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The effect of some probiotic Lactobacillus on physicochemical, rheological, survival and sensory characteristics of dark functional chocolate

Elham Kadhim Nasser[1], Sinan Jawdat Abdul-Abbas[2], Wael Ali swadi[3]

1- Food Science Department-Agriculture College-Basrah University, Basrah, Iraq

2- Ph.D. Assistant prof. Food Science and Biotechnology, Molecular Biology, Basrah , Iraq,

3- Biotechnolog Basrah university, department of food science, College of Agriculture

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*Corresponding Author E-Mail:
elham.nasser@uobasrah.edu.iq

ABSTRACT

The development of functional chocolate is a growing trend in food science, with probiotics offering significant health benefits. However, the harsh processing conditions and low pH of dark chocolate present a major challenge to the viability of probiotic microorganisms, necessitating strain selection and process optimization. This study aimed to investigate the impact of incorporating three microencapsulated strains of probiotic *Lactobacilli* (*L. acidophilus*, *L. casei*, and *L. rhamnosus*) on the key quality parameters of dark chocolate (70% cocoa). Microencapsulated probiotics were added to melted dark chocolate. The resulting chocolates were evaluated for physicochemical properties, rheological behavior, colorimetry, and sensory characteristics over a period of one month .Data SPSS analysis statistical elaborate sunt. The results of the effect of adding Lactobacillus bacteria to dark chocolate and producing functional dark chocolate on the physicochemical properties, color and texture, as well as the acceptance rate showed that adding Lactobacillus bacteria to dark chocolate did not have a negative effect on the chemical characteristics of any of the treatments in different percentages. The biggest concern was the changes in acidity and fatty acid profile, which, according to the chemical test measuring acidity and examining the fatty acid profile of functional chocolate and comparing it with the control sample, we saw no significant changes. Also, the results of texturometric, colorimetric and sensory acceptance tests indicate that there are no inappropriate conditions in functional chocolate and make the production of this product possible. The color of dark chocolate is one of the important factors in attracting the attention of the buyer, and the addition of bacteria did not affect it. Also, no change was observed in its taste. The texture of the functional chocolate was also suitable and acceptable, like the treatment sample.

1-Introduction

Dark chocolate, a globally cherished confectionery, is increasingly recognized for its inherent nutritional profile, rich in polyphenols, flavonoids, and minerals [1]. Concurrently, the growing consumer demand for functional foods products that offer health benefits beyond basic nutrition has spurred significant innovation within the food industry. Probiotics, defined as live microorganisms that confer a health benefit to the host when administered in adequate amounts, stand at the forefront of this trend, primarily associated with gut health and immune modulation [2]. Integrating these beneficial bacteria into popular food matrices, a process known as food fortification, presents a strategic avenue for enhancing public health [3].

The incorporation of probiotics into dark chocolate is a particularly promising yet technically challenging endeavor [4]. Chocolate offers unique advantages as a probiotic carrier: its lipid-rich matrix and low moisture content can potentially shield sensitive microorganisms from gastric acidity, improving their survival and subsequent colonization in the intestine [5]. However, the very properties that confer this protective benefit also pose significant hurdles. The manufacturing process of chocolate involves stages such as conching at elevated temperatures (~50-80°C) and the addition of ingredients with low pH, which can be detrimental to probiotic viability [6]. Furthermore, the inclusion of any novel ingredient, especially a biological agent, risks altering the fundamental qualities that define premium dark chocolate. These include its specific physicochemical characteristics, its critical rheological properties (viscosity and yield stress, which are essential for proper molding, coating, and mouthfeel), and ultimately, its sensory attributes the complex balance of bitterness, sweetness, aroma, and texture that consumers expect [7].

Therefore, the selection of robust probiotic strains, suitable for the chocolate environment, is paramount. Members of the *Lactobacillus* genus are among the most widely studied and commercially utilized probiotics [8]. Yet, not all strains exhibit the same resilience to heat, oxygen, and osmotic stress encountered during chocolate production and storage. A comprehensive evaluation must extend beyond mere viability counts to assess the holistic impact on the product itself [9].

his study specifically selected Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus rhamnosus due to their distinct technological properties. L. casei and L. rhamnosus are recognized for their robust tolerance to oxygen and acid stress, which are critical factors in low-moisture chocolate matrices. Furthermore, these species have shown superior survival rates in previous studies involving non-dairy carriers compared to more sensitive intestinal strains. By examining these interconnected parameters, the research seeks to address a key gap in functional food development: achieving a harmonious synergy where enhanced health functionality does not compromise the intrinsic quality and consumer acceptability of a beloved food product.

2- Materials and Methods

The raw materials used in the research were obtained from the Tehran market. The chemicals used were obtained from the Merck Company (Germany) representative. Chocolate samples were prepared at Kian Chocolate Kimia Company and chemical and microbial tests were performed in the laboratory section of this factory. Textometry and colorimetry tests were performed at the Karaj Agricultural Research Center. Gas chromatography tests were also performed at the Central Laboratory of the Faculty of Agriculture, University of Tehran.

The statistical population for dark chocolate consisted of 900 grams of chocolate (100 grams for each treatment) that was completely homogenized and formulated in such a way that at the end of 8 100-gram

treatments, they contained Lactobacillus casei subsp casei and Lactobacillus rhamnoses with values of 106, 107, 108, 109 and 1010 colonies per gram (cfu/g), respectively, and a 100-gram sample that did not contain the above bacteria and served as a control sample and treatment. Samples were prepared in a completely random manner from homogeneous and uniform treatments. All samples were stored in a refrigerator at 10°C until the specified time of testing. A total of 9 samples were used with 3 replicates for chemical and microbial tests, and 10 samples from each treatment were left for texture, hardness, and colorimetric tests. For sensory testing, 15 samples were taken from 15 individuals from each treatment.
The statistical population for dark chocolate consisted of 900 grams of chocolate that was completely homogenized. Treatments included the addition of microencapsulated *L. casei* and *L. rhamnosus* at targeted concentrations of 10^6, 10^7, 10^8, and 10^9 CFU/g (labeled D6 through D9 respectively), along with a control sample without probiotics. All samples were stored in airtight aluminum-laminated packaging in a refrigerator at 10°C until the specified time of testing

2-1 Experimental Methods

2-1-1 Physicochemical Analyses

Moisture Content: Moisture content was determined using a Sartorius MA35 moisture analyzer (Germany). Samples were placed on the instrument's aluminum pans and allowed to equilibrate to ambient temperature. The analyzer was programmed (PRG mode) to a drying temperature of 105°C for 45 minutes. After initiating the automated cycle, the device displayed the real-time moisture loss, with the final result reported as percent moisture [11].
Ash Content: Acid-insoluble ash content was determined gravimetrically. A 5-10g sample was charred in a porcelain crucible and then ashed in a muffle furnace at 550°C until a constant white ash was obtained. After cooling in a desiccator, 20 ml of 10% hydrochloric acid was added to the crucible, covered with a watch glass, and boiled in a water bath for 10 minutes. The contents were filtered through ashless filter paper and washed with distilled water until acid-free. The filter paper containing the insoluble residue was transferred back to the crucible, re-ashed at 550°C to constant weight, and the ash content calculated [12].

Fat Content: Fat content was determined by cold solvent extraction. A 3-5g grated chocolate sample was weighed into a test tube, mixed with 2 ml distilled water and 5 ml ethyl alcohol, and heated in a 50°C water bath to dissolve. Under a fume hood, 5 ml dimethyl ether and 2 ml 25% ammonia were added, and the mixture was shaken. Subsequently, 5 ml petroleum ether was added and shaken again. After phase separation, the upper ether (fat-containing) layer was carefully pipetted into a pre-weighed Erlenmeyer flask. The extraction was repeated until fat was completely removed. The combined ether extracts were evaporated on a boiling water bath, and the flask was dried at 100°C to remove residual solvents. Fat content was calculated as a percentage of the initial sample weight [12].
Peroxide Value: Peroxide value, an indicator of primary lipid oxidation, was determined by standard chemical extraction and titration methods [13].
Fatty Acid Profile: The fatty acid composition was analyzed using gas chromatography (GC), based on the separation and quantification of individual methyl ester derivatives [13].

2-1-2 Microencapsulation and Probiotic Incorporation Protocol

Probiotic strains were obtained as freeze-dried powders microencapsulated in a matrix of whey protein concentrate (WPC) and gum Arabic using the spray-drying technique (inlet temperature 140°C, outlet 75°C). The powder had a particle size of <50 µm to prevent grittiness in the

mouthfeel. The probiotic powder was added to the dark chocolate mass during the final stage of tempering at a controlled temperature of 32 ± 1°C. The mixture was stirred gently for 3 minutes to ensure homogeneous distribution without incorporating air bubbles, preventing thermal shock to the bacteria.

2-1-3 Probiotic Viability Enumeration

Viability of probiotics was assessed immediately after production (Day 0) and weekly for four weeks. 10g of chocolate sample was homogenized in 90ml of sterile 2% sodium citrate solution (pre-warmed to 37°C) using a stomacher for 2 minutes. Serial dilutions were plated on de Man, Rogosa, and Sharpe (MRS) agar supplemented with 0.05% L-cysteine. Plates were incubated anaerobically (GasPak system) at 37°C for 48-72 hours. Results were expressed as Log_{10} Colony Forming Units per gram (Log CFU/g).

2-1-4 Rheological Analyses

Viscosity: The apparent viscosity of molten chocolate was measured using a rotational viscometer (Brookfield DV-II+ Pro) with a small sample adapter and spindle SC4-27. Samples were tempered and held at 32°C, stirred for 10 minutes to ensure homogeneity, and then transferred to the instrument's sample chamber. The spindle speed was set to 5 rpm. The reading was recorded once the display stabilized, and the value was reported in mPa·s. Note: This measurement represents single-point apparent viscosity. Future studies should consider full flow curves to determine Casson yield stress and plastic viscosity for industrial processing relevance [11].

Texture (Hardness): Hardness was measured using a texture analyzer. Chocolate samples were conditioned at 20°C for 6 hours prior to analysis. A penetration or compression test was performed, and the maximum force (in N or g) required to fracture or deform the sample was recorded as the hardness value [12].

2-1-5 Colorimetric Analysis

Color was measured using a colorimeter in the CIELAB color space. Samples were molded into discs (90 mm diameter, 10 mm thickness) and placed in the sample port. The instrument measured the L* (lightness), a* (red-green), and b* (yellow-blue) values under standardized conditions at ambient temperature (20-25°C). The chroma (C*) was calculated as (a*² + b*²)^(1/2) [13].

2-1-4 Sensory Evaluation

A five-point hedonic scale test (1=Dislike Extremely, 3=Neither Like nor Dislike, 5=Like Extremely) was conducted to evaluate sensory attributes. A panel of five trained experts with prior experience in chocolate profiling evaluated texture, taste, color, and overall acceptability. Evaluations were conducted in individual booths under white fluorescent lighting at ambient temperature (22°C). Panelists were provided with unsalted crackers and room temperature water to cleanse the palate between samples. Note: The data were analyzed on the original 5-point scale without conversion to avoid statistical distortion.

2-2 Statistical Analysis

The tests will be performed in a completely randomized manner with 3 replications. The mean of the data will be evaluated using one-way analysis of variance at a significance level of 0.05. If significant, the Tukey test will be used to determine the difference in means. Data analysis will be performed using SPSS software at a significance level of $p<0.05$.

3- Results and discussion

Since it was found in the initial experiments that all three bacterial treatments (LC, LR, and LL) resulted in the same physicochemical, color, and evaluation results, only treatments containing two bacteria, namely 106 D6 =, 107 D7 =, 108

D8 =, and 109 D9 =, were tested and compared with the control.

3-1 Results of measuring fat content

The results of the analysis of variance showed that there was no significant difference between the treatments at the 5% error level (p>0.05). Also, the effect of time and the interaction between treatments and time were not significant (p>0.05). In other words, the type of treatment and the storage time of the samples did not have a significant effect on the fat content. The results of the experiments are presented in Fig1.

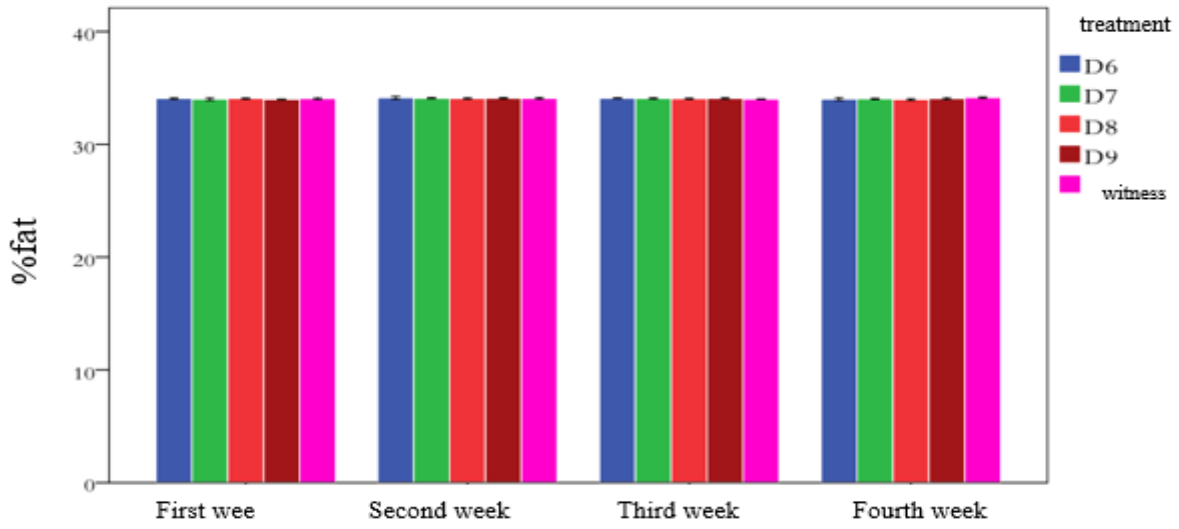

Fig1. Fat content in treatments

*Different lowercase letters indicate statistically significant differences between treatments (p<0.05).

The results of different treatments did not show a significant effect on fat content. Jovanović et al. (2022) showed that the addition of 5 and 10% blueberry extract encapsulated with whey protein reduced the protein content of white chocolate [14]. Poliński et al. (2021) also showed that the addition of elderberry extract and elderberry flower significantly reduced the fat content of the samples [15]. Kaur et al. (2021) showed that, while investigating the effect of raspberry and blueberry extracts on the oxidative, microbial and lipid stability of composite chocolate enriched with calcium and chicken protein, a pattern of fat reduction was observed in chocolate samples with increasing levels of chicken protein powder (0, 10, 20 and 30%) [16].

3-2 Ash Results

The average results of ash content of dark chocolate samples are shown in Figure 2. According to the results, different treatments had a significant effect on ash content (p<0.05). According to the results, the addition of Lactobacillus bacteria significantly increased the ash content of chocolate samples (p<0.05). The highest ash content was 0.185 for treatment D6 in the first week and the lowest ash content was 0.178 for treatment D8 in the fourth week. Fig 2 shows the changes in ash content of treatments over time. The lowest ash content was observed in the control sample (p<0.05).

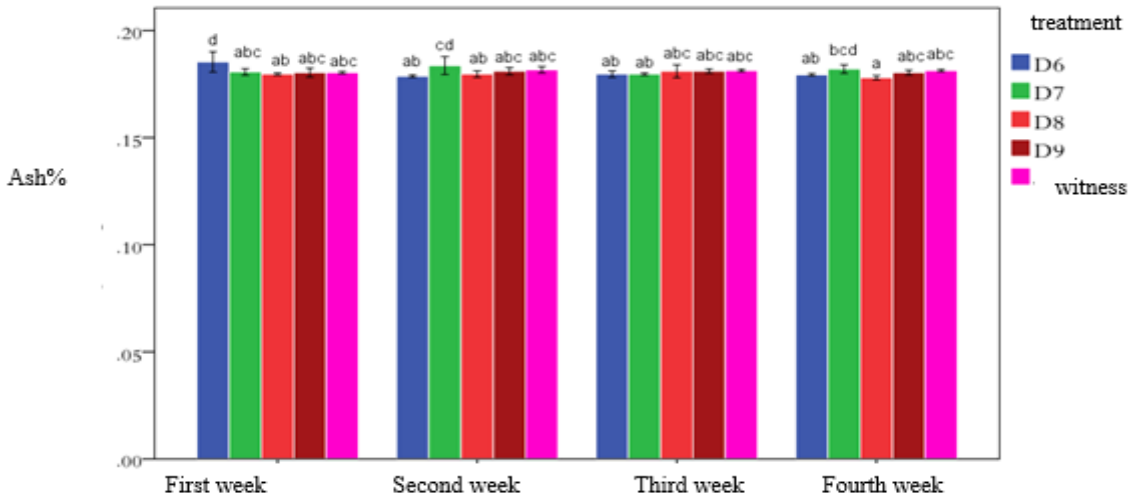

Fig2. ash content in treatments

*Different lowercase letters indicate statistically significant differences between treatments ($p<0.05$).

According to the National Standard 608 (1395), the maximum ash content of chocolate is 0.1%. Accordingly, all the produced samples had ash content higher than the permissible limit of the said standard. This was an expected trend. Aroyeun et al. (2016), while investigating the effects of green tea extract in milk chocolate, showed that increasing the percentage of green tea extract in milk chocolate formulation increased the ash content of the samples [17]. De Oliveira et al. (2021), showed that adding Spirulina microcapsules to milk chocolate increased the ash content of the samples [18]. However, Mirković et al. (2018), reported that adding Lactobacillus plantarum microcapsules did not show a statistically significant difference on the ash content of the samples [19].

3-3 Results of peroxide measurement

The results of analysis of variance showed that there was no significant difference between treatments at the 5% error level ($p>0.05$). Also, the effect of time was not significant ($p>0.05$), but the interaction between treatments and time was significant ($p<0.05$). In other words, the type of treatment and the storage time of the samples had a significant effect on the peroxide value. The results of the post-test using the LSD method are also presented in Fig3. The highest peroxide value was 0.163 for the D8 treatment in the second week and D7 in the fourth week, and the lowest peroxide value was 0.159 for the control treatment in the second week.

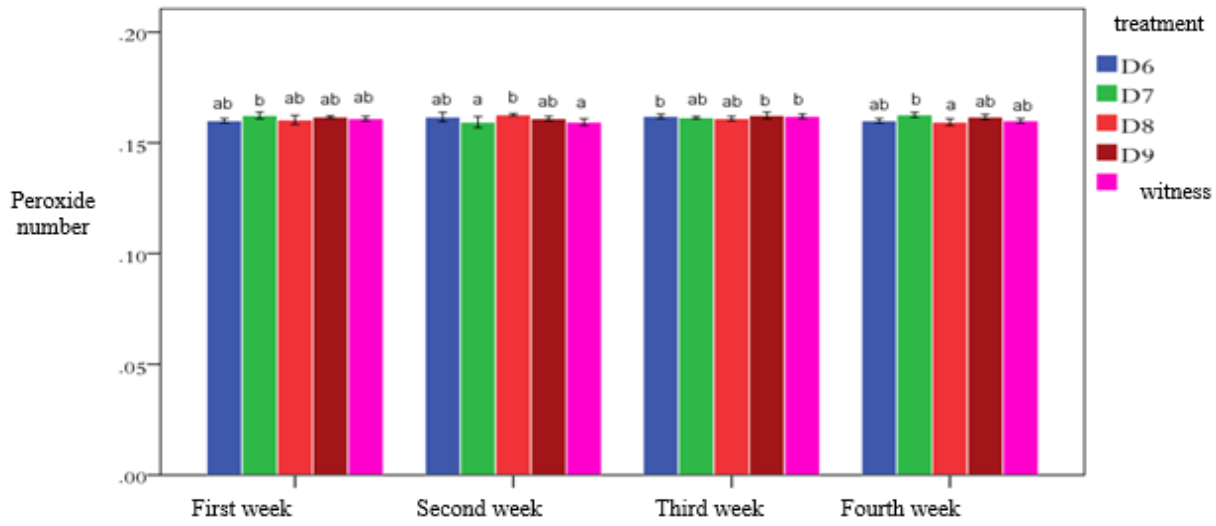

Fig3. peroxide content in treatments

The standard value of the peroxide value of dark chocolate oil is stated as a maximum of 1.5%, which is also shown in Fig3. During the storage period of chocolate, no noticeable change occurred that would lead to peroxide spoilage in chocolate, and the presence of lactobacilli also had no effect on this value. However, according to the data of the section on the study of microbial viability during the storage period, the population of microbes decreased and the acid present in the dead bacteria could lead to an increase in free radicals in chocolate [20], but the presence of natural antioxidants in cocoa powder prevented this from happening and prevented the increase in peroxide value.

3-4 Results of measuring acidity value

The results of performing the analysis of variance showed that there was no significant difference between the treatments at the 5% error level ($p>0.05$). Also, the effect of time and the interaction between treatments and time were not significant ($p>0.05$). In other words, the type of treatment and the storage time of the samples did not have a significant effect on the acidity value. From Fig 4 above, we see that the highest acidity value was 1.34 for the D7 and D8 treatments in the fourth week and the lowest acidity value was 1.29 for the control treatment in the first week. Figure 4 shows the changes in the acidity value of the treatments over time.

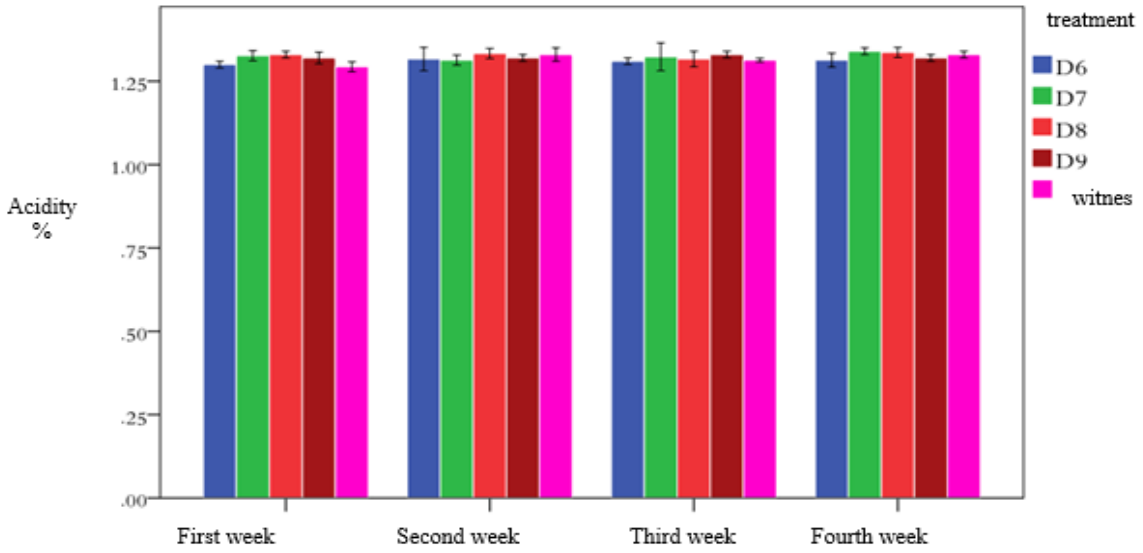

Fig4. acidity content in treatments

According to standard 608 (1395), the acidity of dark chocolate should be a maximum of 1.5%. Therefore, all produced samples had an acidity according to the national Iranian standard. In line with the research results, Shahbazi et al. (2022) showed that adding white tea extract in free and microcapsules significantly reduced the pH of dark chocolate samples. They showed a decrease in the pH of chocolate samples when adding free and microcapsule jujube extract to the formulation of dark cocoa products. These researchers reported a significant decrease in the pH of chocolate samples with an increase in the percentage of free and microcapsules extract [20]. These researchers attributed this to the presence of pectic polysaccharides in jujube. Kaur et al. (2021) showed a decrease in the pH of milk chocolate samples containing blueberry and raspberry extracts. They attributed this to the low pH values of the extracts, which contain high amounts of bioactive phytochemicals such as polyphenols including anthocyanins, proanthocyanidins, and phenolic acids [15].

3-5 Moisture content measurement results

The results of the analysis of variance showed that there was no significant difference between the treatments at the 5% error level ($p>0.05$). Also, the effect of time and the interaction between treatments and time were not significant ($p>0.05$). In other words, the type of treatment and the storage time of the samples did not have a significant effect on the moisture content. The results of the experiments are presented in Fig 5.

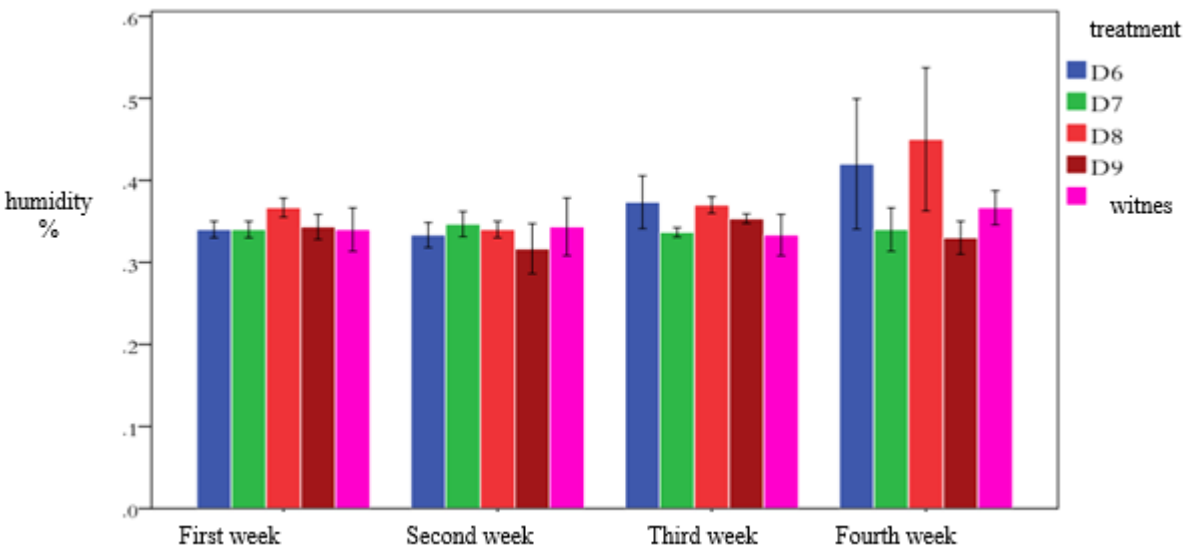

Fig5. Humidity content in treatments

Regarding humidity, as in other cases, based on the data of a, there is no significant difference between the treatments and the control sample, and the presence of these bacteria did not increase humidity, which is mainly due to the correct storage conditions. Packaging and storage in a dry and cool environment are ways to prevent possible moisture from entering the chocolate, which is consistent with the results of research by Afoakwa et al in 2007 and 2010 [21, 11]. They also suggested appropriate packaging with polyethylene materials to maintain the quality of chocolate and keep it away from moisture.

3-6 Probiotic Viability During Storage

The initial viable counts for treatments D6, D7, D8, and D9 were determined to be 6.12 ± 0.15, 7.08 ± 0.10, 8.21 ± 0.18, and 9.15 ± 0.12 Log CFU/g, respectively. Over the 4-week storage period at 10°C, a slight but non-significant decline in viability was observed ($p > 0.05$). By week 4, the counts decreased by less than 0.5 Log CFU/g across all treatments, maintaining populations well above the recommended therapeutic minimum of 10^6 CFU/g (6 Log CFU/g). The survival is attributed to the protective effect of the microencapsulation matrix and the high-fat content of the dark chocolate, which limits oxygen diffusion and moisture migration. This stability aligns with findings by Hossain et al. (2021) who reported that chocolate is a superior vehicle for *Lactobacillus* compared to dairy-based powders.

Table 1. Viability of Probiotic Lactobacilli in Dark Chocolate During Storage (Log CFU/g)

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
D6	6.12 ± 0.15	6.08 ± 0.09	6.01 ± 0.11	5.98 ± 0.14	5.95 ± 0.10
D7	7.08 ± 0.10	7.05 ± 0.12	6.98 ± 0.08	6.95 ± 0.13	6.90 ± 0.11
D8	8.21 ± 0.18	8.15 ± 0.14	8.10 ± 0.09	8.05 ± 0.12	8.01 ± 0.08

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4
D9	9.15 ± 0.12	9.10 ± 0.15	9.05 ± 0.10	9.00 ± 0.11	8.97 ± 0.09

Data represent Mean ± SD of three replicates.

3-7 Results of viscosity measurement

The results of analysis of variance showed that there was no significant difference between the treatments at the 5% error level ($p<0.05$). The results of the experiments are presented in Table1. From the above table, we see that the highest viscosity value of 33.37 was obtained for treatment D7 and the lowest viscosity value of 35.30 was obtained for the control treatment.

Table2. Viscosity values in treatments.

treatment	Viscosity value
D6	36.13 ± 0.51
D7	36.33 ± 0.21
D8	35.37 ± 0.64
D9	35.35 ± 1.25
witness	35.30 ± 0.61

Each data point represents the mean of three replicates ± the corresponding standard deviation.

According to Table 1, no significant difference in viscosity was observed between the bacterial treatments and the control sample, which is due to both the low amount of bacterial powder added to the chocolate (less than 1%) and their dissolution in water and their almost no effect on viscosity.

3-7 Texture Hardness Results

The mean texture hardness of the dark cocoa product samples is shown in Table 2.

According to the results, the different treatments had a significant effect on texture hardness ($p < 0.05$).

Table 2. Penetration value in chocolate.

treatment	Viscosity value
D9	58.45 ± 0.16
witness	9.39 ± 0.25

Each data point represents the mean of three replicates ± the corresponding standard deviation.

Table 2 shows the texture analysis results. Since the presence or absence of bacterial powder had no effect on the texture of the chocolate, only the texture of treatment D9 was measured. No significant difference was observed compared to the control sample. The reason for this is the low

amount used (less than 1%), which did not damage the texture, making this a positive aspect.

3-8 Colorimetric Results

The results of the variance analysis showed that at the 5% error level, there was no significant difference between treatment D9 and the control in terms of the three characteristics L*, a*, and b* ($p > 0.05$). The test results are presented in Table 3.

Table 4-9: Colorimetric Results in the Treatments

treatment	Characteristic L*	Characteristic a*	Characteristic b*
D9	24.61 ± 0.27	53.32 ± 0.02	1.65 ± 0.13
witness	24.56 ± 0.10	50.36 ± 0.03	1.79 ± 0.07

The L* value is defined as brightness, ranging from black (0) to white (100). The chocolate samples showed that the brightness index was affected by different treatments, varying between 23 and 25.

The a* value describes the color from red (positive a*, +120) to green (negative a*, -120), while the b* value describes the color from yellow (positive b*, +120) to blue (negative b*, -120). The results indicated that all samples had positive a* and positive b* values, meaning the color of the dark chocolate, both with and without the addition of probiotic bacterial microcapsules, falls within the red-yellow color range.

Praseptiangga et al. (2019) showed that adding cinnamon bark oleoresin microcapsules increased the brightness of dark chocolate [22]. These researchers demonstrated that as the percentage of microcapsules in the chocolate formulation increased, the redness and yellowness of the samples increased. Tolve et al. (2018) showed that the brightness values of chocolate increased with storage time and microcapsule concentration [23]. Conversely, as expected, brightness decreased with increasing cocoa concentration, as lower brightness values indicate a darker appearance. The redness and yellowness indices increased with microcapsule concentration and decreased with cocoa percentage and storage time. The addition of encapsulated phytosterols increased the brightness values for all chocolates. Zohreh (2020) showed that chocolate enriched with free chlorogenic acids had a lower brightness index than the control sample, but chocolate enriched with encapsulated chlorogenic acids had a higher brightness than the control [24]. There was no difference in redness between all samples (control or enriched). The yellowness of chocolate samples enriched with free chlorogenic acids was higher than that of the control. However, the yellowness of chocolate samples enriched with encapsulated chlorogenic acids was lower than that of the control.

3-9 Sensory Evaluation Results

The results of the non-parametric Friedman test showed a significant difference between the samples evaluated by the 5 panelists in terms of oral acceptance, aroma, texture, break sound (snap), and color ($p < 0.05$). The results of the non-parametric Wilcoxon test for pairwise comparisons between the different samples are also presented in Fig 6 to 10.

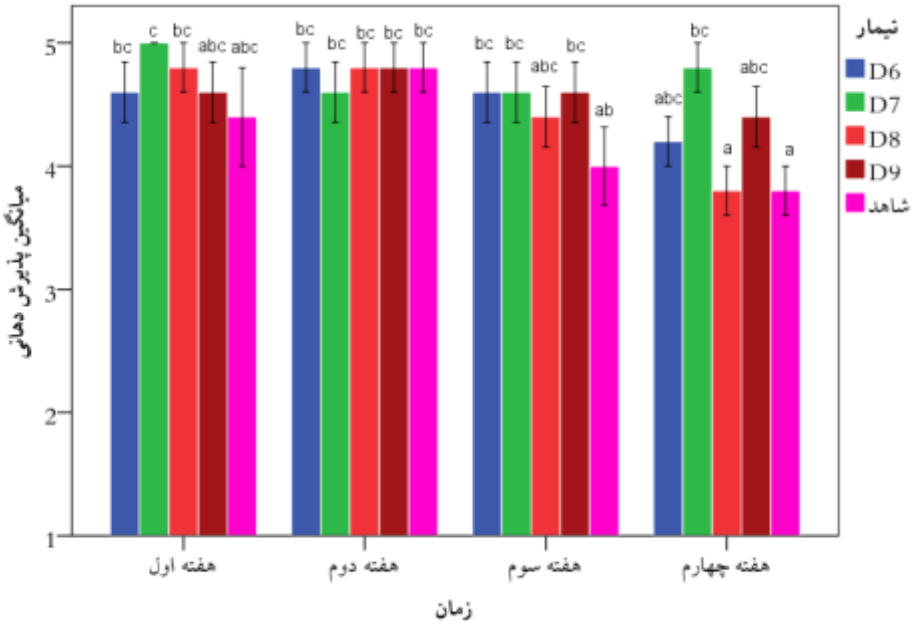

Fig 6. Changes in Oral Acceptance Over Time.

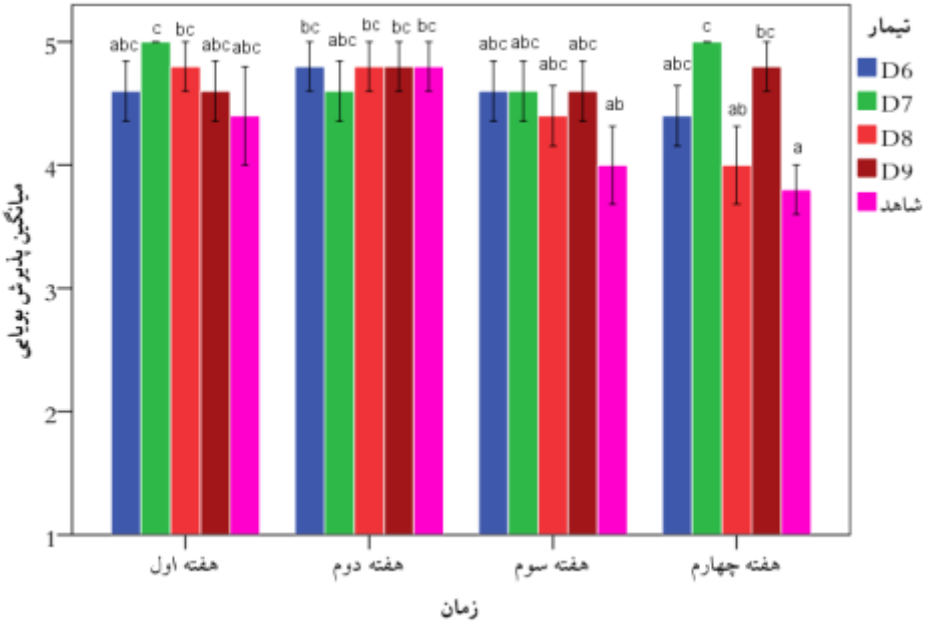

Fig 7. Changes in Olfactory Acceptance Over Time.

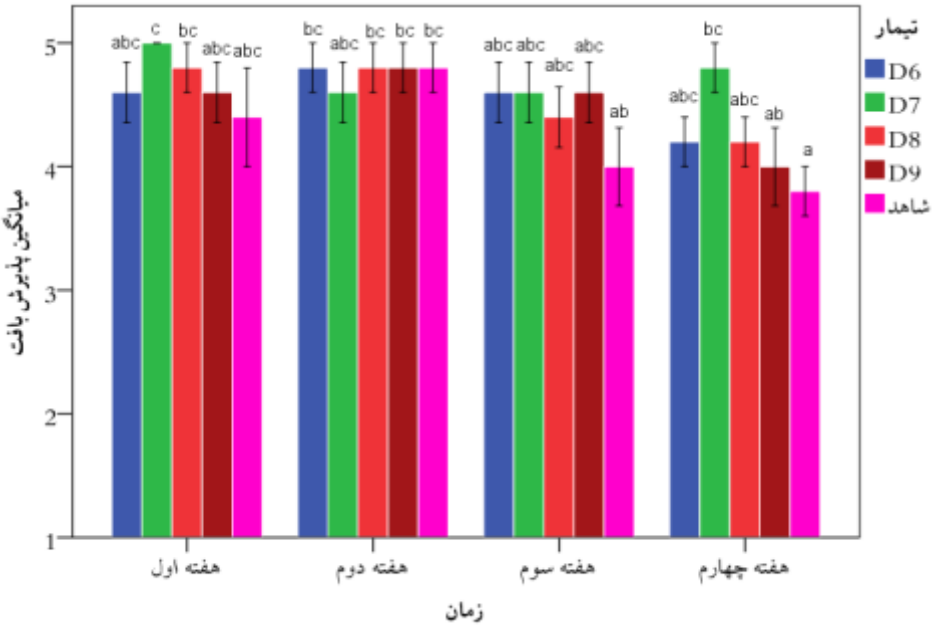

Fig 8. Changes in Texture Acceptance Over Time.

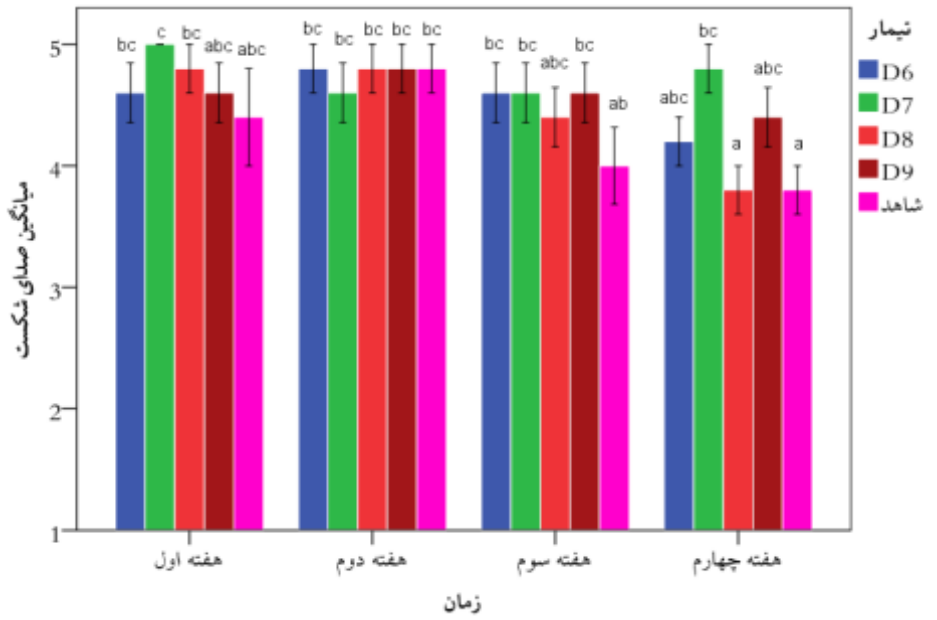

Fig9. Changes in Break Sound (Snap) Acceptance Over Time.

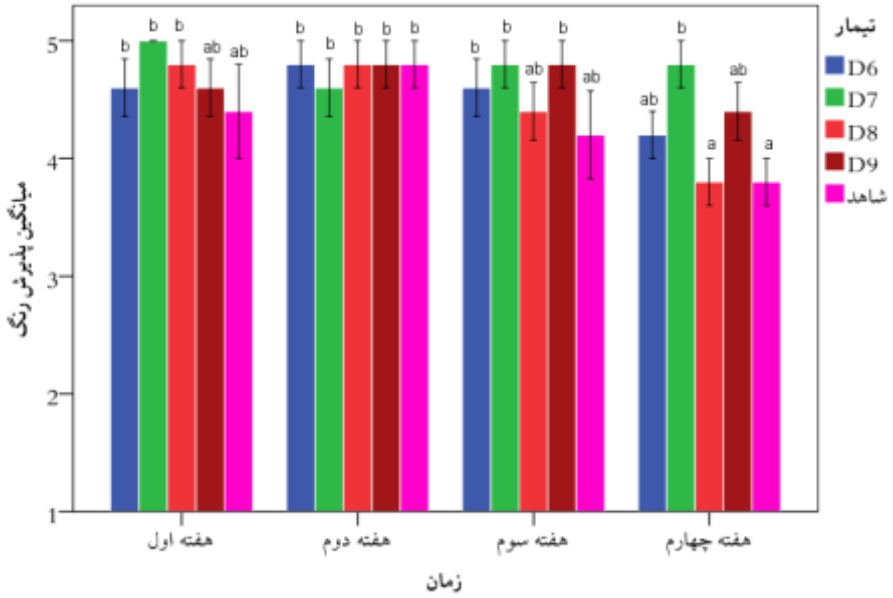

Fig10. Changes in Color Acceptance Over Time.

Fig 6 to 10 shows the opinions of chocolate expert evaluators regarding the functional dark chocolate. In all aspects related to aroma and flavor, olfactory perception, break sound (snap), and surface gloss, the experts noted no significant changes. It can be concluded that the presence of lactobacilli did not cause a decline in chocolate quality, as it did not result in the formation of coarse grains (which can lead to improper tempering and a gritty mouthfeel), nor did it reduce the surface gloss of the chocolate, which are positive points of this product. Furthermore, it did not alter the taste, aroma, or smell of the chocolate. Regarding the acceptance of the breaking sound quality, which is directly related to the chemical bonds between the hydrophilic (sugar) and hydrophobic (fats) components, no significant difference was observed between the treatments and the control sample. Studies have shown that after production and during storage, the metabolic activity of probiotic bacteria in dark probiotic chocolates can affect some textural properties, appearance, flavor, and aroma. Additionally, adding freeze-dried bacterial powder can influence the graininess, appearance, texture, and color of the final product [19]. Mirković et al. (2018) demonstrated similar findings, where encapsulated L. plantarum resulted in no significant sensory deviation from the control, confirming the findings of the present study regarding the neutrality of the probiotic addition on organoleptic properties. (2019) showed in a hedonic test that dark chocolate containing probiotic microcapsules showed no significant difference compared to two commercially available chocolates [25].

3-11 Limitations of the Study and Future Directions

While this study provides valuable insights into the feasibility of probiotic dark chocolate, several limitations should be acknowledged. First, the storage period was limited to one month and conducted strictly at refrigerated temperature (10°C). Future research should extend the storage period to 6-12 months at ambient retail temperatures (20-25°C) to assess long-term viability and lipid oxidation under realistic distribution conditions. Second, while apparent viscosity was unchanged, a full rheological characterization (Casson yield stress) is recommended to ensure industrial compatibility with molding and enrobing equipment. Finally, the protective effect of the chocolate matrix during simulated *in vitro* gastrointestinal digestion was not evaluated. Subsequent studies should

employ dynamic gastric and intestinal models to quantify the actual bioaccessibility and delivery of viable cells to the colon

4-Conclusion

The results regarding the effect of adding *Lactobacillus* bacteria to dark chocolate for producing functional dark chocolate on its physicochemical properties, color, texture, and acceptance level showed that adding *Lactobacillus* bacteria to dark chocolate had no negative impact on the chemical characteristics of any treatments at different percentages. The primary concern was potential changes in acidity. However, based on chemical tests measuring acidity and examining the fatty acid profile of the functional chocolate compared to the control sample, no significant changes were observed. Furthermore, the results from texture analysis, colorimetry, and sensory acceptance tests indicate that no undesirable conditions were created in the functional chocolate, making the production of this product feasible. The color of dark chocolate is an important factor in attracting buyers, and the addition of bacteria had no effect on it. No changes were observed in its flavor either. The texture of the functional chocolate was also appropriate and acceptable, similar to the control sample. This study confirms that microencapsulated *Lactobacillus* strains can be successfully incorporated into dark chocolate as a functional ingredient without compromising quality, provided that proper encapsulation and low-temperature tempering protocols are observed.

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AUTHORS' CONTRIBUTIONS

All activities were performed by author.

COMPETING INTEREST

The author affirm that he has no financial conflicts of interest or competing interests in this study.

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