Development of healthy gummy jellies containing honey and propolis

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Abstract

BACKGROUND: The objective of this work was to develop gummy jellies containing honey and propolis, keeping the natural antioxidant principles of the beehive ingredients, and satisfying the consumer´s requirements.

RESULTS: A gummy jelly containing honey and propolis (HPGJ) was developed. A sensory study with consumers (n= 74) performed an intensity level evaluation test analyzing: color, hardness, adhesiveness, gumminess, sweet taste and honey taste. A penalty analysis indicated that HPGJ was too hard. However, in the global acceptance study more than 90% of consumers gave liking categories.

Regarding the potential functional properties, the antioxidant capacity (AC) of HPGJ was 8.17 ± 0.55 mmol eqTrolox/kg, and up to 40% AC was retained after *in vitro* digestion. Additionally, AC of HPGJ was up to 10 times higher than that of similar commercial products. A storage study at 25°C showed that color and AC significantly increased along 90 days due to the development of Maillard reaction. Storage under darkness allowed keeping low values of global color change up to 45 days. Another positive facet was that the addition of propolis delayed the fungal growth during storage.

CONCLUSIONS: An organoleptically palatable gummy jelly was obtained. Among the positive features, it showed higher AC than similar commercial candies. Additionally, HPGJ offered a high bioaccesible AC input detected upon *in vitro* digestion. Overall, HPGJ could be considered an interesting, appetizing and healthier alternative to regular gummy jellies available in the market. Adequate packaging should be considered in order to extend HPGJ shelf life, reducing browning reactions.

Keywords: Gummy jelly; propolis; honey; antioxidant activity; sensory evaluation.

1. Introduction

The accelerated rhythm of modern life causes an impact on eating habits, whose negative effects on health have been recognized. Food is related to gratification, so the compromise between pleasure and health is a dilemma(1). Considering this, in the last years functional foods have gained importance and the search of knowledge and technologies oriented to these products has increased. Additionally, there is a strong trend of the market to choose natural products and select those with some specific beneficial function for the human body. In this context, the challenge for the candy industry is to develop products incorporating healthy components and reducing the sucrose input. Rodríguez-Zevallos *et al.* (2) developed gummy candies replacing sucrose for honey, seeking to decrease the high glycemic index of the product. Also, tea extracts were added to jelly candies, with the purpose of incorporating polyphenols (3). In this sense, beehive products like propolis and honey are promising ingredients for the formulation of nutritive and functional foods (4).

Propolis is a resinous substance prepared by bees by mixing the resin obtained from plants with their salivary secretions. Propolis composition changes according to the geographical location and the botanical origin (5). Several health related properties of propolis have been worldwide studied, antiviral, anticancer and antitumor properties (6). There are numerous reports indicating that propolis has antioxidant, antibacterial and antifungal properties. Regarding antioxidant activity, Osés *et al*. (7) informed an increase in this property in honey enriched with propolis extracts. Also, Spinelli *et al*. (8) reported an enhancement of the antioxidant properties of fish burgers with the addition of microencapsulated propolis. Considering the antibacterial activity of propolis extracts, it was studied against several bacterial strains (9,10). Also, Miorin *et al*. (11) studied the antibacterial activity of different propolis and reported that the minimum inhibitory concentration for *S. aureus* was 0.36 – 3.65 mg of propolis resin/ml of sample. Another possible application of propolis extracts, proposed by Feas *et al*. (12)

was their use as alternative products for lettuce disinfection, reaching a high efficiency using 20 g/kg EEP.

Honey also has a set of properties that profile it as a captivating raw material for the production of food products. It is known that honey is constituted by a variety of bioactive compounds which, either by themselves or by interaction with other compounds, confer antioxidant properties(13).

The aim of this work was to develop healthy and palatable gummy jellies containing honey and propolis with high antioxidant capacity.

2. Materials and methods

2.1. Materials

Propolis was gently donated by Apícola de Gualeguaychú cooperative (raw propolis was collected from apiaries located in Gualeguaychú, Entre Rios, Argentina). Gummy jellies ingredients (all food grade) were: a) honey (Gala®, Argentina), and b) solid components: unflavored gelatin (Royal®, Argentina), citric acid (RZBC®, China), and stevia (Dulsevia®, Argentina) having a stevia:maltodextrin ratio=10:90.

Commercial products (from the local market of Gualeguaychú, Argentina): gummy candies: Docigoma® (multi fruit flavor:Brand 1), Mogul® (multi fruit flavor: Brand 2), Tembly® (multi fruit flavor:Brand 3); hard candies: Arcor® (honey: Brand 4), Monaca® (propolis: Brand 5); Nutrabit® (propolis: Brand 6); and Monaca® (honey and blueberry: Brand 7).

2.2. Propolis ethanol extracts (EEP)

2.2.1. Extracts preparation

Six extracts were obtained by weighing 5g of raw propolis, mixing with 50 ml of extraction solution (aqueous solutions containing ethanol 70, 80 or 90% v/v), and stirred for 30 min at 30 or 40°C. The liquid was separated and the previous process was repeated with the solid residue. This procedure was repeated 4 times. Next, the

liquid portions were mixed, refrigerated for 72 hours at 4°C and filtered to separate the waxes.

2.2.2 Solid residues of extracts

The extracts were dried at 110ºC (14), and the results were expressed as g of residue per kg of extract. Measurements were made in quintuplicate and reported as the mean ± the standard deviation.

2.2.3 Antioxidant capacity

The antioxidant capacity was determined by TEAC (Trolox Equivalent Antioxidant Capacity) assay according to the procedure proposed by Archaina *et al.* (15), using the 2,2´-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] to produce the cationic free radical $ABTS^{\bullet+}$. A Trolox (Sigma-Aldrich) stock solution 4 mM was used to construct a calibration curve in a range between 2 and 12 mg/ml of Trolox (r^2 = 0.9920). The results were expressed as equivalent mmol of trolox per kg of propolis extract. All measurements were performed in quadruplicate and the results were reported as the mean ± standard deviation.

This measurement was also applied to a 1/25 dilution of the developed gummy jelly, and 1/25 dilution of the soluble fraction obtained after *in vitro* digestion (described in 2.10 section), the results were expressed as mmol eq Trolox/kg of gummy jelly. For the determination of antioxidant capacity on the insoluble fraction from the *in vitro* digestion (described in 2.10 section), the QUENCHER procedure reported by (16) was performed with some modifications. 1.00 mg of lyophilized fraction was added to 10 ml of ABTS and the mixture was vortexed for 2 min and centrifuged at 12,300 G-force for 3 min to facilitate the reaction of the surface with the ABTS reagent. After 30 min, the absorbance was measured at 734 nm. The results were expressed as mmol eq Trolox/kg of gummy jelly.

2.3. Gummy jellies preparation

The preparation procedure was as follows: 1) mixing of the solid components (see 2.1 section); 2) addition of EEP; 3) dissolution of solids in the extract at 100°C for 10 min with shaking; 4) addition of honey and shaking for 5 min at 100°C; 5) molding in silicon molds, and storing at 25°C for 5 hours; 6) unmolding and packing in hermetic transparent bags. The ingredients were employed at different proportions, obtaining five different formulations. The components and the used ranges (g/kg of HPGJ) were: unflavored gelatin (116 - 128), stevia powder (9 - 10), citric acid (5), honey (525 - 704) and the selected propolis extract (73 or 80).

2.4. Ethanol determination

The residual ethanol was determined using the method proposed by Tirado *et al.*(17), with some modifications. 50g of sample were distilled with 200 ml of water. The residual ethanol recovered was brought to volume with distilled water in a 50 ml graduated flask and then filtered through a 0.22 micron nylon membrane filter. A 10 µl aliquot was injected into an Agilent 6890 GC equipped with an Agilent G4513A auto-sampler, a FID detector, a split/splitless injector, a HP 5 capillary column from Altech Agilent Technology (30 mx 0.25 mm x 0.25 µm) and a data processor (Open Lab, Agilent). A calibration curve between 0 and 200 ppm was prepared using HPLC grade ethanol as external standard. The limit of detection (LD) and the limit of quantification (LC) were determined. Each sample was analyzed in triplicate and the results were expressed as the average ± the standard deviation.

2.5. HMF determination

The HPLC method was based on the methodology reported by Madani-Tonekaboni (18). Briefly, the HPGJ samples (1 g each) were diluted in 10 ml of distilled water and 2.5 ml of trichloroacetic acid 40% v/v and shaked for 5 minutes. Then, the solution was placed in a 25 ml flask and taken to volume with distilled water at 35°C,

filtered using a 0.22 µm nylon membrane filter and injected (20 µl) into an HPLC system (Waters 1525, USA) equipped with a photodiode array detector (Waters 2996, USA). The column was a Lichrospher® 100, RP-18e, (250 × 4 mm, 5 µm), and an autosampler (Waters 2707, USA) was used. The HPLC method included an isocratic mobile phase, 80% of a 0.01 molar disodium hydrogen phosphate solution and 20% methanol with a flow rate of 1.0 ml/min, at 30°C. All solvents used were of HPLC grade. The detection wavelength range was 200–450 nm, with specific monitoring at 285 nm. The HMF content of the sample was calculated by comparing the corresponding peak areas of the sample and those of the standard solutions of HMF (Sigma–Aldrich, USA) after correcting for the sample dilution, the results were expressed in mg/kg.

2.6. Sensory analysis

2.6.1. Internal panel

This test was carried out in order to establish the preferred formulation among nine options (containing different proportions of honey, stevia and EEP). The sensorial analysis took into account the appearance, the texture, and the honey taste. The internal panel was composed of 15 panelists(19)from the Universidad Nacional de Entre Rios – Facultad de Bromatología.

2.6.2. Consumer´s panel

The consumer´s panel consisted of 74 people aged between 17 and 65 years old, who attended the Facultad de Bromatología on the scheduled day and time.

Intensity level of attributes evaluation test: this test was used to establish the attributes intensity perceived by consumers (color, hardness, adhesiveness, gumminess, sweet taste and honey flavor) (20). This method uses a three points JAR (Just-About-Right)scale where the intensity is represented as 'too high', 'too low' or 'just' according to the ideal premise each consumer possesses(21).An analysis of penalties was carried out to determine the degree of influence on the global

acceptance of consumers, that present the attributes that were valued at higher and lower intensity levels than the fair point(22). The attributes that have the highest negative impact on taste are found in a "Critical Corner". These are generally those that affect 25-30% or more of the respondents and cause a minimum drop of 1.0 points in the effect on the mean(23).

Global satisfaction degree evaluation: was established by measuring the pleasant or unpleasant sensations produced by the sample using a 7 points hedonic scale(24).

2.6.3 Purchase intention test.

A qualitative sensorial test was performed to evaluate the purchase intention of the HPGJ. 200 volunteer participants received a poll through e-mail and social networks with a photo showing the product, a short description about the HPGJ ("We would like to know the opinion of possible consumers about a functional gummy jelly made with honey and propolis. Propolis is a substance made by bees with a high content of bioactive compounds, which acts as natural preservative and provides antioxidant properties"), and the question "Would you buy it?" accompanied by a structured scale of 3 points ("No", "Yes" and "Maybe").

2.7. Water content

It was determined by the A.O.A.C.950.46 method(25) using an Ionomex stove, MCH (Argentina)at 110°Cup to constant weight. The results were expressed as a percentage (g of water / kg sample) mean ± standard deviation of 3 determinations.

2.8. Water activity (a_w)

It was measured using a Rotronic Hygrolab C1 water activity meter (Bassersdorf, Switzerland). The results were reported as the mean ± standard deviation of quintupled measurements.

2.9. Texture

The Texture Profile Analysis test (TPA) consisted in two consecutive compressions of the food, simulating what would occur in the mouth during chewing (26). The test was performed using an Instron Universal Test Machine with a 5 cm diameter stainless steel tip. The samples were compressed 30% of their height, at 0.5 mm/s. The measurements were performed in tenfold and the results were reported as the mean ± standard deviation.

A typical force–time plot was obtained and the following TPA parameters were calculated: hardness, cohesiveness, adhesiveness, gumminess, elasticity, and chewiness.

2.10. Color

Color was determined using a handheld colorimeter HunterLab MiniScan EZ (Virginia, USA). Color functions were calculated for illuminant D65 at 2º standard observer in the CIELAB uniform color space. L*, a* and b* values were obtained. Global color difference (ΔE_{00}) was calculated according to(27). The opacity of the samples was calculated as the ratio between L* measured with black and white backgrounds. The results were reported as the mean ± standard deviation of ten measurements.

2.11. *In vitro* digestion

Oral, gastric, and intestinal phases were analyzed on two different batches of HPGJ. Samples were incubated at 37°C in a Function Line 7000 drying stove (Heraeus, Germany) under constant stirring with an orbital shaker Vicking M-23 (Buenos Aires, Argentina) at 100 rpm. The *in vitro* digestion was carried out using the standardized method proposed by Minekus *et al* (28). After *in vitro* digestion, the samples were centrifuged at 12,300 G-force, 4ºC for 10 min. Finally, the soluble

fraction “supernatant” and the insoluble fraction “residue” were separated and stored frozen until their use.

2.12. FT-IR

The IR spectra of samples were examined over the range of 450-4000 cm^{-1} using a FT-IR spectrometer model spectrum 400 (PerkinElmer, Inc., Shelton CT, USA). The spectrum of each sample was obtained by taking the average of 64 scans at a resolution of 4 cm^{-1} in a MIRacle single-reflection attenuated total reflectance (ATR) accessory (PIKE Technologies, Inc., Madison WI, USA) with a single reflection diamond/ZnSe crystal plate. Background spectra were obtained and subtracted from each sample IR spectra. The spectral analysis was recorded using the Spectrum software version 6.3.5 (PerkinElmer, Inc.).

2.13. Storage studies

HPGJ samples packed in transparent hermetically sealed bags were stored for 90 days at 25°C under artificial light (C1) or darkness (C2).The lighting was provided by two fluorescent lamps with a value of 7.20 ± 1.24klx measured at the illumination source and 0.38 ± 0.03klx measured at the exposure point of the samples. In order to emulate the light exposure of samples in a foodstore, a time switch was used to interpose 12 hours of illumination with 12 hours of darkness. Samples stored in the darkness (C2) were covered with aluminum foil. Sampling (quintupled) was done every 15 days and the following determinations were made: humidity, a_w, texture, antioxidant capacity, and Maillard reaction development. In the case of Maillard reaction two indicators of the progress of the reaction were considered: 1) 5-hydroxymethyl-furfural (HMF) as an intermediate compound derived from the Maillard reaction, which was followed by HPLC (item 2.5.) and FT-IR(item 2.12), and 2) color, as a final consequence of the reaction development (item 2.10.).

In order to evaluate the effect of propolis on fungal growth, gummy jellies containing several concentrations of propolis extract were assayed (0 g, 30 g, 45 g, 60 g 75 EEP kg ^-1 of formulation). The gummy jellies were packed in transparent bags and stored for 90 days at room temperature. A visual inspection of the samples was performed daily, evaluating presence or absence of macroscopic superficial mycelium.

2.14. Statistical analysis

The results were statistically analyzed by the analysis of variance (ANOVA) to determine significant differences between the samples. The analysis of the means was performed through the LSD Fisher procedure at $p < 0.05$ using the software Infostat v.2008. The principal components analysis (PCA) was applied to the variables that were significant in the differentiation of storage time and condition. The module of Analysis of Principal Components and Classification, based on the correlations and with the option activate cases, of the program InfoStat-Statistical, version 2018e, was used for the data processing.

3. Results and discussion

3.1. Gummy jellies formulation

Gummy jellies were formulated with beehive products (honey and propolis). In the case of propolis, extracts were obtained from the raw material, and the extraction procedure was previously optimized. Six propolis extracts were prepared with different ethanol proportions (70, 80 and 90% v/v), at two treatment temperatures, 30 and 40°C. The criteria to select the extraction conditions were the percentage of extracted solids, and the antioxidant capacity (AC). The selected extract was obtained at 40°C and ethanol 70% v/v, given that high solids extraction 563 ± 32 g soft propolis residue kg ^-1 raw propolis (equivalent to 1.41 ± 0.08 g of resins/100 ml of EEP),and high antioxidant

capacity 488.6 ± 8.4 mmol eqTrolox/kg sample were obtained. Also, the minimum amount of ethanol was desired so that there would be a low ethanol concentration in the gummy jellies. Morgado Schmidt *et al*. (29) analyzed Brazilian propolis extraction using different ethanolic solutions (30, 70, 95% v/v) during 100h at room temperature; they reported that the best extract was obtained with ethanol 30% v/v, showing the highest antiradical activity. However, we cannot compare with our results as we used a different method to assess AA.

In order to define the gummy jelly formulation, an internal panel was used to analyze five formulations containing different proportions of the ingredients. The initial intention was to formulate with honey as only sweetener; however the texture was unpleasant (high adhesiveness) for the panel, therefore stevia was incorporated to increase sweetness. The preferred formulation, named HPGJ (Honey-Propolis-Gummy-Jelly), was selected due to its appearance, texture and honey taste, and contained: 687g honey, 116g unflavored gelatin, 110g water, 73g of the selected propolis extract (containing 1g of soft propolis residue kg ^-1), 9g stevia, and 5g citric acid. Regarding the addition of stevia, JECFA (30) established an ADI of 0-4 mg/kg b.w.. Considering that an adult person (70kg) could consume a portion of 3 gummy jellies per day, the intake of stevia would reach a 3.2% of the established ADI, assuring that there is no impairment on the health benefits upon the addition of this ingredient.

3.2. Sensory analysis

In order to evaluate the potential acceptability of the developed gummy jellies (HPGJ), a sensory study with consumers was performed. **Figure 1a** shows the intensity level of characteristic attributes HPGJ. The color attribute was the most accepted, rated at the just point by 88% of consumers. On the other hand, adhesiveness was rated by 66% of consumers as too low, while for the other attributes there was no clear trend. **Figure 1b** shows the effect of sensory attributes on consumer

acceptability, measured with a penalty analysis, which graphically represents the attributes that turned out to be more intense (+) and less intense (-) than the right point. The attributes of gumminess, adhesiveness, sweetness, and honey taste were perceived with low intensity, while hardness and gumminess were perceived with high intensity. The high hardness showed a negative impact on the acceptability of the gummy jellies. This attribute was located in the "Critical Corner", while the rest did not present incidence.

It is interesting to note that regarding the degree of global satisfaction (**Figure 1c**), more than 90% of the consumers placed the HPGJ within the categories of liking. Despite the fact that attributes intensity levels (JAR) were mostly outside the ideal (**Figure 1a**), and that one attribute was penalized by consumers (**Figure 1b**), the global satisfaction of the product was good and individual attributes appreciations did not impair the global acceptability of the gummy jelly. On the other hand, only 5.4% consumers disliked the product, adducing that they did not like honey.

Some products containing propolis might have strong flavor that affect their acceptability. Oses *et al*. (7,31) studied honey samples with the addition of different proportions of propolis extracts and determined that products with more than 5g soft propolis residue kg^-1 were rejected due to their unpleasant organoleptic characteristics. However, a sensory panel accepted all honeys with 1-5g soft propolis residue kg^-1. Additionally, Narbona *et al.* (32) informed that the addition of 0.5g propolis kg^-1 did not change the instrumental aroma profile nor the descriptive sensory profile of turrón (nougat).The HPGJ obtained in this work contained 73g EEP kg^-1, having only 1g solid propolis residue kg^-1, and were satisfactorily accepted by consumers.

The results obtained through the purchase test were also positive: 45% of the participants answered "I would buy it" because it is an innovative product; while 29%

answered "I would not buy it" because they did not like honey; and 26% of the participants responded “maybe” to the poll.

3.3. HPGJ characterization

Several variables were determined in order to characterize the gummy jelly. HPGJ presented a water content= 216.8 ± 16.8 g H_2O kg ^-1, and a_w= 0.700 ± 0.004. These values were within the range of similar commercial products. The residual content of ethanol in the gummy jellies was 16.81 ± 1.90 ppm. The color parameters of HPGJ were L*= 31.47 ± 1.43, a*= 3.61 ± 0.72 and b*= 6.49 ± 0.77.

In order to compare the HPGJ with two products available in the Argentinean market, a texture analysis was performed on HPGJ, and on two commercial fruit gummy candies (**Table 1**). HPGJ sample was significantly different from the commercial candies in all the studied parameters ($p<0.05$). It was softer, more cohesive, and more elastic. HPGJ also required less effort for chewing, a characteristic linked to hardness and gumminess.

A relevant aspect to evaluate in HPGJ was the antioxidant capacity, given that it was formulated with honey and propolis, both ingredients known to provide antioxidants. AC was determined for HPGJ and for several candy products available in the local market. No commercial gummy candies containing propolis were found in the local market; therefore, AC was determined in regular gummy candies, and in commercial hard candies containing propolis (**Figure 2**). The AC value for HPGJ was 8.17 ± 0.55 mmol eqTrolox/kg, which was significantly different from the values obtained for commercial products, being in some cases up to 10 times higher. Additionally, some hard candies despite having propolis in their formulation had lower AC values than common gummy candies; this could be attributed to the fact that commercial gummy candies, as well as HPGJ, contain citric acid as acidulant, which also provides antioxidant behavior (33).

Additionally, it is relevant to note that the AC (mmol eq Trolox/kg) input of HPGJ was higher than that of kiwi fruit (5.2), wheat bran bread (6.9), whole cereals for breakfast (6.8) and apple (4.8) (34).

Furthermore, we analyzed the AC contribution of HPGJ upon *in vitro* digestion. The AC value obtained for the soluble fraction of the *in vitro* digestion was 3.33 mmol eq Trolox/kg, representing around 40% retention of the product´s AC. This result indicates that an important amount of bioactive components of HPGJ preserved their antioxidant properties upon digestion, being able to provide antioxidant functions. The AC value of HPGJ (mmol eq Trolox/kg) was comparable to other digested products such as steamed spinach (3.2 mmol eq Trolox/kg), and apple (2.2 mmol eq Trolox/kg) (34). On the other hand, the insoluble fraction obtained after digestion retained less than 5% AC.

3.4. Storage studies

Given that HPGJ showed good sensory acceptability and a potential functional input, a stability study was performed. The effect of the storage time at 25°C was evaluated during 90 days under two illumination conditions: the gummy jellies placed in transparent bags were exposed to lighting conditions similar to those found in candy stores (C1), and control samples were stored in the darkness (C2). Various properties were evaluated during storage. Texture and a_w did not present significant changes in the studied period of time. However, color and AC significantly changed throughout storage ($p<0.05$). **Figure 3** shows the changes observed in the color variables throughout storage. An increase in a* and b* was observed (**Figure 3a**), being more relevant for b*. Also, a reduction in luminosity was detected (**Figure 3b**). These changes lead to the darkening of the gummy jellies along storage, probably due to the occurrence of the Maillard reaction, as can also be seen in the photographs in **Figure 3a**.The observed trend for samples stored under illumination condition (C1) and in the darkness (C2) was similar along time, however, by the end of storage, those gummy

jellies stored under light showed an important raise in darkening. As a consequence of these changes, the global color difference (ΔE_{00}) increased throughout storage, reaching values higher than 10 units (**Figure 3c**), being more important in those samples that were exposed to light (C1). According to Keraite and Sivakova (35), ΔE_{00}values lower than 1.0 are not detected by the human eye, ΔE_{00} values between 1 and 2 are perceptible by close observation, and values between 2 and 10 are visible to the naked eye. Taking into account the ΔE_{00}values of HPGJ, the candies developed visible changes during time, which were more marked above 15 and 45 days of storage for the samples exposed to the light and in the darkness, respectively.

On the other hand, HPGJs showed an increase in AC values, which was significant ($p<0.05$) for samples stored above 15 and 60 days of storage for the samples exposed to the light and in the darkness, respectively (**Figure 3c**). Embuscado (36) stated that Maillard's reaction products are natural antioxidants found in foods. This could be the case for our samples, in which the development of non-enzymatic browning during storage was parallel with the increase in AC for both illumination conditions studied.

In order to further investigate the development of non-enzymatic browning during storage, changes in the FT-IR spectra associated with the Maillard reaction were studied. **Figure 4** shows the infrared spectra of C1 and C2before and after 90 days of storage at 25°C.The FT-IR spectra of the HPGJs showed changes in the intensity of absorption of several bands. On one hand, a peak at1643 cm^{-1}, which is characteristic of undissociated carbonyl groups(37,38) was followed. On the other hand, the spectral range from 2900 – 2750 cm^{-1} and 1750 – 1050 cm^{-1} was associated to the different characteristic bands corresponding to stretches of the five-membered heteroaromatic rings with double bond, which have been identified as representative for HMF analysis(29,30).These results were in agreement with the HPLC analysis, where the HMF concentration of HPGJ after storage was 10.9 and 7.8 times higher than the HPGJ at initial time (6.2±0.2 ppm) for C1 and C2, respectively. Significant differences

were observed between illumination conditions, being the concentration of HMF higher for the illuminated samples compared to those stored in the darkness. A similar trend showing the increase in the HMF concentration was reported for honey stored under different lighting conditions(31).

As HPGJ water activity was 0.700, fungal growth could be a limiting factor in the product´s shelf life. Al-Waili *et al*. (41) investigated antimicrobial activity of ethyl alcohol propolis extracts, and their synergistic effect when used with honey. They concluded that propolis prevents the growth of the microorganisms in single and mixed microbial cultures, and has synergistic effect when used with honey. In this sense, we analyzed the influence of the addition of propolis extract in the development of fungal growth. It was observed that gummy jellies with propolis extract concentrations higher than 30g kg^-1 did not show microbial growth during 90 days of storage at 25°C. However, at concentrations lower than 30g kg^-1 EPP, the fungal growth did not differ from the control samples (without the addition of propolis), manifesting superficial mycelium from 50 days of storage.

Figure 5 shows the analysis of principal components obtained from the variables that had a significant effect during storage. The first two components explained 84.7 % of the total variation among the samples, the main component 1 (CP1) is explained by the variables delta E, chromatic coordinates a* and b*, antioxidant capacity (AC), HMF content and the bands corresponding to the vibrations of the groups related to the HMF and carbonyl groups, to the right side; and by luminosity, towards the left side of the plot. Gummy jelly samples corresponding to the beginning of storage were grouped on the left side of the plot, mainly those stored in the darkness. These samples displayed high luminosity values associated with light colors, similar to the color of the honey used to prepare the HPGJ. Along storage, samples were gradually displaced to the right in the plot, together with the appearance of indicators of the Maillard reaction as the increase in the chromatic variables a* and b*, HMF (as detected by HPLC and FT-IR), and AC related to Maillard´s products.

4. Conclusions

This study falls within the current trends of production and consumption of food that can provide additional health benefits. A gummy jelly organoleptically acceptable by more than 90% of consumers was developed. The degree of global satisfaction was not impaired by the high hardness that was negatively evaluated in the penalty analysis.

A positive feature was the successful incorporation of honey and propolis to the gummy jelly, as these products of the beehive are recognized as a source of nutrients and phytochemicals. This new candy could stand out for its high antioxidant capacity compared to similar products available in the market, leading to an additional benefit for health. Moreover, upon *in vitro* digestion 40% retention of the AC was observed, which could be an interesting input for this type of products.

Additionally, it is important to note that even having a water activity value of 0.700, fungal growth was not observed during 90 days at room temperature, therefore the propolis components, and the added citric acid acted as efficient protective agents against microbial deterioration.

Regarding shelf life, a storage study at 25°C indicated that in order to reduce the occurrence of perceptible color changes, HPGJ should be packed protected from light. In these conditions, HPGJ properties like color and texture remained stable during 45 days.

Overall, this healthy and potentially functional gummy jelly could offer an interesting option to regular candies, which is not available in the local market.

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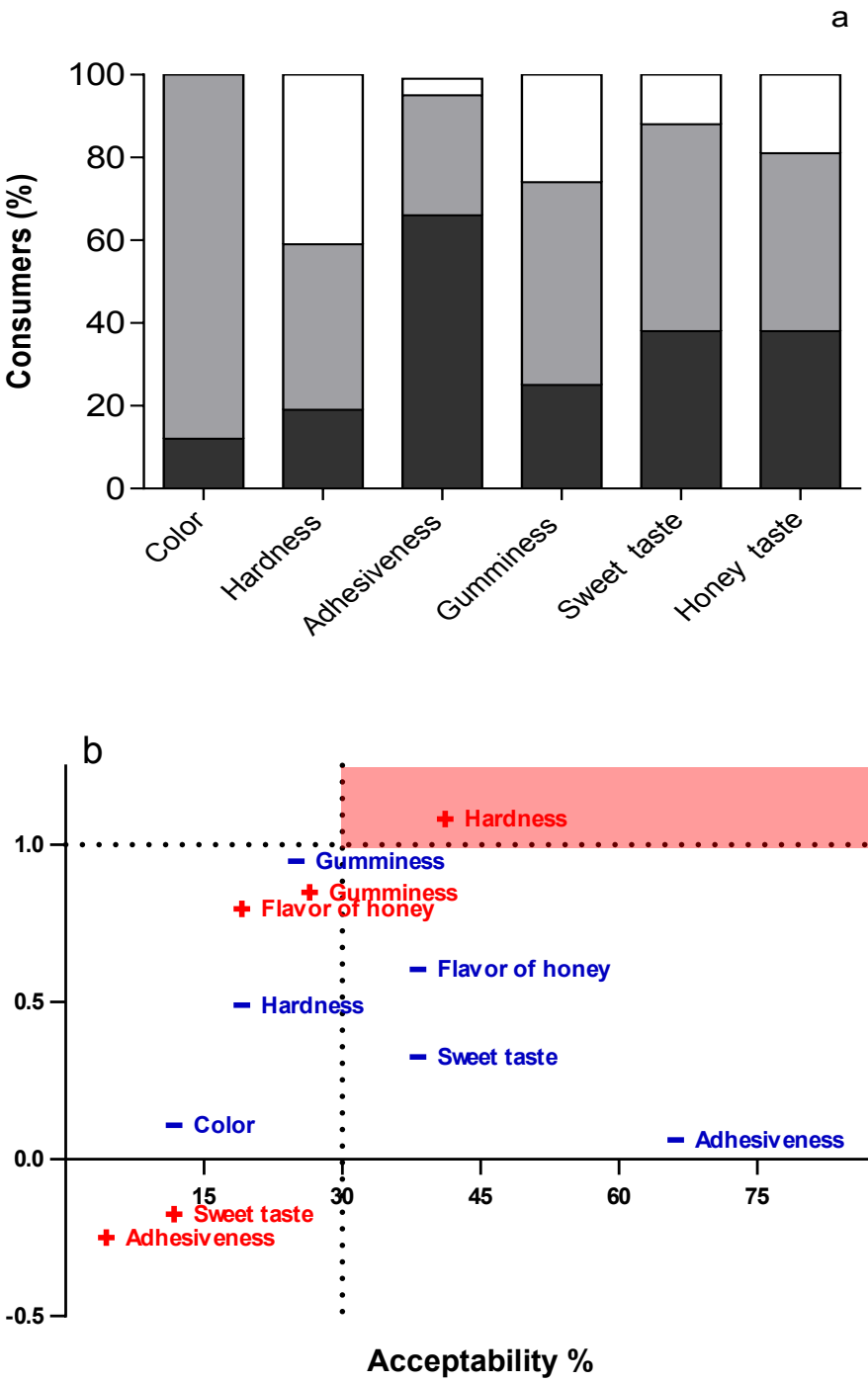
Table 1: Comparison of texture parameters of HPGJ and commercial gummy jellies.

Parameters	Brand 1	Brand 2	HPGJ
Hardness	0.250 ± 0.030[a]	0.077 ± 0.010[b]	0.060 ± 0.005[c]
Cohesiveness	0.51 ± 0.03[a]	0.71 ± 0.01[b]	0.92 ± 0.02[c]
Elasticity	0.81 ± 0.02[a]	0.86 ± 0.01[b]	0.94 ± 0.01[c]
Gumminess	0.13 ± 0.02[a]	0.05 ± 0.01[b]	0.06 ± 0.01[b]
Adhesiveness	-0.004 ± 0.002[a]	-0.004 ± 0.001[a]	-0.003 ± 0.001[a]
Chewiness	0.11 ± 0.01[a]	0.05 ± 0.01[b]	0.05 ± 0.01[b]

Values with different letters in the same row present significant differences ($p<0.05$)



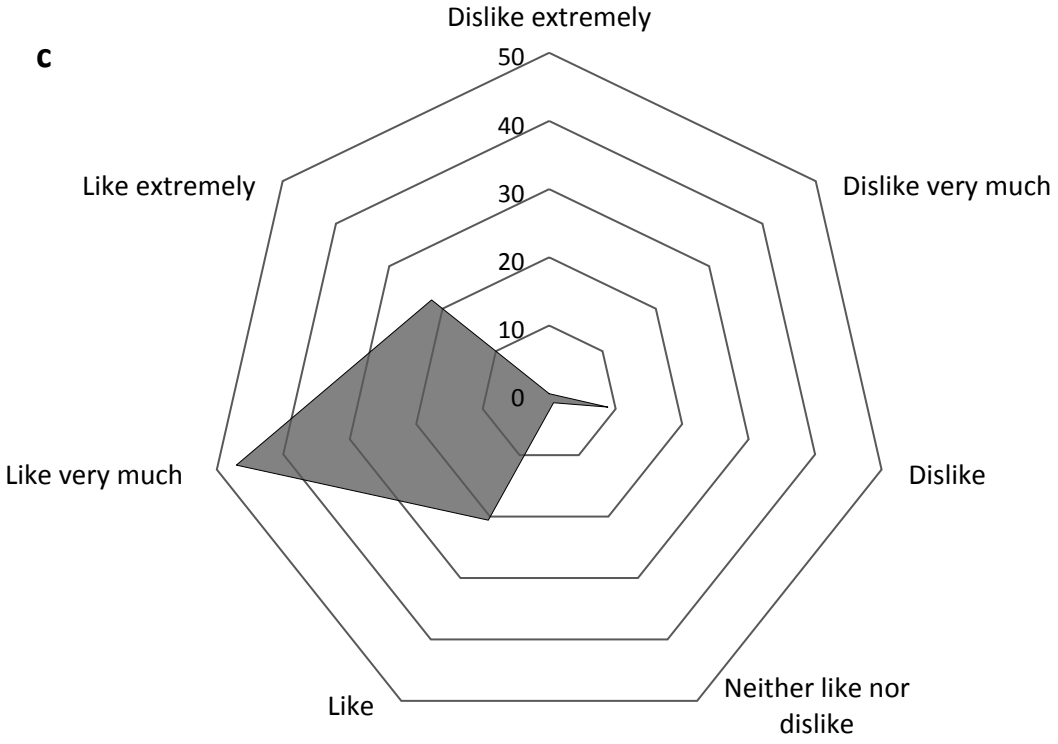


Figure 1: Percentage distribution of JAR (Just-About-Right) for each attribute using the following scale: too high (white), JAR (gray), too low (black) (a). Effect of sensory attributes on consumer´s acceptability, measured with the JAR scale and evaluated with a penalty analysis (b). Evaluation of the global satisfaction degree of HPGC through hedonic scale (c).

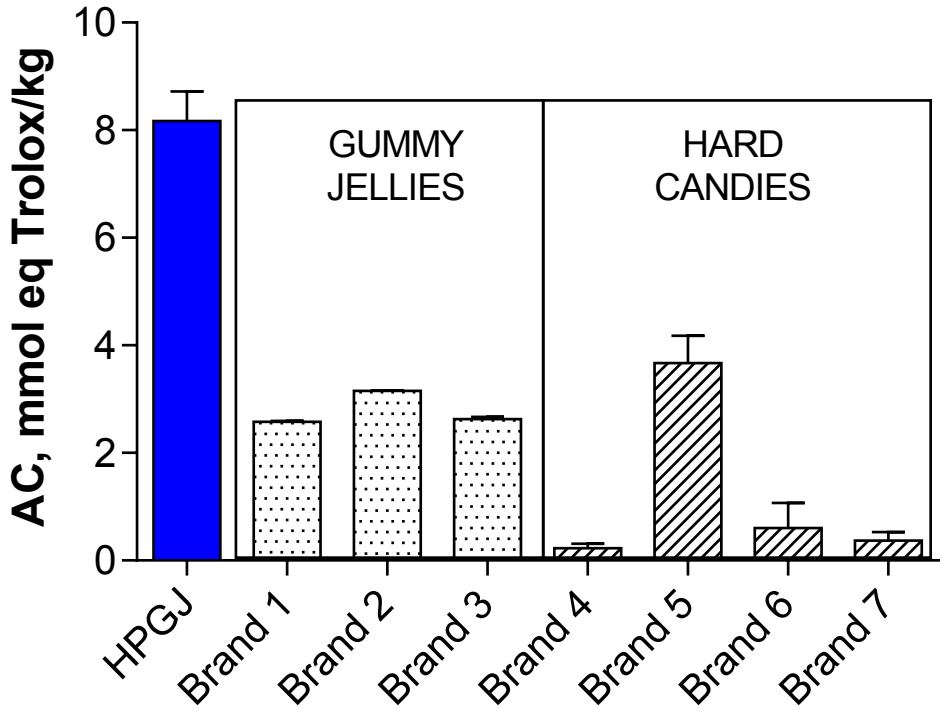


Figure 2: Antioxidant capacity (AC) of HPGJ and commercial candies.

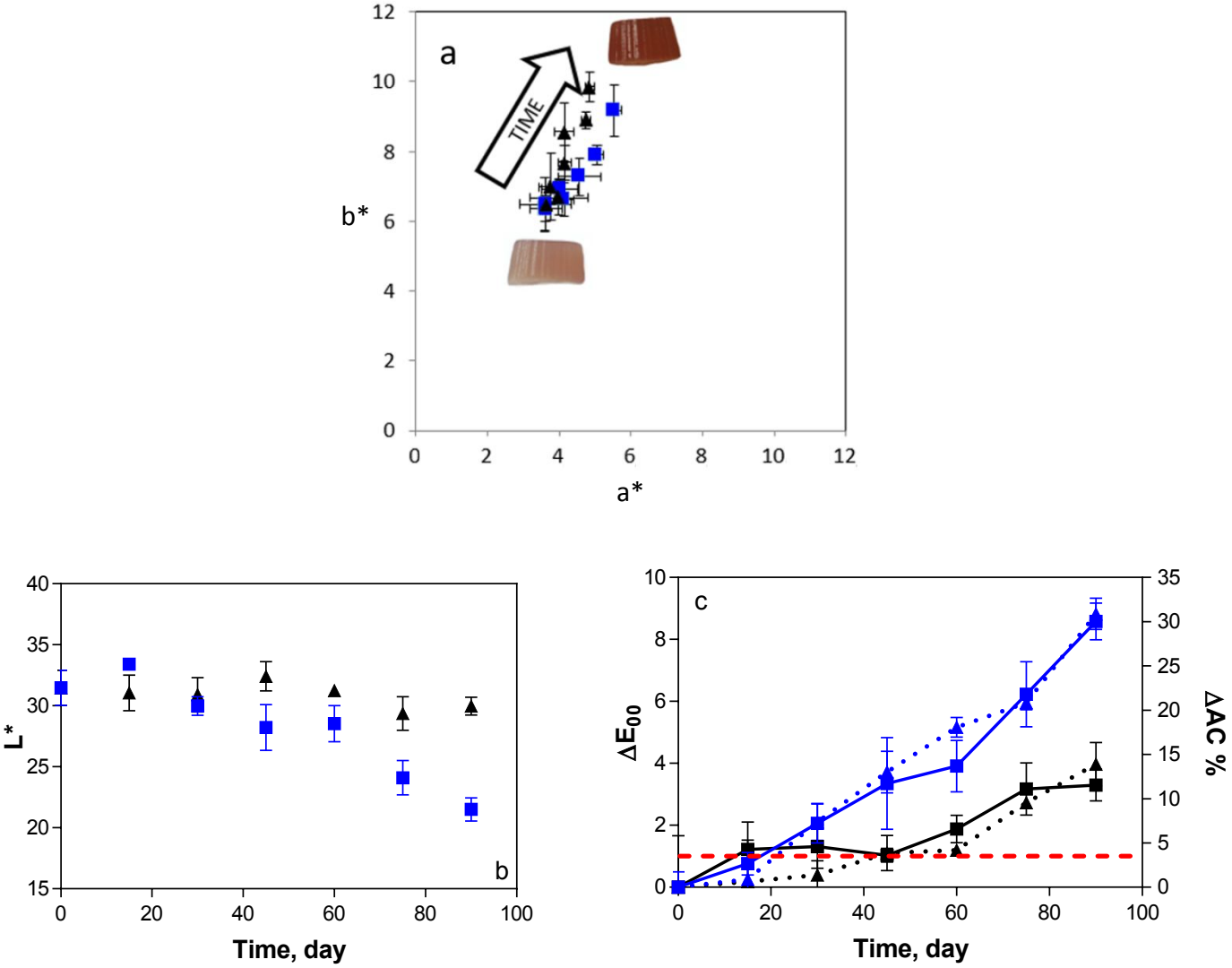


Figure 3: Variation of chromatic variables during storage. (a) b* versus a*; (b) L* versus time; (c) ΔE_{00} versus time (square symbols, solid lines) and variation of antioxidant activity (AC, triangle symbols, dotted lines). The dashed red line is the limit for the human eye perception. Samples stored in darkness (black symbols and lines), and under lighting (blue symbols and lines).

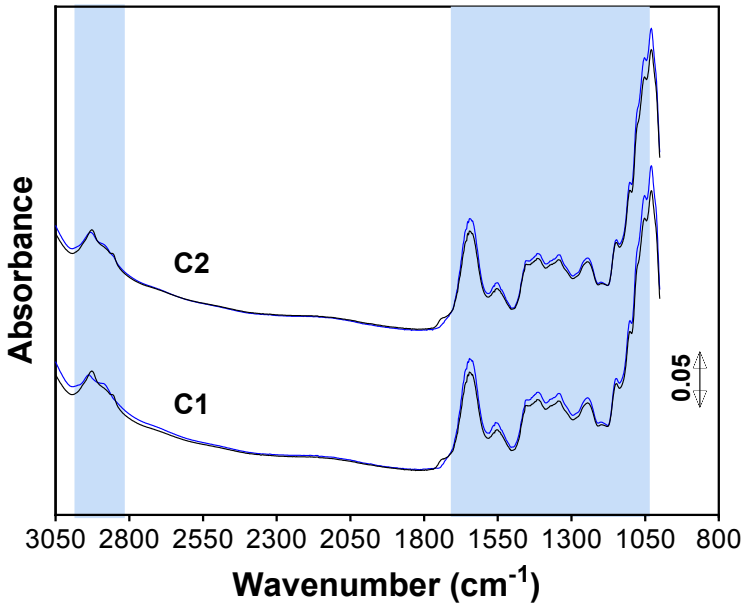


Figure 4: Infrared spectrum of C1 and C2 before (black line) and after (blue line) 90 days of storage at 25°C.

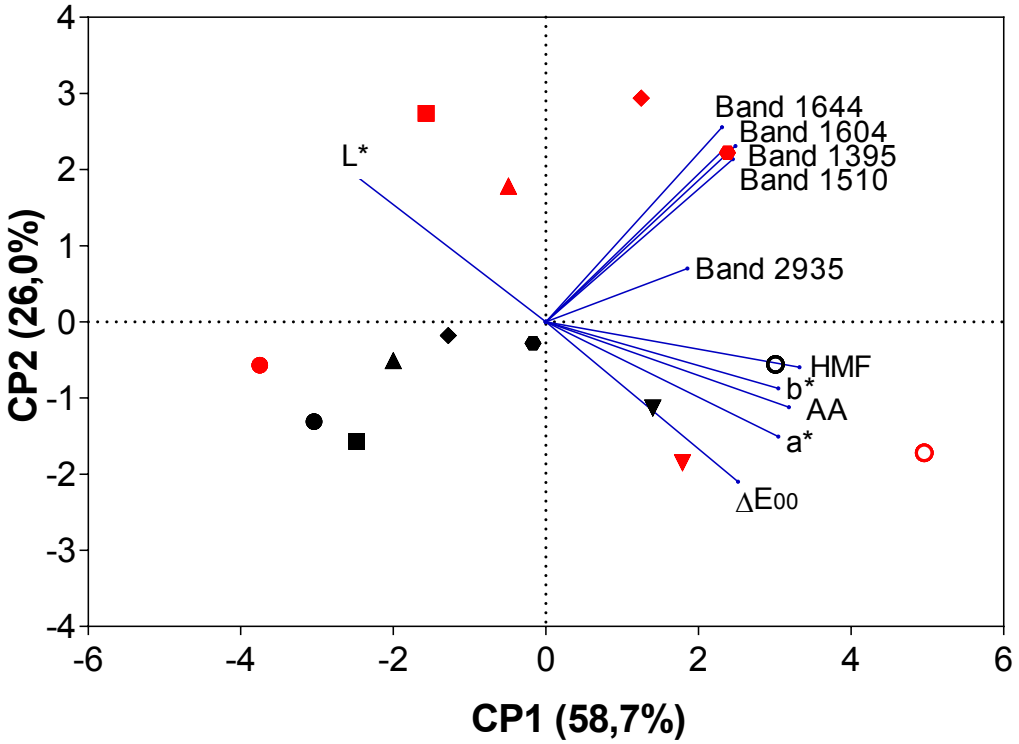


Figure 5: Principal component analysis of samples stored for different times: 0 (●), 15 (■,) 30 (▲), 45 (♦), 60 (⬣), 75 (▼), 90 (○) days. C1: red symbols and C2: black symbols.