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Influence of green pepper extract on the physicochemical, antioxidant, and sensory properties of stirred yogurt

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ABSTRACT

The green pepper has garnered interest in different societies as a functional food and food additive. Numerous studies have indicated that the phytochemicals found in pepper possess antioxidant, anti-cancer, anti-inflammatory, anti-obesity, and anti-arteriosclerotic properties. In this study, green pepper extract was used in yogurt to increase the acceptability of consumption and improve its health properties. For this purpose, green pepper extract was added in concentrations 100, 200, and 300 ppm in the preparation of yogurt. And pH, acidity, total content of phenolic compounds, DPPH inhibition percentage, viscosity, syneresis, and sensory properties were evaluated in 1, 7, 14, and 21 days after production. The results showed that by increasing the amount of extract, the percentage of antioxidant activity and phenolic compounds increased significantly. The effect of storage time on these indicators was also significant ($p < 0.05$). in such a way that over time, there was a decrease in antioxidant activity. But, there was a slight increase in antioxidant activity on the 14th day of storage. Also, the effect of treatment type on some physicochemical characteristics of yogurt containing green pepper extract including pH, acidity, viscosity, and syneresis was significant ($p < 0.05$). During storage, acidity, syneresis, and viscosity increased and pH decreased significantly in all yogurt samples. Regarding the tested sensory attributes, the 200 ppm sample received the highest score among the evaluators, and in terms of overall acceptance, the samples containing the extract were more favorable than the control sample.

1. Introduction

Recently, a growing body of research has focused on providing scientific evidence for the potential health benefits of functional and nutritious foods (Cencic & Chingwaru, 2010). Functional foods are typically referred to as products that are purported to possess specific advantageous physiological effects on the body. The dairy sector, which has a strong association with probiotics, holds the highest market share in the functional food industry, accounting for approximately one-third of the overall market (Granato, Branco, Cruz, Faria, & Shah, 2010). One of these functional foods is yogurt, which is widely consumed as a functional food due to its good taste and nutritional properties (rich in potassium, calcium, protein, and B vitamins) and is an excellent source of probiotics for consumers (Bulut, Tuncturk, & Alwazeer, 2021, Shojaeimeher, Babashahi, Shokri, Mirlohi, & Zeinali, 2023). Many studies show that yogurt has therapeutic and preventive effects on some diseases such as cancers, infections, digestive disorders, asthma, and infectious diarrhea (Abdi-Moghadam et al., 2023). Functional yogurts can incorporate a range of micronutrients, including iron, zinc, iodine, and vitamin A (Sazawal et al., 2013). Additionally, enrichment can be achieved through the inclusion of various plants and fruits such as acacia fiber, sweet pumpkin, black garlic extract, red ginseng extract, and spices (Kim, Ko, Lee, Choi, & Han, 2008). Phenolic compounds, oligosaccharides, and probiotics are constituents of this particular food variety, yet they remain relatively unfamiliar to consumers. Phenolic phytochemicals, which are secondary metabolites derived from plants, make up a significant portion of the diets of both humans and animals (Bahmani, Shokri, Akhtar, Abbaszadeh, & Manouchehri, 2022, Falahi, Delshadian, Ahmadvand, & Shokri Jokar, 2019, Lin et al., 2016, Sadighara, Godarzi, Bahmani, & Asadi-Samani, 2016, Shavali-Gilani,

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Yazdanfar, Jahed-Khaniki, Molaee-Aghaee, & Sadighara, 2023). Oxidative stress and systemic inflammation are the key pathogenic factors that contribute to the progression of metabolic disorder, insulin resistance, β-cell dysfunction, and ultimately diabetes. Phenolic phytochemicals exhibit noteworthy antioxidant activity and possess particular therapeutic properties such as anti-diabetic and anti-hypertensive activity. (Altememy et al., 2022; Abdi-Moghadam et al., 2023b; Shakeri et al., 2022). One of the good sources of phenolic compounds is pepper (Chan, Gan, Shah, & Corke, 2018, Zhang et al., 2015).

Pepper, also known as chili (Capsicum spp.), is commonly used in various diets as a spice, vegetable, and fruit. It possesses diverse bioactive components including capsaicin, ascorbic acids, polyphenols, mineral matters, flavonoids, sugars, fat, and protein (Olatunji & Afolayan, 2018). Green pepper contains a significant amount of functional compounds. It has been reported that green pepper contains two phenolic compounds, 3,4-dihydroxyphenyl ethanol glucoside and 3,4-dihydroxy-6-(*N*-ethylamino) benzamide, which exhibit antibacterial properties (Chatterjee, Niaz, Gautam, Variyar, & Sharma, 2007). In addition, peppers possess the ability to alter fat and energy metabolism in humans, resulting in an anti-obesity effect. This is achieved through appetite control, reduced fat levels in the bloodstream, and inhibition of the formation of white adipose cells (Oh et al., 2023).

The objective of this study was to enhance the acceptability of yogurt consumption and improve its health benefits by incorporating green pepper extract. For this purpose, the physicochemical, sensory, and stability characteristics of stirred yogurt after adding green pepper extract during 21 days of storage were investigated.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and reagents

The sodium hydroxide (NaOH, >98 %), methanol (MtOH, > 99), ethanol (EtOH, > 99), sodium carbonate (Na_2CO_3, >98 %), diphenyl picryl hydrazine (DPPH), phenolphthalein reagen, Folin Ciocaltio reagent (FCR), and gallic acid > 99 were obtained from Sigma-Aldrich. Yogurt, pepper, and raw milk were purchased from markets (Sabzevar, Iran).

2.1.2. Extraction of green pepper

Initially, the peppers were subjected to the processes of drying and grinding. Subsequently, they were combined with methanol at a ratio of 10:1 and placed on a hot plate operating at a speed of 250 revolutions per minute for 24 h. Following this, the samples underwent smoothing using No. 1 filter paper and were concentrated through the employment of a rotary evaporator at a temperature of 40 °C. Lastly, the extracts were subjected to vacuum drying at a temperature of 45 °C for drying (Listantia & Bayani, 2021).

2.1.3. Preparation of stirred yogurt

High-quality, healthy cow's raw milk containing 2.5 % fat and free of antibiotics was pasteurized and homogenized at 85 °C for 15 min and cooled at 45 °C. Then green pepper extract was added to it with a concentration of 100, 200, and 300 ppm. In the next step, after the pH of the samples reached 4.2, the containers were transferred to a cold room with a temperature of 4 °C and were kept for 21 days (Shah, 2003).

2.2. pH and titratable acidity (TA)

To determine the pH of the yogurt, it was first homogenized in a water solution with a ratio of 1 part yogurt to 9 parts water. The pH measurement of the homogenized yogurt was then taken using a digital pH meter. To determine the titratable acidity,10 g of sample was mixed with 100 cc of distilled water and thoroughly stirred. Afterward, 25 cc of the resulting solution was titrated using a 0.1 normal NaOH solution and 0.5 mm of phenolphthalein reagent until a faint pink color became visible. The acidity of the samples was then quantified using the provided formula (Kang et al., 2018).

$$N \times 0.009 \times 100/M.$$

N : *Amount of NaOH consumed*.

M : *Sample weight*.

2.3. Antioxidant activity

The measurement of antioxidant activity was conducted by utilizing the DPPH reagent, a stable radical compound with a distinct purple hue that undergoes a color change to yellow when it is revived by electron or hydrogen donor elements present in antioxidant compounds.

In this method, varying concentrations of essential oil and yogurt extract were mixed with 80 % ethanol and 3.5 mL of a DPPH solution weighing 0.01 g. This process was repeated three times. After being agitated for 30 s and left to incubate at room temperature for 30 min, the optical absorption of the samples was measured at a wavelength of 517 nm. Also, for the control sample, instead of essential oil and extract, 1 mL of 80 % ethanol was used and the percentage of DPPH free radicals was calculated using the following formula (Apostolidis, Kwon, & Shetty, 2007, Rezagholizade-shirvan, Masrournia, Najafi, & Behmadi, 2023, Shokri et al., 2023).

$$Scavaging\ activity = \left(A_{blank} - A_{sample}/A_{blank}\right) \times 100$$

In this formula, the A_{blank} shows the light absorption of the negative control, which is lacking essential oil and extract, and the A_{sample} shows the light absorption of different concentrations of essential oil and extract.

2.4. Total polyphenol content assay

To measure the phenolic content of green pepper extract, Folin Ciocaltio reagent (FCR) solution was used as a reagent, and gallic acid was used as a standard. In this way, after extraction from yogurt, the desired extract was prepared with different concentrations and in 3 repetitions for the measurement of phenolic compounds. Then 1 mL of yogurt extract was mixed with 2.5 mL of FCR. Then, after 3 min, 2 mL of a sodium carbonate solution was added and the resulting mixture was shaken intermittently for 30 s, after 30 min of darkness at room temperature, its absorbance was read at 725 nm and using the same curve method. The standard of gallic acid in the concentration range of 20, 40, 60, 80, and 100 ppm was drawn according to Folin's method in terms of mg/mL and the total amount of phenolic compounds of green pepper extract was calculated in terms of milligrams of gallic acid per gram of extract (Guénard-Lampron, St-Gelais, Villeneuve, & Turgeon, 2019, Rezagholizade-shirvan, Najafi, Behmadi, & Masrournia, 2022).

2.5. Measurement of viscosity

The viscosity of the samples was measured at a temperature of 10.5 °C by a viscometer with a spindle number 4 and a shear speed of 100 rpm. Before measuring the viscosity, the samples were stirred manually for one minute. To determine the classification of the produced yogurt, we examined samples at various shear speeds ranging from 10 to 250 rpm. At each shear speed, we recorded the viscosity value. To investigate the time dependence of viscosity at a cutting speed of 100 rpm, viscosity measurements were taken during 21 days of storage at intervals of 7 days.

2.6. Syneresis concentrations

In the academic experiment, a quantity of 50 g of yogurt was placed onto a filter paper and funnel. The setup was then refrigerated for 2 h. After this period, the water collected from the filtration process was measured in terms of weight. The percentage of Syneresis was subsequently determined using a specific formula (Hong, Son, Kwon, & Kim, 2020).

$$Syneresis(\%) = (Theamountofseparatedwater/Initialweightofthesample) \times 100$$

2.7. Sensory analysis

To assess the quality of the yogurt product and determine the optimal formulation, various yogurt samples containing different concentrations of extract were evaluated by a panel of judges. After receiving preliminary training, three evaluators within the age range of 25 to 29 were selected for this task. Using a hedonic method on a 5-point scale, they assessed the organoleptic characteristics, taste, and appearance of the yogurt samples that were stored at both 4 and 25 °C. (Rezagholizade-shirvan, Kalantarmahdavi, & Amiryousefi, 2023, Rezagholizade-shirvan, Shokri, Dadpour, & Amiryousefi, 2023) The evaluators ranked each sample from excellent to very unfavorable (1–5) during sensory evaluations conducted on the first, seventh, fourteenth, and twenty-first days. All samples were compared with each other in this process.

2.8. Statistical analysis

For the statistical analysis of the samples, it was done in a completely random factorial design. Considering that the most important test in random factorial design is the one-way analysis of variance (ANOVA) test, therefore this test was used for compared between the groups. And experiments with triplicate assays were performed.

The software used was SAS JMP Statistical Discovery version 17 and the corresponding graphs were drawn with Excel 2010. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Antioxidant activity and total phenolic content (TPC)

Through a thorough analysis of the impact of green pepper extract on the potency of free radical inhibition, it was evident that there was a significant enhancement in free radical inhibitory activity as the concentration of the extract increased. Additionally, there was a significant correlation between time and antioxidant activity at the examined levels ($p < 0.05$). As shown in Fig. 1, over time, there was a decrease in antioxidant activity. But, there was a slight increase in antioxidant activity on the 14th day of storage. This increase may be attributed to the activation of enzymes such as catalase and superoxidase after a certain period of storage, as well as the presence of casein and lactic acid bacteria in the milk, which can affect the amount of antioxidant activity (Stobiecka, Król, & Brodziak, 2022). Also, in our study, we observed a significant correlation between the concentration of the extract and storage time with the quantity of TPC present in the sample ($p < 0.05$). In such a way that, as the concentration of the extract increased, there was an increase in the amount of TPC (Fig. 2). Conversely, by increasing the storage time, the amount of these compounds decreased (Fig. 3(. The highest amount of TPC was 721 and 717 mg /GAE, which was observed in samples with 300 ppm and 200 ppm extract The decline in antioxidant activities observed in yogurt during refrigerated storage can be attributed to the increased degradation of TPC which possesses antioxidant properties (Parikh & Patel, 2018). The researchers, Su-Hyun Kang et al., observed a significant increase in the antioxidant activity of yogurt when the amount of red or green pepper juice added to the yogurt was increased. Additionally, they found a direct correlation between the level of antioxidant activity observed in yogurt and the TPC present in

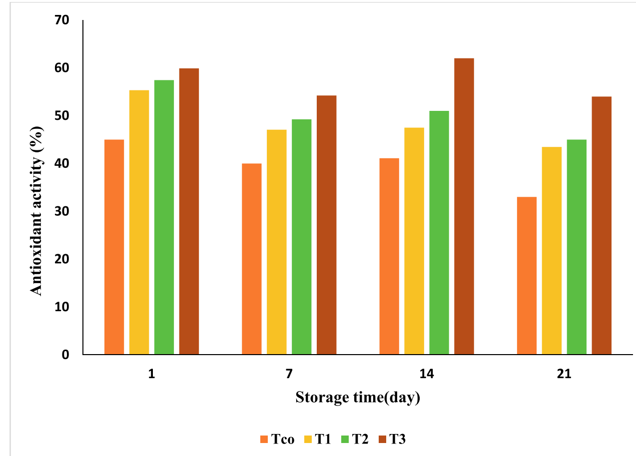

Fig. 1. The mutual effect of time and amount of green pepper extract on the percentage of antioxidant activity. T_1 = 100 ppm, T_2 = 200 ppm, T_3 = 300 ppm, Tco = control sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

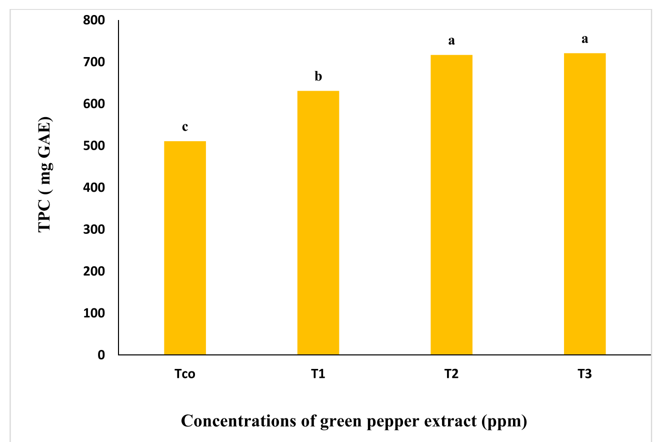

Fig. 2. Total polyphenol content in yogurt containing different concentrations of green pepper extract. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

peppers (Kang et al., 2018). In another study, the objective was to evaluate and compare the antioxidant capabilities of various pepper varieties (red, green, orange, yellow) with synthetic antioxidants. The findings indicated that these peppers displayed comparable levels of antioxidant activity in inhibiting DPPH radicals when used at higher concentrations (Hong et al., 2020). In higher concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups in the reaction medium, the probability of donating hydrogen to free radicals increases, and the inhibitory power of the extract increases (Cho, Kim, Lee, Yeon, & Lee, 2020, Ghandahari Yazdi, Barzegar, Sahari, & Ahmadi Gavlighi, 2019). In their study, Kim, An, Park, Lim, and Kim (2016) found that 100 g of dried green paprika contained 10 mg of carotenoids, 100 mg of tocopherol, and 700 mg of polyphenols. Additionally, it was noted that green paprika exhibited elevated levels of chlorophyll (Kim et al., 2016). All these compounds can be effective in inhibiting free radicals so green pepper can be utilized to fortify yogurt as a bioactive product. Research shows that polyphenols have the potential to provide neuroprotective effects against damage caused by neurotoxins and neuroinflammation (Vauzour, 2012).

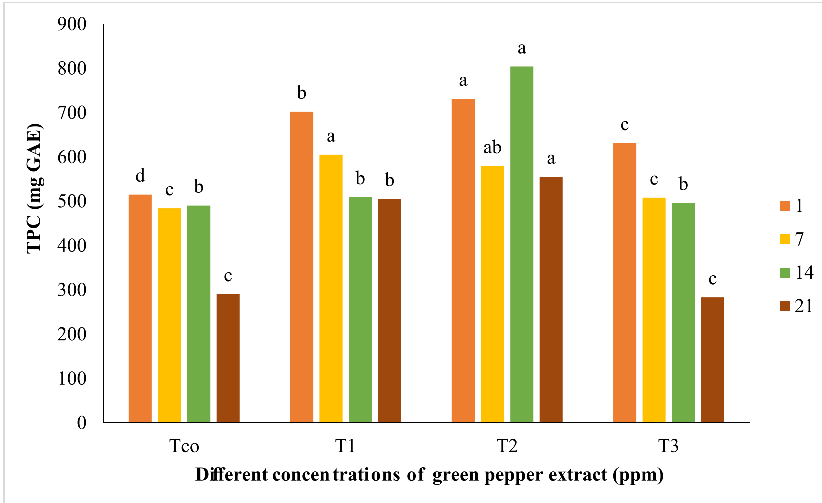

Fig. 3. The interaction effect of storage time (In days 1. 7.14.21 (and concentration of green pepper extract on the amount of TPC. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. pH and titratable acidity (TA)

The analysis of variance revealed a significant relationship between the concentration of the extract and the resulting pH of the final product that with increasing the concentration of the extract, the pH level decreased significantly ($p < 0.05$) (Fig. 4). Also, the pH value was found to be significantly influenced by the passage of time. In each of the three treatments with varying concentrations (100, 200, and 300 ppm) of green pepper extract, the pH value decreased as time progressed. But, in the control sample, there was a notable increase in the pH value on the 21st day. Specifically, the pH value increased from 4.3 to 4.9 during this period (Fig. 5(. During the examination of the concentrations, it was observed that the pH value of the 100 ppm sample remained constant at 4.75 during the storage period. However, for the 200 and 300 ppm samples, there was a significant decrease in pH value from the first day to subsequent days. It can be contended that during the initial stages of storage, the augmentation of green pepper extract and subsequent increase in the available substrate for microbial growth leads to an elevation in bacterial metabolic activity and a consequent decrease in pH levels observed in samples with higher concentrations of the extract (Adel, Ahmed, Babiker, & Yagoub, 2011, Alwazeer, Bulut, & Tunçtürk, 2020). On the other hand, green pepper extract has an acidic pH, which

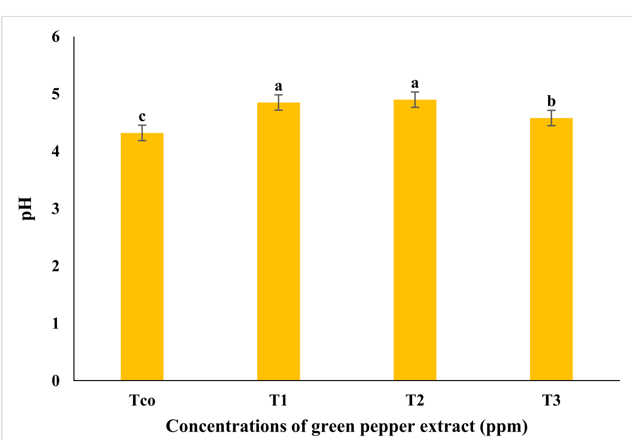

Fig. 4. Changes in pH in yogurt containing different concentrations of green pepper extract. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

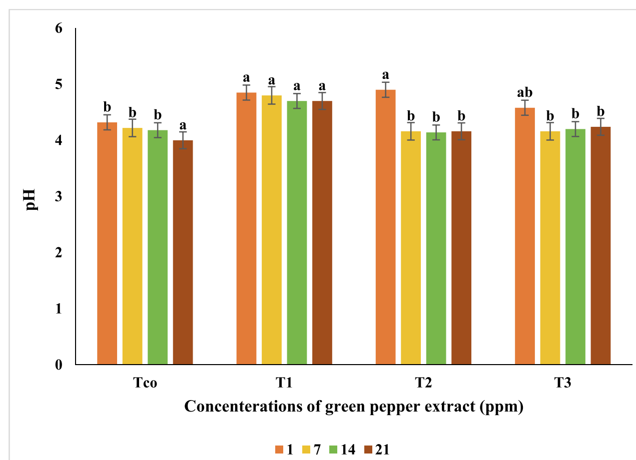

Fig. 5. Changes in pH in yogurt containing different concentrations of green pepper extract during storage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

can affect the pH of samples containing the extract (Yu, Kim, Lee, Son, & Kim, 2014). Examining the trend of acidity changes shows that in yogurt treatments containing green pepper extract, the rate of acidity increase was almost constant during the first days of storage, and this trend increased at a slower rate in the control sample. After the fourteenth day, the increase in acidity in yogurt containing high concentrations of green pepper continued at a faster rate than the control sample, which continued until the twenty-first day (Fig. 6). In the study of Su-Hyun Kang et al., the addition of fermented red pepper juice to yogurt resulted in an increase in TA value from 0.93 % to 0.96 %. Similarly, the addition of fermented green pepper juice to yogurt resulted in an increase in TA value from 0.90 % to 0.93 %. In contrast, the control yogurt without any pepper juice showed a smaller increase in TA value, going from 0.87 % to 0.91 % (Kang et al., 2018). These results are consistent with the results of the study of Amirdivani, S. and A. S. Hj Baba (2011) (Amirdivani & Baba, 2011).

3.3. Syneresis

Figure S1 shows the syneresis changes of yogurt inoculated with different percentages of green pepper extract during the storage period.

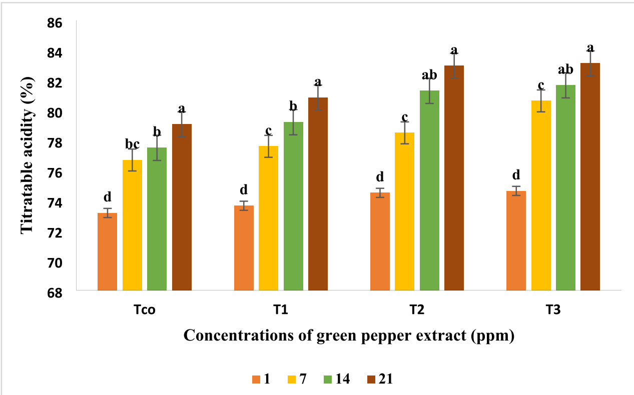

Fig. 6. Changes of TA in yogurt containing different concentrations of green pepper extract during storage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Adding green pepper extract to yogurt increased the amount of syneresis in the samples. Besides, there was a significant difference between the syneresis of samples during 21 days' storage at intervals of 7 days ($p < 0.05$).

With increasing the concentration of green pepper extract in yogurt, the amount of syneresis increased. In fact, with the increase in the activity of lactic acid bacteria followed by a decrease in pH, the strength of the yogurt gel network decreases and as a result, syneresis increases in yogurt (Wajs, Brodziak, & Król, 2023). The decrease in pH changes the natural form of the protein and due to the denaturation of the protein, the water bound to it is released, and Syneresis increases (Lee & Lucey, 2010). In the study of Ziarno and Zarba (2019), decreasing pH increased Syneresis in yogurt samples. Also, the study of Su-Hyun Kang et al showed similar results (Ziarno & Zaręba, 2020). But Mohammadi-Gouraji et al. investigated yogurt enriched with phycocyanin and its antibacterial and physicochemical properties during 21 days of storage. Their results showed that the control sample showed more synergy than phycocyanin-enriched yogurt. While synergy increased during yogurt storage, the rate of increase in phycocyanin-enriched yogurt was lower. Typically, changes in pH during storage can disrupt the texture of yogurt and lead to the separation of protein-bound water. This process naturally results in protein denaturation and an increase in syneresis. The variations in syneresis observed in different studies during the storage period can be attributed to differences in methodology, the exopolysaccharide production abilities of different strains of yogurt starter cultures, and variations in milk composition (Mohammadi-Gouraji, Soleimanian-Zad, & Ghiaci, 2019).

3.4. Viscosity

The data presented in Figure S2 demonstrated statistically significant differences in viscosity among the various treatments ($p < 0.05$). Additionally, a significant difference in viscosity was observed during the 21-day storage period at intervals of 7 days ($p < 0.05$). The findings indicate that increasing the amount of extract in the samples led to an increase in viscosity. This could be attributed to the interaction between phenolic compounds and proteins from both the green pepper extract and yogurt, resulting in the formation of a firmer three-dimensional network and viscous gels (Salehi, Ghorbani, Mahoonk, & Khomeiri, 2021). Mehdizade Moghadam et al. discovered, based on their findings, that the addition of pennyroyal extract to yogurt leads to an increase in the viscosity of the yogurt (Moghadam, Ariaii, & Ahmadi, 2021). The presence of numerous phenolic compounds in pennyroyal extract leads to an interaction with milk proteins, specifically casein, within the yogurt matrix. This interaction ultimately causes the viscosity of the yogurt to be higher compared to the control sample (Limwachiranon, Huang, Shi, Li, & Luo, 2018), in the study of Hong, H., et al. The viscosity of the yogurt containing 2.5 % paprika juice was measured to be 954 cp, while the yogurt with 5 % paprika juice had a viscosity of 2,296 cp. These values were found to be significantly greater than the viscosity of the yogurt without paprika juice (Hong et al., 2020), which was consistent with our study. But the study by Kang et al. (2018) found that the viscosity of yogurt decreased when fermented red or green pepper juice was added. This may be because that the addition of fermented pepper juice can stimulate the growth of LAB as prebiotics and reduce the gel strength by promoting the breakdown of milk solid components or pH-induced alterations in casein micelles, leading to higher acid production. (Kang et al., 2018).

3.5. Sensory evaluation during storage of yogurt

The general results obtained from the sensory tests (including taste, aroma, color, and general acceptance) on the 21st day of the studied treatments are given in Table S1 and Figure S3. The treatments containing green pepper extract had a significant difference from the control sample in terms of taste scores, except for the sample containing 100 ppm extract ($p < 0.05$). The taste score was highest in the sample with a concentration of 300 ppm, specifically measuring 9.29. Following this, the treatment containing 200 ppm had a score of 8.63, which did not show any significant difference. Also, the lowest score was observed in the control sample with a value of 5.11. The findings from the assessment of aroma and smell exhibited a comparable pattern across the final treatments. The sample with 300 ppm attained the highest score (6.85), followed by the sample with 200 ppm (6.83), with no statistically significant difference. Conversely, the control treatment received the lowest score of 6.01. The addition of fermented red pepper juice to yogurt resulted in consistently lower general sensory taste scores, regardless of the length of storage ($p < 0.05$). Similarly, the inclusion of fermented green pepper juice in yogurt also led to slightly decreased scores compared to regular yogurt. On the contrary, in the study of Kang, S. H., et al. (2018) the addition of fermented red pepper juice to yogurt resulted in consistently lower general sensory taste scores, regardless of the length of storage ($p < 0.05$). Similarly, the inclusion of fermented green pepper juice in yogurt also led to slightly decreased scores compared to regular yogurt (Kang et al., 2018).

4. Conclusion

By adding the addition of green pepper extract to the stirred yogurt, the antioxidant activity and the amount of phenolic compounds increased with time. This increase may be attributed to the activation of enzymes such as catalase and superoxidase after a certain period of storage, as well as the presence of casein and lactic acid bacteria in the milk.

Over time, pH decreased and acidity increased. Also, adding green pepper extract to the stirred yogurt did not have a negative effect on the properties of viscosity but the amount of syneresis increased. Regarding the tested sensory attributes, the 200 ppm sample received the highest score among the evaluators, and in terms of overall acceptance, the samples containing the extract were more favorable than the control sample.

CRediT authorship contribution statement

Elham Kovsari: Investigation, Methodology, Writing – original draft, Writing – review & editing. **Parisa Shavali Gilani:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Samira Shokri:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Abdolreza Mircholi Borazgh:** Investigation, Writing – original draft, Writing – review & editing. **Alieh Rezagholizade-Shirvan:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ahmad**

Pedram Nia: Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101070.

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