

EVALUATION AND IMPROVEMENT OF ANTIOXIDANT ACTIVITY AND PHYSICOCHEMICAL PROPERTIES OF YOGURT ENRICHED WITH PERSIAN GUM (*AMYGDALUS SCOPARIA SPACH*) AND FENNEL (*FOENICULUM VULGARE*) EXTRACT

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ABSTRACT

Background. This study aimed to evaluate the physicochemical properties and antioxidant activity of enriched yogurt with different concentrations of fennel extract (0, 1, and 3%) and Persian gum (0, 0.1, and 0.2%) during refrigerated storage (1 and 21 days).

Material and methods. Prepared milks with or without fennel extract and Persian gum were incubated with a mixed starter culture YF-3331 (*Lactobacillus bulgaricus* and *Streptococcus thermophiles*) in a volume of 200 ml in triplicate. The produced yogurt matrix was evaluated for pH/acidity, syneresis, viscosity, total phenolic content (Folin-Ciocalteu), antioxidant activity (DPPH), and sensory properties (12 participants).

Results. The pH, acidity, total phenolic content (TPC), antioxidant activity (AO), and viscosity of enriched yogurt were in the range of 4.29–4.8, 0.74–1.29%, 19.83–46.24% (mg gallic acid/g), 24.62–57.69% and 1329–3825 (cP) respectively. The results showed that increasing the amounts of fennel extract and Persian gum had a significant effect on the pH, acidity, TPC, AO, syneresis (%) and viscosity of enriched yogurt ($p < 0.05$). The sensory analysis of samples revealed that the amount of fennel extract had a significant effect on the general acceptability of yogurt samples ($p < 0.05$), but the effect of Persian gum was insignificant.

Conclusion. The results showed that enrichment of yogurt with fennel extract and Persian gum could improve physicochemical, phenolic compound and antioxidant properties; however, the extent to which additives were used was highly influenced by changes in sensory characteristics and consumer acceptability. In view of this, a yogurt sample containing 1% fennel extract and 0.2% Persian gum was more acceptable than the other samples on the 21st day of storage.

Keywords: yogurt, phenolic compounds, fennel, Persian hydrocolloid, physicochemical

INTRODUCTION

Food enrichment is one of the key processes that can improve the nutrient quality and quantity of food products. Enriched dairy products with essential ingredients can promote public health and significantly reduce

or prevent diseases caused by nutritional deficiencies. Yogurt is one of the most important fermented dairy products. It is attractive to the consumer due to its long shelf life, high nutritional value, favorable sensory

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properties, and digestibility. Yogurt is a suitable carrier for functional ingredients, healthful bacteria, and bioactive compounds in the human body (Ghasempour et al., 2012; Abdi-Moghadam, 2023). Although yogurt has significant nutritional value and is important in human diets, it is not widely considered a major source of phenolic compounds. Plant-based additives have been used to increase the phenolic content of yogurt due to their richness in bioactive compounds such as polyphenols (Gahruie et al., 2015).

Fennel (*Foeniculum vulgare*) is one of the flowering plants, and its seeds contain minerals (13.4%), fat (10%), carbohydrates (42.3%), protein (9.5%), vitamin C, riboflavin, thiamin, niacin and phenolic compounds (18.5%). Fennel contains 23 phenolic compounds such as limonene, fenchone, estragole, and trans-anethole, which have antimicrobial and antioxidant activity. Due to fennel's functional properties, interest in its application as a food additive in food enrichment has grown in recent years (Atwaa et al., 2022). Persian gum is a hydrocolloid extracted from the mountain almond tree and is commonly found in Iran. It is an anionic and fully branched polysaccharide, consisting of arabinose and galactose and possessing a phenolic component. This local gum has no special flavor, smells like confectionery and is affordable in comparison to other natural hydrocolloids (Kadkhodae and Mahfouzi, 2021; Mir et al., 2021).

Several previous studies have applied medical plant extracts and hydrocolloids as bioactive compounds in the production of enriched yogurt. Abdesslen et al. (2019) reported that fennel essential oil improved yogurt stability and reduced syneresis compared to a control sample. In an experiment reported by Tizghadam et al. (2021), set yogurt fortified with dill extract had higher antioxidant activity compared to the control sample. Atwaa et al. (2022) reported that aqueous fennel extract significantly increased the antioxidant activity of probiotic yogurt. Abbasi and Mohammadi (2013) claimed that using Persian gum can improve the stability of milk-orange juice mixture as a functional juice. However, to the best of our knowledge, no study has been carried out to produce functional yogurt enriched with Persian gum (*Amygdalus scoparia* Spach) and fennel extract.

As a response to consumer and food industry demand for enriched yogurt, we aimed to evaluate the

physicochemical (pH, acidity, total phenolic content, syneresis and viscosity), organoleptic (odor, color, taste, and general acceptability), and antioxidant properties of yogurt incorporating aqueous extract of fennel and Persian gum during 21 days of refrigerated storage.

MATERIAL AND METHODS

Materials

Whole milk (3% fat, 3.2% protein, and 4.7% lactose) was purchased from Pegah company in Tehran (Iran). The starter culture (YF-3331) in lyophilized form, comprising a mixed culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, was prepared by Christian Hansen (Horsholm, Denmark). The Persian gum and fennel seeds were purchased from a local market in Tehran, Iran. All chemicals used in this research were of analytical grade and were prepared by Merck (Germany).

Preparation of Persian gum and fennel extract

Lumps of Persian gum were cleaned and powdered using the laboratory miller (A11 IKA, Germany) and passed through a screen (250 μm). Then, the Persian gum particles were collected and stored in a sealed glass container. For the production of fennel extract, 100 grams of fennel seeds were ground using the laboratory miller and added to one liter of boiled distilled water. After 4 hours, the content of the container was filtered and condensed using a rotary evaporator (Heidolph, Germany) until it reached a level of 14% total solids, then kept at 4°C in the refrigerator (Ghosi Hoojaghan et al., 2022).

Set yogurt production

The dry matter of whole milk was standardized by adding skim milk powder (3%) around 14%. According to our theoretical analysis and pre-experiments, Persian gum (0, 0.1%, and 0.2%) and fennel extract (0, 1%, and 3%) were added to the mixture and heated at 90 °C for 10 min. After it had been cooled to 42°C, the starter culture was added to the mixture at a rate of 0.02% (w/v) and incubated at 42°C to reach a pH of around 4.6 (around 4 hours). The samples were cooled down, placed in a 250-mL PET container and stored at 4°C for 21 days (Cho et al., 2020).

pH, titratable acidity and syneresis determination

The pH of the yogurt samples was measured using a digital pH meter (Taiwa, AZ 86502). The titratable acidity (TA) and syneresis were measured according to the method of Rojas-Torres et al. (2021). 30 g yogurt samples were centrifuged at 2800 g for 20 min at 10°C. The clear supernatant was separated and weighed, and the following equation was used to calculate the percentage (w/w) of syneresis:

$$\% \text{Syneresis} = (\text{g supernatant whey} \times 100) / (\text{g sample weight})$$

Viscosity measurement

The viscosity of yogurt samples was measured using the Brookfield DV-III Ultra (Brookfield, Laboratories Inc., USA) with the number 3 spindle. Yogurt samples were poured into the reservoir of the machine, and the number 3 spindle and a shear rate of 30 (1/s) at 4°C were applied (Tizghadam et al., 2021).

Total phenolic content and antioxidant activity

Yogurt samples were centrifuged at 7690 g for 20 min. A syringe filter of 0.45 µm (MSCA, Shanghai, China) was used for sample filtration. To measure the TPC, 300 µl of yogurt supernatant was mixed with 1.5 ml of Folin-Ciocalteu solution, and the mixture was kept at room temperature for 3 minutes. Then 1.2 ml sodium carbonate solution (7.5% w/v) was added to the mixture and kept at room temperature for 30 minutes. The absorbance of the mixture was read at a wavelength of 765 nm with a spectrophotometer (Cecil 7400 UV Visible Spectrophotometer, Cambridge, United Kingdom). The results of TPC were reported as g of gallic acid equivalent (GAE) per liter. For AO determination, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) was applied. 3 ml of 0.1 mM DPPH in ethanol was added to 100 µl of yogurt supernatant, mixed, and kept at room temperature for 60 minutes. Then, the absorption of the samples was measured using a spectrophotometer at 517 nm (Atwaa et al., 2022). The percentage of antioxidant activity was calculated using the following equation:

$$\text{Inhibition (\%)} = (\text{controlled absorbance} - \text{treatment absorbance}) \times 100 / (\text{controlled absorbance})$$

Sensory analysis

Sensory evaluation was performed by 12 panelists (6 women and 6 men, aged 20–30), selected from among the graduate students of Tehran Azad University's Food Science and Technology Department (Tehran, Iran), who received training on the properties of yogurt products. The sensorial properties (color, odor, taste, and overall acceptance) of samples were tested using a five-point hedonic scale ranging from 1 (dislike very much) to 5 (like very much). 20 g yogurt samples were prepared in numbered plates and distributed to the panelists at a temperature of $4 \pm 1^\circ\text{C}$ before a meal (to increase sensory sensitivity). After each test, the panelists rinsed their mouths with water. Because of our domestic policy, sensory tests are exempt from ethical permissions, but participation in the tests and assessments was voluntary (Rojas-Torres et al., 2021).

Statistical analysis

All tests were applied in a completely randomized factorial design with three treatments devised using applied statistical SPSS software version 16 (SPSS, version 22, 2016). The first factor was the fennel concentration in the yogurt (0, 1, and 3%), the second factor was the amount of Persian gum in the yogurt (0, 0.1, and 0.2 %) and the third factor was the storage time (day 1 or 21). As a result, the present study involved nine treatments and three replications. Duncan's multiple range test determined significant differences at the 95% confidential level ($p < 0.05$).

RESULTS AND DISCUSSION

pH and acidity determination

Statistical analysis clarified that the effects of Persian gum and fennel extract on the pH and acidity of fortified yogurt were significant ($p < 0.05$; Fig. 1a, 1b). The incorporation of fennel extract resulted in a noticeable reduction in the acidity of the yogurt sample ($p < 0.05$), yet the addition of Persian gum led to an increase in acidity that was statistically significant ($p < 0.05$). The results indicate that the interaction effects of fennel extract and Persian gum on yogurt acidity were significant ($p < 0.05$). The positive impact of Persian gum on the production of lactic acid bacteria (LAB) might contribute to the availability of indigestible fiber in Persian gum that acts as a prebiotic for

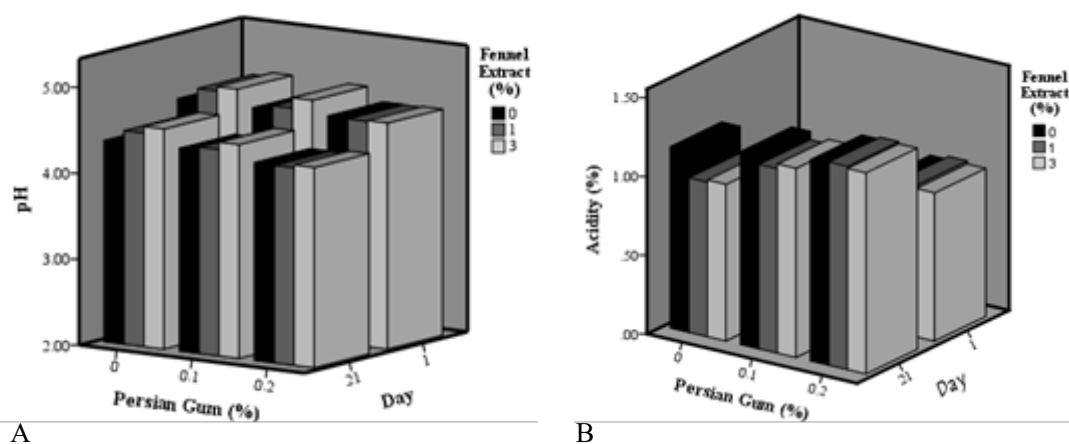


Fig. 1. The effect of Persian gum and Fennel extract on the pH (A) and acidity (B) of fortified yogurt during 21 days of storage

LAB in treated samples, while the antibacterial properties of phenolic compounds in the fennel extract may have restricted the growth of LAB (Atwaa et al., 2022; Vasheghani Farahani, 2022). Similarly, Atwaa et al. (2022) observed a decrease in acidity in yogurt when fennel extract was used. Moreover, an increase in the acidity levels of yogurts prepared with dill extract was observed by Tizghadam et al. (2021). The acidity of samples significantly increased over the storage time, while the pH of samples decreased significantly ($p < 0.05$). The acidic pH of the yogurt may have been due to the synergistic growth of *Streptococcus thermophiles* and *Lactobacillus bulgaricus* during fermentation. Initially, the pH decreased due to the growth of *Streptococcus thermophiles*, which utilized free amino acids in the mixture to provide peptides for *Lactobacillus bulgaricus* growth. As the pH decreased, *Lactobacillus bulgaricus* grew more rapidly and produced a larger quantity of lactic acid, resulting in a significant reduction in pH. Post-fermentation acidification in yogurt is also due to the metabolic activity of the starter cultures during the cold storage period of the product (Sccaro et al., 2009; Rezaei et al., 2023a). A drop in pH during 14 days of storage was also observed in the study of Mendoza-Taco et al. (2022) for sheep yogurt enriched with *Moringa oleifera* leaf extract. Previous studies have stated that the increased trend in acidity is due to the activity of lactic acid bacteria and the production of lactic acid and other organic acids (Rezaei et al., 2023a).

Syneresis of yogurt measurements

The syneresis data for yogurt samples are shown in Fig. 2b. Syneresis is the constriction of the yogurt network, which leads to the excretion of whey from the gel network during storage. The reduction of total milk solids, high acidity, an inappropriate ratio of whey to casein, a long incubation time, and inappropriate transportation are the factors that most significantly affect yogurt stability (Sahan et al., 2008; Rezaei et al., 2023b). In general, the addition of fennel extract at the lowest concentration (1%) decreased the syneresis in yogurt samples, but the difference obtained with higher levels of fennel (3%) was not significant in some treatments ($p > 0.05$). As Figure 2b shows, the addition of Persian gum even at a low concentration (0.1%) reduced syneresis in yogurt samples, and the consistency of yogurt samples was improved by increasing the content of Persian gum from 0.1% to 0.2%. Except for the control sample, all the samples treated with fennel extract and Persian gum showed a significant decrease in syneresis during the storage period ($p < 0.05$). The reduction in syneresis at the lowest concentration of fennel extract in yogurt was related to higher total solids and interactions between fennel carbohydrate and the gel network. However, increased syneresis at higher concentrations of the extract was because of the interrupting effect of fennel extract on yogurt gel formation (Izadi et al., 2015). Generally, one gram of Persian gum can absorb around 12 g of water and increase the yogurt's viscosity. Moreover, Persian gum could

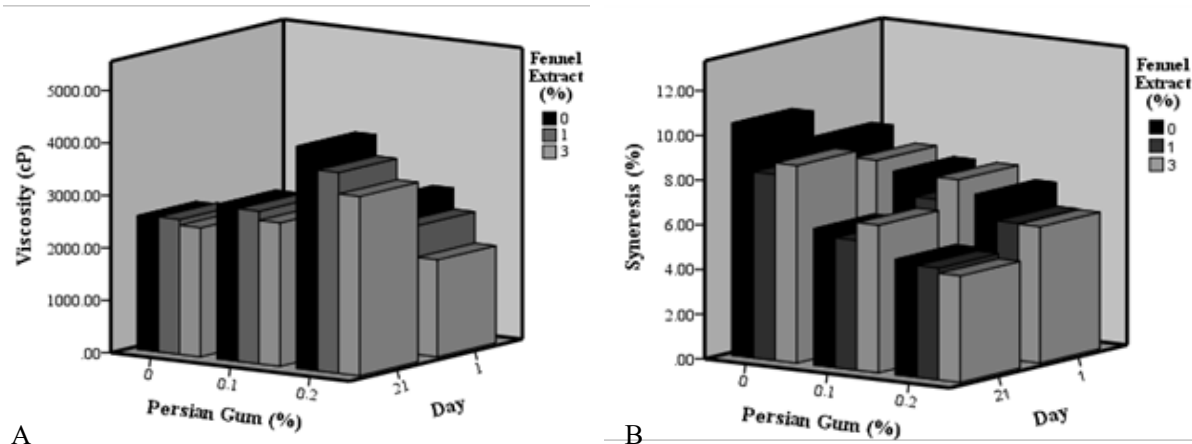


Fig. 2. The effect of Persian gum and fennel extract on the viscosity (A) and syneresis (%) (B) of fortified yogurt during 21 days of storage

interact with whey proteins, reduce the penetration of whey from the casein micelles and increase yogurt stability (Dabestani et al., 2018). Similarly, Tizghadam et al. (2021) reported that adding 5% dill extract to set yogurt decreased the level of syneresis, but a higher level of dill extract (10%) increased syneresis significantly ($p < 0.05$). Hussain et al. (2022) studied the effects of cress seed gum on the syneresis level of non-fat yogurt. The cress seed gum in their research was applied as a food stabilizer, improved water holding capacity, increased viscosity, and reduced syneresis in yogurt. Similarly, Ghosi Hoojaghan et al. (2022) reported that tragacanth gum decreased syneresis in Iranian dough by immobilization of the continuous phase and by improving the capacity of the dough to form a gel structure with casein micelles. The findings of Amirdivani and Baba (2013) contradict our results, as they reported that the presence of 2% green tea extract in yogurt led to a significant increase in syneresis during storage.

Viscosity of yogurt measurements

Yogurt viscosity is influenced by the physicochemical properties of milk, such as dry matter, fat, protein concentration, pH, acidity, and temperature. Moreover, the assessment conditions of viscosity, comprising shear rate, temperature, and spindle number, are important factors (Haji Ghafarloo et al., 2019). As shown in Figure 2a, the viscosity of the yogurt samples was in the range of 1329 to 3825 (cP), and the addition of

Persian gum caused a significant increase in the viscosity of the yogurt samples ($p < 0.05$). However, the viscosity of yogurt samples decreased significantly in the presence of fennel extract ($p < 0.05$). Persian gum with suitable rheological characteristics was applied to enhance the textural properties of dairy products. Persian gum consists of soluble and insoluble fractions; the soluble part produces a transparent solution, and the insoluble part absorbs water and improves the viscosity of dairy products (Dabestani et al., 2018). Furthermore, water is trapped within the gel structure created by casein micelles in yogurt, and the fennel extract hinders the formation of the gel structure in enriched samples, resulting in reduced viscosity (Izadi et al., 2015). Figure 2a shows that storage time positively affects the viscosity of yogurt samples, ($p < 0.05$). The increase in viscosity during storage might be due to the strengthening of the protein-protein interaction and the protein rearrangement in the yogurt structure in the presence of Persian gum. Moreover, the antibacterial effect of phenolic compound in fennel extract limited the degradative effect of LAB on the gel structure of yogurt during storage and improved viscosity (Ghasempour et al., 2012; Barukcic et al., 2022). Similarly, Ghasempour et al. (2012) used Persian gum in low-fat probiotic yogurt, which increased the viscosity and stability considerably. Haji Ghafarloo et al. (2019) reported that increasing the amount of gum arabic from 0 to 1% caused a significant increment in the viscosity of probiotic dough. This result is in agreement with

that of Amirdivani and Baba (2013), who reported that adding 2% green tea extract to yogurt reduced its apparent viscosity. Barukčić et al. (2022) reported that the presence of olive leaf extract led to a reduction in yogurt viscosity, which was more or less proportional to the olive leaf extract concentration. These reports reveal the effect of plant extracts related to plant type and their concentration (Haji Ghafarloo et al., 2019). Similarly, Sahan et al. (2008) observed an increase in the viscosity of non-fat yogurt during storage. Also, Ghosi Hoojaghan et al. (2022) reported that the apparent viscosity of the enriched dough increased during storage. These results contradict the data reported by Salgado et al. (2021), who found that after 14 days of storage, LAB starter fermented milk carbohydrates, so the gel structure of yogurt weakened, and its apparent viscosity was reduced.

Total phenolic content and antioxidant activity

Table 1 indicates that using Persian gum and fennel extract in yogurt samples increased the TPC and AO activity significantly ($p < 0.05$). Table 1 also shows that the addition of 3% fennel extract resulted in a significant increase in AO activity, rising from 31.64% in

the control sample to 47.06%, while the presence of 0.2% fennel extract led to an AO activity of 39.41% in yogurt samples. These results indicate that the interaction effect of fennel extract and Persian gum on the AO activity of yogurt was significant ($p < 0.05$) and the effect of Persian gum on the AO activity was intensified in the presence of fennel extract ($p < 0.05$). As natural antioxidants, Persian gum and fennel extract could absorb free radicals and improve the antioxidant properties of yogurt. The presence of phenolic and flavonoid ingredients in Persian gum and 1,3-O-dicaffeoylquinic acid, 5-O-caffeoylquinic acid, 1,5-O-di-caffeoylquinic acid, 4-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, and 1,4-O-di-caffeoylquinic acid as phenolic acid components in fennel extract led to an increase in the AO activity of fortified yogurt. Following fermentation, the phytochemical content or microbial metabolic activity of plants may result in higher antioxidant activity being observed in enriched samples (Atwaa et al., 2022). Łopusiewicz et al. (2022) reported that adding plant seed gum to the acid whey fermented beverages increased their AO activity significantly ($p < 0.05$). Mir et al. (2021) revealed that an increase in the almond gum concentration of yogurt

Table 1. The effect of Persian gum and Fennel extract on the total phenolic count (mg GAE/g) and antioxidant activity (DPPH (%)) of fortified yogurt during 21 days of storage

Samples	Fennel extract	Persian gum	TPC, mg GAE/g		DPPH, %	
			day 1	day 21	day 1	day 21
C	0	0	21.66 ± 0.81 ^{Ea}	19.83 ± 0.61 ^{Eb}	31.64 ± 1.21 ^{EFa}	24.62 ± 5.43 ^{Eb}
T ₁	0	0.1%	28.23 ± 0.67 ^{DEa}	26.77 ± 0.97 ^{Db}	36.79 ± 4.37 ^{Ea}	27.48 ± 5.38 ^{DEb}
T ₂	0	0.2%	30.20 ± 0.93 ^{Da}	29.65 ± 0.53 ^{CDb}	39.41 ± 3.53 ^{DEa}	30.70 ± 3.57 ^{Db}
T ₃	1%	0	33.13 ± 0.66 ^{CDa}	32.61 ± 0.66 ^{Cb}	41.18 ± 5.39 ^{Da}	37.19 ± 1.64 ^{CDb}
T ₄	1%	0.1%	34.58 ± 0.59 ^{CDa}	32.25 ± 0.89 ^{Cb}	45.24 ± 3.28 ^{Ca}	41.75 ± 1.22 ^{Cb}
T ₅	1%	0.2%	39.88 ± 0.88 ^{Ca}	37.54 ± 0.88 ^{BCb}	48.68 ± 3.43 ^{BCa}	42.72 ± 3.1 ^{BCb}
T ₆	3%	0	35.25 ± 0.83 ^{BCa}	33.73 ± 0.73 ^{Bb}	47.06 ± 1.19 ^{BCa}	40.11 ± 5.09 ^{BCb}
T ₇	3%	0.1%	42.64 ± 0.79 ^{ABa}	38.52 ± 0.96 ^{ABb}	51.74 ± 4.66 ^{Ba}	44.86 ± 2.06 ^{Bb}
T ₈	3%	0.2%	46.24 ± 0.56 ^{Aa}	40.25 ± 0.99 ^{Ab}	57.69 ± 0.66 ^{Aa}	52.42 ± 2.76 ^{Ab}

^aMeans within each column followed by different letters (A–D) show significant differences ($p < 0.05$) between treatments at the same time point.

^bMeans within each row followed by different letters (a–b) show significant differences ($p < 0.05$) between treatments during the storage period.

samples is positively associated with AO activity. Atwaa et al. (2022) reported that an incremental increase in fennel extract content in yogurt samples increased TPC and AO activity significantly ($p < 0.05$). Tizghadam et al. (2021) found that the addition of dill extract to yogurt increased the amount of total phenolic compounds significantly compared to control samples ($p < 0.05$). Table 1 indicates that AO activity decreased during storage in all samples, but the treated samples had higher AO activity compared to the control sample after 21 days of storage ($p < 0.05$). The higher antioxidant activity detected using DPPH in the treated samples could be ascribed to the presence of phenolic and flavonoid components or secondary or subsidiary metabolites produced from them with AO activity that

persisted over 21 days of storage) Soliman and Nasser, (2022). Other researchers, such as Atwaa et al. (2022), have reported that AO activity decreases significantly in all fortified yogurt treated with fennel extract during storage. Similarly, Soliman and Nasser (2022) found that AO activity reduced significantly in stirred yogurt samples with the an increase in the storage period. Lin et al. (2020) reported that the AO activity of *Momordica charantia* and *Momordica charantia* var. *abbreviata* ser juice reduced with storage time.

Sensory evaluation

As presented in Figure 3, the effect of fennel on sensory properties related to yogurt samples on the 1st day of storage was significant ($p < 0.05$), but the effect

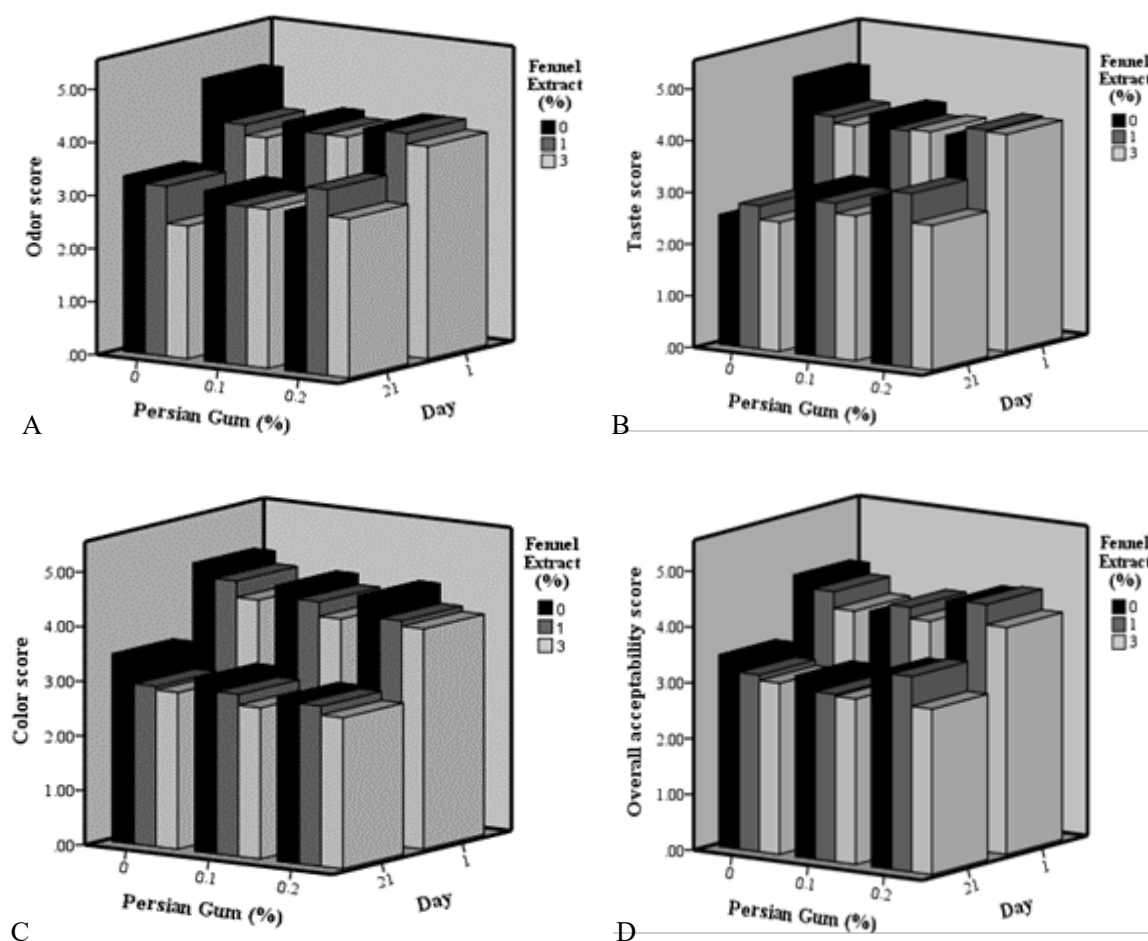


Fig. 3. The effect of fennel extract and Persian gum on the sensory scores of yogurt samples during 21 days of storage; odor (A), taste (B), color (C), and overall acceptability (D)

of Persian gum at the consumed level was insignificant ($p > 0.05$). The sensory analysis of samples revealed that the amount of fennel extract had a significant effect on the odor, color, taste, and general acceptability of the yogurt samples ($p < 0.05$). The presence of fennel extract led to an undesirable taste. Moreover, higher levels of fennel extract could reduce the odor and color scores significantly ($p < 0.05$). Also, the yogurt sample containing 0.1% Persian gum was more acceptable than the other treated samples on the 1st day of storage.

The taste of yogurt is considered one of the most important parameters (Rezaei et al., 2023b). The control sample received the lowest taste score on the 21st day of storage. As Cheng (2010) reported, the production of aldehydes and fatty acids during the storage of yogurt leads to an undesirable flavor. The yogurt sample with 0.2% Persian gum and 1% fennel extract received higher test scores at the end of storage. It seems that the Persian gum used in this study was able to cover the unpleasant flavors produced at the end of storage, although this effect was not significant ($p > 0.05$).

Similarly, Haji Ghafarloo et al. (2019) reported a higher level of ginger extract decreased the taste score of yogurt-based Iranian drinks. The panelists' scores for the odor, color, taste, and general acceptability of yogurt samples decreased significantly with storage time ($p < 0.05$). Salvador and Fiszman (2005) have attributed the reduction in yogurt sensory properties during storage to the acidification and creation of undesirable flavor and the oxidation of color compounds. In general, the yogurt sample containing 1% fennel extract and 0.2% Persian gum was more acceptable than the other samples on the 21st day of storage.

CONCLUSIONS

In this research, a new functional yogurt created by applying fennel extract and Persian gum during yogurt formulation was assessed. The results of this study revealed a significant increment in the TPC and AO activity of yogurt in the presence of fennel extract and Persian gum. Generally, our results recommend the consumption of this enriched yogurt as a functional product for progress in consumer health. The synergism of the enriched yogurt decreased, its stability

increased, and the viscosity of the treated samples improved. However, adding fennel extract had a negative effect on the sensory properties of the yogurt samples, so a study on the production of encapsulated fennel extract in functional yogurt is suggested. The enhanced acidity of yogurt in the presence of Persian gum may suggest that this plant gum has prebiotic properties. The survival of LAB in the presence of Persian gum should be explored in future studies.

DECLARATION OF COMPETING INTEREST

There is no conflict of interest to report.

ACKNOWLEDGMENTS

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