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Development and characterization of mint powder (*Mentha spicata* L.)-fortified functional yoghurt: Physicochemical, sensory properties, and shelf-life assessment

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ABSTRACT

Food fortification has been increasingly used to improve the sensory, functional, and nutritional properties of food products, addressing nutritional deficiencies and helping to prevent diseases in women, children, and the elderly. This study aimed to evaluate the enrichment of yoghurtwith mint extract powder (*Mentha spicata* L.) and assess its effects on yoghurtproperties. Mint powder was added to yoghurtat 0%, 0.1%, 0.2%, and 0.3%. The highest pH was observed on the first day, with the lowest pH recorded after 14 days of storage. The lowest pH was found in the 0.3% mint concentration (F3), followed by 0.2% (F2), and the highest pH was in the control sample. Yoghurts with mint powder showed a smaller decrease in pH compared to the control at the end of storage. Water holding capacity (WHC) increased slightly with higher 8concentrations of mint extract, but the differences were not statistically significant ($p > 0.05$). Syneresis values significantly differed between treatments, with the control sample exhibiting lower syneresis than the fortified yoghurts. Mint powder significantly enhanced the antioxidant capacity of yoghurtby 47.2% compared to the control after 20 days of refrigeration, reducing lipid peroxidation. At 0.2% concentration, a substantial improvement was noted ($p < 0.01$). No significant difference was observed at low mint concentrations (F1), but significant differences were found at higher concentrations (F2 and F3), with the lowest bacterial count recorded in F3 (3.2 log CFU/g).

Keywords**:** Food fortification, Yoghurt, Mint powder, Antioxidant capacity, Shelf life.

INTRODUCTION

Yoghurt is an essential fermented dairy product in the human diet, widely accepted across cultures and offering valuable nutritional benefits. [1, 2]. The fermentation process with *Streptococcus thermophilus* and *Lactobacillus. bulgaricus* produces milk-based food that gains a tangy taste and creamy texture from this bacterial combination [3]. Yoghurt has been consumed for centuries, with its origins traced to Middle Eastern nomadic cultures, leading to modern yoghurt manufacturing techniques. [4]. Yoghurt is widely consumed and valued for its nutritional versatility, as it contains essential vitamins and minerals such as proteins, phosphorus, magnesium, and calcium. [5]. Yoghurt consumption offers several health benefits, including promoting heart health, improving insulin sensitivity, and supporting digestive health and bone density. [6]. The probiotics in yoghurt improve gut microflora, helping to alleviate symptoms of diarrhoea, bloating, and constipation. [6, 7]. The growing demand for yoghurt has increased production, with a broader variety of flavors, health additives, and functional ingredients [8]. Various herbal and functional additives have gained attention due to their health benefits [9]. Mint is well-known in traditional medicine for its anti-inflammatory, digestive, and antimicrobial properties, which enhance yoghurt's flavor. Mint is well-known in traditional medicine for its anti-inflammatory, digestive, and antimicrobial properties, which enhance yoghurt's flavor. Adding mint to yoghurt prolongs its shelf life and improves its physicochemical and sensory qualities [10]. Mint (*Mentha spicata* L.), a member of the Lamiaceae family, includes 42 species, 15 hybrids, and numerous subspecies and cultivars [11]. This research aimed to investigate the effect of peppermint powder supplementation on the physicochemical properties, sensory attributes, and shelf life of functional yoghurt during refrigerated storage.

2. Materials and methods

2.2.1. Production of Yoghurt

Dried mint bought from the local market in Erbil city was blended using a master chef blender, then mixed with fresh milk samples (1, 2, and 3 g/L) at three different concentrations. The milk samples were strained through a muslin cloth and pasteurized (85 C°. for 15 minutes). After cooling to 45°C, the pasteurized mint milk is poured into a vented plastic container. Commercial freeze-dried mixed culture (5g) 'Yogourmet' containing L. bulgaricus, S. thermophilus and L. acidophilus was dissolved in 10 ml sterile warm water to activate the organisms. This active culture was used to inoculate 1 litre (1,000 ml) pasteurised milk at the same temperature of 47 °C. It was then incubated and allowed to stand for 4 hours for fermentation, and the product was placed in a refrigerator at 4 C°. until further testing [12,13].

2.2.2. Determination of pH and Acidity

The pH was determined using a pH meter (model 211, Germany), according to the standard official methods (AOAC) [14]. The acidity of yoghurtsamples was determined as a percentage of lactic acid as follows [14]:

Lactic acid%= V *0.1*0.09/w*100

Where V is the amount of yoghurt consumed in milliliters and w is the weight of the yoghurt.

2.2.3. Determination of moisture content

According to [15], the moisture content in yoghurt samples was measured by drying 5g of yoghurt samples in the oven at 103 ± 5 C°. for 4 hours. The following formula was used to get the percentage moisture content:

$$Moisture\ (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

W1 is the sample's initial weight, and W2 is the sample's weight after drying.

2.2.4. Syneresis determination

Syneresis was measured using the method outlined by [16]. A cup of yoghurt was taken from the refrigerator at 5C°. A syringe with a needle was used to extract the liquid whey from the surface of the sample, and the weight of the cup containing fermented yoghurt was recorded again. The process lasted for less than 10 seconds to avoid further leakage of whey from the yoghurt.

2.2.5. Measurement of water holding capacity

A sample of approximately 20 grams of yoghurt (Y) has been centrifuged at 4 C°. (4000 rpm) for 10 minutes. Then, the separated water (W) was weighed, and the water holding capacity was calculated in grams of separated water per hundred grams of yoghurt, as described by [17]. The following equation was used to calculate WHC:

WHC = (Y −W)/Y × 100

2.2.6. Microbiological Examination

Total bacterial count of yoghurt samples during 0, 7, 14 and 21 days at 4±1 C° was determined according to [18]. Plates were incubated at 3 C°. for 24 hours. The number was calculated and expressed as CFU/g yoghurt.

2.2.7. Sensory Evaluation

The sensory evaluation of yoghurt samples was carried out utilizing 9 grade scale by a set of fourth-stage students (1-dislike highly; 9-liked highly) in the Food Technology Department of the College of Agricultural Engineering Sciences following the method described by [19]. The samples were evaluated for their overall acceptance, appearance, texture, flavour, colour, and smell.

2.2.8. Viscosity

Measurements of viscosity were done with a Brookfield DV-E Viscometer. Spindle No. 3 at 20 rpm was used for all Yoghurt samples, as formerly described by [14]. Viscosity was observed during cold storage.

2.2.9. Antioxidant Activity Assay

The free radical scavenging capability of fortified yoghurt samples was evaluated using the DPPH method described by [20]. Subsequently, 1 ml of each sample was mixed with 3 ml of the methanol DPPH solution (0.1 mM). After being kept in a dark room for 30 minutes, the absorbance of the solutions was measured at a wavelength of 517 nm. Finally, we calculated the antioxidant activity of the samples using the following equation:

$$\text{Free radical scavenging activity} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \qquad \text{(Eq. 3)}$$

Where A_{sample} and A_{blank} represent the absorbance of DPPH solutions with and without additional samples, respectively.

2.2.10. Statistical analysis

In this study, trials were performed in a completely randomized design (CRD) with three treatments (F1 was fortified with 1 g of mint, sample F2 was fortified with 2 g of mint, and sample F3 was fortified with 3 g of mint) and one control sample. A one-way analysis of variance was used to determine the significance of the treatment effects (α=0.05). Furthermore, the mean of the treatments was compared using the Tukey test in Minitab software (version 17), and the graphs were created with Microsoft Office 2013 (Excel).

Results and Discussion

3.1. Physicochemical analysis of mint-fortified yoghurt

3.1.1. Acidity and pH of fortified yoghurt

The pH peaked on the first day of storage and dropped to its lowest on the fourteenth day. The minimum pH was observed for F3, then F2, and the highest level was observed in the control sample Table 1. The findings showed that, in comparison to the control sample, the pH of yoghurts containing mint powder decreased less at the end of the storage period, as shown in Fig. 1. The overall amount of lactic acid produced contributes to the development of the acidity of that product to a large extent. The pH of yoghurt frequently decreases with storage. The reason for this is that the bacteria in the yoghurt, such as Lactobacillus and Streptococcus species, are still digesting the lactose and milk sugar, converting it to lactic acid. As the lactic acid content increases, the yoghurt's pH gradually drops and turns more acidic. The findings of the present study are consistent with another study that showed that the pH change of yoghurt added with spices such as cinnamon, nutmeg, and cardamom demonstrated a similar reduction in pH over storage days. [21] Also, it was found that the pH of foods that contained probiotics dropped during storage because the foods had bacteria in them, either in capsules or in free form. This drop in pH could be because the bacteria are breaking down food faster, using the carbohydrates in the food as fuel.

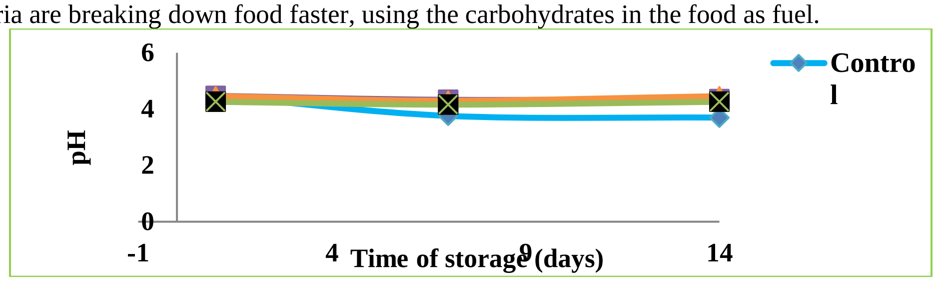

Figure 1. pH changes of fortified and control yoghurt samples during 21-day storage.

As a result, organic acids build up as waste products. [22] also obtained similar results, where the pH for the control was 4.59 and the samples enriched with mint extract were 4.48, 4.46, and 4.47, respectively. However, statistical analysis showed that there was no significant difference (P<0.05) between the pH values of the samples that were left alone and those that were mixed with mint extract. With the passage of time during the storage period, a gradual decrease in the pH values of every sample was observed, and the pH values of the control reached 3.94 after 21 days, while those of the treatments enriched with mint extract reached 3.97, 3.96, and 3.98, respectively. The reason for this decrease is probably due to the glycolysis of lactose and the continued production of starter bacteria. Lactic acid was measured after the fermentation period and during cold storage until the temperature of the yoghurt treatments reached (1±4)°C. [23] also reported the same trend, where they showed that the pH values of yoghurt supplemented with mint, basil, and thyme oil after one day of processing were not significantly different from the control treatment and demonstrated that the pH continued to decrease gradually after storage for 28 days for all treatments as a result of the continued activity of starter bacteria by converting the remaining lactose sugar into lactic acid.

Table 1. Chemical analysis of functional yoghurt samples enriched with mint powder (*Mentha spicata L.*)

Sample	Moisture (%)	pH	Acidity (% lactic acid)
Control	$76.9{\pm}1.2^{A}$	$4.48{\pm}0.020^{A}$	$0.87{\pm}0.01^{A}$
F1	$76.36{\pm}5.3^{A}$	$4.47{\pm}0.02^{A}$	$0.83{\pm}0.08^{AB}$
F2	$77.84{\pm}3.3^{A}$	$4.46{\pm}0.15^{A}$	$0.80{\pm}0.05^{B}$
F3	$78.50{\pm}2.2^{A}$	$4.26{\pm}0.10^{B}$	$0.60{\pm}0.06^{C}$

Means with different letters in each column show substantial differences according to Duncan's test (α=0.05)

3.2. WHC and syneresis analyses

Milk proteins have special properties such as absorbability, greater compatibility with nutrients or affinity for dietary standardization. The water-binding power of a protein is due to a combination of factors resulting from protein-water interactions in food quality. Many reasons have been introduced regarding the water-holding properties of proteins, but this term is still not well understood and a precise definition has not been provided for it. Water-binding capacity is another term used for water-holding capacity. Various methods explain useful information about the hydration and binding of water to proteins. Important functional properties of proteins in the diet are related to the association of water with proteins in the food, among which proteins in milk have significant prospects in compatibility and enhancing synergistic effects on water-holding capacity. Until now, there have been challenges for food scientists to be able to adequately modify and characterize the performance of these materials [25].

Measurement of water holding capacity in the produced yoghurts showed that increasing the concentration of mint extract powder in the enriched yoghurt formulation improved the WHC level slightly. However, these changes were not statistically

significant ($p>0.05$). Different studies have reported different results in this regard. [26] evaluated the impact of Ferulago angulate's ethanolic extract and essential oil on the physicochemical, sensory, and microbial properties of probiotic yoghurt over storage, where the WHC of protein rose compared to the control treatment ($p<0.05$). The ethanolic extract of Ferulago angulate improved the WHCf protein in probiotic yoghurts compared to the essential oil of Ferulago angulate ($p<0.05$), such that the highest WHC protein level was recorded in probiotic yoghurts containing 0.4% extract (4.66 ± 0.80).
[24] A study examined the impact of various plants on yoghurt after 28-day storage and discovered that the WHC of fortified yoghurt increased to 41.25%, indicating that the increase in solids due to the addition of plant components could act as hydrocolloids responsible for increasing WHC. The WHC of yoghurt enriched with *Coriandrum Sativum* decreased, which was explained by the assumption that the bond between water molecules and whey protein was weakened by adding *Coriandrum Sativum to* yoghurt [25].

Table 2. Physical characteristics of functional yoghurt samples enriched with mint powder (*Mentha spicata L.*)

Sample	WHC	syneresis	Hardness
Control	76.9 ± 1.2^{A}	4.48 ± 0.020^{A}	0.87 ± 0.01^{A}
F1	76.36 ± 5.3^{A}	4.47 ± 0.02^{A}	0.83 ± 0.08^{AB}
F2	77.84 ± 3.3^{A}	4.46 ± 0.15^{A}	0.80 ± 0.05^{B}
F3	78.50 ± 2.2^{A}	4.26 ± 0.10^{B}	0.60 ± 0.06^{C}

According to Duncan's test ($\alpha=0.05$), means with different letters in each column indicate significant differences.

Whey accumulation on the gel surface leads to poor consumer acceptance of yoghurt. The whey was easier to remove to the surface due to the lower pH and subsequent contraction of the casein network. This process happened in all treatments, including the control. While the control sample's synergism values were lower than those of the fortified yoghurts, there were significant differences in syneresis values between treatments (Table 2). [27] found that including Annona fruit pulp resulted in better synergism than the control, which is consistent with the findings of the current investigation. This observation could be because of the thermodynamic incompatibility of plant polysaccharides with milk proteins. Another study by [32] confirmed this. They found that adding blue pigment extracts to yoghurt after fermentation increased its synergy more than the control yoghurt. [28], also concur with these results, suggesting that stirred yoghurt may retain excess moisture before yoghurt modification.

3.3. Antioxidant and Microbial Activity Quality

The average results for total bacterial count (TPC) for mint-enriched yoghurt and the control sample are given in Table 3. According to Table 3, at low mint concentrations (F1), there is no significant difference from the control sample. However, at higher concentrations, it is seen that there is a substantial difference between samples F2 and F3 with the control sample, and the lowest bacterial count was recorded for sample F3 (3.2 log CFU/g) as illustrated in Figure 1. These findings inferred that the addition of mint to yoghurt prevents the growth of bacteria due to the presence of natural antimicrobial substances, according to [29]. The antimicrobial properties of extracts derived from aromatic medicinal plants, such as mint and thyme, have been acknowledged as a successful treatment approach against illnesses caused by bacteria, viruses, and filamentous fungi

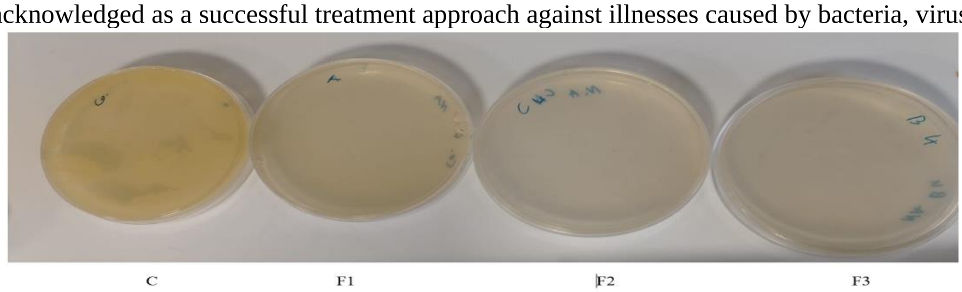

Figure (1): Total bacterial count on solid agar medium, with different concentrations of mint powder, C: control, F1: 0.1%, F2: 0.2%, F3: 0.3%.

Firstly, it is important to note that yoghurt itself has significant antioxidant properties, containing bioactive peptides derived from lactic acid bacteria metabolism, which help enhance antioxidant potential and inhibit lipid peroxidation. The extract powder considerably increased the antioxidant capacity of yoghurt by 47.2% after 20 days of refrigeration compared to natural yoghurt, reduced lipid peroxidation. It resulted in a significant improvement at a level of 0.2% ($p<0.01$).

Table 3. Antioxidant and microbial characteristics of functional yoghurt samples enriched with mint powder (*Mentha spicata L.*) after 21 days of storage

Sample	Antioxidant capacity (%)	Total Plate Count (log CFU/g)
Control	21.9±2.1[A]	4. 8±0.2[A]
F1	25.6±2.3A	4.4±0. 2A
F2	37.8±3B	4.1±0.15B
F3	47.2±3.2C	3.2±0.1C

Duncan's test (α=0.05) identifies significant differences between means in each column with distinct letters.

In another study, lipid peroxidation of experimental yoghurts decreased proportionally (from an average of 1.14 to 0.94, 0.86 and 0.66 mg MDA/kg) with increasing doses (0, 2, 4 and 6). The percentage of hydroethanolic extract of *Mentha piperita* L. appears to provide products with improved fat stability compared to the control [33]. TBARS values increased significantly during storage in all yoghurt products, whether fortified or not. These findings indicate that including mint powder can enhance the fat fraction and extend the product's shelf life during long-term cold storage. Other authors have also stated that adding phenolic extracts from date palm [30], argel [31] and algae [32] substantially lowers lipid peroxidation and significantly extends the shelf life of yoghurts. By the twentieth day of storage, yoghurt enriched with hydroethanolic extract of *Mentha piperita* L -enriched yoghurts maintained better antioxidant activities than the control. The decrease in DPPH and ABTS radical scavenging activities during storage has also been confirmed in many other studies [34]. This decrease is probably partly due to the development of complexes between the polyphenols found in it, as the extract combines with the yoghurt proteins, thereby reducing the recovery of their phenolic compounds and antioxidants. [33].

3.4. Sensory evaluation

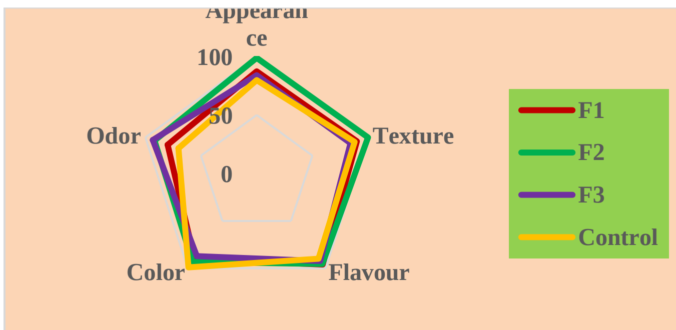

Figure 2. A spider diagram to show the sensory characteristics recorded for the fortified and control samples on the first day of production

Color is the first sensory characteristic of yoghurt that is evaluated by the consumer and thus affects other parameters of yoghurt such as aroma and flavor. The color change of yoghurt is influenced by additives and yoghurt enriched with mint has a greenish color and in short, the color of enriched yoghurt samples depends on the type of additives. The comprehensive characteristics of flavored yoghurts are stronger and healthier than regular yoghurt.

Fifteen sensory evaluators were used to rate the yoghurts on their color, texture, taste, smell, appearance, and general acceptability over 21 days of storage. We examined and recorded the average consumer score for each product, accounting for all characteristics. Therefore, the characteristics of each of the products produced underwent a different trend during the storage period. The added herbal extract powder probably had a positive effect on the scent of the treated samples. At the start of the study, the sample with the average amount of mint extract powder (0.2%) had the strongest smell compared to the others and got the highest score. The control sample, on the other hand, got the lowest score. The control color index also recorded the lowest score on day 1 (Figure 3). Sample F3 with the highest amount of mint powder (0.3%), which ranked first in the favorite color on the first day, also had a high score on the last day of the storage period, but sample F2 with 0.2% mint powder scored higher. A study [35] reported that stirred yoghurt with 10% turmeric rated fewer points lower than the same amount of curcumin, and both were acceptable to consumers. On the first day of storage, blue chickpea had the highest overall acceptability score, followed by control, spinach, soursop, and turmeric; however, toward the end of storage, yoghurt with turmeric had a higher score.

On all evaluation days 1, 7, 14, and 21, the results showed substantial variation between the control sample and the samples enriched with mint powder for all sensory characteristics, including taste, odor, appearance, texture, and overall acceptability, and this difference was more evident for all evaluated traits on the final day. It should be noted that there was no substantial difference between the enriched samples (F1, F2, and F3) during all storage periods and for all sensory properties examined. [36], reported in a similar study that at concentrations above 0.6% mint extract in yoghurt, the shelf life of yoghurt was reduced. This was attributed to the low production of lactate, which contributes to the yoghurt's sour taste, as well as the antibacterial properties of mint extract. However, at 0.2% and 0.4% concentrations, the production of sourness seemed to be sufficient. 0.2% yoghurt performed even better than the control during the post-acidification period, owing to a somewhat sour taste caused by St and Lb's decreased lactate production. In addition to lactate, volatile substances like menthol and menthone, which constitute the components of the experimental extract, when added at a low concentration dose of 0.2%, formed an excellent combination with the typical flavors of yoghurt (acetaldehyde, diacetyl, acetone and acetylone).

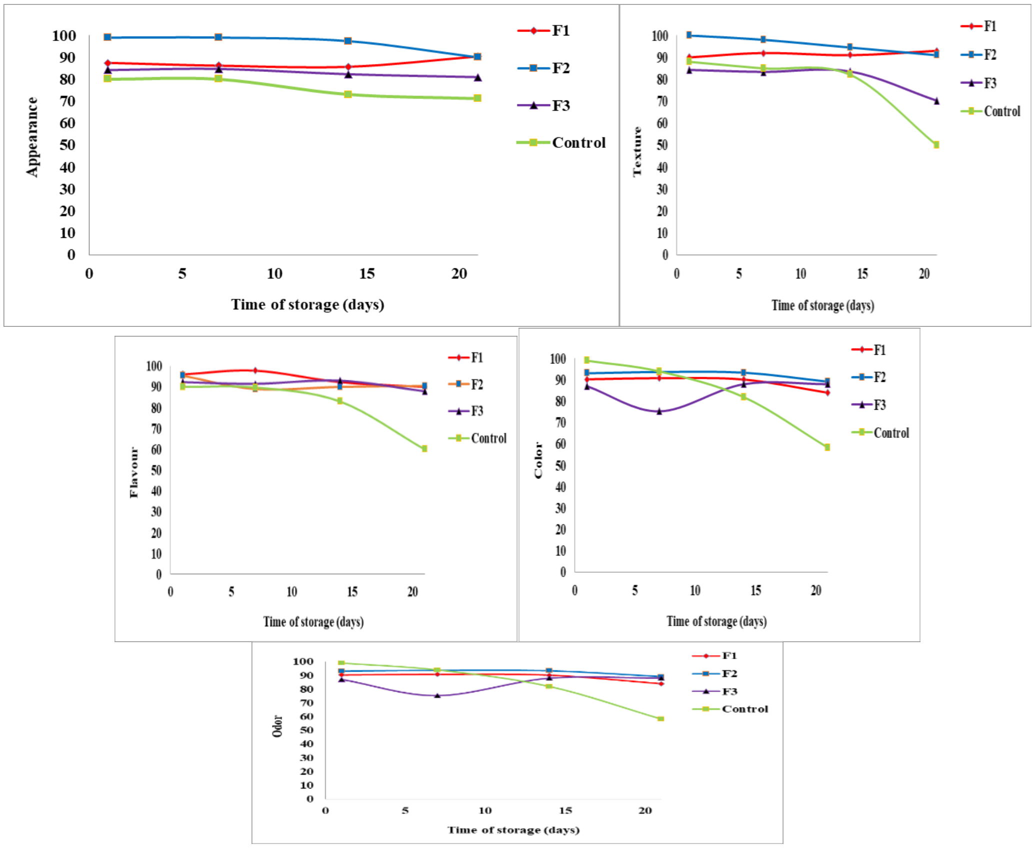

Figure 3. Sensory characteristics recorded for fortified and control samples over a 21-day period

Conclusions

Malnutrition and related diseases can severely affect human health, not just among children but also among the youth and the elderly. With growing awareness of the importance of a nutritious diet, there is an increasing trend to fortify milk and yoghurt products with various additives. This study investigates the impact of Mentha spicata powder on yoghurt and confirms that fermented foods like yoghurt can be enriched with plant extracts for beneficial changes. Yoghurt fortified with Mentha spicata has a longer shelf life, enhanced nutritional value, and greater consumer acceptance. The addition of Mentha spicata, rich in phenolic compounds with antioxidant properties, even at low doses, significantly improves the oxidative and microbial shelf life of yoghurt while maintaining or enhancing its organoleptic qualities throughout storage. Compared to the control, yoghurt prepared with 0.2% mint powder demonstrates better antioxidant properties, improved sensory quality, and increased nutritional content. This makes it a promising new supplement to meet the nutritional needs of consumers, especially children and the elderly. However, further research is needed to optimize yoghurt fortification methods for maximizing its benefits

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تطوير وتوصيف الزبادي المدعم بمسحوق النعناع ((Mentha spicata L.: الخصائص الفيزيائية والكيميائية والحسية وتأثيره على فترة الصلاحية.

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الخلاصة

في الآونة الأخيرة، أصبحت عمليات تعزيز الغذاء تُستخدم لتحسين الخصائص الحسية والوظيفية للمنتجات الغذائية، مما يساعد في تعويض نقص العناصر الغذائية والوقاية من العديد من الأمراض لدى الأطفال والنساء وكبار السن. كان الهدف الرئيسي من هذه الدراسة تقييم تأثير إضافة مسحوق مستخلص النعناع *(Mentha spicata L.)* إلى اللبن الزبادي. تم إضافة مسحوق النعناع بتركيزات مختلفة (0، 0.1، 0.2، و0.3%) إلى اللبن الزبادي وتم مراقبة التغيرات في الخصائص المختلفة خلال فترة التخزين. لوحظ أن أعلى قيمة لدرجة الحموضة *(pH)* كانت في اليوم الأول وأدنى قيمة كانت في اليوم العاشر من فترة التخزين. كانت أقل قيمة لدرجة الحموضة في العينة *F3* (التي تحتوي على 0.3% من مسحوق النعناع) تليها العينة*F2* ، بينما كانت أعلى قيمة في العينة الضابطة. في نهاية فترة التخزين، أظهرت النتائج أن الزبادي المدعمة بمسحوق النعناع سجلت انخفاضًا أقل في *pH* مقارنة بالزبادي الضابط. أما بالنسبة لقياسات سعة الاحتفاظ بالماء*(WHC)* ، فقد أظهرت النتائج زيادة طفيفة في سعة الاحتفاظ بالماء مع زيادة تركيز مسحوق النعناع، ولكن هذه الزيادة لم تكن ذات دلالة إحصائية *.($p>0.05$)* فيما يخص التصفية *(syneresis)*، كانت قيم التصفية في العينة الضابطة أقل مقارنة بالزبادي المدعمة. علاوة على ذلك، أدى إضافة مسحوق النعناع إلى زيادة كبيرة في القدرة المضادة للأكسدة بنسبة 47.2% بعد 20 يومًا من التبريد مقارنة بالزبادي الطبيعي، كما ساعد في تقليل أكسدة الدهون بشكل ملحوظ. في التركيزات العالية *(F2 وF3)*، لوحظ فرق كبير مقارنة بالعينة الضابطة، حيث سجلت العينة *F3* أعلى مستوى في عدد المستعمرات الميكروبية *(3.2 لوغ CFU/جم)*.

الكلمات المفتاحية: تعزيز الغذاء، الزبادي، مسحوق النعناع، مدة الصلاحية.