

Research Article

# Survival of Free and Encapsulated Probiotics in Healthy Gummy Candy as a Carrier for Probiotic Capsules

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## Abstract

Probiotic gummy candy is an innovative food that can be used as a carrier to deliver probiotics. This study aimed to manufacture healthy gummy candy (HGC) as a carrier for probiotic capsules. The ingredients' effect on the viability of probiotic capsules and free cells was evaluated for 48 days under refrigerated conditions. Also, the textural properties, overall acceptability of products, and physicochemical properties were tested. The encapsulation probiotics in sodium alginate and chitosan provided a high efficiency of 88.16%. At the same time, the encapsulated cells-based HGC (HGC-C) had higher counts of probiotics than free cells-based HGC, and it remained above the 3 log CFU/g at the end of the 48-day storage period in HGC-C, while the free cells reached zero at 30 days. The HGC product showed a low hardness value and high antioxidant capacity as well as, and the colour coordinates for the product showed more yellow, with an intense red colour. Using mango pulp and probiotics in gummy candies is an innovative and viable alternative to the confectionery industry; therefore, combining fruits as a source of prebiotics and probiotic bacteria could be a promising formula for probiotics gummy supplement preparation.

## More Information

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**Keywords:** Probiotics; Antioxidant activity; Gummies; Capsule; Polyphenols



## Introduction

In recent decades, synthetic additives have been less accepted because of food safety issues and consumers' concerns about their harmful effects on their health [1]. Combinations of probiotics and natural materials confer health benefits to the host [2,3]. Moreover, regular consumption of probiotics has many significant therapeutic effects on human health, such as protection against diarrheal diseases, inflammatory disorders, and hypercholesterolemia, as well as exhibiting anti-cancer, anti-diabetic, and antioxidant effects by enhancing host immunity and strengthening the intestinal barrier [4,5]. *Lactobacillus plantarum* and *Lactobacillus paracasei* are well known to have several properties, including antimicrobial and anti-adhere activities against several pathogen productions of organic acids, hydrogen peroxide, and bacteriocins [6,7]. In addition, it can modulate the human gut microbiota by suppressing growth and opportunistic invasion [8]. Various factors may reduce the beneficial effects of probiotics, e.g., during food processing, storage conditions, and gastrointestinal

transiting; in line with this, encapsulation is a crucial way to deal with these issues and enhance the viability of probiotics in the human gastrointestinal tract [9,10]. Confectionery products such as chewing gum and gummies are trendy among consumers [11]. Bartkin, et al. [12] and Lele, et al. [2] reported that gummy candies are a good choice as a carrier for essential oils and probiotics. Using natural juices, fruit purees, or fruit by-products improves gummies and jellies' sensory properties and produces healthy formulations with antioxidant properties [13-15]. According to Pauliuc, et al. [16], thyme honey contains many sugars, including fructose, glucose, sucrose, turanose, maltose, trehalose, melezitose, and affinose. At the same time, ripened mango fruit is a good source of glucose, fructose, and sucrose sugars [17]. Lime is an excellent ascorbic acid source with antioxidant properties [18,19]. However, there are no reports of this combination of natural juice, fruit, and honey being used in candy or gummy candies as carriers of probiotic capsules. Therefore, this study aimed to produce probiotics-based healthy gummy candy by replacing sweeteners, flavorings, and colorings with honey, mango pulp, and lemon juice and evaluating



the product' physicochemical and sensory properties and viability of probiotics capsules.

## Materials and methods

### Culture probiotics preparation

*Lactobacillus plantarum* PTCC 1058 was obtained from the Persian Type Culture Collection center, *Lactobacillus paracasei* subsp. *tolerans* IBRC was obtained from the Iranian Biological Resource Center. The lyophilized *Lactobacillus plantarum* PTCC 1058 and *Lactobacillus paracasei* subsp. *tolerans* M11230 were activated separately in MRS broth at 37 °C for 24 h. Gram staining, catalase, and oxidase tests were performed to determine the purity of culture; after that, cells were centrifuged at 4000×g for 10 min at 4 °C and washed twice with sterile physiological serum (SPS) (NaCl<sub>2</sub> 0.85% w/v) then, re-suspended in 1 mL of SPS to obtained a suspension containing  $9.72 \pm 0.62$  Log CFU/ mL and enumerated by the spread plate method onto MRS agar and incubated for 2 days at 37 °C.

### Preparation of sodium alginate solution

The sodium alginate (ALG) solution preparation was modified from Gul and Dervisoglu [20]. The formula consisted of 6 g of ALG and 100 mL of boiling distilled water. The solution was vigorously stirred at room temperature for 20 min with the help of a magnetic bar; then, after being autoclaved at 121 °C for 15 min, it was refrigerated overnight.

### Preparation of chitosan solution

The chitosan solution was prepared by adding 4 g of chitosan (Ch) to 1 L of acetic acid (1 M) and it was left on a stirrer at room temperature for 1 hour. Then, 5 M NaOH was added to adjust the pH to 6, and it was sterilized in an autoclave for 15 min at 121 °C [21].

### Preparation of sodium alginate hydrogel capsules loaded with probiotics

ALG capsules loaded with probiotics were prepared according to Li, et al. [22] using an extrusion method with slight modification. 0.5 mL *Lactobacillus plantarum* with 0.5 mL *Lactobacillus paracasei* suspension was well mixed with 9 mL sterilized ALG solution. Then, the mixture was stirred using a magnetic bar at room temperature for 20 min at 100 rpm until a homogenous solution was obtained. The ALG-bacteria mixture was added dropwise through a 6 mL syringe (22-gauge needle) into 200 mL of 3% w/v CaCl<sub>2</sub>. The distance between the syringe and the CaCl<sub>2</sub> solution was 10 cm and left for 30 min to harden the ALG hydrogel capsules; the capsules were separated by a sieve and rinsed with an SPS solution. The capsules were kept in 0.1% peptone water at 4 °C for further use.

### Preparation of sodium alginate coated-chitosan capsules

ALG capsules (4.5 g) were transferred into 0.5 mL Ch

solution. The capsules were stirred gently with a magnetic bar for 1 hour at 100 rpm; after that, they were rinsed with an SPS solution and kept in peptone water (0.1 % w/v) at 4 °C.

### Viable cells count

The viable encapsulated and free probiotics cells were counted using the spread plate method [23]. ALG/Ch capsules (1 g) were dissolved in 9 mL 1% w/v sterile sodium citrate under constant stirring at 100 rpm for 10-15 min at ambient temperature. Serial dilutions were prepared in (0.2%) peptone water and spread on MRS agar. After 24-48 h incubation at 37 °C, the viable probiotic cells were counted and expressed in log CFU/mL.

### Encapsulation efficiency % measurement

The encapsulation efficiency percentage (EE%) was calculated according to Equation 1 as proposed by De Araújo Etchepare, et al. [24].

$$EE \% = \frac{N}{N_0} \times 100 \quad (1)$$

N is the Log (CFU/ mL) of viable cells released from the hydrogel beads.

N<sub>0</sub> is the initial Log (CFU/ mL) of the viable cells used for encapsulation.

### Capsules' size measurement

The diameters of the wet capsules were measured with the help of a compound microscope (Carl Zeiss, Germany) and Image J processing software. An average of 3 capsule diameters was measured.

### Production of capsules/ free cells- based healthy gummy candy

All the ingredients have been purchased from the local market in the Islamic Republic of Iran. Healthy gummy candy (HGC) production was modified by Kamil, et al. [25]. Bovine gelatin sheets were soaked in 50 mL of cold water for 10 minutes to bloom, and then the gelatin solution was entirely dissolved by heating at (70 °C – 75 °C) in a water bath for 15 minutes. Next, water, lemon juice, thyme honey, and mango pulp were mixed in a blender, and then the fruit solution and blooming gelatin were blended until there were no lumps.

To prepare the probiotic capsules-based HGC (HGC-C), the gummy mixture was cooled to 45 °C then, poured into silicone molds (1x1 cm). 5 probiotic capsules were placed in each mold well; the molds were then placed in the refrigerator for 2 hours. The HGC-C pieces were removed from the molds and placed on plastic wrap to dry in the refrigerator for three days at 4 °C.

To prepare the free cells-based HGC (HGC-F), 1 mL of

probiotics suspension was added to 9 mL of the gummy mixture (45 °C) under constant stirring for 10 min at ambient temperature. Then, the molds were placed in the refrigerator for 2 hours. The HGC-F pieces were removed from the molds and placed on plastic wrap to dry in the refrigerator for three days at 4 °C.

### Physicochemical characteristics of HGC

PH, TSS %, titratable acidity %, protein, and moisture % were measured using the methods of AOAC [6]. pH and total soluble sugars were measured using a pH meter (Consort C830, Belgium) and a refractometer (Bellingham+Stanley Limited, England). TA estimation was measured as citric acid %, and the sample was titrated with (0.1 N) NaOH and 3-5 drops of 1% phenolphthalein indicator. The percentage TA was calculated using Equation 2.

$$\text{TA\% (citric acid \%)} = \frac{V \times 0.1 \text{ NaOH} \times 0.064 \times 100}{m} \quad (2)$$

V is a titer volume of NaOH

m is a mass of HGC (g)

0.064 is a milliequivalent of citric acid

The moisture content was estimated by heating the sample using an oven and sitting at 105 °C for 4 hours. It was weighed once more until it reached a constant weight after cooling in a desiccator. The following equation (Equation 3) was used to calculate the % of moisture content [26].

$$\text{Moisture \%} = \frac{w1 - w2}{w1} \times 100 \quad (3)$$

W1 is the weight of the sample before treatment (g).

W2 is the weight of the sample after treatment (g).

The percentage of total nitrogen was estimated using the Kjeldahl method. The sample was digested using concentrated sulfuric acid with catalysts, followed by distillation and titration with hydrochloric acid. Then, the total nitrogen in the sample was measured and multiplied by a conversion factor (6.25).

### Determination of total phenolic content (TPC) and antioxidant activity %

**Preparation of HGC extract:** The extract was prepared with slight modifications. 1 g of HGC-C sample mixed with 5 ml of 80% methanol, then left on stirrer at room temperature in the dark for two hours. The mixture was filtered through Whatman No.1 filter paper to obtain a clear supernatant; afterward, it was centrifuged at 6000 x g for 10 min and used to determine total phenolic content and antioxidant activity % [27].

### Total phenolic contents measurement

The total phenolic content (TPC) of the methanolic crude extracts of HGC was estimated using the Folin–Ciocâlteu reagent described by Ben Rejeb, et al. [27]. Suspended 300 µL of a crude extract with 300 µL of 10% Folin–Ciocâlteu reagent and vortexed for 1-2 min, followed by the addition of 1 mL of 7% Na<sub>2</sub>CO<sub>3</sub>, then the mixture was vortexed and stood for 30 min at room temperature in the dark for colour development. The mixture was filtered through Whatman filter paper No. 1. Absorbance was measured at 760 nm wavelength. The final result was expressed as mg GAE per g of HGC weight.

### Stock gallic acid solution

The stock solution was prepared by dissolving 0.500 g gallic acid in 10 mL of 80% methanol and then, making the volume 100 mL with distilled water in a 100 mL volumetric flask to get the concentration of 5 mg/mL [28].

### Preparation of standard Gallic acid curve

The calibration curve was plotted by adding 0, 0.2, 1, 2, 3, 5, and 10 mL aliquots of stock gallic acid solution into 100 mL volumetric flasks and then diluting to volume with distilled water to get 0.01, 0.05, 0.1, 0.15, 0.25, and 0.50 mg/mL concentration of gallic acid; every concentration was mixed with 300 µL of 10% Folin–Ciocâlteu reagent and vortex for 1-2 min followed by 1mL of 7% of Na<sub>2</sub>CO<sub>3</sub> and vortexed, the absorbance was measured at 760 nm.

The amount of TPC in the HGC-C samples was obtained using the following equation (Equation 4) [29].

$$\text{TPC} = \frac{C \times V}{m} \quad (4)$$

TPC is the total phenolic content (mg/g) in gallic acid equivalent (GAE)

C is the concentration of gallic acid obtained from the gallic acid standard curve (mg/mL)

V is the volume of extract (mL)

m is the mass of extract (g)

### Antioxidant activity %

Crude extract (2 mL) was added to 2 mL of freshly prepared DPPH (0.0024 mg DPPH in 100 mL 80% methanol), then well vortexed and allowed to stand for 30 min in the dark. The absorbance was measured at 517 nm (Equation 5) [30].

$$\text{DPPH \% inhibition} = \frac{A0 - A1}{A0} \times 100 \quad (5)$$

A0 = is a DPPH absorbance

A1 = is a sample absorbance

## Texture profile analysis of HGC

The texture profile analysis (TPA) was performed with an AMETEK Brookfield texture analyzer by Herrero, et al. [31]. The texture profile parameters hardness, adhesiveness, resilience, fracturability, cohesiveness, springiness, gumminess, and chewiness were determined. Block shape HGC with dimensions of approximately 8 × 9 mm (W × H) and a depth of 10 mm. The HGC sample was positioned in the sample testing region; then, the flat cylindrical probe (TA4/1000) was lowered to the sample with a load of 10,000 grams, compressed, and penetrated the sample at a speed of 2 mm/s and pretest speed of 2 mm/s, then returned to its starting point at 2 mm/s.

## Colour intensity

The color of the top and bottom HGC samples was measured using a color analyzer; their values of lightness (L\*), redness/greenness (+ a\* / - a\*) and yellowness/blueness (+b\* / - b\*) was determined [32].

## The survivability % of probiotics in the HGC

The survivability % of free and encapsulated probiotics cells during refrigeration storage for 48 days for HGC samples was determined. The count of surviving encapsulated and free cells on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 36, 42 and 48 were assessed. HGC-C sample (~1g, 1 block) was suspended in 1% sodium citrate, followed by vigorously stirring at 30 °C for 15-20 min. Tenfold was serially diluted in 0.2% peptone water, plated on MRS plates using the spread plate technique, and incubated for 48 h at 37 °C. The bacteria were enumerated as colony-forming units per gram (CFU/g). All samples were counted in triplicates (Equation 6) [33].

$$\text{Survivability \%} = (\text{Nt} / \text{N0}) \times 100 \quad (6)$$

N0 = The Log (CFU/mL) of the viable cell counts at the zero time

Nt = The Log (CFU/mL) of the viable cells counts at various exposure times (t)

HGC-F sample (~1g, 1 block) was suspended in 0.2% peptone water, then stirred at 30 °C for 10-15 min. A set of serial dilutions was made with peptone water, and the colony forming units (CFU/g) were determined by spread method on MRS agar plates and incubation for 48 h at 37 °C. All samples were counted in triplicates [34].

## Sensory properties tests

Ten assessors conducted a sensory analysis of the HGC-C after one week of refrigerated storage. Supplement sample attributes, including taste, odor, color, mouthfeel, aftertaste, and overall acceptability, were scored on an increasing scale ranging from 1 (extremely dislike) to 5 (extremely like).

## Statistical analysis

Data were subjected to analysis of two-way variance (ANOVA) using GeneStat Release 10.3 DE Software. The mean value and ± standard deviation at least triplicate were calculated. Differences were identified as significant at  $p \leq 0.05$ .

## Results and discussions

### The capsules' morphology, sizes, and encapsulation efficiency %

*Lactobacillus plantarum* and *Lactobacillus paracasei* were gram positive, short to long rod shape, catalase and oxidase negative. All capsules were spherical and had a whitish-yellow color due to the natural color of the alginate and chitosan materials employed in the coating [35]. In addition, the capsule size is a crucial factor in the encapsulation technique; the chitosan-alginate capsule size was  $2.13 \pm 0.10$  (Table 1).

### Physicochemical characteristics of HGC

The HGC samples had a pH value of  $4 \pm 0.1$  (Table 2). The added lime juice to HGC samples decreased the pH of the final product. These results agreed with the results of Niam, et al. [36], who found that the pH value of gummy candies was 4.26–4.57. In confectionery factories, pH is frequently determined to control the degree of hydration; furthermore, it plays a crucial role in preventing the growth of microorganisms [37]. TSS includes the total amount of sugar and negligible soluble proteins, amino acids, and other organic materials [38]. The total soluble solid (TSS) content of the HGC samples was  $44 \pm 1.5$  °Brix lower than commercial gummy candies (74–80 °Brix) (Table 2) [39]. It is worth noting that the main sweeteners in gummy candy products are sucrose and glucose syrup, as the types of sugars and their concentrations might affect the percentage of TSS [40]. The titratable acidity % (TA%) rating indicates total acid content. It is a better indicator of the impact of acid contents

**Table 1:** The capsules' sizes and encapsulation efficiency %.

Formulation	Initial loading of the cells Log (CFU/mL) before encapsulation	Final loading of cells Log (CFU/ mL) after encapsulation	Capsules' size average (mm)	EE %
ALG-Ch capsules	$9.72 \pm 0.62^A$	$8.57 \pm 0.34^A$	$2.13 \pm 0.10$	88.16

<sup>A</sup>Means having the same letters in the row are not significantly different ( $p > 0.05$ )  
<sup>EE</sup>% = Encapsulation efficiency %.

**Table 2:** Physicochemical composition of HGC.

Physicochemical composition of HGC	
pH	$4 \pm 0.1$
*TA%	$1.02 \pm 0.01$
*TSS%	$44 \pm 1.5$
Protein%	$2.77 \pm 0.01$
Moisture%	$28.43 \pm 0.03$
*TPC	0.06 mg GAE /g
Antioxidant activity %	$91 \pm 1 \%$



on flavor than other methods, such as pH [41]. The HGC titratable acidity was  $1.02 \pm 0.01$  (% as citric acid) (Table 2), which is higher than the result of Chalak, et al. [42] who found the titratable acidity range from 0.08% to 0.43%. Our result agreed with Abu Shama, et al. [43] work. Who reported that red peel candy's titratable acidity (% citric acid) was 1.052% after 4 weeks of cold storage ( $8 \pm 2$  °C).

Gummy candies' water content impacts their stability, texture, shelf life, and microbial growth [44]. The moisture of the HGC sample was  $28.43 \pm 0.03\%$  (Table 2), and it had a high moisture content compared to commercial products. Ergun, et al. [45] stated that gummy candies typically have a moisture content of 8% to 22% or less. Gummy candies become harder as moisture level decreases, usually leading to a longer shelf life [45]. On the other hand, the quality, texture, and shelf life of gummy candies can be significantly influenced by their final moisture [39].

The higher moisture content in HGC compared to commercial gummies was due to corn syrup or glucose use in commercial gummy candies, while in HGC, honey was used as a sweetener. Corn syrup can absorb moisture when increasing dextrose equivalent (DE) [46]. Gummies' main ingredients are gelatin, agar, and pectin. Gelatin is a water-soluble polypeptide with a high molecular weight derived from the partial hydrolysis of collagen [47]. In addition, gelatin has unique properties such as water-holding, gelling, and thickening agents in food materials [48]. The HGC protein percentage was  $2.77 \pm 0.01\%$  (Table 2). The low protein content was due to using a low amount of gelatin in the HGC formula; lime juice, thyme honey, and mango pulp are also low in protein content [49]. A Teixeira-Lemos, et al. [50] study showed that the protein concentration in 100 g of orange and honey gummy jellies and berries mix gummy jellies was 4.04 g and 3.25 g, respectively.

### Total phenolic content and antioxidant activity %

The biological effects of phenolic compounds are varied, including anti-inflammatory, antibacterial, vasodilator effects, anticarcinogenic, antiviral, antithrombotic, antiallergic, and hepatoprotective [51]. TPC content and antioxidant activity of the prepared HGC are given in Table 2; TPC content was 0.06 mg GAE/g, and high antioxidant activity % was  $91 \pm 1\%$  at pH 4. Similar results were found by Ruenroengklin, et al. [52] and Ozelik, et al. [53], the extract exhibited the highest antioxidant activity at pH 4.0 compared to other pH conditions (6.5 and 8.5).

The high value of antioxidant activity might be due to using lime juice in the HGC formula. Citrus fruit is a valuable source of antioxidant compounds, including flavonoids, phenolic compounds, and ascorbic acid, which are crucial in reducing cancer risk [54]. The high antioxidant activity % was noted in contrast to the concentration of total phenolic content, which was low. A decrease in TPC does not always lead to a decrease

in antioxidant activity, as degradation products of phenolic compounds can sometimes have higher antioxidant activity than the original products [55,56]. Madalagiri, et al. [57] reported a lower level of antioxidant activity in mango pulp due to the lower level of total polyphenols and flavonoids, which are relatively higher in the peel. In addition, thyme honey is a good source of phenolic compounds with high antioxidant activity % (60.96%) [58]. Moreover, citric acid, mainly acid in Persian lime juice, acts as an antioxidant [59].

### Probiotics survivability % in HGC

The number of encapsulated cells decreased from  $6.98 \pm 0.06$  (100%) Log CFU/g to  $3.88 \pm 0.13$  (55.42%) after 48 days of refrigeration storage (Table 3). On the other hand, the number of free cells decreased from  $7.13 \pm 0.10$  (100%) Log CFU/g to 0.00 after 30 days of refrigeration storage. There was a significant difference ( $p < 0.05$ ) in the encapsulated and free cells' numbers during storage. Encapsulated probiotic organisms showed much higher survival in HGC-C compared to HGC-containing free probiotic bacteria. The decrease in the cell's survival might be due to the low pH of Lime juice and oxygen in the HGC product. Therefore, to enhance the survival of probiotic bacteria in low-pH products, encapsulation was an effective method to protect cells from mild heat treatment and low-pH [60]. According to Sheehan, et al. [61] low-pH fruit juices, with a pH range between 2.5 and 3.7, increased bacterial sensitivity to stressful conditions. In addition, the presence of probiotic cells in low pH environments (below 4.5) leads to increased energy consumption to maintain the intracellular pH, which leads to a deficiency of ATP for other vital functions and cell death [62]. Oxygen can also lead to the formation and accumulation of toxic metabolites in cells, resulting in cell death due to oxidative damage [63].

### Texture parameters

Compression test results comparing the texture of HGC with the control (commercial gelatin-based candy) showed

**Table 3:** Survival of encapsulated and free probiotics cells in the HGC during refrigeration storage ( $\sim 4$  °C) for 48 days.

Days	The number of free cells Log (CFU/g)	Survivability %	The number of encapsulated cells Log (CFU/g)	Survivability %
0	$7.13 \pm 0.10^A$	100	$6.98 \pm 0.06^A$	100
3	$6.17 \pm 0.13^B$	88.14	$6.74 \pm 0.22^A$	96.28
6	$5.06 \pm 0.28^C$	72.28	$6.56 \pm 0.04^B$	93.71
9	$4.39 \pm 0.09^D$	68.71	$6.53 \pm 0.08^B$	93.28
12	$4.16 \pm 0.15^D$	59.42	$6.43 \pm 0.11^B$	91.85
15	$3.57 \pm 0.07^E$	51.00	$5.87 \pm 0.26^C$	83.85
18	$2.74 \pm 0.49^F$	39.14	$5.65 \pm 0.26^C$	80.71
21	$2.47 \pm 0.38^F$	35.88	$5.52 \pm 0.27^C$	78.85
24	$2.15 \pm 0.03^G$	30.71	$5.33 \pm 0.05^D$	76.14
27	$1.47 \pm 0.15^H$	21.00	$5.27 \pm 0.46^D$	75.28
30	0.00	0	$5.09 \pm 0.19^D$	72.71
36	0.00	0	$4.75 \pm 0.26^E$	67.85
42	0.00	0	$4.24 \pm 0.38^E$	60.57
48	0.00	0	$3.88 \pm 0.13^F$	55.42

<sup>A-H</sup> Means having the same letters in the column are not significantly different ( $p > 0.05$ ).



that there was no significant effect ( $p > 0.05$ ) on resilience (elasticity), and cohesiveness was observed. On the other hand, there are significant differences ( $p < 0.05$ ) in hardness, adhesiveness (stickiness), fracturability, springiness, gumminess, and chewiness (Figure 1). According to our results, all of the used ingredients (probiotics, mango pulp, lime juice, and thyme honey) reduced the hardness of the HGC texture [2]. The hardness value of HGC is lower than that of control gummy candy due to sugar being replaced with honey. It has been found in recent studies that adding sweeteners (xylitol) at a relatively low concentration (3% - 5%) increases the firmness and hardness of the gelled structure [64]. Higher energy is required to deform commercial gummy candy, and this is an undesirable characteristic for consumers, especially adults. Therefore, reducing the hardness led to greater acceptance of the product.

The low variability between the two sides indicates that the HGC samples produced were uniform (Table 4). Teixeira-Lemos, et al. [50] reported an excellent uniformity between the two sides of the gummy jellies made with honey and fruits. The HGC has lower resilience than the control sample; this might be related to the difference in the ingredients of the two products [65]. In line with this, honey in gelatin systems will influence the product structure and rheological properties [66-68]. At the same time, adhesiveness or stickiness refers to the effort required to overcome attractive forces between the food surface and the surface of the substance with which the food is in contact (such as the

tongue, teeth, and palate) [13]. Surface properties and the combined effect of adhesive and cohesive forces play a role in determining these values [69]. The use of glucose syrup and sucrose in commercial gummies led to a higher in the values of hardness and gumminess in the gummies [70], unlike the use of honey in the HGC, which had lower values of hardness, gumminess, and chewiness. The results showed a decrease in the gumminess of samples compared to the control due to fruits and honey being formulated, which meant less energy was required for breaking HGC. Our results are consistent with Moghaddas Kia, et al. [71] results, who observed a reduction in the gumminess of samples when gellan gum and red beet extract were formulated.

Color intensity

Figure 2 a, b, and c show the color coordinates of the HGC on both sides analyzed, top and bottom; the results on the two sides were very similar. The color coordinates  $a^*$  showed positive values for the HGC samples (15.9-15.38). The color coordinates  $b^*$  showed positive values for both sides of the sample (22.94-23.75). The values of  $L^*$  were positive (24.07-26.18). In general, the results of the color evaluation confirmed that the HGC was clear, more yellow, with a more intense red color. Also, no (non-enzymatic browning reactions) were observed during HGC production due to the moderate heat used. The uniformity of HGC between the two sides has been observed. Previous results for gummy jellies made with a mix of berries and oranges with honey have also shown good uniformity between the two sides of the product [50]. Moreover, carotenoid pigments found in ripe mango fruit provide an alternative to artificial colorings and have additional beneficial health effects [72].

Sensory evaluation

The fundamental values for sensory evaluation of HGC are taste, odor, color, mouthfeel, after-taste, and overall acceptability shown in Table 5. Taste, mouthfeel, and overall

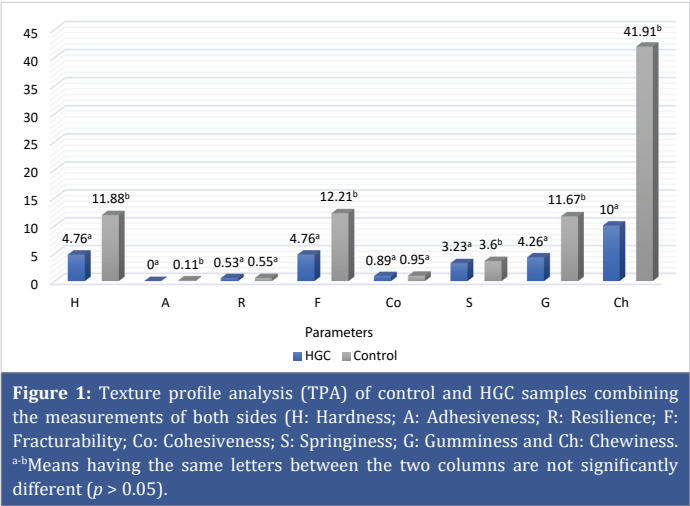


Figure 1: Texture profile analysis (TPA) of control and HGC samples combining the measurements of both sides (H: Hardness; A: Adhesiveness; R: Resilience; F: Fracturability; Co: Cohesiveness; S: Springiness; G: Gumminess and Ch: Chewiness. <sup>a-b</sup>Means having the same letters between the two columns are not significantly different ( $p > 0.05$ ).

Table 4: Texture profile analysis (TPA) on both sides of the control and HGC product.

Texture parameters	HGC		Control	
	Top	Down	Top	Down
Hardness N	4.65 ± 0.23	4.88 ± 0.13	11.97 ± 0.32	11.79 ± 0.98
Adhesiveness mJ	0	0	0.13 ± 0.05	0.10 ± 1.69
Resilience	0.55 ± 0.05	0.51 ± 0.08	0.55 ± 0.02	0.56 ± 0.03
Fracturability N	4.65 ± 0.23	4.88 ± 0.13	12.15 ± 0.45	12.27 ± 0.96
Cohesiveness	0.93 ± 0.02	0.85 ± 0.04	0.96 ± 0.04	0.94 ± 0.05
Springiness mm	3.18 ± 0.04	3.28 ± 0.04	3.58 ± 0.18	3.62 ± 0.24
Gumminess N	4.36 ± 0.26	4.16 ± 0.17	11.74 ± 0.56	11.61 ± 1.06
Chewiness mJ	10 ± 0.00	10 ± 0.00	41.93 ± 0.51	41.90 ± 2.12

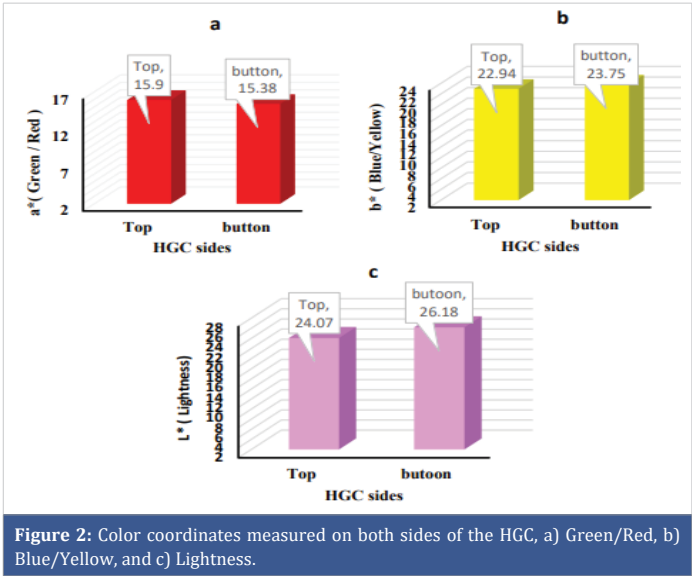


Figure 2: Color coordinates measured on both sides of the HGC, a) Green/Red, b) Blue/Yellow, and c) Lightness.

**Table 5:** Sensory evolution of HGC product.

Parameters	Scores average
Taste	4.1 ± 0.73
Odor	3.6 ± 0.69
Color	3.9 ± 0.56
Mouthfeel	4 ± 0.47
After taste	4.2 ± 0.63
Overall acceptability	4.1 ± 0.31

acceptability had the highest scores and were chosen by panelists. Respondents knew the benefits of probiotics inspired by fermented dairy, and nutritional value was essential to evaluating the HGC. HGC product targets all consumer categories and provides an ideal dessert for children and adults interested in healthy sweets. The scores for color and odor were the lowest compared with other parameter scores due to using natural materials without any artificial food additives [73].

## Conclusion

Natural materials and probiotics-based functional food are well accepted and have a higher market demand. Incorporating healthy ingredients such as fruits as natural flavors and colorings and honey as a natural sweetener to improve the low health profile of gummy confectionery products is a promising strategy. In the current study, encapsulated probiotics in sodium alginate and chitosan provide a high encapsulation efficiency of 88.16%. Moreover, chitosan-coated alginate capsules in HGC products indicated maximum viability and minimum log reduction during storage than free cells. The natural ingredients significantly affect the textural properties ( $p < 0.05$ ). Our research has shown that it seems possible to obtain gummy candies with acceptable sensory properties, which can also be marketed as healthy gummy candies without artificial or additive materials, as well as a good source of probiotics to restore microbial balance in the human digestive system. In addition, the HGC product was considered an excellent carrier of probiotics and a good source of antioxidants. Further research is needed to explore different formulations and improve sensory attributes, which could enhance the shelf life and effectiveness of probiotic-based gummies over time.

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## Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Raghda Abdulhussain Kareem, Seyed Hadi Razavi, and Zeinab Mousavi. Seyed Hadi Razavi wrote the manuscript's first draft, and all authors commented on previous versions. All authors read and approved the final manuscript.

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