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ORIGINAL PAPER

Production and characterization of probiotic jelly candy containing *Bacillus* species

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Abstract

In this study, probiotic soft jelly candy was developed using *Bacillus coagulans*, *Bacillus clausii* and *Bacillus subtilis* as probiotics and the rheological, textural, microbiological and sensory characteristics of jellies were determined. In terms of probiotic features, the viability and in vitro gastrointestinal tract (GIT) durability of probiotics were determined during 3 months of storage. No alteration in the physicochemical properties of the soft candies was observed with the usage of probiotics ($p > 0.05$) in comparison to the control samples. Utilization of the probiotic strains resulted in the increment of the total phenolic content and antioxidant capacities of soft candies. The flow behavior characteristics of the soft candies did not change with the addition of probiotics and they showed similar viscosity values. Jelly candies (P1, P2, EG and BB2) containing strains of *Bacillus subtilis*, *Bacillus coagulans* and *Bacillus clausii* preserved their probiotic properties until the end of the 90th day as the *Bacillus* numbers were higher than 6 log cfu/g. In the in vitro GIT test, the survival rate of *Bacillus* strains was over 90.36% at the end of the storage period. The produced probiotic jelly candies presented desired sensorial acceptability. These findings revealed that using of probiotic *Bacillus* strains is excellent alternative for production of functional confectionery.

Keywords *Bacillus* probiotics · Probiotic soft jelly candy · In vitro gastrointestinal tract durability

Introduction

The confectionery industry has made great progress from the past to the present with the developing technology, and a great increase has been observed in the trade and consumption of sugar with industrial-scale production. Nowadays, it has a market value of approximately 82.3 billion USD [1]. The confectionery industry has a wide range of products such as hard candy, fondants, caramel, fudge, toffee, jellies, gummies, licorices. Soft jelly candies have significant place in the confectionery sector and they are important product group in terms of carrying functional components, as they are frequently consumed by adults and especially children [2]. Importantly, soft jelly gummies, candies and chewing gums are food matrices suitable for the transportation of functional components such as vitamins, antioxidants, fiber and probiotic microorganisms [3, 4]. Studies have shown that with consumers giving more importance to the relationship between health and nutrition, the trend towards healthier and functional products has increased in confectionery products [5]. Among the functional ingredients, probiotics have an important place in food systems and have also gained popularity in the confectionery industry [5–7]. Probiotics are beneficial bacteria that strengthen the intestinal barrier and support the maintenance of the digestive system and immunity, so they have been used as enrichments in many confectionery products such as chocolate, chewing gum and gummy candy [6, 8]. However, it is necessary to ensure a high survival rate of probiotics during processing and production, and to achieve known health benefits, the shelf life of final products must be extended to contain sufficient numbers of viable microorganisms at the time of consumption.

Jelly candies are usually produced with high temperature (> 100 °C) applications and acidity is also at high levels [9] so direct use of probiotics is not appropriate. The viability of commonly used probiotic bacteria such as *Lactobacillus* and

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Bifidobacteria can be adversely affected by various processing steps such as production method and storage conditions [10]. In addition, these bacteria are very sensitive to normal physiological conditions such as the high acidic environment of the stomach and bile salts, and their survival rate under these conditions has low values of approximately 20–40% [11]. For this reason, an extra successful encapsulation process is required in order to use these species during jelly production process. The application of biopolymers for the encapsulation of probiotics and bioactive compounds is a very common method [12], and alternative gelatin sources are also prominent in this field with their various barrier properties and their use as a matrix of bioactive compounds [13–15]. However, when *Bacillus* species are used, encapsulation process is not required thanks to their spores that are resistant to harsh conditions. An important group among *Bacillus* species are probiotics with beneficial effects on health, and the most studied are *Bacillus subtilis*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus laterosporus*. Due to the resistance to tough environment conditions as well as their high level of gastrointestinal tract durability, the tendency to use *Bacillus* probiotics has increased in recent years and their applications in different food matrices have been suggested for alternative productions [16].

Various probiotics, bioactive components, vitamins etc. have been used in the production of functional jelly confectionery so far [6, 7, 17, 18]. However, there is no comprehensive study investigating the addition of different probiotic *Bacillus* species spores to the production of soft jelly confectionery. In order to meet the demand for functional products in the confectionery industry, probiotic *Bacillus* containing jelly candies can be an effective way of carrying probiotics. In this context, this study was aimed to produce probiotic soft candies using *Bacillus* bacteria that can withstand harsh digestion and environmental conditions, and to determine the rheological, physicochemical, textural, sensorial and microbial properties of the produced functional soft candies.

Materials and methods

Bacterial strains and *Bacillus* sporulation

In order to obtain spores from *Bacillus* strains (commercial strains: *Bacillus subtilis* DE111, *Bacillus coagulans* BC99, *Bacillus clausii* Enterogermina® and two isolates: *Bacillus subtilis*: BB2 and *Bacillus coagulans*: FC) (Kahraman et al. submitted) cultures were first grown in Nutrient broth (NB) at 37 °C for 2 days and active cultures were transferred to 50 mL of NB media and incubated for overnight at 37 °C. Then, the cultures were transferred to 250 mL sporulation media (0.2% Glucose, 0.05% $MgSO_4 \cdot 7H_2O$, 0.6% KH_2PO_4, 0.5% Meat extract, 0.3% Peptone) and incubated at 37 °C for 5 days [19–21]. After incubation, the cultures were maintained at 80 °C for 15 min, and then washed with distilled water. For this purpose, cultures were centrifuged at + 4 °C, 5000 rpm for 15 min and the spores were precipitated. Afterwards, the supernatant was discarded, the pellet was washed with 10 mL of sterile distilled water and centrifuged again. This process was repeated three times. Afterwards, the obtained pellet was dissolved in 5 mL of sterile distilled water and stored at + 4 °C to be used in production.

Probiotic soft jelly candy production

Soft jelly confectionery production studies were carried out using three different *Bacillus* commercial strains and two *Bacillus* isolates and in total of six soft jellies were produced including the bacteria-free control sample. For this purpose, firstly, spores were obtained from cultures as stated above. To produce jelly candies, firstly 26 ± 4% (w/w) crystal sugar, 45 ± 5% (w/w) clear, colorless, thick maltose syrup (It was used to prevent crystallization) 3 ± 1% (w/w) water was cooked at 115 °C until brix 83. After that, 18 ± 2% gelatin (with a bloom grade of 250–270) solution (gelatin:water 1:2 w/w) was added for gelling agent to the cooked syrup prepared and mixed at 80 °C. Then, 0.8% (w/w) coloring agent (natural black carrot concentrate) and 0.12% (w/w) flavoring agent (natural strawberry flavor) were added to the total mixture. Finally, the mixture was prepared by adding 3.6% (1:1 w/w) citric acid solution for the control sample and 2.4% (w/w) for the mixture containing bacterial cultures. As the last step, bacterial spores (10^8–10^{10} *Bacillus* spores to 500 g of the product to obtain at least 10^6 spores per g) (Table 7) were added to the mixture separately at a temperature of 45–50 °C. Soft confectionery mixtures were transferred to squeezing bags, deposited into molds containing starch and left to dry at 25 °C for 2 days. The dried soft jelly candies were first cleaned of their starch. Then, oil coating (palm oil, carnauba wax, beeswax) was applied to the outer parts and transferred to plastic containers and plastic bags for storage at room temperature (Fig. 1).

Rheological analysis of jelly samples

In order to determine the flow behavior properties of the samples, the steady state rheological properties were examined. The rheological properties of jelly candy mixtures before gelation were determined at 50 °C without solidification. This temperature was selected considering depositing temperature during production. Rheological measurements were performed using a stress/strain-controlled rheometer device (Anton Paar MCR 302, Austria) equipped with a parallel plate system. The distance between the two plates was adjusted to be 0.5 mm and shear rate was applied to the

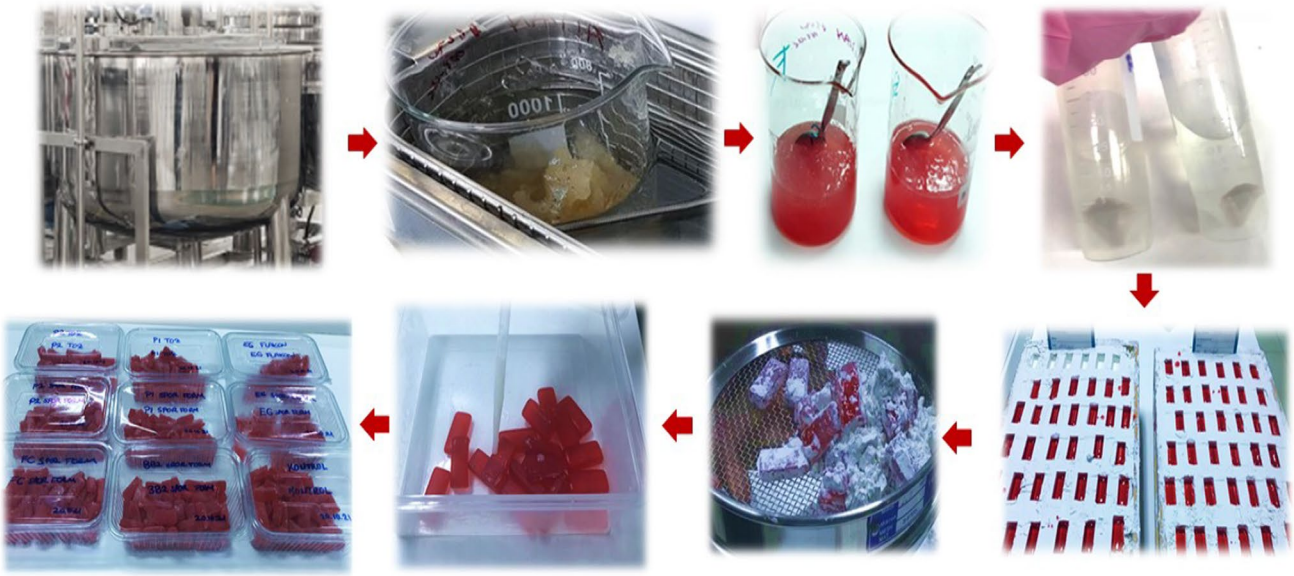


Fig. 1 Probiotic jelly candy production process

sample in the range of 0.1–100 s^{-1} during the measurement. Herschel Bulkley model (Eq. 1) was used to describe flow behavior.

$$\sigma = \sigma_0 + K\dot{\gamma}^n \quad (1)$$

where σ is the shear stress (Pa), K is the consistency coefficient (Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}), n is the flow behavior index (dimensionless), σ_0 is the yield stress (Pa).

Textural analysis of jelly samples

Texture profile analysis (TPA) was performed with a texture analyzer (Micro Stable TA-TX Plus, UK) equipped with a 5 kg load cell, using P2 cylindrical probe and candy holder body. Samples were subjected to double compression with the probe allowing the sample to deform without puncturing. The TPA test was used with the following input data; TPA option, strength in grams, strain distance format, 1.7 mm/s pre-test, test and post-test speeds, 73% strain. The hardness (N), stickiness, gumminess and resilience values of the jelly candy samples were calculated from the TPA graph. Texture analysis was performed during the storage of jelly candies.

Determination of the physicochemical characteristics

The pH value (Inolab, pH 7110, WTW, Germany), brix (Optical Refractometer, Abbe 5, Germany) and the water activity (a_w) (LabTouch-aw, Novasina, Switzerland) of the jelly candies were determined according to AOAC [22]. Color properties of the jelly candy samples were determined using colorimeter (Konica-Minolta CR-400, Japan).

Determination of bioactive properties of jelly samples

Total phenolic contents

Bioactive components were extracted from jelly candies with 80% (v/v) methanol–water. In the extraction process, 5 g of candy put into 30 mL of solvent and the dispersion was shaked for 24 h at room temperature with shaker (Heidolph Rotamax 120, Germany) equipped with orbital platform. Then the mixture was filtered. The total phenolic content was determined according to the Folin–Ciocalteu spectrophotometric method described by [23] with some modifications. First, 0.5 mL of extract was mixed with 2.5 mL of tenfold diluted Folin–Ciocalteu's phenol reagent. Then 2 mL of Na_2CO_3 aqueous solution (%7 w/v) was added to the mixture and shaken. After 30 min of reaction at room temperature, the mixture was centrifuged (3000 rpm for 5 min), filtered (with the 0.22 µm pore size filter) and the absorbance was read with a spectrophotometer (Optizen Pop Bio UV/Vis Spectrophotometer, S. Korea) at 765 nm. The measurement was compared to a standard curve of gallic acid (GA) solution. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per kilogram (mg GAE/kg). All measurements were performed in triplicate.

Antioxidant capacity

The antioxidant activity of the samples was determined using to the method described by [24]. For this purpose, 0.1 mL of the extract was mixed with 4.9 mL of 0.1 mM DPPH solution and the mixture was incubated at 27 °C for 20 min. Then the mixture was centrifuged (3000 rpm for 5 min) and filtered (with the 0.22 µm pore size filter). The absorbance of the samples was measured at 517 nm using a UV–vis spectrometer (Optizen Pop Bio UV/Vis Spectrophotometer, S. Korea). The results expressed µmol Trolox equivalent per gram (µmol TE/g). All measurements were performed in triplicate.

Evaluation of the viability of *Bacillus* strains

Probiotic *Bacillus* strains were quantified in the Nutrient Agar (TSA, Merck Millipore, USA). The diluted samples were plated in nutrient agar by the drop plate technique, and the Petri dishes were incubated at 37 °C for 24 h for *Bacillus subtilis* species and 48 h for *Bacillus coagulans* and *Bacillus clausii* species. The viability of *Bacillus* species was determined during the storage period.

"In vitro" simulation of gastrointestinal conditions

In vitro gastrointestinal tract (GIT) durability analysis of produced probiotic jelly candies was determined according to the method recommended by Buriti et al. [25] and Liserre et al. [26]. The samples (10 g) were exposed to the treatment of pepsin (3 g/L) and lipase (0.9 g/L) solutions that had a pH of 2.0 set with the aid of 1 M sterile hydrochloric acid (HCl) solution to simulate the gastric phase. For enteric I phase simulation, solutions of bile salts (10 g/L) and pancreatin (1 g/L) were then added and the pH was adjusted to 5.6 using alkaline solution (150 mL 1 M NaOH per L, 14 g $PO_4H_2Na \times H_20$). Finally, to simulate the enteric II phase, 10 g/L bile salt and 1 g/L pancreatin solution were added to the samples again after the pH was adjusted to 7.2. Each solution of the three phases were kept at 37 °C in a shaker for 2 h. To count spores, 1 mL of food matrix (jelly candies) exposed to simulated gastrointestinal conditions was collected at 0. and 6. h and plated to nutrient agar for counts. The survival rates of probiotic strains at t_0 and t_f were calculated according to the equivalence stated below (Eq. 2).

$$\%Survival = \frac{Nlogfinaltime(6h)}{Nloginitialtime(0h)} \times 100 \quad (2)$$

In vitro gastrointestinal tract durability analysis was performed during the storage period.

Sensory evaluation

The sensory properties of the probiotic jelly candies produced were determined by the scoring test based on the grading of the quality characteristics of the samples, such as color, smell and taste. Sensory profiling was carried out by 20 semi-trained panelists informed for this purpose. Panelists evaluated the products for color, smell, chewiness, taste characteristics and general acceptability. Panelists rated the product on a scale of 1 (extremely disliked) to 10 (extremely liked) for each sensory trait. Sensory analysis was performed during the storage period.

Statistical analysis

Statistical analysis was carried out to evaluate the data differences of the *Bacillus* treated and the control samples. The data obtained were subjected to analysis of variance. The results were subjected to one-way analysis of variance. Variance analyzes were performed using the JMP (release 6.0) package program. Significance levels between the means were determined by the Tukey test.

Results and discussion

Rheological properties of jelly samples

Steady state rheological properties were determined in order to determine the flow behavior properties of the jell candy samples at the deposit temperature (50 °C). A decrease in viscosity was observed with increasing shear rate in both the control sample and the samples containing probiotics (Fig. 2). This result was in the expected characteristic due to the pseudoplastic structure of the jelly candies produced. Applied forces during shearing can result in deformation in shear direction, disentanglements of the structures occurred, orientation of particles in flow direction, disintegration of particles and also deformation of the particles as a result of mechanical forces generated during shear can result in decreasing viscosity of the samples. Apparent viscosity of the probiotic samples was found to be very close to control sample at the same shear rate values. The parameters of the Herschel–Bulkley model obtained by fitting of the shear rate versus shear stress data to the model Eq. 1 are given in Table 1. Yield stress and consistency coefficient values of the samples changed between 26.91–30.52 Pa and 4.16–4.49 Pa s^n. Slight differences were observed among the yield stress and consistency coefficient of the samples. These two parameters are important in depositing of the samples during moulding process, mixing of the samples during addition of aroma and acid, and spreading of the samples in the mould. During production process of the samples, there

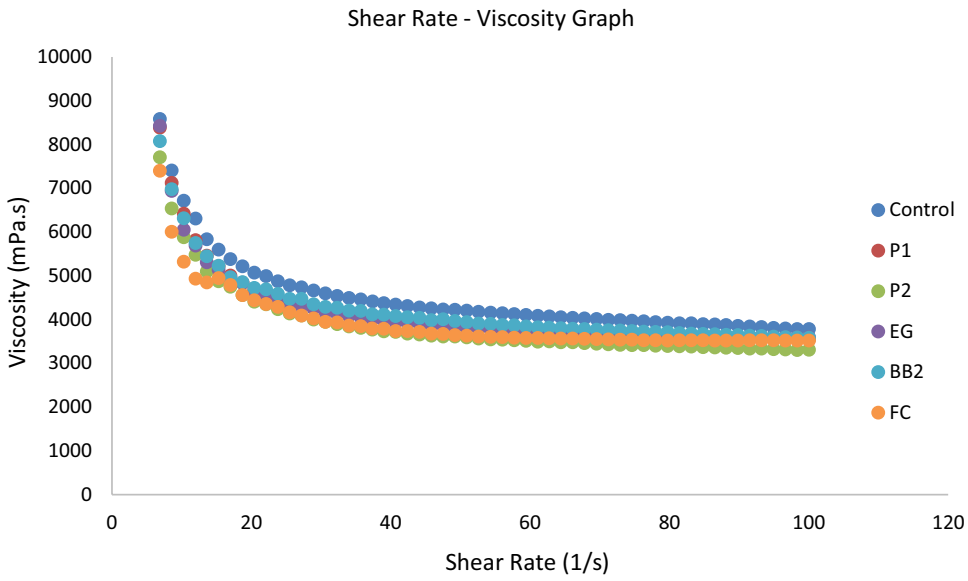


Fig. 2 The shear rate–viscosity graph of the jelly candies

Table 1 Herschel–Bulkley function parameters used in determining the flow behavior in jelly candy mix

	σ_0 (Pa)	K (Pa s^n)	n	R^2
Control	26.91 ± 7.25^a	4.49 ± 0.65^a	0.945 ± 0.04^a	0.99 ± 0.00^a
P1	30.52 ± 4.36^a	4.18 ± 0.61^a	0.955 ± 0.02^a	0.99 ± 0.00^a
P2	27.29 ± 7.79^a	4.48 ± 0.66^a	0.945 ± 0.04^a	0.99 ± 0.00^a
EG	27.51 ± 2.94^a	4.40 ± 0.16^a	0.955 ± 0.01^a	0.99 ± 0.00^a
BB2	26.76 ± 1.75^a	4.16 ± 0.55^a	0.960 ± 0.03^a	0.99 ± 0.00^a
FC	27.43 ± 0.81^a	4.43 ± 0.17^a	0.950 ± 0.01^a	0.99 ± 0.00^a

Averages marked with the same lowercase letter indicate that there is no statistical difference between products ($p < 0.05$)

Jelly candy samples coded according to the probiotic bacteria contains

P1 Bacillus subtilis DE111, *P2 Bacillus coagulans* BC99, *EG Bacillus clausii* Enterogermina®, *BB2 Bacillus subtilis*, *FC Bacillus coagulans*

was no problem encountered. Rheological properties of all the samples were suitable for machinability.

Textural properties

The textural properties of soft jelly candies on day 1 are given in Table 2. Hardness, cohesiveness, gumminess and resilience values obtained as a result of TPA analysis were examined. In addition, the textural properties of all soft candies produced were measured for 90 days and similar results were obtained within the tested samples. Table 3 shows the texture results of the sample P1 which was selected as the most preferred sample by sensory evaluation. Hardness is one of the most important sensory parameters obtained from TPA analysis. It is used to evaluate the sensation of food in the mouth and is defined as the force that must be applied to

Table 2 Textural properties of soft jelly candies on the 1st day

	Hardness (N)	Cohesiveness	Gumminess (N)	Resilience
Control	5.14 ± 0.53^a	0.91 ± 0.01^a	4.50 ± 0.19^a	0.59 ± 0.03^a
P1	5.18 ± 0.59^a	0.92 ± 0.02^a	4.56 ± 0.58^a	0.57 ± 0.03^{abc}
P2	5.05 ± 0.57^a	0.88 ± 0.02^b	4.46 ± 0.52^a	0.54 ± 0.01^{bc}
EG	5.02 ± 0.10^a	0.91 ± 0.00^a	4.89 ± 0.41^a	0.58 ± 0.02^{ab}
BB2	5.04 ± 0.17^a	0.90 ± 0.01^{ab}	4.39 ± 0.24^a	0.54 ± 0.02^{bc}
FC	5.08 ± 0.56^a	0.91 ± 0.00^a	4.40 ± 0.14^a	0.53 ± 0.00^c

Averages marked with the same lowercase letter indicate that there is no statistical difference between products ($p < 0.05$)

Table 3 Textural properties of P1 coded soft jelly candy with *Bacillus subtilis* addition during storage

	Hardness (N)	Cohesiveness	Gumminess (N)	Resilience
Day 1	5.18 ± 0.59^A	0.92 ± 0.02^A	4.56 ± 0.58^A	0.57 ± 0.03^A
Day 15	5.55 ± 0.99^A	0.91 ± 0.01^A	5.54 ± 0.86^A	0.58 ± 0.02^A
Day 45	5.60 ± 1.34^A	0.87 ± 0.01^B	5.06 ± 0.87^A	0.55 ± 0.01^{AB}
Day 90	6.03 ± 1.47^A	0.87 ± 0.03^B	5.71 ± 0.45^A	0.51 ± 0.03^B

Averages marked with the same capital letter indicate no statistical difference due to storage in the same product ($p < 0.05$)

bring about a certain pre-determined deformation [27, 28]. No significant difference ($p > 0.05$) was observed between the control group and probiotic added candies. This can be evaluated as the addition of probiotics did not significantly change the firmness in the structure. Although the hardness values of the samples on the 1st day varied between 5.18 ± 0.59 N and 5.02 ± 0.10 N, a non-significant increase ($p > 0.05$) was observed depending on storage (Table 3).

In other studies, hardness values of soft jelly candies were altered between 07.15 and 17.30 N potentially due to varying gelatin ratios and product contents [9, 29]. At the 90th day of storage, the hardness values of the samples ranged from 6.73 ± 0.59 (BB2) to 5.94 ± 0.96 (FC). The reason for this increase in hardness was attributed to small moisture losses in the products during storage.

The cohesiveness value is used to evaluate the extent to which the product withstands a deformation applied a second time after the first deformation, compared to the first deformation (using the area under the force time diagram). At low stickiness values, the soft confectionery is easy to chew and swallow. Cohesiveness has been accepted as an important factor among the acceptance criteria for many years [30]. The control sample and the samples containing probiotics were found to have similar cohesiveness values with values varying between 0.78 and 0.94 in the literature [9, 29, 31]. While the cohesiveness values of the soft candies produced varied between 0.88 ± 0.00 and 0.92 ± 0.02 on the 1st day (Table 2), they changed between 0.84 ± 0.03 and 0.88 ± 0.02 on the 90th day. During the storage period, there was a decrease in cohesiveness in products in comparison to control sample.

Gumminess, is previously defined as "the energy necessary to breakdown a semisolid food product into a state suitable for swallowing, and it is related to the basic characteristics of hardness and cohesiveness" [32]. This secondary parameter, as can be seen, only applies to semisolid materials with a low hardness. The gumminess value of the P1 coded sample was found to be closest to the control sample, with a value of 4.56 ± 0.58 (Table 2). No significant difference ($p > 0.05$) was observed during storage (Table 3).

Resilience values are a measure of the extent to which food is restored in terms of speed and strength when deformation is eliminated. The resilience values of the samples on the 1st day varied between 0.59 ± 0.03 and 0.53 ± 0.00. In terms of resilience, significant differences were observed between the control sample and the jelly candies containing probiotics ($p < 0.05$). In general, the resilience values of the control jelly candies were found to be higher (Table 2). Depending on the storage, a decrease was observed in the resilience values of the samples in general (except FC coded probiotic jelly candy containing *B. coagulans*). All these findings on the textural properties of the jelly samples demonstrated that, no alteration in the textural properties of jelly samples were observed in *Bacillus* containing jelly samples in comparison to the control group which suggest the suitability of jelly samples as a probiotic *Bacillus* carrier.

Physicochemical characteristics of jelly samples

The °Brix, aw, and pH values of the jellies at day 1 are given in Table 4. The brix values of the jelly candies after production ranged from 83.50 ± 0.42 to 83.15 ± 0.07, and no significant difference was observed between them ($p > 0.05$). Physical parameters such as °Brix and pH are usually measured in confectionery factories to control the degree of hydration [33]. Brix content of jelly candies is generally higher than 65° Brix, but these values are concentrated in the range of 75–85 in the literature [33–35]. The pH value of jelly candies containing probiotics was slightly higher than the control group. The reason for this difference in pH is due to the fact that the citric acid added during production is less added to probiotic productions in order to prevent the probiotics from being damaged. When the probiotic jelly candies pH values were compared among themselves, no statistically significant difference was observed ($p > 0.05$). Regarding water activity, there was no significant difference between the evaluated groups ($p > 0.05$). These values are safe for the final product quality and should be between 0.55 and 0.75 [36]. Water activity indicates the fraction of total moisture of a product that is free and therefore suitable for different chemical reactions that can affect microbial growth and product stability [37]. Thus, according to the results obtained, jelly candies can be classified as acidic foods with water activity between 0.9 and 0.6 [38, 39].

Color characteristics of food products, especially jell candy products, are important quality parameters that can determine the first impression as a vital component of consumer acceptability. As can be seen in Table 5, no significant

Table 4 Physicochemical properties of the soft jelly candies

	°Brix	pH	a_w
Control	83.35 ± 0.21[a]	2.94 ± 0.007[b]	0.63 ± 0.004[a]
P1	83.35 ± 0.35[a]	3.13 ± 0.007[a]	0.63 ± 0.013[a]
P2	83.15 ± 0.07[a]	3.15 ± 0.071[a]	0.63 ± 0.002[a]
EG	83.35 ± 0.07[a]	3.20 ± 0.021[a]	0.62 ± 0.004[a]
BB2	83.35 ± 0.07[a]	3.19 ± 0.007[a]	0.62 ± 0.004[a]
FC	83.50 ± 0.42[a]	3.17 ± 0.014[a]	0.62 ± 0.007[a]

Averages marked with the same lowercase letter indicate that there is no statistical difference between products ($p < 0.05$)

Table 5 Color characteristics of the soft jelly candies

	L*	a*	b*
Control	32.63 ± 1.56[a]	6.85 ± 1.13[a]	− 0.54 ± 0.51[a]
P1	31.49 ± 1.96[a]	9.33 ± 1.63[a]	0.00 ± 0.49[a]
P2	32.34 ± 0.63[a]	8.82 ± 1.19[a]	1.02 ± 1.65[a]
EG	31.94 ± 0.90[a]	8.85 ± 0.37[a]	− 0.53 ± 0.42[a]
BB2	31.55 ± 1.22[a]	8.92 ± 1.60[a]	− 0.27 ± 0.92[a]
FC	32.31 ± 1.81[a]	8.03 ± 0.52[a]	− 0.24 ± 0.17[a]

Averages marked with the same lowercase letter indicate that there is no statistical difference between products ($p < 0.05$)

difference ($p > 0.05$) was observed in terms of L* value when the control group and soft jelly candies containing probiotics were compared. It was observed that the addition of probiotics did not cause a significant change in the darkening of soft jelly candies. The fact that there was no significant difference ($p > 0.05$) between the a* and b* values suggests the close characteristics of the jellies in terms of color. Similar color properties were also observed in sensorial test (Fig. 3).

Bioactive properties

The bioactive properties of jelly candies are given in Table 6. The addition of probiotics had a positive effect on the phenolic content of the samples. In the 1st day measurements, the phenolic content of the control sample was 180.60 ± 6.51 mg GAE/kg, while the highest P1 coded jelly candy was 202.80 ± 2.26 mg GAE/kg produced with the addition of *Bacillus subtilis*. Likewise, the antioxidant content was positively affected by the addition of probiotics. The 1st-day DPPH radical scavenging activities of probiotic-containing jelly candies was ranged from 3.861 to 4.424 µmol trolox/g. Studies have shown that bioactive compounds, which are very important for foods, increase with various functional raw materials added to foods [15, 40, 41]. Our findings are in accordance with previous suggestion as soft jelly candies can be specified as a suitable formulation for supplementation with different bioactive compounds such as nutraceuticals and antioxidants as well as for probiotics [42].

Table 6 Total phenolic content and antioxidant capacity of the soft jelly candies

	Total phenolic (mg GAE/kg)	DPPH (µmol trolox/g)
Control	180.60 ± 6.51^{b}	3.861 ± 0.042^{d}
P1	202.80 ± 2.26^{a}	4.412 ± 0.04^{a}
P2	200.00 ± 6.79^{ab}	4.070 ± 0.034^{c}
EG	192.40 ± 5.09^{ab}	4.166 ± 0.051^{bc}
BB2	200.40 ± 5.09^{ab}	4.424 ± 0.025^{a}
FC	197.40 ± 3.11^{ab}	4.316 ± 0.076^{ab}

Averages marked with the same lowercase letter indicate that there is no statistical difference between products ($p < 0.05$)

Viability of *Bacillus* species probiotics

Bacterial counts of the soft jelly candies produced are given in Table 7. As expected, a decrease of approximately 2 log10 units was observed during the production process (Table 7). The general opinion is that the minimum number of probiotic microorganisms required to provide health benefits to the consumer should be higher than 10^6 cfu/g [14, 43–45]. According to the bacterial count results on the 1st day, it was seen that all jelly candies samples achieved the required

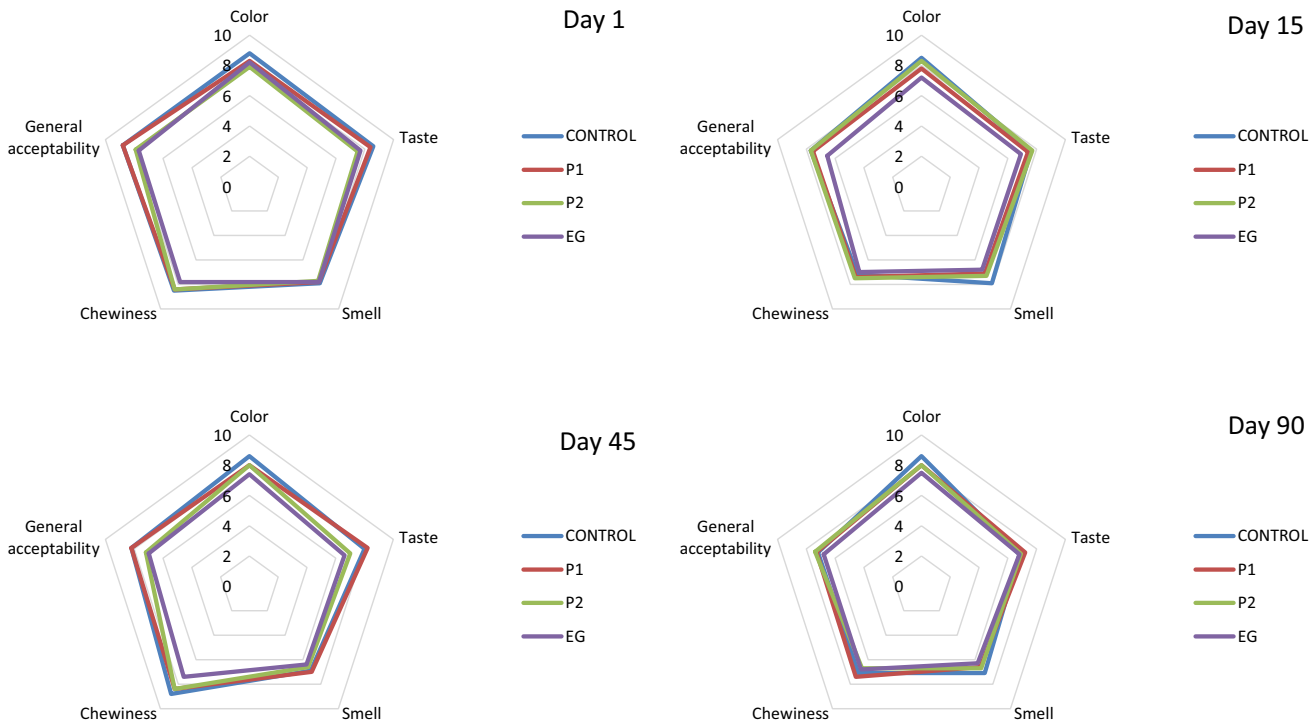


Fig. 3 Sensory properties of soft jelly candies during storage (Color figure online)

Table 7 Viability of *Bacillus* probiotics in soft jelly candies during storage

Jelly candy product	Amount of mixture (g)	The amount of bacteria added to the mix (log cfu/g)	The amount of bacteria in the final product (log cfu/g) during storage days			
			Day 1	Day 15	Day 45	Day 90
Control	600	0	–	–	–	–
P1	600	10.15 ± 0.11	8.09 ± 0.31^{Aa}	7.81 ± 0.30^{ABa}	7.63 ± 0.06^{ABa}	7.02 ± 0.07^{Ba}
P2	600	10.01 ± 0.05	7.56 ± 0.72^{Aab}	7.51 ± 0.21^{Aa}	6.96 ± 0.16^{Ab}	6.74 ± 0.04^{Ab}
EG	600	9.43 ± 0.14	7.26 ± 0.24^{Aab}	7.09 ± 0.36^{Aa}	6.46 ± 0.15^{Bc}	6.06 ± 0.10^{Bc}
BB2	400	9.42 ± 0.37	7.39 ± 0.21^{Aab}	7.33 ± 0.25^{Aa}	7.40 ± 0.02^{Aa}	7.19 ± 0.01^{Aa}
FC	400	8.40 ± 0.07	6.52 ± 0.47^{Ab}	5.99 ± 0.09^{ABb}	5.90 ± 0.17^{ABd}	5.80 ± 0.17^{Bc}

Averages marked with the same capital letter indicate no statistical difference due to storage in the same product. Averages marked with the same lowercase letter indicate that there is no statistical difference between products on the same day ($p < 0.05$)

viable microbial number to carry probiotic properties. Jelly candies containing strains of *Bacillus* P1, P2, EG and BB2 preserved their probiotic properties throughout the storage period. However, *Bacillus* numbers of candy sample produced with *Bacillus coagulans* FC was below this limit. Since the production of soft jelly candy is carried out by high temperature and acidity, it is important to preserve the durability of the bacteria during production and storage. Bacterial spores are produced as a means of survival in extreme environmental conditions [46]. Their thermal stability and ability to survive in harsh conditions have made *Bacillus* species attractive for their usage as food additives [16]. According the our findings, these high survival values of *Bacillus* species make them important candidates for the production of functional probiotic soft confectionery.

Durability of *Bacillus* species in jellies under simulated GIT conditions

Resistance to simulated gastric and enteric fluids are among the frequently recommended in vitro tests for evaluating probiotic potential of microbial strains [25, 47]. Controlling the tolerance of probiotic microorganisms in the final product to gastrointestinal conditions can help to select an appropriate food matrix, particularly for bacteria more sensitive to GIT conditions, and may contribute to their survival [25, 48]. The survival rates of microbial strains with probiotic properties added to soft confectionery products exposed to simulated GIT fluids were determined during the storage period of each product (Table 8). Survival percentages of probiotic *Bacillus* species in soft candies were found as higher than 90% under GIT conditions. In a study in which probiotic bacteria were used as carriers in various food matrices, it was found that *Bacillus* probiotics showed higher viability than probiotic *Bifidobacterium* and *Lactobacillus* strains regardless of the food examined, and the survival rate of *Bacillus* strains was always above 83% under GIT conditions [49]. When the samples were compared on the 1st day, it was determined that there was no significant difference between them ($p > 0.05$). Depending on the storage, a slight decrease was observed on the 15th, 45th and 90th days in the P1 coded sample, while the other soft candies did not differ significantly depending on storage. These findings demonstrated that jellies could be an important food matrix not only to carry *Bacillus* species during their shelf life but also to potentially increase their survival under GIT conditions.

Table 8 In vitro gastrointestinal tract durability of *Bacillus* probiotics in soft jelly candies during storage

	% Survival			
	Day 1	Day 15	Day 45	Day 90
P1	96.20 ± 1.65^{Aab}	94.74 ± 0.81^{ABab}	94.93 ± 0.60^{ABa}	93.22 ± 0.24^{Ba}
P2	96.93 ± 1.06^{Aa}	95.01 ± 2.42^{Aab}	94.93 ± 3.95^{Aa}	94.46 ± 1.01^{Aa}
EG	90.13 ± 1.97^{Ab}	91.40 ± 0.79^{Aab}	91.32 ± 1.16^{Aa}	90.36 ± 2.62^{Aa}
BB2	96.88 ± 1.18^{Aa}	95.60 ± 1.56^{Aa}	94.95 ± 2.01^{Aa}	93.45 ± 1.43^{Aa}
FC	92.20 ± 4.58^{Aab}	90.84 ± 1.93^{Ab}	90.56 ± 3.73^{Aa}	91.37 ± 3.16^{Aa}

Averages marked with the same capital letter indicate no statistical difference due to storage in the same product. Averages marked with the same lowercase letter indicate that there is no statistical difference between products on the same day ($p < 0.05$)

Sensorial properties

Sensory analysis results of soft candies are given in Fig. 3. General acceptability was found mostly in the control soft candy and P1 coded product containing *Bacillus subtilis*. The overall acceptability ranged from 8.8 ± 1.40 to 6.6 ± 1.42, and it was seen that the addition of probiotics did not adversely affect the sensorial properties of soft confectionery. Although the taste of soft candies was appreciated, P2 and P3 coded soft candies containing *Bacillus coagulans* and *Bacillus clausii* had the lowest scores. The chewiness properties of the soft candies produced were reflected in the results in a way that there was no difference in the averages of different products on the same days in accordance with the texture analysis results. The smell characteristics of the soft candies were appreciated and no significant difference was observed between them ($p > 0.05$). The color likability of the probiotic soft candies was found to be very close to that of the control sample. According to the results of the statistical analysis between the samples, no significant difference was observed between the samples on the same storage day and no difference was observed in terms of color, taste and smell during storage ($p > 0.05$).

Conclusion

As consumers become more conscious about healthy nutrition, they prefer functional foods, such as probiotic enriched products. This study showed that a probiotic soft jelly candy containing *Bacillus* species with desired textural, sensorial quality could be produced. Thanks to the resistant spores of *Bacillus* probiotics, the probiotics in the product showed high viability and in vitro GIT durability during 90 days of storage, and the product showed an excellent carrier property. Importantly no alteration in the characteristics of the jelly was observed with the usage of *Bacillus* species. More studies are underway to produce *Bacillus* containing probiotic candies enriched with prebiotics or fruit extracts.

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Author contributions In this research, all authors contributed effectively. BK conducted the experiments, collected the data and contributed to writing. KK assisted in conducting laboratory analyzes and collecting the data. DD provided technical support for the produce of jelly candies. ÖST and ED contributed to the planning, conducting and evaluation of the experiments. MA, supervised the research project, gave suggestions for experimental design and revised the manuscript. All authors read and approved the final manuscript.

Data availability All data interests and material in this study support published claims of the authors and comply with field standards.

Code availability No software application and custom code was available in this study.

Declarations

Conflict of interest The authors have declared no conflict of interest for this article.

Consent to participate All authors have consent to participate in this study.

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