Study of the qualitative and sensory properties of yogurt manufactured by adding medicinal and aromatic plant extract(mint)

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Abstract

This research aimed to study yogurt, to which an aqueous extract of the medicinal and aromatic plant (mint) was added to manufacture a new dairy product to improve and treat many different digestive system problems. They were using the lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). The yogurt product was manufactured by adding an aqueous extract of mint with three concentrations of (1%), (2.5%), and (4%) (v/v) mint, and they were represented by N1, N2, and N3 treatments, respectively, as well as the control (C) treatment in which the yogurt was prepared without any addition. The treatments were stored for 21 days at (5±1)°C. The physicochemical and rheological properties were studied, which included measurement (pH, total acidity, viscosity, syneresis, and hardness), microbiological tests, as well as sensory evaluation on the first day of manufacture and after 7, 14, and 21 days of cold storage. The results showed no significant differences in the values of pH and total acidity, While significant differences were found in the values of syneresis, viscosity, and hardness. It was also observed that the numbers of starter bacteria varied for all treatments immediately after manufacturing, but during storage, a gradual decrease in their numbers was observed for all treatments, and the N3 treatment recorded the highest numbers of starter bacteria compared to the other treatments. Also, the results showed superiority of N2 and N3 treatments in the sensory evaluation of flavor, taste, texture and general acceptance.

Keywords: Yogurt, rheological properties, mint, plant extract

Introduction

Since ancient times, milk and its derivatives have been a significant part of the human diet, playing a prominent role [1]. Yogurt is one of the most popular fermented milk products produced worldwide and is widely accepted by consumers due to its nutritional value and potential health benefits [2]. It contains many nutrients, such as vitamins, proteins, calcium, phosphorus, Magnesium, and zinc, etc., necessary for a healthy life for humans and all age groups [3].

Recent years have witnessed a broad trend in developing dairy products and producing foods with new flavors. With the advent of fortified foods, health awareness and interest in adding herbs and spices as important food additives in dairy and food products has increased worldwide [4]. Mint is an important medicinal and aromatic plant; its genus contains between 25 and 30 species [5]. It has been used traditionally in folk medicine and is a rich source of iron and Magnesium, which play an essential role in human nutrition [6]. Mint belongs to the Lamiaceae (Labiatae) family, a large family of annual or perennial herbs and is widely grown worldwide to benefit from its herbal properties [7]. This plant is widely used in folk remedies and traditional medicine to treat gastrointestinal and nervous disorders due to its anti-inflammatory and antimicrobial properties and to reduce cramps, gastrointestinal pain, loss of appetite, nausea, and diarrhea [8]. In addition to its nutritional importance, it is known that adding plant extracts such as mint to yogurt can cause a chemical reaction with various food components, leading to changes in the physical and chemical properties that are important for the quality characteristics of yogurt. Dairy products have become a unique delivery vehicle that has been successfully used to deliver phytochemicals and other nutrients with health benefits into our diet [9].

Therefore, the current study aimed to produce a therapeutic milk product (yogurt) using a lactic acid bacteria starter with the addition of medicinal and aromatic plant extracts (Mint). Moreover, we are studying the effect of adding aqueous mint extract on the physicochemical, rheological, microbiological, and sensory properties of yogurt, as well as studying the effect of adding mint extract on prolonging the shelf life of this product.

Materials and Methods

Materials *Milk:* Full-fat powdered cow's milk was used in the manufacturing of yogurt, produced by the Irish company Dairygold Food, co.cork, and purchased from the local market in Baghdad.

Starter: The strains of *Streptococcus Salivarius Subsp thermophiles* and *Lactobacillus delbrueckii Subsp bulgaricus* type Y / 330 produced by the Italian company (SACCO), which are added directly to the milk mixtures prepared for the manufacture of yogurt.

Mint leaves: Fresh mint was purchased from local markets in Babylon Governorate and classified by a specialized professor in the Department of Field Crops/College of Agriculture/Al-Qasim Green University. The stems of the mint leaves were removed and carefully cleaned manually to remove dirt and damage, then dried in a drying oven, turning them into a fine powder. The resulting powder is used to prepare the aqueous extract of mint.

Preparation of plant extracts: the aqueous extract of mint was prepared according to Farhan, [10] where 5 g of mint powder was weighed and 50 ml of distilled water was added to it in a ratio of (1:10, solids: solvent) and then the solution was stirred using a

hot plate (magnetic stirrer) at a temperature of 100C°, after which the extract was filtered through a Whatman No. 1; then the filtrate was subjected to centrifugation for 10 minutes to obtain a clear aqueous extract. Finally, the extract was collected in dark-colored bottles and kept in cold storage at four °C until use.

Yoghurt Preparation: the yoghurt was made according to the method of Tamime and Robinson, [11]. Reconstituted full-fat milk powder (13%) was used, and 4 liters was prepared. Then, the homogenization process was carried out, and the milk was subjected to heat treatment at 90 °C for 10 minutes, then the treatments were cooled to 42 °C. Then they inoculated with the starter culture consisting of *Streptococcus salivarius subsp thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus* by direct addition and with the quantity indicated by the manufacturer at the rate of 0.01 g per L. The prepared quantity was divided into 4 sections, and the first section was left without addition and used in the manufacture of yogurt for the control treatment C as for the other three sections, aqueous mint extract was added to each liter at concentrations of (1%),(2.5%),(4%) (v/v). Then they were mixed well and filled into plastic containers with a capacity of 125 ml and incubated at a temperature of 42 ± 2 °C until the coagulation was complete, about 4-5 hr. Furthermore, until the pH decreased to 4.6, it was taken out of the incubator and transferred to the refrigerator for cooling and preservation at (5±1) °C until the necessary tests were performed after 1, 7, 14, and 21 days after manufacture.

Physicochemical Analysis:

Estimation of pH and total acidity(T.T.A.): The pH of the yogurt treatments was estimated after 1, 7, 14, and 21 days of manufacture by placing the pH meter sensor, type HQ 411 d model 211 of German origin, directly into the yogurt . A total acidity test (T.T.A.): The total acidity was estimated based on the value of lactic acid in the product was carried out according to what was stated in A.O.A.C. [12], Where the percentage of total acidity was calculated according to the following equation:-

$$\text{Lactic acid } \% = \frac{\text{Consumed volume of NaOH x 0.1 x 0.09}}{\textit{sample weight}} \text{ x } 100$$

Rheological examinations: The apparent viscosity of yoghurt samples was estimated at a temperature of 10 °C after 1, 7, 14 and 21 days of cold storage, according to Donkor et al.,[13] using a Brookfield DVII+ viscometer (Brookfield Engineering Lab Inc., Stoughton, Mass.). Hardness of yogurt parameters was estimated using a tissue analyzer (CT3,4500 Brookfield engineering lab) with a carrying force of 5 kg, according to what was stated in Joon et al., [14]. Meanwhile, syneresis was measured using the method Amatayakul et al., [15].

Microbiological tests of yogurt:

Microbiological tests included estimation of the total number of starter bacteria, and numbers of *Escherichia coli* from day 1, 7, 14, and up to 21days of cold storage at a temperature of (5 ±1) ° C . 1 ml of the samples were transferred to a test tube

containing 9 ml of sterile peptone water to obtain a dilution of 10^1. Then the contents were mixed by (Vortex electrophoresis), and the rest of the dilutions were completed until the desired dilution was reached; 0.1 ml of the dilution was transferred to Petri dishes and then poured onto the appropriate media depending on the required essay.

Estimation of the total number of starter bacteria:

M17 agar was used to estimate *Streptococcus salivarius subsp thermophilus* and De Man, Rogosa and Sharpe agar (M.R.S. agar) was used to estimate *Lactobacillus delbrueckii ssp.bulgaricus*. Then the dishes were incubated under anaerobic conditions at a temperature ranging between (45-42)°C for (48-72) hr.

Calculating the total number of coliform bacteria:

MacConkey agar medium was used to estimate total coliform bacteria by the pour plate method, then the dishes were incubated at 37°C for (24-48) hr.

Sensory evaluation of yogurt: Sensory evaluation was conducted by several specialized professors at the College of Food Sciences /Al-Qasim Green University. The evaluated characteristics of yogurt treatments were: flavor, texture, acidity and external appearance, according to Nelson & Trout, [16].

Statistical analysis: the Statistical Analysis System -S.A.S.[17] was used when analyzing the data to study the effect of various factors on the studied traits according to the complete random design (C.R.D.), and the significant differences between the means were compared through the Least Significant Difference-LSD test.

Results and Discussion

pH measurement: Fig. (1). shows the pH values of N treatment for yoghurt supplemented with aqueous mint extract. The pH values of yoghurt after storage for one day at (5 ± 1)°C , for control was (4.68), and this is consistent with Jasim, [18], Who found that pH of yoghurt was 4.68. The pH values of N1, N2, and N3 treatments that were supplemented with aqueous mint extract were 4.69, 4.67 and 4.65, respectively. The results of the statistical analysis of N treatment showed no significant differences ($P<0.05$) between the pH values of the control and the treatments to which mint extract was added. These results showed that the addition of aqueous mint extract has no effect on the pH of the yoghurt as it does not affect the starter bacteria , this is consistent with what Al-Shawi [19] found, which showed that there were no significant differences between the pH values of the control treatment and treatments in which aqueous and alcoholic mint extract were added to yoghurt fortified with probiotics and prebiotics (synbiotic). These results are also consistent with (Kumar [20], who found that adding mint at a rate of 2%, 4%, and 6% in curd production did not significantly affect the pH value of the resulting yogurt. As the storage period progresses, we notice a gradual decrease in the pH values of all treatments as well as the pH values of the control after

21 days, which reached 4.53, and for N1, N2 and N3, treatments, they were 4.52, 4.50 and 4.49, respectively. The low pH may be due to the ability and persistence of starter bacteria to convert the lactose sugar into lactic acid and other organic acids [21].

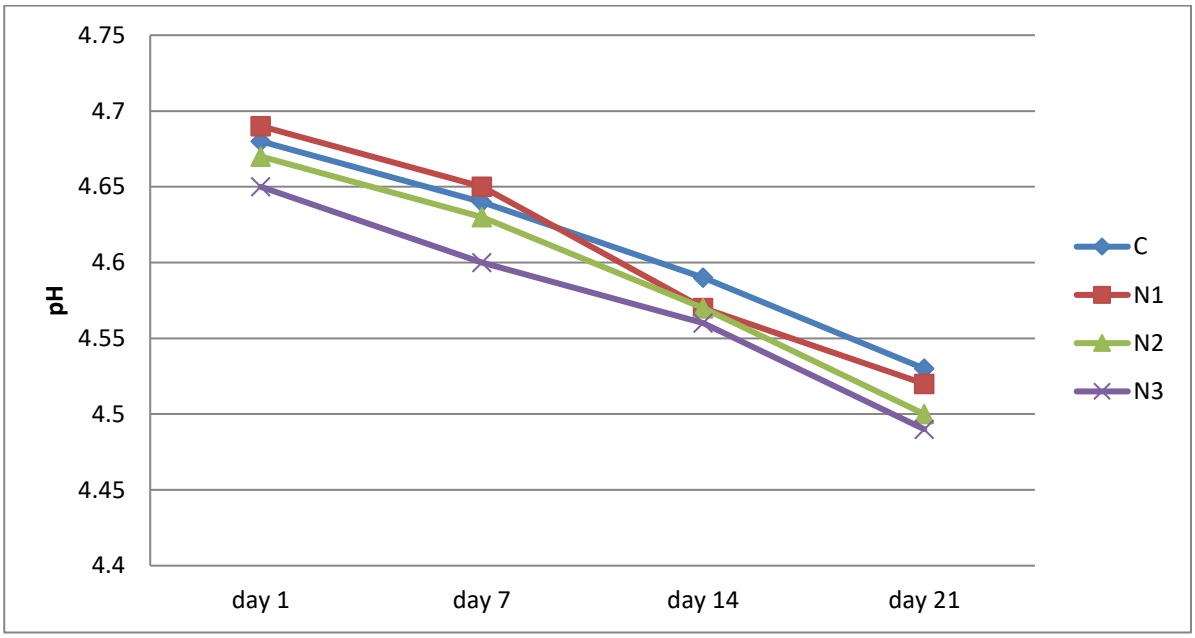

Figure (1): pH values of yogurt prepared with different concentrations of aqueous mint extract (N) and stored for 21 days at (5±1) °C.

Total Titratable Acidity (T.T.A.)

Figure (2) shows the values of acidity of yogurt, as the values of acidity after storage for one day at (5±1)°C for control was 0.84 % , and this result is close to Hatem [22], Who found that acidity of yoghurt was 0.86%, but it was lower than what Alshawk [23] found for the control treatment, which was 0.89% . As for the acidity values of N1, N2 and N3 treatments were 0.82, 0.85, and 0.87, respectively. After storage for 21 days, an increase in the total acidity values was observed for the control, which was 0.95 %, and for N1, N2, and N3 treatments, which were 0.95, 0.97 and 0.98 %, respectively. These results agree with what Al-Shawi [19] indicated, as he indicated an increase in the total acidity values of yoghurt fortified with aqueous and alcoholic mint extract after storage as a result of the increased activity of starter bacteria and their continued production of lactic acid. The results of the statistical analysis of yogurt treatment showed no significant differences ($P<0.05$) between the values of the acidity of the control and the treatments to which aqueous mint extract was added immediately after manufacturing and during the storage period. This is attributed to the lack of effect of adding the mint extract on the total acidity of the yogurt, because it does not affect the work of the starter bacteria responsible for producing lactic acid [19].

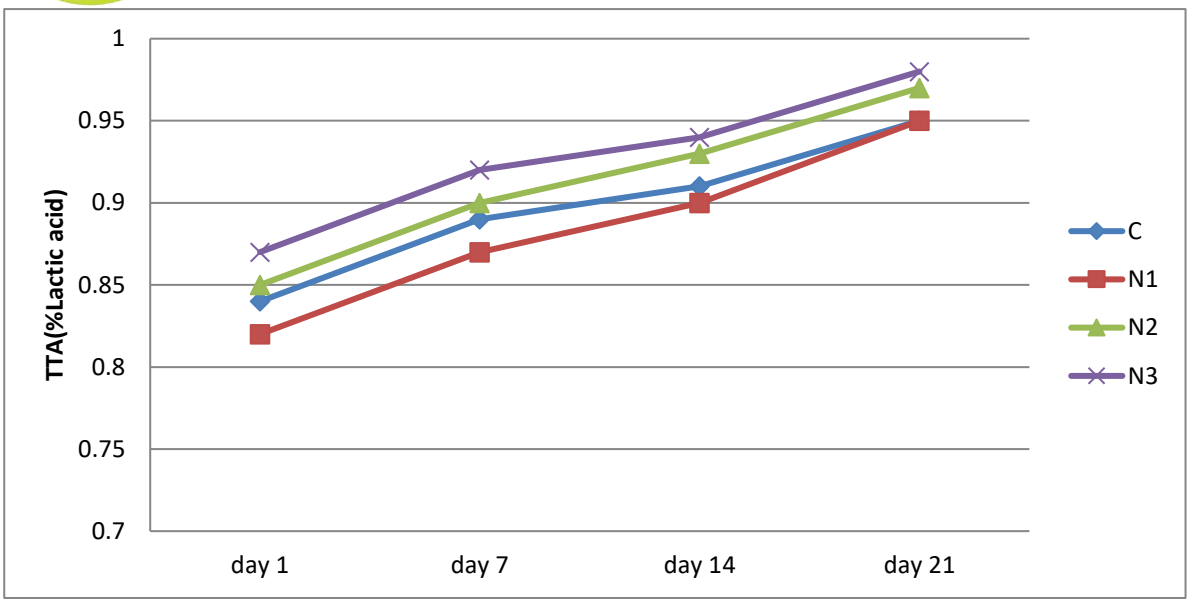

Fig (2): T.T.A. values of yogurt prepared with different concentrations of aqueous mint extract (N) and stored for 21 days at (5±1) °C.

Viscosity: Fig. 3. shows the viscosity values of yogurt after a day of manufacturing. The control was 2300 centipoise, while the viscosity values for the N1,N2, and N3 treatments were 2380, 2360, and 2280 centipoise, respectively. We note from the viscosity results that high concentrations of mint extract had a negative effect, such that the addition of treatment N3 caused a reduction in viscosity values compared to the control treatment. The results show that the higher the percentage of aqueous mint extract added to the yogurt, the lower the viscosity. This is consistent with El- Said et al., [24], who stated that with an increase in the concentration of added plant extracts, in general, the products' consistency decreased due to a decrease in the ability of proteins to bind to water. But after storage 21 days, there was an increase in the viscosity values for all treatments. The value of viscosity for control was 2830 centipoise, and for N1, N2 and N3 treatments, were 2870, 2860 and 2800 centipoise, respectively. This is consistent with Shaghaghi et al., [25], who indicated that the viscosity of yogurt treatment increased immediately after manufacturing from 2123 centipoise to 2244 centipoise during storage for 14 days. Results of this study showed significant differences ($P<0.05$) in the viscosity values after 21 days of storage between the control and the treatments to which aqueous mint extract was added.

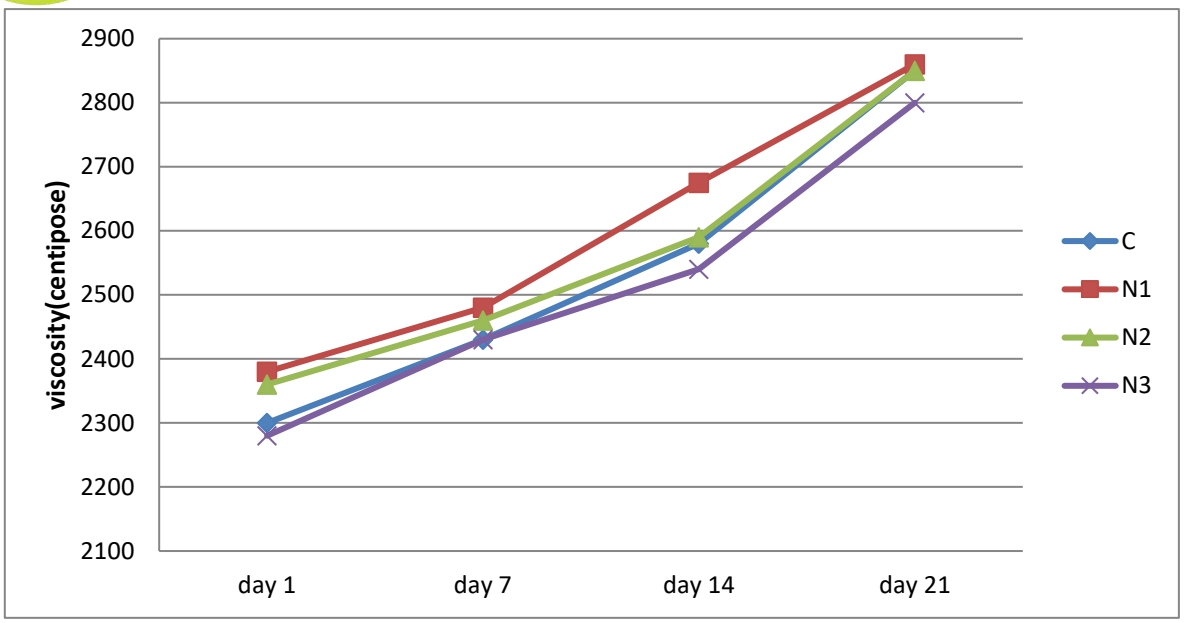

Figure (3): Viscosity values of yogurt prepared with different concentrations of aqueous mint extract (N) and stored for 21 days at (5±1) °C.

Syneresis : Table (1). shows the syneresis values of yogurt after a day of manufacturing, the control was 3.23 ml, which is less than Hatem found [22]. At the same time, the syneresis values for the N1, N2, and N3 treatments were 2.97, 3.18 and 3.28 ml, respectively. The statistical analysis results showed significant differences ($P<0.05$) between the levels of syneresis for the control and N1, N2, and N3 treatments throughout the storage period. We note that adding aqueous mint extract at concentrations less than 4% led to a reduction in the level of syneresis in yoghurt. As for the 4% concentration of the N3 treatment, this treatment was the highest in the syneresis, and the reason for this may be due to rearranging the protein structure and reducing the retention of total solids in the protein network, thus obtaining an unstable protein network that allows water to penetrate [26]. After 21 days of storage, the syneresis levels decreased from the first day for all treatments, as the control was 2.04 ml, and the N1, N2, and N3 treatments were 1.94, 1.98 and 2.11 ml, respectively, This is consistent with Celik [27], who indicated that the level of syneresis of yoghurt decreased from 055.8% on the first day of manufacturing to 53.3% on the 14th day of refrigerated storage. The low levels of syneresis may be due to the continued metabolic activity of the starter bacteria. This can also be attributed to the decrease in net pressure inside the protein matrix, which leads to a decrease in syneresis as the storage period advances [28].

Table (1): syneresis and hardness values of different yoghurt treatments immediately after manufacturing and during storage at (5 ± 1) °C for 21 days.

Treatment	Storage period (day)	Syneresis ml/50 ml	Hardness /g
C	1 day	3.23	97.5
	7 day	2.81	102.9
	14 day	2.54	107.4
	21 day	2.04	116.0
N1	1 day	2.97	102.4
	7 day	2.68	106.3
	14 day	2.40	112.6
	21 day	1.94	120.3
N2	1 day	3.18	100.8
	7 day	2.75	105.7
	14 day	2.49	110.1
	21 day	1.98	118.5
N3	1 day	3.28	96.8
	7 day	2.85	99.4
	14 day	2.59	105.9
	21 day	2.11	115.8
L.S.D.	---	0.933 *	15.495 *
*(P≤0.05).			

C: control , N1 : 1% mint extract , N2 : 2.5 % mint extract , N3: 4% mint extract .

Hardness: Table (1). Shows the hardness values of yogurt after a day of manufacture for N treatment; The control was 97.5 g, and for N1, N2, and N3 treatments it was 102.4, 100.8 and 96.8 g, respectively. We note that the hardness of yogurt added to mint extract N1 and N2 increased compared to the control treatment. The condition of reaching the N3 concentration began to decrease, which may be due to the weakening of the protein network and affecting the cohesion of the casein molecules among themselves. Mousavi et al. [29] indicated that the hardness of yogurt depends on the nature of the contents of the additives, the level of starter bacteria, and the time of incubation, and stated that the level of starter bacteria can increase the hardness of the yogurt. In another study, Mudgil et al., [30] indicated that incubation time did not significantly affect the hardness of yoghurt. After 21 days of storage, the hardness values increased for all treatments, reaching 116.0 g for the control , and for N1, N2, and N3 treatments were 120.3, 118.5 and 115.8 g, respectively, These results are consistent with what was found by Mustafa and Albadawi [31], who indicated an increase in the hardness values of the yogurt for the control treatment immediately after manufacturing from 71 g to 110 g at the end of the 21-day storage period. The reason for the increase in hardness throughout storage may be attributed to the activity of starter bacteria, which work to lower the pH of the yoghurt, which leads to an increase in its hardness, and then an increase in its viscosity, and this is consistent with what was mentioned Walstra [32].

The statistical analysis results showed significant differences ($P < 0.05$) in the hardness values of yoghurt after storage for 21 days between the control treatment and N1, N2, and N3 treatments.

Microbiological Tests Of Yogurt

Table (2). shows in numbers of the starter, and coliform bacteria in yogurt supplemented with aqueous mint extract immediately after manufacturing and during storage at (5 ± 1) °C for 21 days. We found that the total number of starter bacteria in the yogurt after storing it for one day for the control treatment was 70×10^7 CFU/ml , and this result is close to Sadiq [33] for yogurt amounting to 69×10^7 CFU/ml, which is within limits set by the International Organization (WHO/FAO,1997;IDF/ FIL, 1997), which stipulates that the number of live cells of the starter bacteria should not be less than 10^7 colony-forming units /ml. At the same time, the total number of starter bacteria for N1, N2, and N3 treatments were 65×10^7, 69×10^7 and 73×10^7 CFU/ml , respectively. The results of the statistical analysis for the N treatment show significant differences ($P<0.05$) between the total number of starter bacteria for the control and the treatments to which mint extract was added. The N3 treatment recorded the highest percentage of starter bacteria compared to the control and other treatments. These results show that adding aqueous mint extract improves the proteolytic activity of the starter bacteria in the yoghurt because it does not affect the starter bacteria. This is consistent with what was found by Al-Shawi [19], who studied the effect of adding aqueous and alcoholic mint extract to yoghurt fortified with probiotics and prebiotics (synbiotic) on the effectiveness of starter bacteria, and found that these extracts improve the proteolytic activity of L. acidophilus bacteria, He also indicated that the highest percentage of L.A.B. bacteria was in yoghurt to which alcoholic mint extract was added, followed by aqueous mint extract throughout the storage period. It is also consistent with what was found by Marhamatizadeh et al., [34] who studied the effect of mint addition on L.A.B. growth, They indicated that the numbers of Lactobacillus acidophilus and Bifidobacterium bifidum increased with increasing mint concentration. The reason may be due to phenolic compounds found in herbal extracts that stimulate and enhance the growth of starter bacteria in yogurt [35] and probiotic bacteria [36]. As for during storage for 21 days , a gradual decrease in the numbers starter bacteria was observed for all treatments, where was for control treatment 20×10^7 CFU/ml, and for N1, N2, and N3 treatments were 23×10^7, 23×10^7, and 25×10^7 CFU/ml, respectively. This decrease may be due to the development of acidity in the yogurt and the decrease in pH. This is consistent with what was found by Dave and Shah [37], who indicated a decrease in yogurt starter bacteria from 99.1 x 10^7 CFU/g immediately after manufacturing to 65.3. $\times 10^7$ CFU/g on day 15 of storage.

Table (2): microbiological tests for different yogurt treatments immediately after manufacturing and during storage at (5 ± 1) °C for 21 days.

Treatments	Shelf life of storage (day)	starter bacteria (CFU /ml)	*E.Coli* (CFU /ml)
C	1 day	70×10^7	-
	7 day	80×10^7	-
	14 day	44×10^7	-
	21 day	20×10^7	-
N1	1 day	65×10^7	-
	7 day	78×10^7	-
	14 day	46×10^7	-
	21 day	23×10^7	-
N2	1 day	69×10^7	-
	7 day	80×10^7	-
	14 day	45×10^7	-
	21 day	23×10^7	-
N3	1 day	73×10^7	-
	7 day	84×10^7	-
	14 day	47×10^7	-
	21 day	25×10^7	-
L.S.D.	---	25.04 *	NS

•Reading is an average of three repetitions.

Regarding the numbers of coliform bacteria, we note from Table (2) that the results were close for all treatments, as no contamination was recorded in the presence of coliform bacteria immediately after manufacturing and during storage for 21 days in all treatments. The reason for this may be due to the high pasteurization temperature, good manufacturing and production conditions, and adherence to the necessary health conditions. This is consistent with what was found by Nawar [38], who indicated that coliform bacteria were not present in all yogurt treatments immediately after manufacturing and during the storage period. He attributed this to the hygienic practices that are followed during manufacturing and storage.

Sensory evaluation: The results of the sensory evaluation of yogurt treatments prepared by adding different concentrations of the aqueous mint extract showed a difference in flavor, taste, texture, and the scores of total acceptance for all treatments. Table (3). shows the total sensory scores the raters gave to the yogurt immediately after manufacturing at a temperature of 5 ± 1 °C. The sensory evaluation score given to the control treatment immediately after manufacturing was 92.6, and for N1, N2, and N3 treatments were 92.95, 95.23, and 95.0, respectively.

Table (3): The sensory evaluation of different yogurt treatments immediately after manufacturing and during storage at (5 ± 1) °C for 21 days.

Treatment	Storage period (day)	Flavor 45˚	Texture 35˚	Acidity 10˚	Appearance 10˚	Total 100˚
C	1 day	42.3	32.30	9	9	92.6
	7 day	41.8	32.00	8	9	90.8
	14 day	41.5	31.50	8	9	90.0
	21 day	40.81	31.00	8	9	88.81
N1	1 day	42.00	32.95	9	9	92.95
	7 day	41.10	32.15	9	9	91.25
	14 day	40.55	32.00	9	9	90.55
	21 day	40.22	31.90	10	8	90.12
N2	1 day	43.13	33.10	9	10	95.23
	7 day	42.70	32.78	9	9	93.48
	14 day	42.56	32.15	9	9	92.71
	21 day	41.60	32.03	9	9	91.63
N3	1 day	43.0	33.00	9	10	95.0
	7 day	42.95	32.89	9	9	93.84
	14 day	42.77	32.42	9	9	93.19
	21 day	42.63	32.13	8	9	91.76
L.S.D.	---	3.29 *	2.18 NS	1.76 *	1.83 *	5.37 *
*(P≤0.05).						

C: control , N1 : 1% mint extract , N2 : 2.5 % mint extract , N3: 4% mint extract .

As for the total sensory evaluation scores granted to the treatments during 21days of refrigerated storage at (5 ±1) °C, it was 88.81 for the control, and N1, N2, and N3 treatments were 90.12, 91.63, and 91.76, respectively, of the evaluation scores above, N2 and N3 treatments got the highest evaluation scores 91.63 and 91.76, respectively, followed by the N1 treatment and control, which were close during the storage periods. Since the higher concentration of added mint extract obtained higher scores compared to the control, it was noted from the results that the N3 treatment was superior in all the sensory evaluation characteristics of flavor, taste, acidity, and appearance, which the raters described as more receptive. This is consistent with what Farhan et al.,[10] found, as they indicated that adding mint improved the sensory qualities of yoghurt.

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