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Incorporation of Microencapsulated and Free *Lactiplantibacillus plantarum* to Bitter Chocolate: Sensory and Survival Analyses

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Abstract

Background and Objective: Chocolate is a trendy food consumed by various age groups. It has been hypothesized that shocolate can become a significant functional product by incorporating probiotics into it. In this study, chocolate was used as a food matrix to transfer probiotic microorganisms to it. Bitter chocolate was chosen due to its preference by the consumers. Therefore, free and microencapsulated probiotic cultures were prepared.

Material and Methods: *Lactiplantibacillus plantarum* was used as the probiotic microorganism and calcium-alginate gel capsule was used for microencapsulation. The number of microorganisms and sensory characteristics of free, microencapsulated probiotic culture and culture-free bitter chocolates were assessed after 60 d of storage at 18 °C.

Results and Conclusion: Based on the results, count of the microorganisms in probiotic chocolate was 5.8×10^7 CFU g^{-1} on Day 0, while it decreased to 1.7×10^7 CFU g^{-1} on Day 60. Although decreases were seen in the level of probiotics, it has been shown that shocolates included sufficient counts of probiotics to be reported as probiotic chocolates. The microbial count of probiotics in microencapsulated probiotic chocolate (2.1×10^7 CFU g^{-1} on Day 0) decreased significantly to 2.4×10^5 CFU g^{-1} on Day 60. The highest microbial count was observed in samples containing free probiotic cultures after 60 d. However the microbial count did not decrease significantly in samples containing free cultures, 2-log decreases were observed in microencapsulated cultures. Thus, chocolate can be used as matrix for the probiotics. For sensory analysis, sample containing free culture was the most preferred after 60 days of storage regarding the overall acceptability.

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1. Introduction

Consumption of functional products, including probiotic foods, has increased worldwide. Pursuit of healthy eating and lifestyle has affected humanity in recent years, leading to health-conscious individuals to turn to functional foods. Probiotics, live microorganisms with positive health effects when consumed appropriately, are incorporated into foods, enhancing nutritional and technological characteristics of the foods. These functional probiotic foods promote intestinal health by increasing beneficial microorganisms, preventing diarrhea and inhibiting harmful pathogen colonization. Other benefits include lowering blood cholesterol, strengthening the immune system and neutralizing cancer-causing compounds. Addition of probiotics to foods is critical for the human health [1-4]. *Lactiplantibacillus plantarum* (*L. plantarum*) is a microorganism belonging to the probiotic microorganism group [5-7]. Addition of probiotic microorganisms to food products can lead to decreases in the number of probiotic cultures due to the stressful environments. To minimize these barriers, techniques such as the selection of bacterial strains, regulation of food processing processes and microencapsulation have been developed and used to protect probiotic bacteria. Microencapsulation is the process of entrapping microorganisms with appropriate carrier support materials. This creates a film layer around the cells, protecting the cell viability against the barriers. The most studied technique in this method includes extrusion coating based on the forming calcium-alginate gel capsules [7].

Alginate is used in the microencapsulation method due to several advantages. These include being non-toxic to the body, having characteristics that easily encapsulate bacteria, being safe for foods, being inexpensive and being soluble in the intestines. Size and shape of the beads formed in the microencapsulation method depend on the diameter of the needle; through which, alginate is transferred, density of the alginate and distance; to which, the alginate is transferred [8-12]. Probiotic foods include approximately 60-70% of the functional food market. Although a majority of the probiotic products are yogurts and fermented dairy products, production of non-dairy probiotic products such as chocolates has increased in recent years [10,13]. Cocoa butter, sugar and cocoa particles include basic components of the chocolates. Researchers have reported that chocolate has characteristics that can carry probiotic microorganisms and tolerate adverse effects of the gastrointestinal system [14,15]. It has been reported that *Bifidobacterium lactis, Lactobacillus* (*L.*) *acidophilus, L. paracasei, L. casei* and *L. rhamnosus* probiotics have successfully been used in production of chocolate and cocoa desserts. Based on a similar study results, no difference was found in sensory characteristics and the products could be used as carrier matrices for the probiotics, comparing probiotic-added products with control samples [16].

The primary aim of the present study was to decrease digestive problems caused by the changes in the intestinal microbiota due to the changes in the current diet systems and frequent uses of fast ready-to-eat meals. Reactions can develop against the probiotic isolates, especially in childhood. The target includes development of intestinal microbiota by adding probiotic cultures to foods such as chocolates, which are loved by the people of all age groups. So, aim of this study was to add *L. plantarum* probiotics (microencapsulated and free) to bitter chocolate samples that provided them functional characteristics. A probiotic strain, which was a food supplement in capsule form, was used in the study. This was a novel approach for the chocolate matrix. In addition, temperature value assessed as the storage temperature in functional chocolate experiments was a temperature value that was not used previously. Changes in the number of microorganisms in chocolates turned into functional products at the end of the storage and their sensory characteristics were assessed by trained panelists. Another aim included assessment of the matrix characteristics of chocolates in carrying probiotic cultures.

2. Materials and Methods

Bitter chocolate was purchased from Solen Cikolata Gida Sanayi, Gaziantep, Türkiye. The probiotic microorganism used included *L. plantarum* 299v. This microorganism (later named *L. plantarum*) was isolated and used from a commercially available probiotic food supplement (Probest, Abdi Ibrahim Ilac Sanayi ve Ticaret Anonim Sirketi, Istanbul, Türkiye). McFarland Unit Cell Densitometer (Biosan SIA, Latvia) is used to measure cell concentrations. All the chemicals were purchased from Merck, Germany.

2.1. Chocolate

Chocolate production includes several stages. In the final stage, semi-finished chocolate is tempered, molded and packaged. In the tempering process, semi-finished chocolate is melted at 50 °C and then cooled down to 28 °C. This process is repeated 4-5 times. As a result of the process, chocolate is poured into molds. With the tempering process, chocolate becomes much smoother and shiny. Semi-finished chocolate was used in the current study and no tempering process was used to assess the matrix formation characteristics of chocolates for the probiotic microorganisms. Chocolate production was designed in three various ways. The first sample included normal chocolate without microbial culture, the second sample contained free-form probiotic microorganism culture (*L. plantarum*) and the third sample included microencapsulated probiotic *L. plantarum* culture (Figure 1). In preparation of the chocolate, semi-finished chocolate was melted at 50 °C using water bath and then transferred to sterile molds [17]. Molded chocolate was cooled down at room temperature and set to harden. Chocolate was stored at 18 °C until analysis. Characteristics of bitter chocolate used as semi-finished product in the study are listed in Table 1.

2.2. Addition of Probiotic Microorganisms to Chocolates

In this study, *L. plantarum* probiotics were used. The *L. plantarum* was cultured in MRS media at 37°C for 24 h. To quickly assess the probiotic culture for the addition to chocolates, bacterial count was set to 1 McFarland (approximately 3.0×10^8 CFU ml^{-1}). In assessment of the exact number of the bacteria, necessary dilutions were prepared and the number of microorganisms was assessed using spreading method.

Table 1. Characteristics of the chocolates used as semi-finished products

Characteristics	Levels
Humidity (%)	max 1.5
Water activity (a_w at 25 °C)	max 0.6
Ash (%)	max 2.5
Total cocoa mass (%)	min 35.0
Fat-free cocoa mass (%)	min 14.0
Cocoa butter (%)	min 18.0

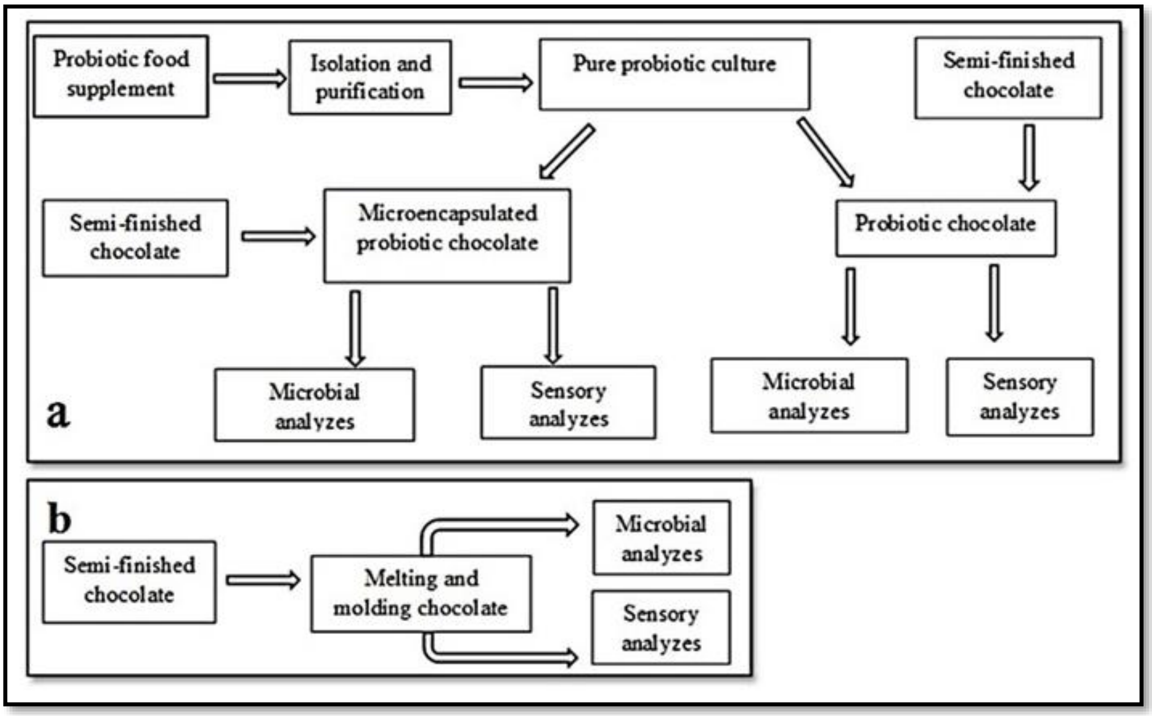

Figure 1. Schematic representation of the chocolate production. Probiotic chocolates and microencapsulated chocolates (a), and chocolates without probiotics (b).

Probiotic and microencapsulated cultures were added to the chocolates and the chocolates were then transferred to sterile packages. The mixture was allowed to cool down at RT until the desired hardness was achieved. The chocolate packages were sealed and stored at 18 °C using incubator. Number of microorganisms was assessed on Days 0, 30 and 60 of storage and sensory analyses were carried out [18,19]. All studies were carried out in two parallels and three replications.

2.3. Microencapsulation of Probiotic Microorganisms

Extrusion technique was used for the microencapsulation of probiotic microorganisms. During preparation of the probiotic cultures, cultures were centrifuged at 3000 g after incubation in MRS media at 37 °C for 24 h). After discarding the supernatant, cultures were dissolved and concentration of the microorganisms increased using 1 McFarland (approximately 3.0×10^{8} CFU m-^{l}). Microorganisms were added into a previously prepared 1% sterile sodium alginate solution using syringe. The homogenized alginate with bacterial mixture was transferred to a 1% (w v^{-1}) sterile $CaCl_2$ solution [18]. The alginate with the bacterial mixture was homogenized in $CaCl_2$ solution using magnetic stirrer and then solidified. Beads were filtered using Whatman no. 4 filter papers and transferred to sterile Petri dishes [20]. During chocolate production, microencapsulated culture from these Petri dishes was homogeneously added to the chocolates [21].

2.4. Assessment of the Number of Probiotic Microorganisms

The *L. plantarum* probiotic was homogenized using sterile dilutions containing 0.85% (w v^{-1}) salt and 0.1% (w v^{-1}) peptone. Briefly, 1 ml of the homogenized sample was diluted with dilution fluid. Diluted samples were spread plated on de Man, Rogosa and Sharpe (MRS) agar and incubated at 37 °C for 48 h [22]. After incubation, number of the microorganisms was assessed. To assess number of the microencapsulated *L. plantarum*, sterile sodium citrate buffer was added to the sample and homogenized for 15 min using magnetic stirrer. Then, 1 ml of the homogenized chocolate was used to prepare sufficient dilutions [21]. Number of the microorganisms was assessed using spreading method. After incubation at 37 °C for 48 h, number of the microorganisms was assessed. All studies were carried out in two parallels and three replications.

2.5. Sensory Analysis of Probiotic Chocolates

For sensory analysis, a panel of ten people was selected and trained. Samples were assessed for appearance (smoothness-appearance, brightness and blooming), aroma (cocoa flavor and off-flavor), taste (cocoa taste and sweetness), texture during the first bite (hardness and brittleness) and chewing (strength, smoothness-texture, melting level, stickiness, spread and mouth covering), color (brown color) and overall acceptability. Participants rated each characteristic on a scale of 1-9. Numbers representing the scale were established as 9, extremely like; 5, neither like nor dislike and 1, extremely dislike [17].

2.6. Statistical analysis of Probiotic Chocolates

Findings of this study were reported as means of triplicate data with standard deviations (SD). Analysis of data was carried out using ANOVA. Significant differences ($p<0.05$) in individual means were assessed using Tukey's HSD test. The SPSS software v.22.0 (IBM, Chicago, USA) was used to analyze the results. Results were expressed as means ±SD.

3. Results and Discussion

In the study, number of the probiotic microorganisms in each chocolate (chocolate without probiotics, probiotic chocolate and microencapsulated chocolate) after storage was assessed and sensory characteristics of the chocolates were analyzed.

3.1. Microbiological Analysis

With the microbial analysis results, potential of the bitter chocolate as a matrix for *L. plantarum* was assessed. Results of the microbiological analysis are present in Table 2. Based on Table 2, it was assessed that chocolate without added probiotics did not contain microorganisms on Days 0, 30 and 90. Chocolate is generally not a product; in which, microorganisms can multiply. Moreover, it was assessed that the microorganism counts of chocolate with probiotics were 5.8×10^7 CFU g^{-1} on Day 0, 2.5×10^7 CFU g^{-1} on Day 30 and 1.7×10^7 CFU g^{-1} on Day 60. It was shown that number of the microorganisms did not change significantly ($p<0.05$) after storing probiotic chocolate for 60 d. It was concluded that chocolate could be used as a matrix for probiotics as the number of microorganisms was 1.7×10^7 CFU g^{-1} on Day 60. When free form of *L. plantarum* was added to chocolates, no significant changes ($p<0.05$) were observed in the number of microorganisms for 60 d. Although addition of high quantities of the culture was initially carried out to achieve a high culture count in the final products, high quantities of the free culture could adversely affect taste of the chocolate. For the probiotic microorganisms to include beneficial effects on human health, probiotics should be present in foods at a level of 10^6-10^7 CFU g^{-1}(or ml^{-1}) [23,24]. Regarding the results, it has been demonstrated that the measured free probiotic culture was included in these values with beneficial effects. Several studies support these results. A study reported bitter chocolate as an appropriate matrix for the transport of *B. breve* NCIM5671 strain [25]. Another study stated that the number of probiotic bacteria added to chocolates was constant [26].

Based on Table 1, 2.1×10^7 CFU g^{-1} microorganisms were inoculated into the chocolate in production of microencapsulated chocolates. Number of the microorganisms was 6.0×10^5 CFU g^{-1} on Day 30 and 2.4×10^5 CFU g^{-1} on Day 60. Furthermore, number of the microorganisms decreased significantly ($p<0.05$) at the end of Day 30. Number of the microorganisms did not change significantly ($p<0.05$) within 30 and 60 d of storage. In a study by Erginkaya et al., [17], it was stated that bitter chocolates stored at 4 and 25 °C for 60 d showed differences in physical characteristics due to storage at various temperatures. It was also stated that a storage temperature of 25 °C caused significant decreases in the microbial count. In the present study, a storage temperature of 18 °C was set, which was within the temperature range in the highlighted study and the commercial storage temperature for chocolate. Studies showed that storage temperature caused decreases in microbial counts at 4 °C and probiotic cell concentration decreased by 1-2 log CFU g^{-1}. At 25 °C, microbial counts decreased by 4-7 log CFU g^{-1} [14,18,27]. Based on the results, it was clear that chocolate could be used as a matrix to include probiotics. Several studies support this conclusion [14-17].

The global market for probiotic foods, supplements and probiotic foods is growing significantly. Therefore, cell encapsulation is emerging as an alternative for the incorporation of probiotics into various food matrices. [28]. In the present study, alginate concentration used for the capsule formation was set at 1% (w v^{-1}); thus, avoiding adverse effects on the appearance of chocolates. However, capsules formed within the gel concentrations did not sufficiently protect the probiotic cultures. In addition to the studies supporting these results, there are studies indicating that the microencapsulation process is an effective method for protecting probiotics. In a study, although starch-alginate capsules included the highest survival rate within the encapsulated cells, less than 1 log CFU g^{-1} of loss occurred [29,30].

Table 2. Survival of microencapsulated and free probiotic *Lactiplantibacillus plantarum* in bitter chocolates during two months of storage

Sample	0. day (log CFU g^{-1})	30. day (log CFU g^{-1})	60. day (log CFU g^{-1})
Chocolate without probiotic	ND	ND	ND
Probiotic Chocolate	7.76±0.40^a	7.40±0.54^a	7.22±0.70^a
Microencapsulated Chocolate	7.33±0.71^a	5.78±0.56^b	5.38±0.20^b

ND:Not detected. a, b: The averages shown with different exponents on the same row are different from each other ($p<0.05$)

In another study, probiotic strain of *Limosilactobacillus reuteri* DSM 17938 and non-probiotic strain of *L. plantarum* 48M were microencapsulated in alginate matrix using emulsion technique. Survival of microorganisms in microcapsules was assessed against gastric conditions and heat stress. Results showed that the microencapsulation process increased viability of *L. plantarum* 48M cells following exposure to gastric conditions, resulting in similar survival to *L. reuteri* DSM 17938. Additionally, it was stated that microencapsulation could not protect *L. reuteri* DSM 17938 and *Lactiplantabacillus plantarum* 48M cells when exposed to heat treatment [31]. There are studies indicating that microencapsulation is effective in protecting probiotics against adverse environmental conditions [5,6]. The fact that the microencapsulation process cannot protect microorganisms exposed to heat in the results shows that decreases in the number of microorganisms as a result of the microencapsulation process may be a normal result.

Studies have demonstrated that chocolate can be used to transport probiotics without the need of microencapsulation process [18,32-35]. İn contrast, there are several studies indicating that the use of probiotics in foods via micro-encapsulation method is more effective than the use of free probiotics [11,13,23,30,36-40]. Chocolate enriched with encapsulated probiotics (probiotic-chocolate) and control (chocolate with non-encapsulated probiotics) were stored under aseptic conditions at 25 and 4 °C for 120 d. Relatively, when the protective effects of probiotic chocolates were compared, encapsulated chocolates includ-ed further probiotics (at least 2.0 log) after 120 d, compared to non-encapsulated probiotics. This verified that the encapsulates were protective under the two storage conditions [41]. Despite the encapsulation process, probiot-ics could be affected by stress conditions and the microencapsulation process could be affected by several parameters. These parameters were assessed as the size of microcapsules [29, 42], encapsulation method and coating materials as well as the concentration [9,18,24,28,37,40,43]. In production of capsules, encapsulation is based on alginate materials and addition of prebiotics to alginate increases protection of the capsules [28,29,40]. Studies have shown that the survival rates of probiotics depend on the type of probiotic, type of chocolate, storage temperature and time [18,32,43,44].

In a study by Sedefoglu et al. [7], ice cream samples were encapsulated with alginate to assess bacterial counts. They concluded that encapsulation did not provide additional protective effects to the probiotics. In cases; where the storage duration exceeded 120 d, microorganisms in microencapsulated samples were still viable. In a study, encapsulated *L. plantarum* 564 and commercial probiotic *L. plantarum* 299v were added in the production of bitter chocolates and it was seen that the probiotic bacteria survived very well after the production and during storage [45]. Although effects of alginate encapsulation on the survival of lactic acid bacteria in the food matrix has been studied, uniformity in the encapsulation procedure has not been identified in studies yet. Studies vary in capsule size, alginate concentration, calcium chloride concentration, hardening time of the capsules in calcium chloride and the initial number of the cultures, leading to differences in the survival of encapsulated bacteria. In summary, a higher number of microorganisms were detected in chocolates produced using free probiotic culture than in those with added probiotic cultures through microencapsulation. Additionally, it has been reported that chocolate can be used as a matrix to include probiotics.

3.2. Sensory Analysis Results

Sensory analysis results of the probiotic bitter chocolate samples during storage are provided in Tables 3 and 4. Description of the characteristics is as follows. Smoothness-appearance, smooth appearance of the product's surface without lumps of grits; brightness, intensity of light reflection in the product, opposite of opaque; bloom, white-gray layer of the visible lipid/sugar crystals on the surface; cocoa aroma, aroma of the cocoa powder (from none to very); off-flavor, unpleasant and unwanted aroma in the product; cocoa taste, intensity of the taste of cocoa in the product; sweetness, taste quality most often associated with sucrose (none to very); hardness, force needed to cut the food using central incisor teeth; brittleness: breakage level of the piece of chocolate in first bite; strength, force needed to compress samples between tongue and palate; smoothness-texture, levels of even and consistent continuity of the product in mouth; melting level, time needed to melt half of the sample while chewing (slow to fast); stickiness, level of stickiness to molar teeth; spread, level of covering surface of the mouth; mouth covering, the after-feel film, which covers the mouth surface; brown color, light brown to dark brown; general acceptability, acceptability of the product in general with all of its sensory characteristics [7].

As seen in Table 3, no significant level (p<0.05) was detected in sensory characteristics of the chocolates without probiotics, probiotic chocolates or microencapsulated chocolates on Days 0 and 30. Sensory characteristics of the chocolate samples on Day 60 showed significant differences (p<0.05). From daily parameters, the highest smoothness value was assessed in the microencapsulated chocolate sample. This was due to the structure of the chocolate changing after a certain time. It was assessed that the brightness, blooming, cocoa flavor, off-flavor, cocoa taste [similar importance as probiotic chocolate (p<0.05)], hardness, stickiness and brown color parameters of the chocolates without probiotics were significant (p<0.05).

Table 3. Sensory analysis results of chocolates without probiotics, probiotic chocolates and microencapsulated chocolates during 60 days of storage

	0. day			30. day			60. day		
Parameter	N	P	E	N	P	E	N	P	E
Smoothness-appearance	6.6±1.1^{a}	4.9±1.7^{a}	7.4±0.9^{a}	6.4±0.4^{a}	5.9±1.4^{a}	6.6±1.9^{a}	6.2±0.6^{b}	5.6±0.6^{c}	8.4±0.9^{a}
Brightness	6.1±1.7^{a}	5.1±1.3^{a}	7.4±0.7^{a}	6.3±0.5^{a}	5.5±1.4^{a}	6.4±2.2^{a}	7.0±0.4^{a}	5.6±0.4^{c}	6.8±0.7^{b}
Blooming	5.1±1.0^{a}	6.2±1.1^{a}	4.5±1.9^{a}	5.6±0.9^{a}	5.6±1.8^{a}	5.3±1.6^{a}	7.0±1.0^{a}	6.0±0.6^{b}	6.0±0.3^{b}
Cocoa flavor	6.7±0.8^{a}	6.7±0.4^{a}	6.9±0.7^{a}	6.9±0.6^{a}	6.6±0.9^{a}	6.8±0.5^{a}	6.6±0.8^{a}	6.2±0.8^{c}	6.4±0.4^{b}
Off-flavor	2.9±0.7^{a}	3.0±0.6^{a}	2.6±0.6^{a}	4.2±1.3^{a}	3.9±0.8^{a}	3.6±0.9^{a}	3.4±0.4^{a}	3.2±0.5^{b}	3.2±0.8^{b}
Cocoa taste	6.7±0.4^{a}	6.6±0.1^{a}	6.7±0.5^{a}	6.7±0.6^{a}	7.0±0.5^{a}	6.8±0.3^{a}	6.4±0.5^{a}	6.4±0.6^{a}	5.8±0.5^{b}
Sweetness	6.5±0.8^{a}	6.3±0.7^{a}	6.6±1.0^{a}	6.4±0.4^{a}	5.8±0.7^{a}	6.8±0.2^{a}	6.6±0.3^{b}	6.8±0.9^{a}	6.2±0.6^{c}
Hardness	6.2±0.7^{a}	7.3±0.9^{a}	6.8±0.4^{a}	6.5±1.2^{a}	7.0±0.4^{a}	7.1±0.6^{a}	6.4±1.1^{a}	5.8±1.4^{b}	5.6±1.1^{c}
Brittleness	7.6±0.3^{a}	7.0±0.5^{a}	7.4±0.5^{a}	6.3±0.9^{a}	6.3±0.8^{a}	6.6±0.9^{a}	5.2±1.2^{c}	6.4±1.1^{a}	6.2±1.3^{b}
Strength	6.9±0.3^{a}	7.1±1.1^{a}	6.8±0.3^{a}	6.3±1.6^{a}	7.0±0.3^{a}	6.8±0.6^{a}	6.2±0.6^{b}	6.6±0.4^{a}	6.6±0.5^{a}
Smoothness-texture	7.2±0.5^{a}	6.8±0.6^{a}	7.5±0.6^{a}	6.5±0.7^{a}	6.1±0.2^{a}	7.2±0.3^{a}	6.0±0.4^{c}	6.2±0.2^{b}	7.6±0.7^{a}
Melting level	6.7±0.6^{a}	6.6±0.6^{a}	6.7±0.6^{a}	6.5±1.2^{a}	6.3±0.9^{a}	6.8±0.4^{a}	6.8±0.6^{b}	7.2±0.8^{a}	6.2±0.9^{c}
Stickiness	6.5±0.8^{a}	6.3±0.7^{a}	5.7±1.0^{a}	6.3±0.9^{a}	6.3±0.6^{a}	6.2±0.7^{a}	6.8±1.2^{a}	6.6±0.6^{b}	6.6±0.6^{b}
Spread	7.1±0.1^{a}	6.8±0.7^{a}	6.5±0.9^{a}	6.4±1.0^{a}	6.4±0.2^{a}	6.7±0.4^{a}	6.4±0.6^{b}	6.8±0.7^{a}	6.4±0.1^{b}
Mouth covering	6.7±0.4^{a}	6.3±0.7^{a}	6.7±1.0^{a}	6.5±1.1^{a}	6.4±0.5^{a}	6.8±0.2^{a}	6.2±0.7^{c}	6.6±1.0^{b}	6.8±0.3^{a}
Brown color	6.9±0.1^{a}	6.6±0.4^{a}	7.5±0.5^{a}	6.7±0.9^{a}	6.8±0.8^{a}	6.8±0.4^{a}	7.2±0.5^{a}	7.2±0.9^{a}	7.0±0.4^{b}
General acceptability	7.1±0.5^{a}	7.2±0.4^{a}	7.7±0.5^{a}	6.9±0.5^{a}	6.8±0.7^{a}	7.3±0.9^{a}	6.6±0.6^{c}	8.0±0.3^{a}	7.6±0.6^{b}

N: Chocolate without probiotic, P:Probiotic Chocolate, E: Microencapsulated Chocolate. a, b, c: The averages shown with different exponents on the same row are different from N, P and E at same day ($p<0.05$)

It was also stated that cocoa taste, sweetness, brittleness, strength, melting level, spread and brown color [similar importance as chocolate without probiotic ($p<0.05$)] were significant as well ($p<0.05$) in probiotic chocolates. In microencapsulated probiotic chocolate, smoothness-appearance, strength [similar importance as probiotic addition ($p<0.05$)], smoothness-texture and mouth covering characteristics were statistically significant ($p<0.05$). Various characteristics of chocolates were assessed through sensory analyses. On Day 60, it was reported that eight sensory parameters of the chocolates without probiotics, eight sensory parameters of the chocolates with probiotics and four sensory parameters of the microencapsulated probiotic chocolates were significant ($p<0.05$). It has been assessed that chocolates with probiotics were different significantly ($p<0.05$) from chocolate with microencapsulation and chocolate without probiotics addition in general acceptability. This is one of the important characteristics in sensory analyses. At the end of 60 d of storage, the most favored chocolate was that containing free culture, followed by the microencapsulated chocolate. Graphical representation of the results is provided between Figures 2 and 3. Table 4 presents sensory analysis results of the probiotic bitter chocolate samples over the storage, based on the added cultures.

Based on Table 4, sensory analysis results on Days 0, 30 and 60 were provided based on the added cultures. In chocolate samples without probiotics, the brittleness parameter included a significant level ($p<0.05$) on Day 0, whereas this characteristic decreased on Days 30 and 60. Regarding sensory parameters of the probiotic chocolate, hardness showed significant decreases ($p<0.05$) over time. In general acceptability parameter, the highest value was reached after 60 d. It was recorded that cocoa taste and hardness parameters of the chocolate containing microencapsulated probiotics decreased significantly on Day 60. Based on the general acceptability results, chocolate containing probiotics was acceptable at a significant level ($p<0.05$) depending on the 60-d storage. Studies indicate that the addition of probiotics to affect functional characteristics of the chocolates does not make changes in sensory characteristics [16,18,32-36,45]. In a study, sensory and physiological characteristics such as color, texture, rheology and melting profile of bitter chocolates with added probiotic cultures and control bitter chocolate samples without probiotics were compared. Results showed that physiological characteristics of the chocolate containing probiotics and control chocolate did not differ significantly [25].

Other studies reported that chocolates with microencapsulated probiotics received similar scores in sensory evaluations, compared to those containing free probiotics [18,35]. In another study, it was assessed that microcapsules containing bitter chocolate included no differences with two commercially available chocolates based on the hedonic sensory assay [26]. Although it was stated that adding probiotics to chocolate varieties did not include negative effects based on the sensory assessment results; however, the study reported significant decreases in the general liking scores of all chocolate samples after 60 d of storage [18]. In another study, chocolates were investigated for a_w, pH, surface color and morphology, hardness, microbiological quality, sensory acceptance and probiotic viability. After 120 d of storage at 25 °C, probiotic populations in chocolate decreased by a maximum of 1.4 log due to the stress environment.

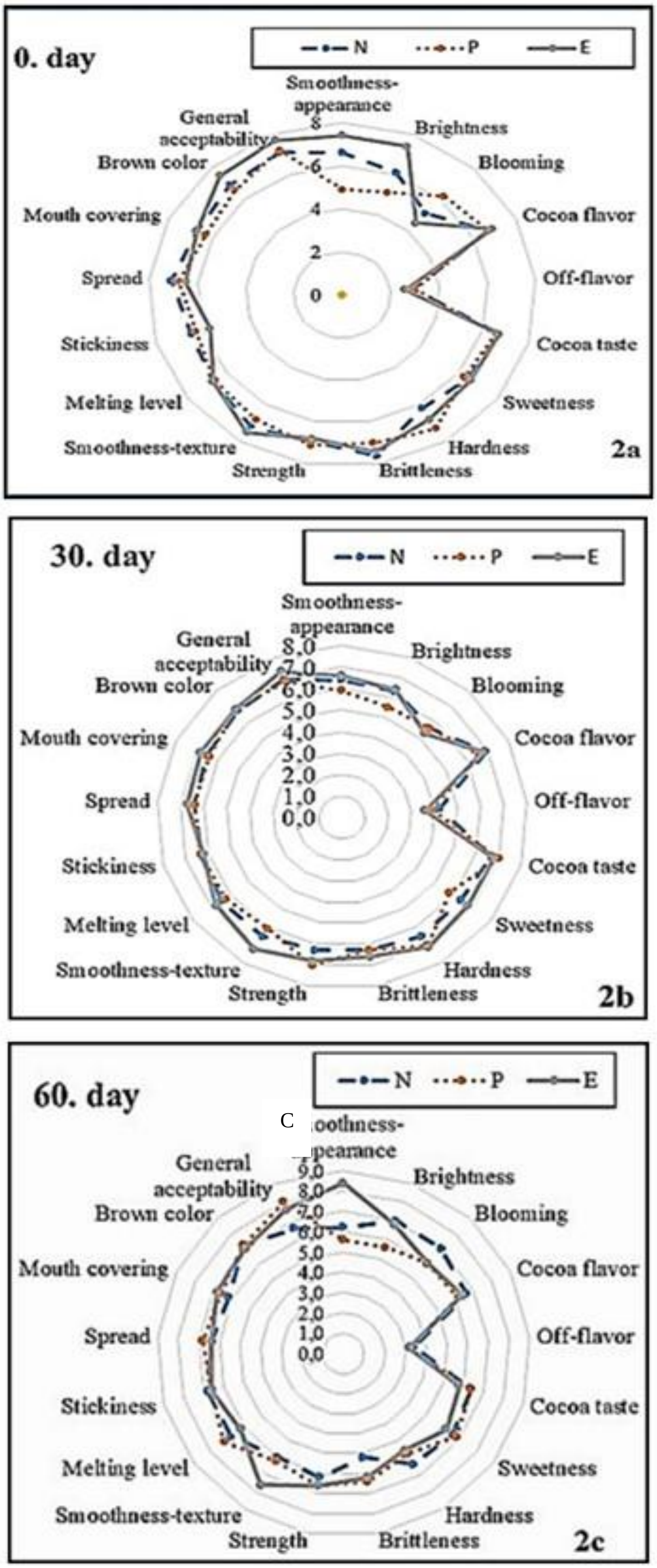

Figure 2. Sensory analysis results based on Days 0 (2a), 30 (2b) and 60 (2c) of storage

Table 4. Sensory analysis results based on the storage time of "chocolate without probiotics", "probiotic chocolate" and "microencapsulated chocolate"

	N			P			E		
Parameter	0. day	30. day	60. day	0. day	30. day	60. day	0. day	30. day	60. day
Smoothness-appearance	6.6 ± 1.1^{a}	6.4 ± 0.4^{a}	6.2 ± 0.6^{a}	4.9 ± 1.7^{a}	5.9 ± 1.4^{a}	5.6 ± 0.6^{a}	7.4 ± 0.9^{a}	6.6 ± 1.9^{a}	8.4 ± 0.9^{a}
Brightness	6.1 ± 1.7^{a}	6.3 ± 0.5^{a}	7.0 ± 0.4^{a}	5.1 ± 1.3^{a}	5.5 ± 1.4^{a}	5.6 ± 0.4^{a}	7.4 ± 0.7^{a}	6.4 ± 2.2^{a}	6.8 ± 0.7^{a}
Blooming	5.1 ± 1.0^{a}	5.6 ± 0.9^{a}	7.0 ± 1.0^{a}	6.2 ± 1.1^{a}	5.6 ± 1.8^{a}	6.0 ± 0.6^{a}	4.5 ± 1.9^{a}	5.3 ± 1.6^{a}	6.0 ± 0.3^{a}
Cocoa flavor	6.7 ± 0.8^{a}	6.9 ± 0.6^{a}	6.6 ± 0.8^{a}	6.7 ± 0.4^{a}	6.6 ± 0.9^{a}	6.2 ± 0.8^{a}	6.9 ± 0.7^{a}	6.8 ± 0.5^{a}	6.4 ± 0.4^{a}
Off-flavor	2.9 ± 0.7^{a}	4.2 ± 1.3^{a}	3.4 ± 0.4^{a}	3.0 ± 0.6^{a}	3.9 ± 0.8^{a}	3.2 ± 0.5^{a}	2.6 ± 0.6^{a}	3.6 ± 0.9^{a}	3.2 ± 0.8^{a}
Cocoa taste	6.7 ± 0.4^{a}	6.7 ± 0.6^{a}	6.4 ± 0.5^{a}	6.6 ± 0.1^{a}	7.0 ± 0.5^{a}	6.4 ± 0.6^{a}	6.7 ± 0.5^{a}	6.8 ± 0.3^{a}	5.8 ± 0.5^{b}
Sweetness	6.5 ± 0.8^{a}	6.4 ± 0.4^{a}	6.6 ± 0.3^{a}	6.3 ± 0.7^{a}	5.8 ± 0.7^{a}	6.8 ± 0.9^{a}	6.6 ± 1.0^{a}	6.8 ± 0.2^{a}	6.2 ± 0.6^{a}
Hardness	6.2 ± 0.7^{a}	6.5 ± 1.2^{a}	6.4 ± 1.1^{a}	7.3 ± 0.9^{a}	7.0 ± 0.4^{ab}	5.8 ± 1.4^{b}	6.8 ± 0.4^{a}	7.1 ± 0.6^{a}	5.6 ± 1.1^{b}
Brittleness	7.6 ± 0.3^{a}	6.3 ± 0.9^{ab}	5.2 ± 1.2^{b}	7.0 ± 0.5^{a}	6.3 ± 0.8^{a}	6.4 ± 1.1^{a}	7.4 ± 0.5^{a}	6.6 ± 0.9^{a}	6.2 ± 1.3^{a}
Strength	6.9 ± 0.3^{a}	6.3 ± 1.6^{a}	6.2 ± 0.6^{a}	7.1 ± 1.1^{a}	7.0 ± 0.3^{a}	6.6 ± 0.4^{a}	6.8 ± 0.3^{a}	6.8 ± 0.6^{a}	6.6 ± 0.5^{a}
Smoothness-texture	7.2 ± 0.5^{a}	6.5 ± 0.7^{a}	6.0 ± 0.4^{a}	6.8 ± 0.6^{a}	6.1 ± 0.2^{a}	6.2 ± 0.2^{a}	7.5 ± 0.6^{a}	7.2 ± 0.3^{a}	7.6 ± 0.7^{a}
Melting level	6.7 ± 0.6^{a}	6.5 ± 1.2^{a}	6.8 ± 0.6^{a}	6.6 ± 0.6^{a}	6.3 ± 0.9^{a}	7.2 ± 0.8^{a}	6.7 ± 0.6^{a}	6.8 ± 0.4^{a}	6.2 ± 0.9^{a}
Stickiness	6.5 ± 0.8^{a}	6.3 ± 0.9^{a}	6.8 ± 1.2^{a}	6.3 ± 0.7^{a}	6.3 ± 0.6^{a}	6.6 ± 0.6^{a}	5.7 ± 1.0^{a}	6.2 ± 0.7^{a}	6.6 ± 0.6^{a}
Spread	7.1 ± 0.1^{a}	6.4 ± 1.0^{a}	6.4 ± 0.6^{a}	6.8 ± 0.7^{a}	6.4 ± 0.2^{a}	6.8 ± 0.7^{a}	6.5 ± 0.9^{a}	6.7 ± 0.4^{a}	6.4 ± 0.1^{a}
Mouth covering	6.7 ± 0.4^{a}	6.5 ± 1.1^{a}	6.2 ± 0.7^{a}	6.3 ± 0.7^{a}	6.4 ± 0.5^{a}	6.6 ± 1.0^{a}	6.7 ± 1.0^{a}	6.8 ± 0.2^{a}	6.8 ± 0.3^{a}
Brown color	6.9 ± 0.1^{a}	6.7 ± 0.9^{a}	7.2 ± 0.5^{a}	6.6 ± 0.4^{a}	6.8 ± 0.8^{a}	7.2 ± 0.9^{a}	7.5 ± 0.5^{a}	6.8 ± 0.4^{a}	7.0 ± 0.4^{a}
General acceptability	7.1 ± 0.5^{a}	6.9 ± 0.5^{a}	6.6 ± 0.6^{a}	7.2 ± 0.4^{ab}	6.8 ± 0.7^{b}	8.0 ± 0.3^{a}	7.7 ± 0.5^{a}	7.3 ± 0.9^{a}	7.6 ± 0.6^{a}

N: Chocolate without probiotic, P:Probiotic Chocolate, E: Microencapsulated Chocolate. a, b, c: The averages shown with different exponents on the same row are different from days of N, P and E ($p<0.05$)

Based on these results and a good acceptance of all samples by the panelists, semisweet chocolate can be addressed as a good matrix for probiotics [33].

4. Conclusion

This study concludes that chocolate is an appropriate food for carrying probiotic cultures. Additionally, importance of chocolates containing probiotic cultures emerges due to their popularity within children and difficulty in supplementing children foods with probiotic cultures. By adding probiotic cultures to chocolates, functional foods can be prepared. The fact that chocolates containing free culture did not show decreases in the number of microorganisms indicates that cultures can be added without the microencapsulation process. No changes in sensory characteristics were observed after adding probiotic cultures to the chocolates and their general acceptability values were higher than those of other samples. High values were achieved in microbial and sensory analysis results by adding free probiotic cultures to the chocolates without microencapsulation. This eliminates needs of further labors, times and costs for the microencapsulation process. Thus, chocolates can be added with functional characteristics using added free probiotics.

Several parameters were effective in low detection of probiotics as a result of the microencapsulation process. Microencapsulation method used in the present study included container material, $CaCl_2$ volume and alginate section, where various microencapsulation blockers were stored. As a result of the study, it is concluded that chocolate can be used as a matrix in distribution and formation of probiotic cultures. The current study suggests further studies to carry out. These studies can involve various probiotics, adjusted storage temperatures and durations and modified parameters in microencapsulation processes

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6. Conflict of Interest

The author declares no conflict of interest.

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افزودن *لاکتی‌پلانتی‌باسیلوس پلانتاروم* ریزپوشانی‌شده و آزاد به شکلات تلخ: ارزیابی حسی و بررسی زنده‌مانی

هدایت ساگلام

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چکیده

سابقه و هدف: شکلات یک ماده غذایی محبوبی است که در گروه های سنی گوناگون مصرف می شود. فرض بر این است که شکلات می‌تواند با افزودن زیست‌یارها[۱] به آن، به محصول فراسودمند[۲] مهم تبدیل شود. در این مطالعه از شکلات به عنوان ماتریس غذایی برای انتقال میکروارگانیسم های زیست‌یار استفاده شد. شکلات تلخ به‌دلیل ترجیح مصرف کنندگان انتخاب شد. بنابراین، کشت‌های زیست یار ریزپوشانی شده و آزاد تهیه شد.

مواد و روش ها: از لاکتی پلانتی باسیلوس پلانتاروم به عنوان میکروارگانیسم زیست‌یار و کپسول ژل آلژینات - کلسیم برای ریزپوشانی استفاده شد. تعداد میکروارگانیسم‌ها و ویژگی‌های حسی کشت‌های زیست‌یار آزاد، ریزپوشانی‌شده و شکلات‌های تلخ فاقد کشت پس از ۶۰ روز نگهداری در دمای ۱۸ درجه سلسیوس ارزیابی شد.

یافته‌ها و نتیجه‌گیری: بر اساس نتایج به‌دست آمده، تعداد میکروارگانیسم‌های موجود در شکلات زیست‌یار در روز صفر $5/8\times10^{7}$ CFU g^{-1} بود، درحالی‌که در روز ۶۰ به $1/7\times10^{7}$ CFU g^{-1} کاهش یافت. علی‌رغم مشاهده کاهش تعداد زیست‌یارها، شکلات‌ها حاوی تعداد کافی زیست‌یاربودند تا به‌عنوان شکلات زیست‌یار تعریف شوند. شمارش میکروبی شکلات حاوی زیست‌یار ریزپوشانی شده ($2/1\times10^{7}$ CFU g^{-1} در روز صفر) به‌طور معنی‌داری به $2/4\times10^{5}$ CFU g^{-1} در روز ۶۰ کاهش یافت. بیشترین شمارش میکروبی در نمونه های حاوی کشت‌های زیست‌یار آزاد پس از ۶۰ روز مشاهده شد. . با این حال شمارش میکروبی در نمونه‌های دارای کشت سلولی ازاد به‌طور معنی‌داری کاهش پیدا نکرد، کاهش ۲ لگاریتمی در کشت‌های ریزپوشانی مشاهده شد. بنابراین، شکلات می‌تواند به‌عنوان ماتریس برای زیست‌یارها استفاده شود. برای ارزیابی حسی، پس از ۶۰ روز نگهداری نمونه فاقد کشت بیشترین پذیرش کلی و ارجحیت را داشت.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- فرآورده فراسودمند
- میکروریزپوشانی
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هدایت ساگلام

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[۱] probiotics
[۲] functional