

Characterization of a Probiotic Whey–apple Juice Fermented Beverage Enriched with Licorice Extract and Caffeine

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Abstract

Background and Objective: Probiotic whey-apple juice fermented beverages are becoming popular for their health benefits and incorporating bioactive ingredients such as licorice extract and caffeine enhances their functionality and consumer appeal. This study aimed to assess the physicochemical, microbial and sensory characteristics of a whey-apple juice based probiotic fermented beverage enriched with licorice extract and caffeine during storage.

Material and Methods: A probiotic beverage was formulated with various licorice extract (0–0.6%) and caffeine (60–100 mg.100 ml⁻¹) levels and stored for 1, 15 and 30 d. This was analyzed for pH, total soluble solids, viscosity, color, phenolic content, antioxidant activity, probiotic viability and sensory traits. A three-factor factorial design with statistical analysis was used for all experiments.

Results and Conclusion: Higher licorice extract and caffeine levels significantly increased *Lactobacillus plantarum* viability (from 6.27 to 7.79 CFU.ml⁻¹), phenolic content (from 125.87 to 164.16 mg GAE.g⁻¹), antioxidant activity (IC₅₀: from 145.46 to 112.26 µg.mg⁻¹) and viscosity (from 142.62 to 168.36 mPa.s⁻¹) while decreasing pH (from 4.26 to 4.17) and lightness (from 53.74 to 46.1) (p<0.05). The two additives altered color values, with a significant increase in redness only for licorice extract. The sample with 0.3% licorice extract and 80 mg caffeine included the highest sensory acceptability. Licorice extract and caffeine can improve the functional, physicochemical and sensory qualities of probiotic beverages during storage.

Keywords: Antioxidants, Caffeine, Fermented beverage, *Glycyrrhiza*, *Lactobacillus plantarum*, Probiotics

What is “already known” on this topic:

- Probiotic whey–fruit juice beverages are popular for their health benefits, and research explores adding functional ingredients to enhance them.
- Bioactive compounds like plant extracts can improve the antioxidant properties and functionality of fermented foods and beverages.
- The stability of probiotic viability and product quality during storage is a critical factor in developing successful functional beverages.

What this article adds:

- This study provides specific formulation data, showing that licorice extract and caffeine together significantly increase *Lactobacillus plantarum* viability, phenolic content, and antioxidant activity during 30-day storage.
- It identifies a novel optimal combination (0.3% licorice extract, 80 mg caffeine/100ml) that maximizes sensory acceptability while improving functional and physicochemical properties.
- The research quantifies how these additives alter color and viscosity, offering practical insights for formulating stable, appealing, and enhanced probiotic beverages.

1. Introduction

Whey, a by-product of global cheese and casein production, represents environmental pollutant and valuable nutritional resource due to its high production volume and organic load. Rich in essential amino acids, peptides and proteins, whey offers numerous health benefits, including antihypertensive, antioxidant, anti-inflammatory and antimicrobial effects. Efficient use of whey supports sustainable waste management and creation of value-added functional foods [1-3]. Apple juice is a nutrient-rich beverage containing natural sugars, vitamins particularly vitamin C, minerals such as potassium and antioxidants such as polyphenols. Combining whey protein with apple juice not only improves the beverage taste but also enhances its nutritional profile by adding natural sugars, vitamins and antioxidants. Additionally, whey serves as an excellent base for probiotic beverages, promoting gut health and overall wellness [4, 5].

Licorice (*Glycyrrhiza uralensis*) is a widely used medicinal herb characterized by a diverse array of bioactive compounds, including prominent flavonoids such as liquiritigenin and isoliquiritigenin. Liquiritigenin, a primary constituent, shows multiple pharmacological activities encompassing antioxidant, anti-inflammatory, antibacterial, antidepressant and anxiolytic effects. Caffeine, the principal active ingredient in energy beverages, acts as a central nervous system (CNS) stimulant, enhancing alertness and cognitive function. The incorporation of licorice extract and caffeine significantly augments the functional attributes of the beverage. Collectively, these ingredients exert a synergistic effect, enhancing health-promoting potential and sensory qualities of the formulation [6-8].

Previous studies have documented the development and quality assessment of apple-whey based herbal functional ready-to-serve beverages [4,9]. For example, Sharma et al. [4] formulated a low-calorie beverage by mixing 75% apple juice with 25% whey protein, achieving the desired protein content. Additionally, kombucha beverages incorporating licorice and ginger have demonstrated strong antioxidant capacities and increased phenolic content, enhancing their functional characteristics [6]. Moreover, a non-alcoholic mixed beverage, containing coconut water, cashew apple juice and caffeine, has successfully been developed [10]. Despite the growing interest in functional probiotic beverages, no study has investigated the integration of probiotic cultures into whey-apple juice systems enriched with licorice extract and caffeine. This gap creates unresolved questions regarding microbial stability, physicochemical characteristics, sensory acceptance and health-promoting potential. Conventional formulations often suffer from decreased probiotic viability due to acidic fruit components and lack of natural synergistic stabilizers.

The present study addressed this challenge by developing a novel probiotic apple-whey beverage fortified with licorice extract (0, 0.3 and 0.6%) and caffeine (60, 80 and 100 mg). This innovative combination affected licorice polyphenols and caffeine stabilizing effects to enhance probiotic viability, while assessing physicochemical, microbial and sensory attributes during storage.

2. Materials and Methods

2.1. Materials

Caffeine powder and carboxymethylcellulose (CMC) were purchased from Merck, Germany and licorice root was purchased from Shirin Darou, Shiraz, Iran. *Lactobacillus plantarum* (*L. plantarum*) PTCC 1058 cultures were provided by the Iranian Research Organization for Science and Technology (IROST, Tehran, Iran). The lyophilized strain ($\geq 10^8$ CFU.ml⁻¹) was propagated in MRS broth for 24 h at 37 °C using CO₂ incubator (Memmert, Munich, Germany). Man, Rogosa and Sharpe (MRS) agar and broth were supplied by Merck, Darmstadt, Germany. All reagents in this study included analytical grade and were purchased from Merck, Germany.

2.2. Licorice Extract Processing

Licorice root was washed, dried and ground into powder, which was then extracted with 70% ethanol through a 72-h shaking process. The extract was filtered to remove residues, concentrated using rotary evaporation and dried to achieve the final extract [11].

2.3. Apple Juice Processing and Conservation

The apple juice was prepared by thoroughly washing and sorting the fruits, followed by cutting and grating. Apple juice was achieved using electric juicer and then passed through a muslin filter. The extracted juice was pasteurized in glass containers via heating and stored at room temperature (RM) until further use [9].

2.4. Whey Preparation from Cow Milk

Fresh cow milk was processed using method of Sharma et al. [9]. The milk was heated to 82 °C for 10 min, then cooled to 70 °C and acidified with 2% citric acid under continuous stirring to induce casein coagulation. The clotted mixture was passed through a double layer of muslin cloth and the achieved whey was centrifuged at 5000 rpm for 10 min to eliminate residual fat prior to its use in product formulation.

2.5. Production of Probiotic Beverage

Apple juice and whey were heated to 70 °C for 15 min and filtered and then mixed at 60:40 ratios. Carboxymethyl cellulose (0.5 g per 100 ml), caffeine (60, 80 and 100 mg per 100 ml) and licorice extract powder (0, 0.3 and 0.6%) were



added to the mixture. The mixture was cooled to 37 °C and inoculated with a fresh *L. plantarum* probiotic culture at an initial level of approximately 10^9 CFU.ml⁻¹. Fermentation was carried out at 37 °C until the beverage reached a target pH of 4.5. The final pH after fermentation was assessed at 4.3 ± 0.1 . After fermentation, samples were stored at 4 °C. Experiments were carried out on Days 1, 15 and 30 to monitor physicochemical characteristics, microbial viability and sensory attributes of the beverage [12].

2.6. Physicochemical Analysis of Probiotic Beverage

During the storage time, probiotic beverage samples were analyzed for pH, total soluble solids (TSS) and viscosity. The pH and TSS were assessed using digital pH meter (AZ 86502, Taiwan) and digital refractometer (RX-7000 α , India), respectively. The apparent viscosity was assessed using Brookfield viscometer (DV II + LV, Brookfield, Middleboro, MA, USA) equipped with an LV2 spindle. Viscosity was expressed in mPa.s⁻¹ and assessed at 20 °C within fourteen spindle speeds ranging 1.5-2000 rpm; the primary reported values corresponded to 60 rpm. Assessments were carried out in triplicate to enhance reproducibility [9].

2.7. Color Assessment of Probiotic Beverage

The color characteristics of the probiotic beverage were assessed using HunterLab UltraScanvis US-Vis 1,310 device, USA, which assessed L*, a* and b* color values. The lightness parameter (L*) ranged from 0, representing black, to 100, representing white, a* scale assessed color on a spectrum from +127 (red) to -128 (green), while the b* scale ranged from +127 (yellow) to -128 (blue) [13].

2.8. Assessment of Phenolic Levels and Antioxidant Potential

Probiotic beverage samples were centrifuged and filtered prior to analyzing total phenolic content (TPC) using Folin-Ciocalteu method. The TPC was assessed using Folin-Ciocalteu method. A calibration curve was prepared with Gallic acid standards (0–200 mg.l⁻¹) and results were expressed as Gallic acid equivalents (GAE) per liter. Each assessment was carried out in triplicate. Antioxidant activity was assessed using DPPH radical scavenging assay. Sample extracts at various concentrations were reacted with 0.1 mM DPPH solution and absorbance was assessed at 517 nm after 30 min incubation in dark. The inhibition percentage was calculated and IC₅₀ values were achieved from a dose-response curve generated by plotting inhibition percentages against sample concentrations. All assessments were carried out in triplicate to ensure reproducibility [9].

2.9. Assessment of Probiotic Bacterial Survival

For microbiological assessment, 10 ml of the sample was mixed with 90 ml of sterile saline to achieve a 10⁻¹ dilution,

followed by preparation of successive serial dilutions. Enumeration of *L. plantarum* was carried out by spreading the diluted samples onto MRS agar supplemented with vancomycin and incubating at 37 °C for 72 h using CO₂ incubator. The results were reported as logarithmic colony-forming units (Log CFU) per milliliter [14-15].

2.10. Sensory Analysis

A sensory analysis was carried out with a panel of 12 trained participants (six men and six women, aged 20–30 y) using five-point hedonic scale ranging from 1 (“extremely dislike”) to 5 (“extremely like”). The panelists assessed the probiotic beverage samples for color, flavor, texture and overall acceptability during a 30-d storage time. For each session, 20-ml portions of the beverage were present on coded plates, set at 4°C \pm 1 and panel members rinsed their mouths with water between the assessments [16].

2.11. Statistical Analysis

All experimental trials were carried out using completely randomized factorial design with three variables of caffeine concentration, licorice powder level and storage time to assess their individual and combined effects on the physicochemical, microbiological and sensory characteristics of the probiotic drink. Data analysis was carried out using SPSS software v.22 and significant variations within the treatments were identified using Duncan’s multiple range test at a 95% confidence threshold ($p < 0.05$).

3. Results and Discussion

3.1. The pH and Probiotic Viability Assessment

Figure (1a, 1b) shows the pH levels and *L. plantarum* viability in probiotic beverages formulated with various quantities of licorice extract powder and caffeine through storage. The pH values of all samples ranged 4.13-4.32, which set within the acceptable range for apple juice (3.2–4.2) according to the Iranian National Standardization Organization (INSO) (ISIRI 14345, 2011), though the use of whey increased the highest pH. The ANOVA revealed significant major effects of licorice ($p < 0.001$) and caffeine ($p < 0.001$) on pH, as well as a significant interaction between the licorice and caffeine ($p < 0.001$). Similarly, *L. plantarum* viability was significantly affected by licorice ($p < 0.001$) and caffeine ($p < 0.001$), with a strong interaction effect ($p < 0.001$). The two parameters decreased significantly over the storage time ($p < 0.05$).

These results verified that licorice and caffeine acted synergistically to enhance probiotic viability while decreasing pH. Additionally, caffeine and associated phenolic compounds affected *L. plantarum* metabolic pathways through enzymatic activity, potentially increasing bacterial viability.



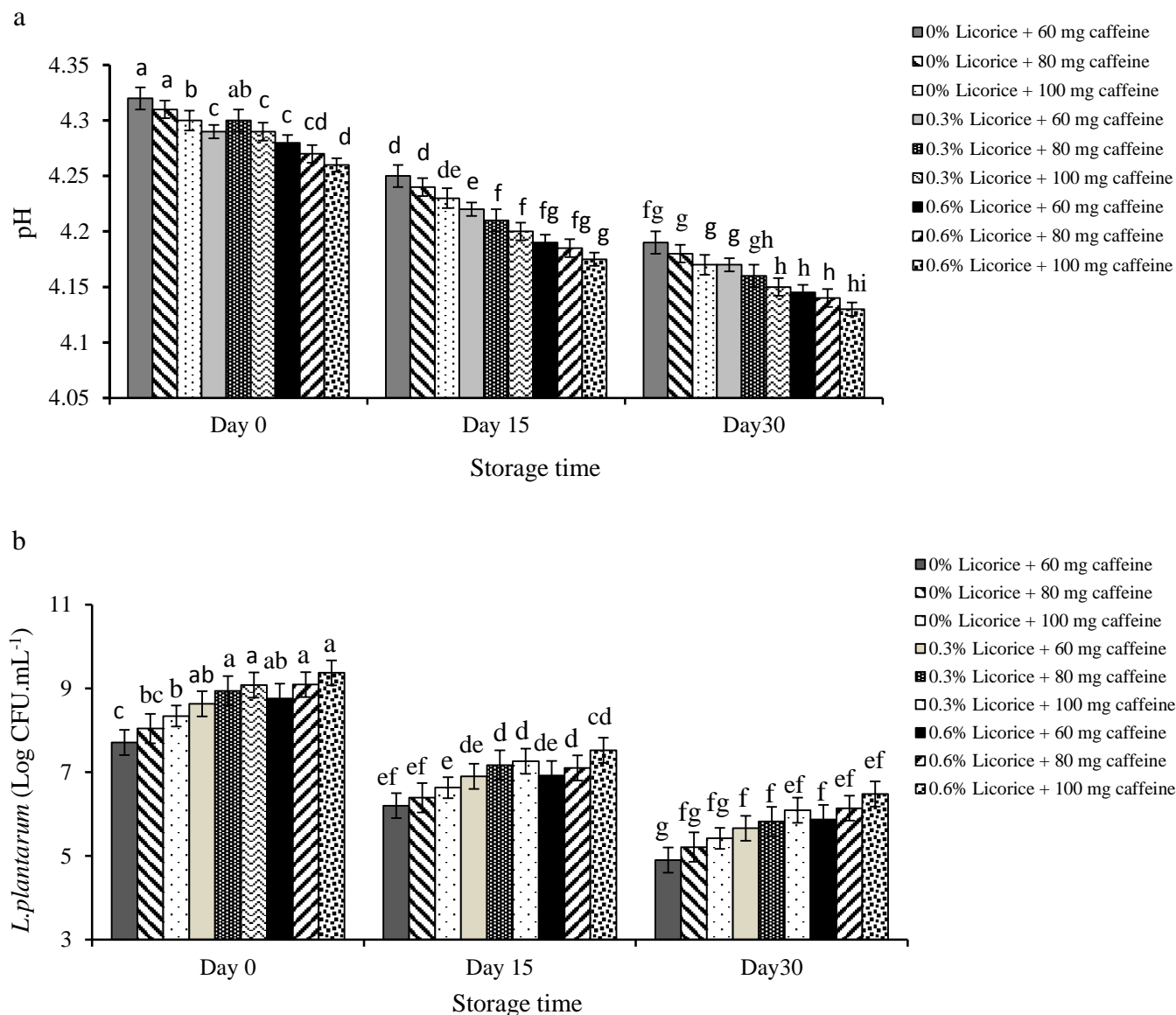


Figure 1. The effect of licorice extract powder (0%, 0.3%, and 0.6%) and caffeine concentration (60, 80, and 100 mg) on (a) pH and (b) viable count of *Lactobacillus plantarum* (Log CFU·mL⁻¹) in probiotic beverage during 30 days of storage

The observed decrease in pH with higher licorice and caffeine concentrations reflected intensified fermentation by *L. plantarum*, resulting in greater production of organic acids such as lactic acid that further promoted probiotic function. Caffeine positive effect might be linked to its potential degradation by these bacteria into methylxanthines, which could play an important role in cultivating beneficial gut microbiota such as *Lactobacillus* species. Methylxanthines might stimulate lactic acid bacteria (LAB) through nutritional enhancement, serving as possible carbon sources; thereby, creating a further favorable environment for bacterial proliferation. This combination of biochemical interactions suggested the potential beneficial effects of licorice extract and caffeine on probiotic viability and activity [14,17]. Over the storage time, a significant decrease in *L. plantarum* viability and pH was typical for probiotic beverages. As nutrients decreased

and metabolic waste accumulated, probiotic cell numbers decreased. Acid production might decrease or stabilize over time, affecting pH measurements. The initial pH decrease was linked to active bacterial metabolism during early storage, but extended time led to lower bacterial activity and viability; thereby, decreasing the two parameters significantly ($p < 0.05$). This decrease highlighted the importance of optimizing storage conditions and duration for maintaining probiotic efficacy in commercial products [18]. Similarly, a study on Iranian ultrafiltered white probiotic cheese containing caffeine detected that the addition of caffeine improved the survival of *L. fermentum* [19]. Tsurunichenko and Kretova [14] reported that licorice root was a valuable source of probiotic-supporting substances, particularly fructans. They identified that a 1% concentration of licorice root extract represented the minimum effective dose needed to significantly stimulate



the growth of probiotic microorganisms. In addition, Aleman et al. [20] studied the effects of functional ingredients on yogurt and detected that adding licorice root improved the viability of *L. bulgaricus* during storage.

3.2. Total soluble solids and Viscosity Assessment

Figure (2a, 2b) illustrate the TSS and viscosity of probiotic beverages formulated with varying concentrations of licorice extract powder and caffeine during storage. The TSS values of the samples ranged 10.3–11.85 °Brix, while viscosity values varied 141.21–171.43 mPa.s⁻¹. The ANOVA results showed that caffeine concentration included no significant effect on TSS ($p = 0.20$) or viscosity ($p = 0.11$). Licorice extract concentration included a non-significant effect on TSS ($p = 0.42$), but significantly increased viscosity ($p = 0.004$). Storage time significantly affected TSS ($p = 0.002$) and viscosity ($p = 0.001$). Importantly, the interaction between licorice and caffeine was not significant for TSS ($p = 0.292$) but was significant for viscosity ($p = 0.018$), indicating that higher licorice levels amplified the thickening effect of caffeine. These

findings suggested that while variations in caffeine concentration might affect TSS and viscosity, the absence of statistical significance was likely attributed to the relatively low caffeine levels used in the formulation. The statistically significant increase ($p < 0.05$) in the viscosity of the probiotic beverage with increasing licorice extract concentration could be linked to several factors inherent to the licorice composition. Licorice extract is rich in soluble solids and crude fibers, which act as natural thickeners, enhancing the beverage viscosity. Additionally, these components improve the water-holding capacity, contributing to a thicker further stable texture [20]. The significant increase in TSS and viscosity of samples during storage could be attributed to several factors. Over time, the evaporation of water leads to a concentration effect, increasing TSS, which can contribute to higher viscosity. Additionally, licorice extract contains carbohydrates that may interact with other components in the beverage matrix, enhancing thickening and water retention during storage.

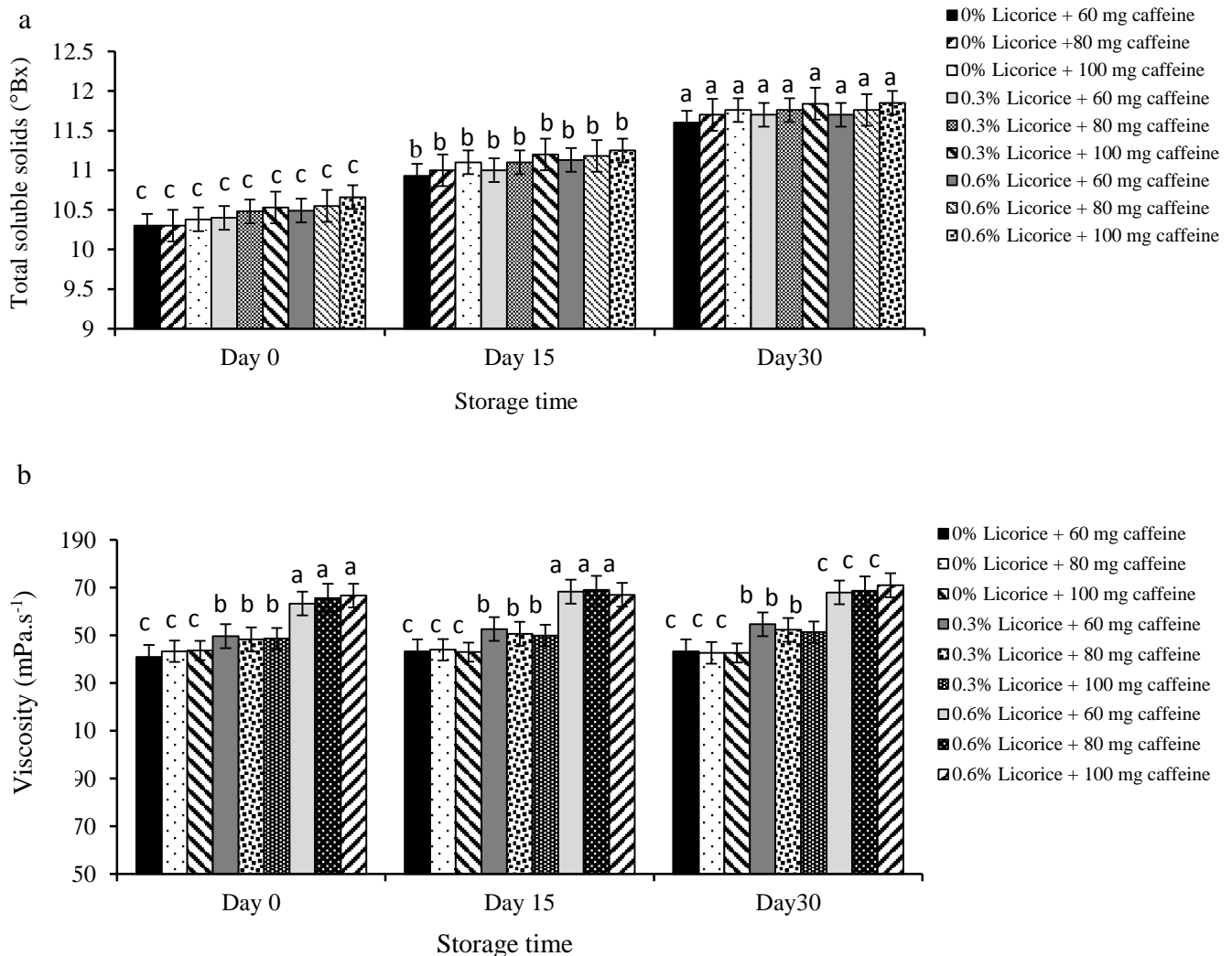


Figure 2. The effect of licorice extract powder (0%, 0.3%, and 0.6%) and caffeine concentration (60, 80, and 100 mg) on (a) total soluble solids (TSS, °Brix) and (b) viscosity (cP) of probiotic beverage during 30 days of storage.



The presence of caffeine and bioactive compounds from licorice affects microbial activity and biochemical reactions during fermentation, potentially increasing the production of organic acids and other metabolites that modify the beverage rheological characteristics [17]. De Carvalho et al. [7], in their study on developing a mixed beverage made from coconut water and cashew apple juice with caffeine, detected that the TSS content of their samples ranged 10.6-11.6, closely similar to the current findings. Similarly, Mohamed et al. [22] reported that formulations containing licorice extract showed significantly greater viscosity than the control samples, suggesting that licorice extract enhanced the thickness and texture of health drinks. Additionally, Khoshdouni et al. [23] reported a slight increase in TSS values over time, corresponding to the results achieved in the current study.

3.3. Total Phenolic Content and Antioxidant Activity (IC_{50})

Fig. (3a, 3b) show the effect of licorice extract powder and caffeine on TPC ($mg\ GAE \cdot g^{-1}$) and antioxidant activity (IC_{50} , $\mu g \cdot mL^{-1}$) of probiotic beverages during storage. The TPC values ranged 116.12-171.53 $mg\ GAE \cdot g^{-1}$, while IC_{50} values varied 108.25-156.74 $\mu g \cdot mL^{-1}$ within the treatments and storage times. Moreover, ANOVA results indicated that the licorice extract concentration significantly increased TPC ($p < 0.001$) and antioxidant activity ($p < 0.001$). Caffeine concentration included a non-significant effect on TPC ($p = 0.27$), but significantly improved antioxidant activity as assessed by IC_{50} ($p < 0.001$). Importantly, the interaction between licorice and caffeine was significant for TPC ($p = 0.031$) and IC_{50} ($p = 0.024$), indicating that higher licorice levels amplified the antioxidant-enhancing effect of caffeine. The TPC and antioxidant activity decreased significantly over the storage time (TPC, $p < 0.001$; IC_{50} , $p < 0.001$), reflecting the gradual degradation of phenolic compounds and decreased bioactivity during prolonged storage.

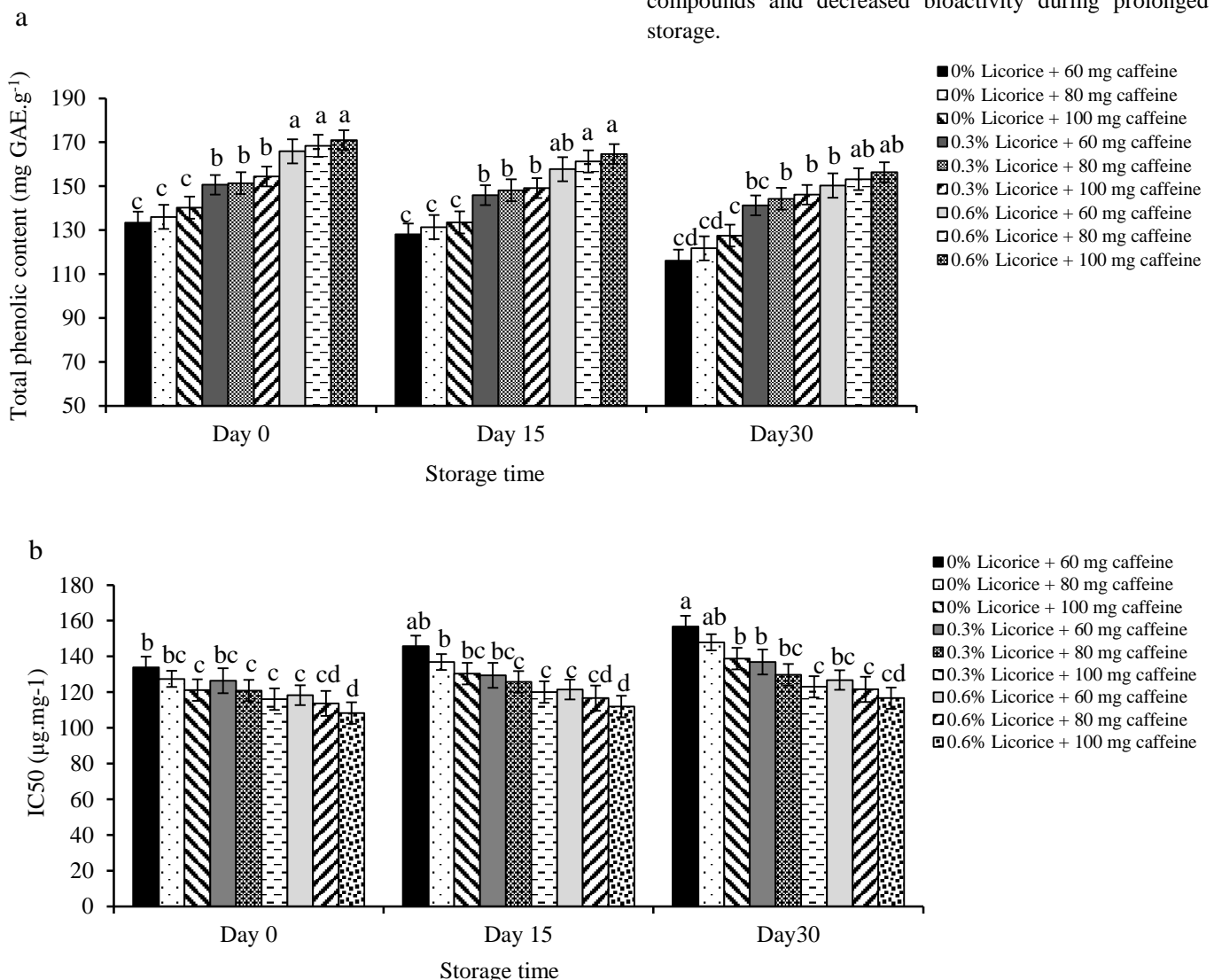


Figure 3. The effect of licorice extract powder (0%, 0.3%, and 0.6%) and caffeine concentration (60, 80, and 100 mg) on (a) total phenolic content ($mg\ GAE \cdot g^{-1}$) and (b) antioxidant activity measured as IC_{50} ($\mu g \cdot mL^{-1}$) of probiotic beverage during 30 days of storage.



Licorice extract contains abundant phenolic compounds and flavonoids, which confer strong antioxidant potential. From its significant constituents are phenylflavonoids such as dehydroglyasperin C (DGC), recognized for their effectiveness in neutralizing free radicals and safeguarding cells against oxidative injuries. The antioxidant activity of licorice is primarily attributed to its ability to quench reactive oxygen species, limit lipid peroxidation and enhance the function of antioxidant enzymes. These mechanisms highlight licorice reported health-promoting characteristics, including anti-inflammatory and anti-aging effects and highlight its value as a natural ingredient for improving the antioxidant profile of foods and beverages. Collectively, the diverse bioactive molecules in licorice provide substantial protection against oxidative stress and may help decrease the risk of associated disorders [24]. Incorporation of caffeine into probiotic beverage formulations has been shown to progressively increase antioxidant activity. Caffeine shows antioxidant capacity through radical scavenging and moderation of oxidative stress. Within a probiotic system, caffeine can act synergistically with other bioactive compounds; thereby, enhancing the overall ability of the beverage to counteract reactive oxygen species. This gradual improvement is likely associated with interactions between caffeine, probiotic microorganisms and phenolic constituents, ultimately strengthening the functional attributes of the product over time [25]. A significant decrease in phenolic content and antioxidant activity during storage ($p < 0.05$) is commonly observed in probiotic beverages. Throughout storage, factors such as exposure to oxygen and light, fluctuations in temperature and changes in pH contribute to the breakdown of phenolic compounds. Additionally, biochemical reactions and interactions between phenolics and other components within the beverage can further destabilize these antioxidants, resulting in decreased antioxidant effectiveness over time [26]. Quintana et al. [27] reported that the TPC in licorice root extract ranged 48.47–180.06 mg GAE·g⁻¹ while assessing its antioxidant activity. Similarly, Vieira et al. [25] demonstrated that caffeine effectively scavenged hydroxyl radicals and investigated the mechanisms behind its antioxidant potential. Fatima et al. [28] observed that antioxidant activity and free radical scavenging capacity gradually decreased during the storage of functional fruit and vegetable-based drinks containing herbal and spice extracts. Although licorice and caffeine significantly enhanced the phenolic profile and antioxidant activity of probiotic beverages, limitations should be addressed. The storage time was relatively short (30 d), which restricted conclusions on long-term stability; future studies should extend to 60 d to provide further comprehensive shelf-life data. Quantitatively, the TPC values observed in this study (116–171 mg GAE·g⁻¹) were within or slightly higher than the values reported by

Quintana et al. (48–180 mg GAE·g⁻¹), highlighting the strong contribution of licorice extract. Importantly, published studies on probiotic beverages containing caffeine were limited, underscoring the novelty of this study and the need of further investigations in this area.

3.4. Color Assessment of the Probiotic Beverage

Figure (4a, 4b, 4c) shows the changes in the L*, b* and a* color indices of the probiotic beverage containing licorice extract and caffeine over the storage time. The L* values ranged 44.61–56.45, the a* values ranged 14.42–25.76 and the b* values ranged 17.23–26.4 within the treatments and storage times. The ANOVA results indicated that increasing licorice extract and caffeine concentrations significantly decreased L* ($p = 0.012$) and significantly increased b* ($p = 0.008$). The two factors increased a* values, but this increase was significant only for licorice extract ($p = 0.021$). Storage time significantly affected all three indices ($p < 0.001$). Importantly, the interaction between licorice and caffeine was significant for L ($p = 0.033$), and b* ($p = 0.027$), but not for a* ($p = 0.29$). These findings verified that higher licorice levels amplified the color-modifying effect of caffeine, leading to darker and further saturated hues during the storage.

This decrease in lightness could be linked to the inherent color compounds present in licorice extract and caffeine such as polyphenols and alkaloids, which created a deeper color. Additionally, interactions between these compounds and the beverage matrix during storage might enhance pigment formation or aggregation, further decreasing brightness. The significant change at $p < 0.05$ verified that this effect was statistically robust, suggesting a clear effect of these ingredients on the visual appearance and potentially consumer perception of the product [29–30]. Increasing the quantities of licorice extract and caffeine causing significant increase in the b* (yellow-blue) value could be explained by the yellowish pigments naturally present in licorice, primarily flavonoids such as liquiritin and glabridin, which contributed to its characteristic yellow color. Studies indicate that licorice flavonoid content is responsible for this yellow hue, which can intensify as concentration increases, thus increasing the b* value towards yellow tones [31]. The significant increase in a* (red-green) values for licorice extract might be attributed to the presence of reddish-brown compounds such as melanoidins, which were formed during processing or storage through Maillard reactions. These polymeric brown pigments accumulated with heat treatment, contributing to darkening and shift towards red color. Additionally, heat treatment could increase the TPC and antioxidant activity of licorice extracts, affecting their color characteristics [32–33]. Kang et al. [31] demonstrated that during storage, licorice extract created the formation of brown melanoidin pigments, resulting from Maillard reaction between sugars and amino acids. Similarly, Ardalanian and Fadaei [33] observed that adding ginseng



extract to doogh samples caused increases in a* and b* color values. In an associated study, Jooyandeh and Alizadeh Behbahani [32] reported that the inclusion of pepper extract

in functional yogurt led to a decrease in L* (lightness) with increases in a* (red-green) and b* (yellow-blue) values.

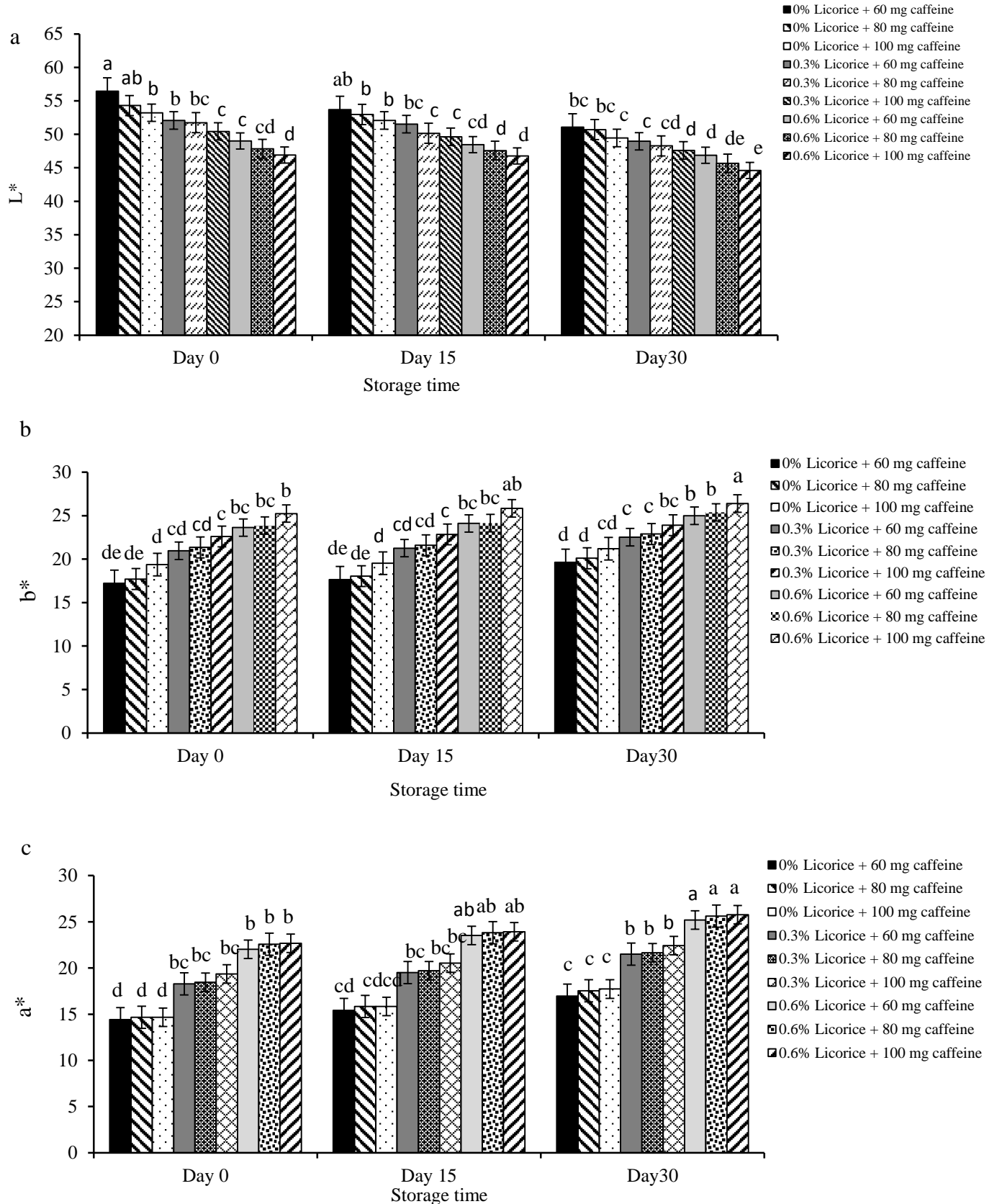


Figure 3. The effect of licorice extract powder (0%, 0.3%, and 0.6%) and caffeine concentration (60, 80, and 100 mg) on (a) total phenolic content (mg GAE·g⁻¹) and (b) antioxidant activity measured as IC₅₀ (μg·mL⁻¹) of probiotic beverage during 30 days of storage.



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3.5. Sensory Assessment of Probiotic Beverage

Figure (5) presents the sensory evaluation outcomes of probiotic beverage samples assessed by the panel on Day 30 of storage, considering attributes such as taste, aroma, color, texture and overall acceptability. Statistical analysis indicated that variations in licorice extract and caffeine levels significantly affected taste scores ($p < 0.05$). The combination of 0.3% licorice extract with 80 mg of caffeine improved taste perception, whereas higher concentrations resulted in decreased ratings. Caffeine content showed no significant effect on aroma or color, while 0.3% licorice extract enhanced color scores ($p < 0.05$). Texture was favorably affected by 0.6% licorice extract, with no significant effect from caffeine. The sample containing 0.3% licorice extract and 80 mg of caffeine achieved the highest overall acceptability.

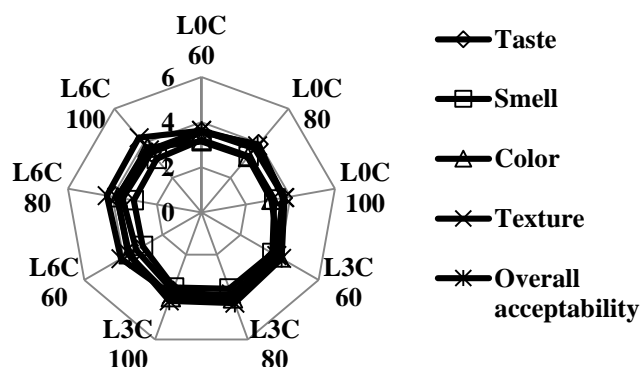


Figure 5. The effect of licorice extract powder (0%, 0.3%, and 0.6%) and caffeine concentration (60, 80, and 100 mg) on sensory attributes of probiotic beverage on the 30th day of storage. Sensory evaluation was performed using a 5-point hedonic scale (0 = dislike extremely, 5 = like extremely) for five attributes: taste, smell, color, texture, and overall acceptability.

Similarly, Azami et al. [34] developed a dairy beverage with licorice extract and cocoa powder and detected that moderate licorice levels improved taste and aroma, but excessive quantities led to decreased acceptability due to bitterness and off-flavors. Mahmoudi et al. [35] produced a functional non-dairy probiotic beverage containing fermented jujube extract, reporting that addition of extract enhanced aroma and taste, contributing to higher acceptability scores. Similarly, Ghosi Hoojaghan et al. [36] studied the effect of fennel extract in doogh, finding that low concentrations included insignificant effects on flavor, but higher concentrations decreased flavor scores.

4. Conclusion

This research formulated a functional probiotic drink enriched with licorice extract and caffeine, which showed improvements in probiotic survival, antioxidant potential and sensory attributes. Licorice extract significantly enhanced phenolic content, viscosity and antioxidant

capacity, whereas caffeine contributed to antioxidant activity and probiotic viability. Appropriate concentrations of these components optimized flavor, appearance, texture and overall consumer acceptance. Overall, the results indicate that licorice extract and caffeine act synergistically to strengthen the health-promoting characteristics and sensory quality of probiotic beverages, highlighting their promise as valuable functional ingredients.

5. Declaration

5.1. Acknowledgements

Contribution of Islamic Azad University, Iran, Tehran, is deeply appreciated.

5.2. Declaration of competing interest

The authors declare no conflict of interest or finance.

5.3. Authors' Contributions

Aydin Ali Bigelow: Writing original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Funding acquisition. Marjaneh Sedaghati: Writing–review and editing, Supervision, Methodology, Resources, Projected administration, Conceptualization. Mozghan Emtyazjoo: Supervision, Resources, Projected administration, Conceptualization.

5.4. Using Artificial Intelligent Chatbots

AI chatbot was used only for grammar correction.

5.5. Ethical Consideration

No ethical approval was required for the conduct of this study.

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خصوصیات نوشیدنی تخمیری پروبیوتیک بر پایه آب پنیر-آب سیب غنی شده با عصاره شیرین بیان و کافئین

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چکیده

سابقه و هدف: نوشیدنی‌های تخمیری پروبیوتیک بر پایه آب پنیر-آب میوه به دلیل فواید سلامتی بخش و دربرداشتن ترکیبات زیست‌فعال محبوبیت یافته‌اند، زیرا سبب افزایش خواص عملکردی و جذابیت برای مصرف کنندگان می‌شوند. هدف این مطالعه بررسی خواص فیزیوشیمیایی، میکروبی و حسی نوشیدنی تخمیری پروبیوتیک بر پایه آب پنیر-آب سیب غنی شده با عصاره شیرین بیان و کافئین در طول دوره نگهداری است.

مواد و روش‌ها: نوشیدنی پروبیوتیک با غلظت‌های مختلف عصاره شیرین بیان (۰-۶۰٪) و کافئین (۰-۱۰۰ میلی‌گرم در ۱۰۰ میلی‌لیتر) فرموله شد و برای مدت ۳۰ روز نگهداری شد. pH، مواد جامد محلول، ویسکوزیته، رنگ، محتوی فنلی، فعالیت آنتی‌اکسیدانی، زنده‌مانی باکتری پروبیوتیک و خصوصیات حسی نمونه‌ها مورد ارزیابی قرار گرفت. طرح فاکتوریل سه‌عاملی جهت آنالیز آماری نمونه‌ها مورد استفاده قرار گرفت.

یافته‌ها و نتیجه‌گیری: غلظت بالاتر عصاره شیرین بیان و کافئین سبب افزایش معنی‌دار زنده‌مانی لاکتوباسیلوس پلانتروم (از ۶/۲۷ CFU.ml⁻¹ به ۷/۷۹)، محتوی فنلی (از ۱۲۵/۸۷ mg GAE.g⁻¹ به ۱۶۴/۱۶)، فعالیت آنتی-اکسیدانی (IC₅₀: از ۱۴۵/۴۶ µg.mg⁻¹ به ۱۱۲/۲۶) و ویسکوزیته (از ۱۴۲/۶۲ mPa.s⁻¹ به ۱۶۸/۳۶) گردید، درحالی‌که منجر به کاهش pH (از ۴/۲۶ به ۴/۱۷) و روشنایی نمونه‌ها (از ۵۳/۷۴ به ۴۶/۱) شد (p<۰/۰۵). هر دو افزودنی سبب تغییر شاخص‌های رنگی شد اما افزایش معنی‌دار در قرمزی تنها در حضور عصاره شیرین بیان مشاهده گردید. نمونه‌هایی که حاوی ۰/۳٪ عصاره شیرین بیان و ۸۰ میلی‌گرم کافئین بودند بیشترین امتیاز پذیرش حسی را کسب کردند. طبق نتایج حاصل، افزودن عصاره شیرین بیان و کافئین سبب بهبود خواص عملکردی، فیزیوشیمیایی و حسی نوشیدنی پروبیوتیک تخمیری در طول دوره نگهداری می‌گردد.

واژگان کلیدی: آنتی‌اکسیدان‌ها، کافئین، نوشیدنی تخمیری، عصاره شیرین بیان، لاکتوباسیلوس پلانتروم، پروبیوتیک

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