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Impact of Mint and Clove Extract (Nanoformulations) on Functional Yoghurt Properties

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ABSTRACT

Study investigates the effect of nanoformulations of clove and mint extract on functional yoghurt's microbiological, phenolic, and sensory properties. Clove and mint extract nanoformulations have particle sizes of 5.97±0.48 nm and 196.1±11.14 nm, respectively. Clove extract nanoformulations (CENFs) showed the highest relative antioxidant activity, ferric-reducing antioxidant power, total phenolic, and total flavonoid components as compared to mint extract nanoformulations (MENFs). The yoghurt sample with 0.025% clove extract nanoformulations had the highest GAE content of 114.18 µg/mg, while the sample with 0.0125% mint extract nanoformulations had the highest content of 94.62 µg/mg after 15 days in a refrigerator. Microbial analysis showed significant effects on lactic acid bacteria, yeast, and mould counts in yoghurt prepared with clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) and storage periods at refrigeration temperatures degree up to 15 days. Total bacterial counts in yoghurt did not differ significantly from the control sample. The coliform group not detected in any samples during the storage period. The sensory evaluation showed significant effects on the appearance and flavor of yoghurt with different concentrations of clove extract nanoformulations (CENFs) (0.0125, 0.025, and 0.05%) compared to the control sample. However, body and texture evaluations showed no significant difference. Significant differences were observed between yoghurt prepared with mint extract nanoformulations (MENFs) and the control sample in appearance, body, texture, and flavor. The sensory score was significantly reduced with the addition, and with increasing MENF concentration, a lower sensory score was observed.

Keywords: Clove, mint, nanoformulations, total phenolic, functional yoghurt

INTRODUCTION

One of the most important methods for improving the nutritional value and quality of food is food fortification (Ondetti and Cushman, 1982). Lactobacillus delbrueckii subsp. Bulgaricus and Streptococcus thermophilus collaborate in the production of yoghurt, a fermented dairy product (Beal et al., 1999; Küçükçetin et al., 2011 and Zhang et al., 2019). It is a popular dairy product with high nutritional value that has been used as a successful matrice for the production of a variety of functional foods that promote health (Zhang et al., 2019). "Nanotechnology is the understanding and control of matter at dimensions of approximately 1 to 100 nanometers, where unique phenomena enable novel applications" according the National Nanotechnology Initiative (2006) and GuhanNath et al. (2014). Nanotechnology is the process of reshaping agents or materials to nanoscale dimensions or shapes (Nile et al. 2020). Nanotechnology has an impact on a variety of industries, including pharmaceuticals, agriculture, textiles, electronics, and food (Cerqueira and Pastrana, 2019 and Pathakoti et al., 2017).The use of nanotechnology in the food industry has resulted in improved sensory characteristics, bioactive component absorption, increased availability of nutraceutical agents, and more consistent food packaging materials. (Sahani and Sharma, 2021). The use of nanostructured materials in food processing encourages the development of novel nano-foods that are more acceptable than genetically engineered foods. Anticaking, antibacterial, antioxidant, and shelf-life properties of nanostructured food additives may be improved. (Ezhilarasi et al., 2013).

There is a growing interest in acquiring bioactive substances from natural sources such as medicinal plants, herbs, and spices due to numerous health dangers and limits on utilizing synthetic additions. Medicinal plants have gotten a lot of press as a major source of physiologically active chemicals bioactive chemicals derived from medicinal plants have been proven in numerous studies to have a wide range of biological effects, including antioxidant and antibacterial characteristics.(Wojdyło et al., 2007; Khoobchandani et al., 2022). As a result, these bioactive molecules have a good chance of being employed as natural antioxidants and antimicrobials.

Bioactive chemicals found in mint leaves have applications in the food industry. As a result, mint is a viable option for a variety of food preparations. Due to its health claims and therapeutic values, in addition, one of the most valuable spices, clove (Syzygium aromaticum), has long been used as a food preservative and for a variety of therapeutic uses. For polyphenolic compounds like flavonoids, hydroxycinnamic acids, and hydroxybenzoic acids, it is an important source. There is evidence that clove and essential oils exhibit antibacterial, antimycotic, yeast-inhibiting, and Brownian enzyme-inhibiting activities (Shahabi Ghahfarrokhi et al., 2013; Daraei Garmakhany et al., 2017 and Aghajani and Garmakhany, 2021).

So, in the present study, clove and mint extract nanoformulations were incorporated into milk with the aim of

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producing functional yoghurt as well as examining its effects on the total phenolic, microbiological, and sensory properties of the resulted yogurt. In addition, to evaluate the antioxidant activity, ferric-reducing antioxidant power assay, total phenolic content, and total flavonoid content of mint and clove extract nanoformulations.

MATERIALS AND METHODS

Materials

The dairy processing plant at the Faculty of Agriculture, Mansoura University, Egypt provided the bovine milk. The starter culture containing Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus from the DVS ThermophilicYoFlex® (Chr. Hansen, Horsholm, Denmark) was used for producing yoghurt. All microbiological media including tryptone soy agar (TSA), MacConkey agar, and potato dextrose agar were purchased from Thermo Fisher Scientific (Cairo, Egypt).

Methods

preparing nanoformulations of mint and clove extract

Clove and mint extracts were utilised to prepare NFs using the hydrothermal squeezing method as described by Shin et al. (2007). After being soaked in 60 mL of analytical grade ethanol in a 100 mL Teflon beaker for about 10 hours at 70 psi without stirring, dry clove or mint leaves (10 g) were autoclaved. The extract was obtained from the leaves under the effect of the pressure and temperature of the autoclave. After being separated, the extracts were collected, centrifuged, and stored in a cool, dark space until needed.

Extract ultramorphology

The NFs extracts were examined morphologically using transmission electron microscopy (TEM) (JEOL-JEM-2100, JEOL Ltd, Tokyo, Japan). This test was carried out in the EM-Unit at Mansoura University at 160 kV. In conclusion, a sample of nanoformulations (NFs) extracts (1 mL) were sonicated for 2 minutes in an ultrasonic bath. A drop of the diluted nanoformulations (NFs) was placed to a carbon-coated copper grid, and the excess materials were removed before a thin film was stretched over the holes and dried (at room temperature). The image was obtained using Gatan software (Version 2.11. 1404.0), While using the scale bar provided by the image capture device, the Nano Measurer 1.2.5 software recorded the average diameters of 250 particles each of the mint extract nanoformulations (MENFs) and clove extract nanoformulations (CENFs) in each extract.

Making of fortified yoghurt with clove and mint extract nanoformulations:-

The fat percentage of raw bovine milk was adjusted to 3 %, followed by heating at 94°C for 15 min, followed by cooling to 40°C. Nanoformulation extractions were added in 3 levels (0.0125, 0.025, 0.05 %) at 60°C. The 3% of yoghurt starter mother culture was added to all the treatments. The mix was incubated at 42°C until the pH value reached 4.6–4.5, at which point the mixture completely coagulated. Yogurt samples were refrigerated immediately after the acid coagulation of milk and cooled overnight for analysis on zero time, 7, and 15 days, respectively.

Chemical properties of fortified yoghurt with clove and mint extract nanoformulations

According to AOAC (2003), the fortified yoghurt with clove and mint extract nanoformulations' protein, fat, and total solids (T.S) contents were determined. The moisture contents of the fortified yoghurt clove and mint extract nanoformulations were determined using the hot air oven drying method. The Kjeldahl method was used to determine the protein percentage of samples. Using the Soxhlet extraction method with petroleum ether for 6 hours, the percentage of fat in different samples was determined (AOAC, 2003).

Antioxidant activity of the tested nanoformulation extracts (CENFs) and (MENFs).

According to (Brand-Williams et al., 1995), the 1.1-diphenyl-2-picrylhydrazil (DPPH) reagent was used to evaluate the free radical scavenging activity. A volume 2.9 ml of 60 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol solution were vortexed with 100 µl of the sample solution. The reaction mixture was stirred and the absorbance at 517 nm was measured after 30 minutes in complete darkness. As a blank, methanol was used. The Trolox calibration curve was shown to be a function of the percentage of DPPH radical scavenging activity. Micromoles of Trolox equivalents (TE) per 1 g (mol TE 100 ml-1) were used to express the final results.

Ferric reducing antioxidant power measurement (FRAP)

After being combined with 2.5 ml of 10% trichloroacetic acid (TCA), the mixture was incubated at 50°C for 20 minutes with 0.5 ml of the solution, 2.5 ml of 0.2 M buffer phosphate (pH 6.6), and 2.5 ml of 1% potassium ferric cyanide. An amount 2.5 ml of 0.1% ferric chloride (FeCl3), 2.5 ml of distilled water, and ml of the solution's top layer were added to this mixture, which was then centrifuged for 10 minutes at a constant speed of 3000 rpm. After being left at room temperature for 10 minutes, the absorbance was measured at 700 nm (Tometri et al., 2020).

Determination of total flavonoids content (TF) and total phenolic (TP) content

According to Herald et al., 2012, the total flavonoid contents of clove extract nanoformulation (CENFs) and mint extract nanoformulation (MENFs), as well as the total phenolic (TP) content of clove extract nanoformulation (CENFs), mint extract nanoformulation (MENFs), and a water extract of fortified yoghurt with clove extract nanoformulation (CENFs) and mint extract. At 510 and 630 nm, respectively, the total flavonoid and phenolic contents were measured.

Microbiological analyses

By combining 10 ml of each yoghurt with 90 ml of saline solution, a serial dilution was created. Then, each batch's microbiological enumeration (count) was assessed using the following the following standards:

The tryptone soy agar (TSA) medium was used to evaluate the total bacterial counts, which were then incubated for 48 hours at 30°C under aerobic conditions (IDF, 1997). MRS agar was used to count lactic acid bacteria (LAB) for 72 hours at 30°C. Using MacConkey agar media, the total count of coliforms was determined. At 37°C, the plates were incubated for 18 hours (Marshall, 1992). With the use of potato dextrose agar media, the number of yeasts and moulds was counted. The plates were incubated upside down for 5 days at 22°C. (Baggerman, 1981). The log cfu/g values from each microbiological analysis were tallied twice and expressed.

Sensory evaluation

Samples of yoghurt with clove and mint extract nanoformulations sensory properties were evaluated during storage at 4°C for 15 days by staff members of Mansoura University, Faculty of agriculture. According to (EN ISO 13299 ,2016) samples were given sensory evaluations for outer appearance (10 points), body and texture (40 points), as well as Flavor (50 points).

Statistical analysis

The Shapiro-Wilk test and Lieven's test have both been used to examine the homogeneity of variance and distribution of all numerical data. SAS (2008) was used to statistically analyse the data as a two-way analysis of variance to investigate the effect of treatment and storage period. Tukey's test at $P < 0.05$ was used to distinguish the significant differences.

RESULTS AND DISCUSSION

The average composition of yoghurt treatments was 85.76 %, 2.62 % and 3.20 % for moisture, protein, and fat, respectively.

Particle size analysis

Using a transmission electron microscope (TEM), the average particle size of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) was measured. Fig. 1 shows an illustration of particle size data. The particle size of clove and mint extract nanoformulations were recorded 5.97±0.48 nm and 196.1±11.14 nm, respectively. Jeevanandam et al. (2018) demonstrated that Nanomaterials (NMs) are generally characterized as having a diameter between 1 and 100 nm. In principle, they are defined as materials with lengths of 1-1000 nm in at least one dimension. (Salama et al., 2022), who reported that clove EO nanoemulsions were formulated into a water-based nano-emulsion using a low-energy auto-emulsification method and that the particle size was 18.1 nm ± 0.1. At that point, we'd like to talk about the worry that consuming dairy products that include nanoparticles could be toxic (Naseer et al., 2018). As some nanoparticles are typically found in what we consider to be "safe food," such milk, this worry may not be correct (Rogers, 2016). Examples include the folding of globular proteins like b lactoglobulin in milk and the ability of casein micelles to self-assemble. β-lactoglobulin nanofibers (4.0 nm) are regularly generated during yoghurt processing under the effect of heat and pH change, resulting in the production of the traditional elastic gel network of yoghurt (Guy et al., 2011). These examples demonstrate how we constantly come into contact with various types of nanoparticles, which are present naturally in milk and other dairy products. Due to their extraction from natural plants and species, the clove and mint extract nanoformulations in our study have a natural identity. Additionally, the clove and mint extract nanoformulations are allowed in food and utilised in very modest levels (0.125, 0.25, and 0.5 mg /kg yoghurt), as well as within the daily intake level of 25 mg/kg b.wt./day (Joint FAO/WHO, 1974). This gives the artificially produced functional yoghurt enhanced with nanoformulations of clove and mint extracts an important margin of safety. This scientific investigation can be followed by a supplemental toxicological study to confirm this statement.

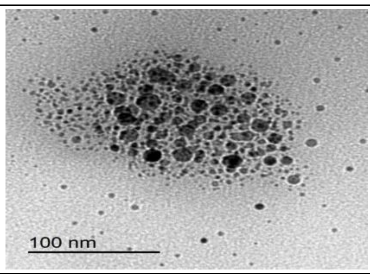
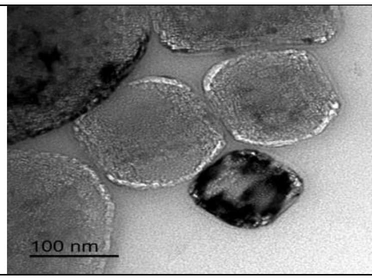
	CENFs	MENFs
		
Particles Size (Mean±SE)	5.97±0.48 nm	196.1±11.14 nm

Fig. 1. By transmission electron microscopy, the ultramorphology of the NFs extracted. CENFs: clove extract nanoformulation and MENFs: mint extract nanoformulation.

Antioxidant activity, Ferric reducing antioxidant power assay, total phenolic and total flavonoids content of herbs and species extracts nanoformulations

Table (1) shows the antioxidant activity, Ferric reducing antioxidant power assay, total phenolic and total flavonoids content of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs). Clove extract nanoformulations (CENFs) showed the highest relative antioxidant activity, ferric reducing antioxidant power, total phenolic, and total flavonoid components (2439.55±35.51, 1613.97±86.81, 329.35±24.84 and 71.30±6.33, respectively) as compared to mint extract nanoformulations (MENFs), which contained (1053.59±28.67, 1312.19±79.97, 164.11±13.84 and 64.59±4.95, respectively). (Ramos et al., 2017), who reported that the clove aqueous extract showed higher antioxidant activity values.Shan et al. (2005) showed that for polyphenolic compounds like flavonoids, hydroxycinnamic acids, and hydroxybenzoic acids, clove is an essential source. The main bioactive component of clove is eugenol. Gallic acid is found in higher concentrations than the other phenolic acids (783.50 mg/100 g fresh weight)**.** Moghadam et al. (2021) found that the amount of DPPH free radical activity increased as the concentration of free and microencapsulated extract pennyroyal (*Mentha pulegium.* L) increased. Plant extracts' phenolic components give them antioxidant activity. The principal reason of phenolic compounds' antioxidant activity is their ability for redox oxidation. In addition, Moghadam et al. (2021) reported that as the values of ferric reducing power (FRAP) increased together with the concentration of FRAP values. The extract had the highest antioxidant activity at 1000 ppm, and at this concentration, it had a ferric reduction power (FRAP) that was higher to the synthetic antioxidant BHA (280.84 mol fer/g) ($P < 0.05$). The ferric reduction power (FRAP)was also increased by using nano-extracts.

Table 1. Antioxidant activity, Ferric reducing antioxidant power assay (FRAP), total phenolic and total flavonoids content of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) (mean ± SD).

	DPPH (μM Trolox eq/mg extract)	FRAP (μM trolox eq/mg extract)	Total Phenolics (μg GAE/mg extract)	Total Flavonoids (μg RE/mg extract)
Clove extract nanoformulations	2439.55±35.51	1613.97±86.81	329.35±24.84	71.30±6.33
Mint extract nanoformulations	1053.59±28.67	1312.19±79.97	164.11±13.84	64.59±4.95

GAE= gallic acid equivalent. RE= Rutin equivalent.

Total phenolic of yoghurt fortified with clove extract nanoformulations (CENFs) and mint extract nanoformulation (MENFs)

As previously mentioned in the method section, three different concentrations of both clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) were used to fortify functional yoghurt. Two concentrations of clove extract nanoformulations and mint extract nanoformulations (0.025 and 0.0125 percent, respectively) were chosen based on their high total scores from the sensory evaluation as compared to the other yoghurt samples prepared with clove extract nanoformulations and mint extract nanoformulations and compared with control without addition clove extract nanoformulations (CENFs) and mint extract nanoformulation (MENFs). The results of the total phenolic, which will be discussed below, are therefore based on these two concentrations. The total phenolic content of yoghurt fortified with clove extract nanoformulations (CENFs) and mint extract nanoformulation (MENFs), are shown in Table (2).

Table 2. Total phenolic content of yoghurt (Y) fortified with clove extract nanoformulations (CENFs) and mint extract nanoformulation (MENFs) (mean ± SD).

Treatment	Total Phenolics (μg GAE/mg) Storage period (day)		
	0	7	15
Control (Y)	79.66±2.11	94.57±6.97	95.22±7.70
Y+ 0.025 % CENFs	102.16±6.51	114.41±3.92	114.18±6.99
Y+ 0.0125% MENFs	76.33±4.71	88.43±2.87	94.62±3.27

The lowest phenolic content was found in the control sample (Y) as compared to the yoghurt sample prepared with 0.025 % of clove extract nanoformulations and 0.0125% of mint extract nanoformulations. Furthermore, the yoghurt sample prepared with 0.025 % clove extract nanoformulations had a higher total phenolic content than the other treatment. Moreover, the total phenolic content of yoghurt samples prepared with 0.025% and 0.0125% clove extract nanoformulations and mint extract nanoformulations, respectively, increased during refrigerator storage periods of up to 15 days. The yoghurt sample prepared with 0.025 % of clove extract nanoformulations recorded the highest content of 114.18 μg GAE/mg, whereas the yoghurt sample prepared with 0.0125% of mint extract nanoformulations recorded the highest content of 94.62 μg GAE/mg after 15 days of storage in a refrigerator compared to zero time. Ramos et al. (2017) demonstrated that the greatest mean values for the total phenolic content (TPC) during the refrigerated storage were found in fermented milk containing the optimised herbal extract ($p < 0.05$), and the level remained stable over time. This result is similar to those obtained by (Farhan et al., 2020) comparing control TY samples made with and without Aqueous extract of mint leaves prepared, the control TY sample with no Aqueous extract of mint leaves prepared was found to have the lowest phenolic content. As Aqueous extract of mint leaves prepared concentrations increased, so increased the phenolic content. Additionally, after 14 days of storage, TY samples fortified with various Aqueous extract of mint leaves prepared concentrations had higher phenolic component contents than the control TY and other treatment.

Microbiological evaluation of yoghurt fortified with clove extract nanoformulations (CENFs) and mint extract nanoformulation (MENFs)

The evaluation of the microbiology of yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) during storage at 4±1°C for 15 days is shown in Table (3 and 4). The growth of total bacterial counts in yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) did not significantly differ from the control sample (Table 3). In contrast, the results of a statistical analysis of the influence of different concentrations (0.0125, 0.025, and 0.05%) of mint extract nanoformulations (MENFs) on the dependent variable according to log CFU/g in yoghurt indicated that the growth of the total bacterial counts was significantly affected by the overall concentrations of mint extract nanoformulations (MENFs) ($P < 0.05$). With concentrations of 0.0125, 0.025, and 0.05% of mint extract nanoformulations (MENFs), the ANOVA test revealed that the total bacterial counts in yoghurt were significantly decreased ($P < 0.05$) when compared to the control sample (Table 4). In addition, the total bacterial counts were reduced to 7.15 log cfu/ml on the 15th day of storage (Table 4). (Salama et al., 2022), who indicated that the total bacterial count (which includes all aerobic bacterial load in stirred yoghurt other than the starting cultures) was between 7.16 and 7.50 log cfu/ml in all samples using essential oil nanoemulsion for flavouring, including clove. (Thabet et al., 2014), who reported that in comparison to the control samples, the total viable count (TVC) decreased in the presence of mint oils. The results could be attributed to the essential oils' antimicrobial properties during storage period. In addition to (Salama et al., 2022), who found that after 15 days of storage, the overall bacterial counts decreased to 6.22–6.85 log cfu/ml. The metabolic activity of the lactic acid bacteria is what causes the decrease (El-Shafei et al., 2018 and Assem et al., 2019).

Lactic acid bacteria (LAB) count results of yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) during storage at 4±1°C for 15 days are presented in Table (3 and 4). The Lactic acid bacteria (LAB) count was significantly affected by the overall concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) and storage periods at refrigeration temperature of up to 15 days ($P < 0.05$). Joung et al. (2016) who indicated that Supplementing with plant extract improved the LAB of the starter's viability.

The results of the current study showed that concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) and storage periods at refrigeration temperature of up to 15 days had significant effect on the quantity of mold and yeast in yoghurt samples ($p<0.05$) (Table 3 and 4). Additionally, the mold and yeast count of yogurt containing different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) decreased significantly ($P<0.05$) with increasing love extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs). The highest yeast and mould count was found in the control sample (Y) as compared to the other samples. In contrast, no yeast and mould were found in yoghurt with two concentrations of clove extract nanoformulations and mint extract nanoformulations (0.025 and 0.05 percent, respectively) during the storage period. Yangilar and Yildiz (2018) reported that thyme essential oil has antifungal and antibacterial properties. The best alternative to using chemical preservatives to preserve yoghurt is to use clove extract nanoformulations (CENFs) and mint extract nanoformulation (MENFs), according to the findings of this study.

This study's microbiological examination of shelf life includes an evaluation of coliform growth. This microorganism didn't appear in all samples during the 15 days of storage because of the hygiene process followed during processing and packaging (Assem et al., 2019).

Table 3. Microbiological Evaluation of yoghurt prepared with different concentrations of clove extract nanoformulations (CENFs) during storage at 4±1°C for 15 days.

Treatment		**Total bacterial count**	**Lactic acid bacteria**	**Mold & Yeast**
Effect of treatment				
Control (Y)		6.84±0.04	8.33±0.00[a]	3.61±0.08[a]
Y+ 0.0125% CENFs		6.90±0.02	8.33±0.01[a]	2.92±0.09[b]
Y+ 0.025% CENFs		6.82±0.02	8.30±0.01[c]	0.00±0.00[c]
Y+ 0.05% CENFs		6.87±0.04	8.32±0.01[b]	0.00±0.00[c]
Storage period (day)		Total bacterial count	Lactic acid bacteria	Mold & Yeast
		Effect of Storage Time		
0		6.87±0.03	8.30±0.01[c]	1.58±0.49
7		6.86±0.02	8.32±0.01[b]	1.66±0.51
15		6.84±0.03	8.34±0.00[a]	1.65±0.51
Treatment	Storage period (day)	Total bacterial count	Lactic acid bacteria	Mold & Yeast
		Effect of interaction		
Control (Y)	0	6.79±0.05	8.32±0.01	3.66±0.06
	7	6.81±0.09	8.33±0.00	3.46±0.23
	15	6.91±0.05	8.34±0.00	3.71±0.05
Y+ 0.0125% CENFs	0	6.88±0.08	8.32±0.00	2.67±0.03
	7	6.90±0.02	8.33±0.00	3.19±0.12
	15	6.92±0.02	8.35±0.00	2.90±0.10
Y+ 0.025% CENFs	0	6.85±0.04	8.27±0.01	0.00±0.00
	7	6.86±0.02	8.29±0.01	0.00±0.00
	15	6.75±0.03	8.33±0.00	0.00±0.00
Y+ 0.05% CENFs	0	6.95±0.04	8.29±0.00	0.00±0.00
	7	6.88±0.05	8.32±0.00	0.00±0.00
	15	6.77±0.07	8.33±0.00	0.00±0.00

Means in the same column followed by different lowercase letters were significantly different (P < 0.05).

Table 4. Microbiological Evaluation of yoghurt prepared with different concentrations of mint extract nanoformulation (MENFs) during storage at 4±1°C for 15 days.

Treatment		**Total bacterial count**	**Lactic acid bacteria**	**Mold & Yeast**
		Effect of treatment		
Control (Y)		7.32±0.01[a]	8.18±0.01[c]	3.71±0.06[a]
Y+ 0.0125% MENFs		7.30±0.01[a]	8.34±0.01[a]	3.14±0.09[b]
Y+ 0.025% MENFs		7.17±0.04[b]	8.27±0.01[b]	0.00±0.00[c]
Y+ 0.05% MENFs		7.07±0.05[c]	8.27±0.01[b]	0.00±0.00[c]
Storage period (day)		Total bacterial count	Lactic acid bacteria	Mold &Yeast
		Effect of Storage Time		
0		7.27±0.02[a]	8.22±0.02[c]	1.67±0.51
7		7.22±0.04[ab]	8.27±0.02[b]	1.67±0.51
15		7.15±0.05[b]	8.30±0.02[a]	1.79±0.55
Treatment	Storage period (day)	Total bacterial count	Lactic acid bacteria	Mold & Yeast
		Effect of interaction		
Control (Y)	0	7.32±0.01	8.13±0.00	3.58±0.04
	7	7.34±0.02	8.19±0.00	3.64±0.07
	15	7.31±0.03	8.23±0.00	3.90±0.03
Y+ 0.0125% MENFs	0	7.31±0.03	8.29±0.00	3.12±0.06
	7	7.29±0.03	8.35±0.00	3.03±0.20
	15	7.30±0.03	8.37±0.00	3.26±0.18
Y+ 0.025% MENFs	0	7.24±0.05	8.24±0.00	0.00±0.00
	7	7.19±0.06	8.26±0.01	0.00±0.00
	15	7.07±0.03	8.31±0.00	0.00±0.00
Y+ 0.05% MENFs	0	7.22±0.07	8.23±0.01	0.00±0.00
	7	7.06±0.04	8.29±0.00	0.00±0.00
	15	6.93±0.03	8.28±0.01	0.00±0.00

Means in the same column followed by different lowercase letters were significantly different (*P* < 0.05).

Sensory evaluation

The most critical factor affecting customer acceptance and demand for this commodity is the sensory properties of yoghurt. The sensory evaluation of yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) and mint extract nanoformulations (MENFs) and stored at 4 °C for 14 days is shown in Figures 2 and 3.

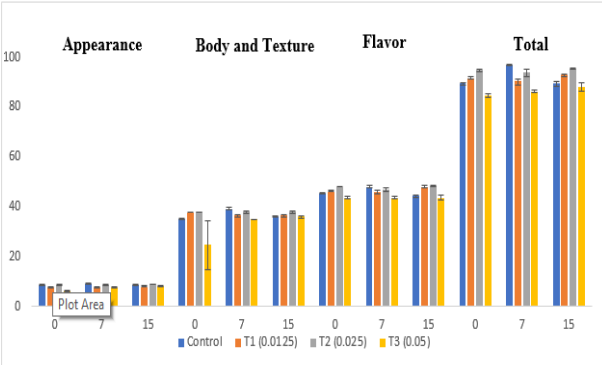


Fig. 2.Sensory evaluation of yoghurt prepared with different concentrations of clove extract nanoformulations (CENFs). Note at the bottom: (Bars show the average ± SD.)

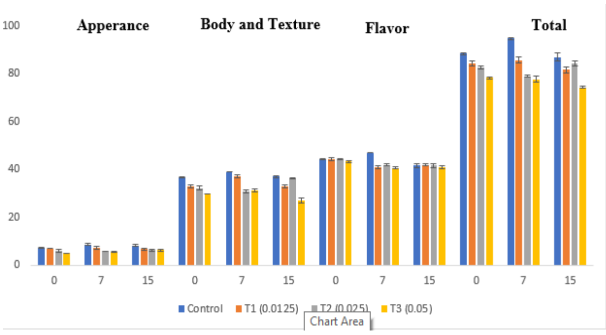


Fig. 3. Sensory evaluation of yoghurt prepared with different concentrations of mint extract nanoformulation (MENFs). Note at the bottom: (Bars show the average ± SD.)

The characteristics that were evaluated were those of appearance, body, and texture, and flavor. Figure 2 showed that when compared to the control sample, clove extract nanoformulations (CENFs) at different concentrations (0.0125, 0.025, and 0.05%) significantly affected how yoghurt appearance and flavor. On the contrary, the yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs) did not significantly differ from the control sample on the evaluation of body and texture. However, the storage periods at a refrigerator temperature of up to 15 days showed a significant effect ($P < 0.05$) on the appearance of the yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs). On the other hand, the storage periods at a refrigerator temperature of up to 15 days showed no significantly negative effect ($P < 0.05$) on the body, texture, and flavor of the yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of clove extract nanoformulations (CENFs). Importantly, the yoghurt sample prepared with 0.025 % of clove extract nanoformulations recorded the highest total scores from the sensory evaluation compared to yoghurt samples prepared with 0.0125 and 0.05% of clove extract nanoformulations. On the other hand, Figure 3 showed significant differences ($P < 0.05$) between the yoghurt prepared with various concentrations (0.0125, 0.025, and 0.05%) of mint extract nanoformulations (MENFs) and the control sample in terms of appearance, body, and texture, and flavor. With the addition, and with increasing the concentration of the mint extract nanoformulations (MENFs), a lower sensory score was observed in terms of appearance, body, texture, and flavour. However, the storage periods at a refrigerator temperature of up to 15 days showed a significant effect ($P < 0.05$) on the body, texture, and flavor of the yoghurt prepared with different concentrations (0.0125, 0.025, and 0.05%) of mint extract nanoformulations (MENFs). On the other hand, the appearance of the yoghurt containing various concentrations (0.0125, 0.025, and 0.05%) of mint extract nanoformulations (MENFs) was unaffected ($P > 0.05$) by storage periods at a refrigerator temperature for up to 15 days. Additionally, the total scores from the sensory evaluation increased gradually over the course of the seven days of storage for all treatments and then decreased at the end of the storage period. Importantly, the yoghurt sample prepared with 0.0125% of mint extract nanoformulations recorded the highest total scores from the sensory evaluation compared to yoghurt samples prepared with 0.025 and 0.05% of mint extract nanoformulations. These results agree with those reported by (Thabet et al., 2014), who found that in comparison to the untreated control, there were considerable and significant differences ($P< 0.05$) in the samples treated with mint. Additionally, Moghadam *et al.* (2021) found that the sensory score in treatments with pennyroyal extract (*Mentha pulegium*. L) was generally lower than the sensory score in treatments with pennyroyal nano extract (*Mentha pulegium*. L). Moreover, with the addition of extract from pennyroyal (*Mentha pulegium*. L), the sensory score was greatly decreased, and a lower sensory score was seen when the extract's concentration was increased. On the other hand, these results are in line with those reported by Salama *et al.* (2022), who found that the stirred yoghurt flavouring mint and clove EO nanoemulsions decreased some of their initial appearance, flavour, body, texture, and overall characteristics after 15 days of storage compared to previously.

CONCLUSION

This study's objective was to investigate how clove and mint extract nanoformulations affected the microbiological, and sensory properties of yoghurt. clove and mint extract nanoformulations showed the relative antioxidant activity, ferric-reducing antioxidant power, total phenolic, and total flavonoid components. Also, the total phenolic content of yoghurt was improved by the addition of clove and mint extract nanoformulations. In the current research, two concentrations of clove extract nanoformulations and mint extract nanoformulations (0.025 and 0.0125 %, respectively) were chosen based on their high total scores from the sensory evaluation as compared to the other yoghurt samples prepared with clove extract nanoformulations and mint extract nanoformulations and showed also that the yoghurt sample prepared with 0.025 % of clove extract nanoformulations recorded the highest content of 114.18 µg GAE/mg, whereas the yoghurt sample prepared with 0.0125% of mint extract nanoformulations recorded the highest content of 94.62 µg GAE/mg after 15 days of storage in a refrigerator compared to zero time. In conclusion, the production of yoghurt as a functional food that contains clove and mint extract nanoformulations offered dairy consumers a new choice that not only has the desired flavor but also has good medicinal and nutrient value when consumed and according to the results of this study, utilising clove extract nanoformulations (CENFs) and mint extract nanoformulation (MENFs) as preservatives instead of chemicals preservatives is the best option for preserving yoghurt.

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تأثير مستخلص النعناع والقرنفل (تركيبات نانوية) على خصائص الزبادي الوظيفي

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الملخص

في هذه الدراسة، تم دراسة تأثير مستخلص القرنفل والنعناع (الصيغ النانوية) (CENFs & MENFs) على الخصائص الميكروبيولوجية والفينولية الكلية والحسية للزبادي الوظيفي. كان حجم جزيئات مستخلصات القرنفل والنعناع النانوية 5.97 ± 0.48 نانومتر و 196.1 ± 11.14 نانومتر، على التوالي. أظهرت التركيبات النانوية لمستخلص القرنفل (CENFs) أعلى نشاط نسبي لمضادات الأكسدة، وقوة مضادات الأكسدة على خفض الحديديك، ومكونات الفينول الكلية، ومكونات الفلافونويد الكلية عند مقارنتها بالتركيبات النانوية لمستخلص النعناع .(MENFs) علاوة على ذلك، فإن عينة الزبادي المحضرة باستخدام 0.025٪ CENFs تحتوي على محتوى فينولي إجمالي أعلى من المعاملات الأخرى. في التقييم الحسي، كان للتركيزات المختلفة (0.0125 و 0.025 و 0.05٪) من تركيبات نانوية من مستخلص القرنفل تأثير معنوي على مظهر و نكهة الزبادي مقارنة بعينة الكنترول. في المقابل، عند تقييم القوام والتركيب، فإن الزبادي المحضر بتركيزات مختلفة (0.0125 ، 0.025 ، و 0.05٪) من التركيبات النانوية لمستخلص القرنفل (CENFs) لم يختلف بشكل كبير عن العينة الكنترول من ناحية أخرى، كانت هناك فروق معنوية (P< 0.05) بين الزبادي المحضر بتركيزات مختلفة (0.0125 ، 0.025 ، و 0.05٪) لمستخلص النعناع (MENFs) والعينة الكنترول من حيث المظهر والقوام والتركيب، والنكهة. بالإضافة إلى ذلك، من حيث المظهر والقوام والملمس والنكهة ، انخفضت الخواص الحسية بشكل كبير مع الإضافة، ومع زيادة تركيز تركيبات نانوية لمستخلص النعناع(MENFs) ، لوحظ انخفاض درجات التقييم الحسي.