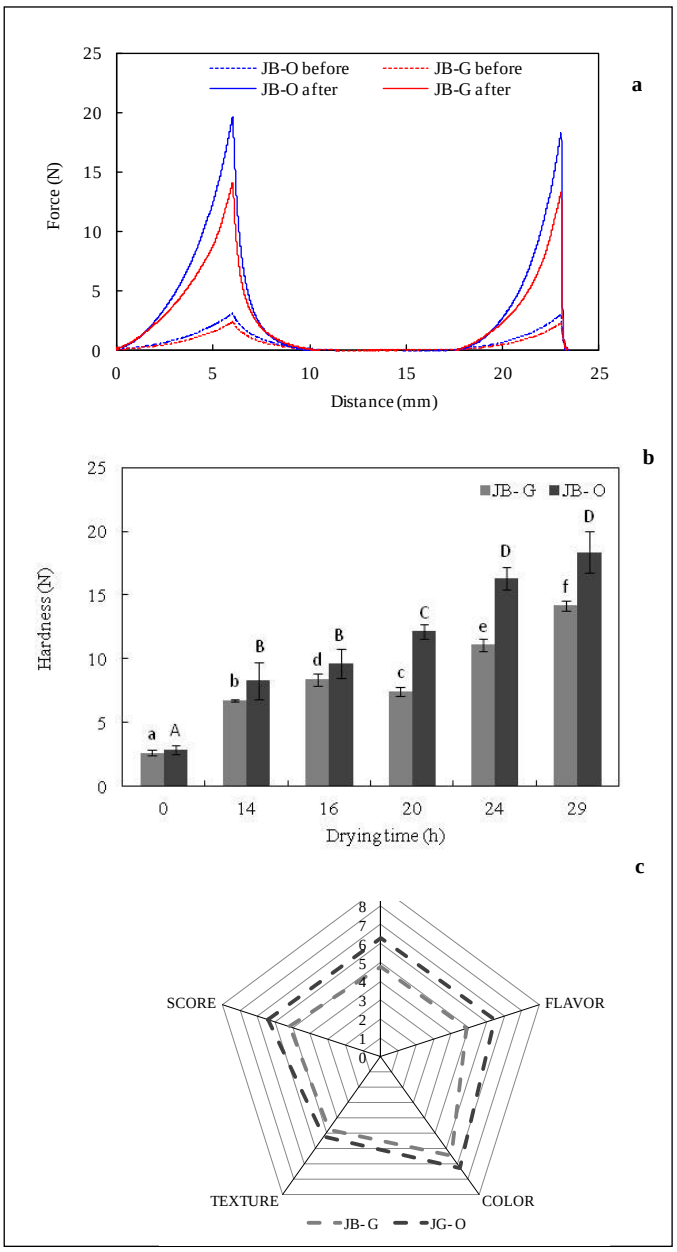

Fig. 4. *a) Texture profile analysis (TPA) curves before and after the drying step; b) hardness as a function of drying time of jelly beans containing grapefruit (JB-G) or orange (JB-O) cremogenates and c) Sensory evaluation of jelly beans: average values of the analyzed attributes (color, texture, flavor and acceptability) as well as the average score obtained.*

Although, cremogenate type significantly ($p<0.05$) modified jelly beans hardness, obtaining the higher values for formulations containing orange one (Fig. 4b). Moreover, developed jelly beans showed no adhesiveness, both formulations were found to be completely elastic (springiness = 1). In candy gums, springiness is the distinctive property which determines the sensory attributes of this kind of products and it mainly depends on the quality and proportion of the different ingredients and additives included in the formulation. Changes in other relevant texture descriptors particularly applicable to gelled confections were also evidenced since chewiness, gumminess and cohesiveness increased after drying (Table 4). These observations can be explained by a combined effect of gelation mechanism and dehydration.

The obtained behavior from small deformation tests involved dynamic oscillatory rheometry are in close agreement with those found from large deformation test (TPA). According to Saha and Bhattacharya [4], the rheological characterization of the sample correlates to the textural attributes of the food, which determines its sensory characteristics and consumer acceptability.

D. Nutritional contribution

The content of ascorbic acid evaluated by HPLC of freshly prepared jelly beans was 19.99 and 18.33 mg of AA per 100g of dry matter for samples containing orange and grapefruit cremogenates, respectively. After drying the AA content of JB-O was 14.79 mg of AA per 100 g of dry matter experienced a reduction of 26%. Meanwhile, the AA content of JB-G was 16.85 mg of AA per 100g of dry matter being in this case the reduction 8%. This result could be explained considering the protective effect of developed matrices combined with the proper active compounds of each cremogenate.

According to Argentinean normative CAA [30] the recommended daily intake (RDI) of Ascorbic acid for children aged 1 to 9 years is in the range 30 to 35 mg, depending on their age. 8-10 gummies consumption (considering a weight of 4.25 g per sample unit) covers 20% of this requirement, so according to the CAA this product could be considered within the category of fortified foods. Meanwhile, the calcium contents in the cremogenates were similar, being the obtained values 50.3 and 51.8 mg per 100 g cremogenate for grapefruit and orange, respectively.

TABLE 4 TEXTURE PROFILE ANALYSIS PARAMETERS OF JELLY BEANS FORMULATED BEFORE AND AFTER THE DRYING PROCESS

Jelly bean formulation		Hardness (N)	Cohesiveness (dimensionless)	Gumminess (N)	Chewiness (N)
JB- O	before	2.9 ± 0.3 ^{a}	0.577 ± 0.005 $^{a, b}$	1.7 ± 0.2 ^{a}	1.7 ± 0.2 ^{a}
	after	18.1 ± 2.1 ^{c}	0.595 ± 0.006 ^{c}	5.9 ± 0.6 ^{b}	5.9 ± 0.6 ^{b}
JB- G	before	2.6 ± 0.2 ^{a}	0.575 ± 0.001 ^{a}	1.5 ± 0.1 ^{a}	1.5 ± 0.1 ^{a}
	after	14.2 ± 0.4 ^{c}	0.584 ± 0.003 ^{b}	8.3 ± 0.3 ^{c}	8.3 ± 0.3 ^{c}

Nomenclature used: JB-O: jelly bean containing orange cremogenate, JB-G: jelly bean containing grapefruit cremogenate. Informed values correspond to the average obtained ones ± the standard deviation. Different letters within the same column indicate significant differences ($p<0.05$).

Meanwhile the calcium content in jelly beans were 191.9 and 171.9 mg Ca /100g sample for those obtained with orange and grapefruit cremogenates, respectively. These results demonstrate that during the chemical gelation of pectin, Ca^{+2} is incorporated in the matrix, allowing the development of healthy products. In this regard considering that according to the CAA Ca RDI for children 1 to 9 years is in the range between 500 and 700 mg, depending on their age, 12-14 jelly beans consumption (considering a weight of 4.25 g per unit jelly beans) cover 20% of this requirement, so according to the C.A.A this product could be considered within the category of fortified foods.

Taking into account the international specifications for these micronutrients, the Codex Alimentarius Commission [31], through the Codex guidelines define a set of Nutritional Reference Values for this population segment considering 60 mg and 800 mg/100 g of product for Ascorbic acid and calcium, respectively [32].

E. Sensory evaluation

After all, sensory quality conditioned the product consumers' acceptance. The results indicated that the formulations containing orange cremogenate obtained higher scores for attributes tested than those of grapefruit (Fig. 4c). With regard to general acceptability, 50% of the panellists evaluated it with scores between 4 and 6; while respect to taste, 54% preferred formulations containing orange.

Fig. 4c shows the results for color attribute of jelly beans and again noted that formulations containing orange cremogenate received higher scores than those of grapefruits. Sensory evaluation of color has the decisive importance as it is prior to other sensory parameters and therefore can be exclusive (33). In the evaluation, samples presented a rated "like extremely" color directly associated with citrus fruits from which they derive.

One of the most important parameters that determined the consumer acceptability is the product texture. However, the best assessment of the texture will be through the sensations in the mouth. Panellists qualified the jelly beans texture as "regular", no significant differences ($p>0.05$) were detected between formulations containing different cremogenates.

Regarding to general acceptability, the average score obtained were 6.84±1.6 for orange formulations and 5.72±2.1 for grapefruit ones. Frequent consumers of jelly beans are panellists between 20-30 years of age, who have accepted the developed products regardless the flavor. It was also observed that both men and women qualified formulations containing orange cremogenate better than those containing grapefruit one.

Finally, in recent years studies related to the well-being or emotional attributes that can be obtained from sensory tests have begun to become relevant. It is generally recognized that human eating choices are affected by and associated to emotions [34]. Within this context, jelly beans have been described as a colorful, soft, rich, fresh, fruity, citrus and novel sweet candy. Likewise, panellist also pointed out that they perceived "the true flavor of the fruit" as a product distinctive characteristic.

IV. Conclusions

The design of functional system based on blend of hydrocolloids formulations functionalized with active principles extends the possibilities of use of cremogenates as a source of bioactive compounds, minerals, and vitamins. Thus, it was possible to develop healthy treats based on grapefruit and orange cremogenates and hydrocolloid mixture.

Viscoelasticity studies confirmed the type-gel structure of the formulated systems. Likewise, rheological results correlated with TPA profiles of jelly beans. Jelly beans TPA profiles allowed to monitor the drying time and to estimate the required time for structural stabilization, which was 24 h in orange and grapefruit formulations, being hardness the most affected parameter.

Composite developed systems have a protective effect of ascorbic acid avoiding thermal degradation exhibiting a slight reduction during the drying step especially in the case of samples containing grapefruit cremogenate.

The innovative nature of this research is to be highlighted since no similar products are available on the market, offering new flavor like grapefruit, without the addition of additives such as flavorings and colorings. The results of the current research reveal that the developed jelly beans emerge as healthy alternatives and had a good global acceptability by consumers. The functionalization of the matrix with calcium, involved in the chemical gelation mechanism of pectin, can incorporate this mineral in the final product, being this relevant from a nutritional point of view.

Conflict of interest statement

We declare that we have no conflict of interest.

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