

Improving the sensory and rheological properties of yogurt using aqueous extract of basil (*Ocimum basilicum* L.)

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Abstract

This study aims to produce a therapeutic milk product (yogurt) to improve and treat many different digestive problems, using the lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) supplemented with aqueous extract of the plant (basil) with three concentrations of 1%, 3% and 5% [v/v] basil, they were represented by R1, R2 and R3 treatments respectively, as well as the control [C] treatment in which the yogurt was made without any addition. The treatments were stored for 21 days at $[5\pm 1]$ °C. The physicochemical properties were studied, which included measurement [pH, total acidity, syneresis, viscosity, and hardness] and microbiological examinations, as well as sensory evaluation on the first day of manufacture and after 7, 14 and 21 days of cold storage. The results showed that there were no significant differences in pH values immediately after manufacturing for all treatments. As for the percentage of total acidity (TTA), it was close to immediately after manufacturing for all the different yogurt treatments, while during storage, a clear increase in their values was observed for all transactions. Results showed a significant difference in syneresis, viscosity and hardness immediately after manufacturing and during cold storage for all treatments. R1 and R2 treatments improved the viscosity, hardness and syneresis of yogurt. The R2 and R3 treatments recorded the highest numbers of starter bacteria compared to the other treatments. Also, the results showed that the superiority of R2 treatment in the sensory evaluation of the characteristics of taste, flavor, texture and general acceptance.

Keywords: Yogurt, Physicochemical properties, Basil, plant extract

Introduction

Yogurt is a popular dairy product all over the world, not only for its unique texture and flavor, but also for its health benefits [1]. It contains many nutrients, such as proteins, vitamins, phosphorous, calcium, magnesium, zinc and others, which are essential for a healthy life human, and for all age groups [2]. In general, yogurt is produced by fermenting raw milk use of microbial cultures for *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [3]. Recent years have witnessed a wide trend to develop dairy products, and produce foods with new flavors. and with the advent of fortified foods,

health awareness increased worldwide and interest in adding herbs and spices as important food additives in dairy and food products [4]. Sweet basil *Ocimum basilicum* is an annual herbaceous plant belonging to the mint family *Lamiaceae* and an essential component of many culinary traditions and practices [5]. Basil has many pharmacological effects because it contains biologically active factors that help prevent diseases such as cancer, reduce aging, antiviral, and has antimicrobial properties [6]. Phenols and flavonoids are the main phytochemical compounds in basil, and basil contains many secondary metabolites, such as polyphenols, flavonoids and terpenes with recognized potential biological effects, identified in basil, and due to its strong aroma and valuable pharmaceutical potential, sweet basil is synonymous with the nickname “Aromatic King” among medicinal and aromatic herbs [7, 8]. it is widely used in medicines and foodstuffs [9], In addition to the nutritional importance, it is known that the addition of plant extracts such as basil to yogurt can cause a chemical reaction with different nutritional components that lead to changes in the physicochemical properties important for the quality characteristics of yogurt. Therefore, the current study aimed to produce a therapeutic milk product [yogurt] using a lactic acid bacteria starter with the addition of a medicinal and aromatic plant extract (sweet basil). and study the effect of adding aqueous basil extract of on the physicochemical, rheological, Microbiological and sensory properties of yogurt, as well as studying the effect of adding basil extracts on prolonging the shelf life of this product.

Materials and methods:

Preparation of plant extracts

the aqueous extract of basil was prepared according to a previous method [10] where 5 g of basil 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 100°C, 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 4 °C until use.

Yogurt Preparation

The yogurt was made according to a previous method [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 a temperature of 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 in to 4 sections, 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 basil extract was added to each liter, at concentrations of (1, 3, 5%) (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, and until the pH decreased to 4.6, it was taken out of the incubator and transferred to the refrigerator for



cooling and preservation at a temperature of $(5 \pm 1) ^\circ\text{C}$ until the necessary tests are performed after 1, 7, 14 and 21 days after manufacture.

Physicochemical Analysis

The pH of the yogurt treatments was estimated after 1, 7, 14, and 21 days of manufacture by placing the sensor of the pH meter type HQ 411 d model 211 of German origin, directly into the yogurt treatment. A total acidity test (TTA) The total acidity was estimated based on the value of in the product, was carried out according to AOAC method [12], Where the percentage of total acidity was calculated according to the equation below:

$$\text{Lactic acid \%} = \text{Consumed volume of NaOH} \times 0.1 \times 0.09 / \text{sample weight} \times 100$$

Rheological examinations

The apparent viscosity of yogurt samples was estimated at a temperature of $10 ^\circ\text{C}$ after 1, 7, 14 and 21 days of cold storage [13] using a Brookfield DVII+ viscometer (Brookfield Engineering Lab Inc., Stoughton, Mass.). Syneresis was measured [14], While the hardness of yogurt parameters was estimated using a tissue analyzer (CT3,4500 Brookfield engineering lab) with a carrying force of 5 kg [15].

Microbiological examinations of yogurt

Microbiological examinations included estimation of the total number of starter bacteria, numbers of *Staphylococcus aureus*, and *Escherichia coli* numbers from day 1, 7, 14, and up to 21 days of cold storage at a temperature of $(5 \pm 1) ^\circ\text{C}$. 1 ml of the samples was transferred to a test tube containing 9 ml of sterile peptone water to obtain a dilution of 101. Then the contents were mixed with the (Vortex electrophoresis) and then the necessary decimal dilutions were performed by transferring 1 ml of the first dilution to several test tubes containing 9 ml of sterile peptone water until the desired dilution was reached, 0.1 ml of the dilution was transferred to Petri dishes and then poured on to the appropriate media according to 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 (MRS agar) was used to estimating *Lactobacillus delbrueckii* ssp. *bulgaricus*, then the dishes were incubated in anaerobic conditions at a temperature ranging between $(45-42)^\circ\text{C}$ for (48-72) hr.

Estimation 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

Calculating of the number of *Staphylococcus aureus* bacteria

Mannitol Salt Agar was used to estimate the number of *Staphylococcus aureus*, then the dishes were incubated at 37°C for 24 hr.

Sensory evaluation of yogurt

Sensory evaluation was performed by a number of specialized professors in the College of Food Sciences / Al-Qasim Green University. the treatments were evaluated according to the sensory evaluation form created previously [16]

Statistical analysis

The Statistical Analysis System -SAS [17] was used when analyzing the data to study the effect of different factors on the studied traits according to the complete random design (CRD), and the significant differences between the means were compared through the Least Significant Difference-LSD test.

Results and Discussion

pH measurement

The results in Figure (1) shows the pH values of R treatment for yogurt supplemented with aqueous basil extract. the pH values of yogurt after storage for one day at a temperature of $(5 \pm 1)^{\circ}\text{C}$, for control was (4.65), and this result is close to Jasim [18], Who found that pH of yogurt was 4.68. As for the pH values of R1, R2 and R3 treatments that supplemented with aqueous basil extract, were 4.63, 4.62 and 4.59, respectively. 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 where reached 4.44 , and for R1, R2 and R3, treatments they were 4.42, 4.40 and 4.39, respectively. the reason for the low pH may be attributed to the ability and persistence of the activity of the starter bacteria in converting lactose sugar to lactic acid [19], these results are in agreement with Gürkan et al., [20], where he indicated that the pH values of yogurt with purple basil in the form of powder and aqueous extract were not significantly different from the control after one day of processing, he indicated that it continued to gradually decrease after storage for 21 days for all treatments as a result of the continuation of the activity of the starter bacteria by converting the residues of lactose sugar into lactic acid. The results of the statistical analysis of R treatment showed that there were no significant differences ($P < 0.05$) between the pH values of the control and the treatments to which basil extract was added, these results showed that the addition of aqueous basil extract has no effect on the pH of the yogurt as it does not affect the starter bacteria .shows the effect of using the aqueous extract of pomegranate peel powder and dried Moringa leaves and their mixture on the weekly body weight rates of broilers. It is noted from the table that there are no significant differences between all experimental treatments in the first week of chicks' life. In the second week, the two treatments showed T2 that T5 was significantly superior ($P \leq 0.01$) to the control treatment. While there were no significant differences between treatments T3, T4, and T6 on the one hand, and the control treatment on the other hand. At the third, fourth, fifth, and sixth weeks of bird-life, all additional treatments were significantly ($P \leq 0.01$) outperformed compared to the control treatment.

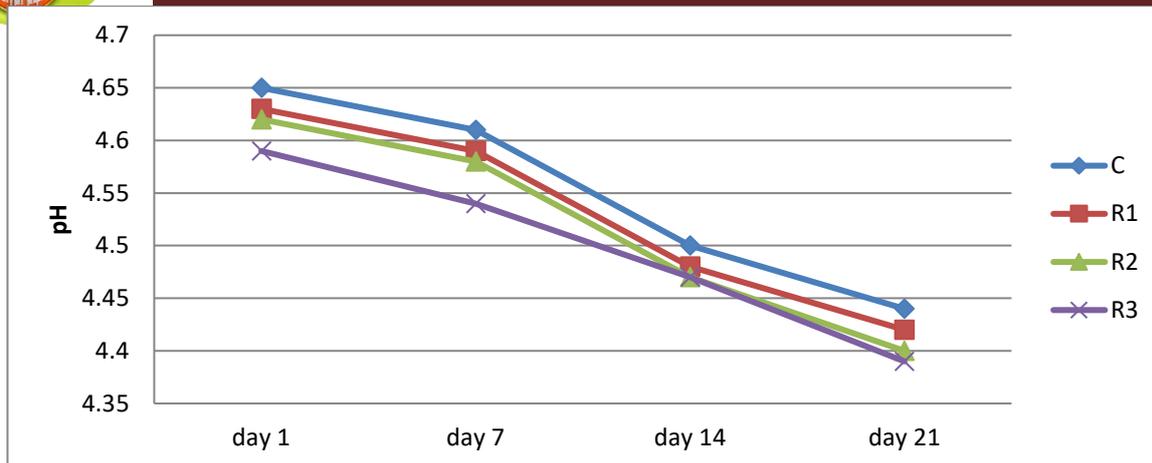


Figure (1): pH values of yogurt prepared with different concentrations of aqueous basil extract (R) and stored for 21 days at (5 ± 1) °C.

Total Titratable Acidity (TTA)

It shows in Figure (2) the values of acidity of yogurt, as the values of acidity after storage for one day at (5 ± 1) °C for control was 0.81 % , As for the acidity values of R1, R2 and R3 treatments were 0.80%, 0.82% and 0.83% , respectively. After storage for 21 days, an increase in the total acidity values was observed for the control, which was 0.92 % and for R1, R2 and R3 treatments were 0.91, 0.93 and 0.95 % , respectively. These results are in agreement with Amirdivani and Baba, [21] where indicated that the high acidity of the yoghurt after storage as a result of the continued production of lactic acid by the starter culture bacteria in the produced medium because it was not affected by the added basil extract. The results of the statistical analysis of yogurt treatment showed that there were no significant differences ($P < 0.05$) between the values of the acidity of the control and the treatments to which aqueous basil extract was added immediately after manufacturing and during the storage period. This shows that adding aqueous basil extract had no effect on the total acidity percentage due to the basil extract not having an effect on the starter bacteria, and this is consistent with Gürkan, [20].

First transaction T1 (control): no addition. The second treatment: T2 was added to the aqueous extract of pomegranate peel powder at a level of 50 ml/liter of water. The third treatment T3: adds the aqueous extract of moringa leaves powder at a concentration of 75 ml/liter of water. Fourth treatment T4: adding an aqueous extract of pomegranate peel powder at 75% and dried moringa leaves powder at 25%. Fifth treatment: T5 Addition of aqueous extract of pomegranate peel powder at 50% and dried moringa leaf powder at 50%. Sixth treatment: adding an aqueous extract of pomegranate peel powder at 25% and dried moringa leaf powder at 75%. NOS indicates that there are no significant differences between the means of the treatments. * * The different letters within the same column indicate that there are significant differences between the groups at the 0.01 probability level.

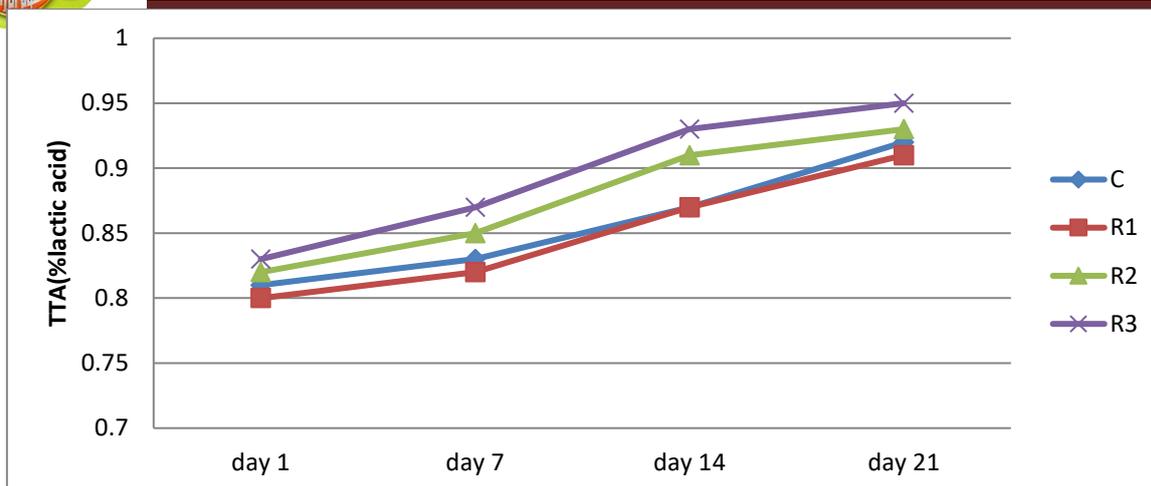


Figure (2): TTA values of yogurt prepared with different concentrations of aqueous basil extract (R) and stored for 21 days at (5 ± 1) °C.

Viscosity

The results in Figure (3) displays the viscosity values of yogurt after a day of manufacturing, the control was 1890 centipoise, while the viscosity values for the R1, R2 and R3 treatments were 1940, 1900 and 1815 centipois, respectively. Results of this study indicated that the addition of aqueous basil extract improved the viscosity property, and the R1 treatment recorded the highest viscosity values compared to the other treatments. the reason for this may be due to the presence of some compounds that have the ability to interact with milk proteins, such as polyphenols [22]. As for the high concentrations, it had a negative effect on the viscosity characteristic, as the R3 treatment led to a decrease in the viscosity values compared to the control, this decrease in viscosity could be due to the instability of the structural structure of casein particles in the yogurt during storage [23]. But after storage 21 days, there was an increase in the viscosity values for all treatments, the value of viscosity for control was 2430 centipoise, and for R1, R2 and R3 treatments were 2450, 2440 and 2380 centipoise, respectively. This increase in viscosity could be due to the stability of the casein structures and the increased intra-casein binding during storage (24). the reason for this may be attributed to the activity of the starter bacteria and the production of exogenous polysaccharides that interfere with the protein content of milk, raise its viscosity and improve its quality characteristics. Results of this study showed that there was significant differences ($P < 0.05$) between the viscosity values of the control and R1, R2 and R3 treatments immediately after manufacturing and during storage for 21 days.

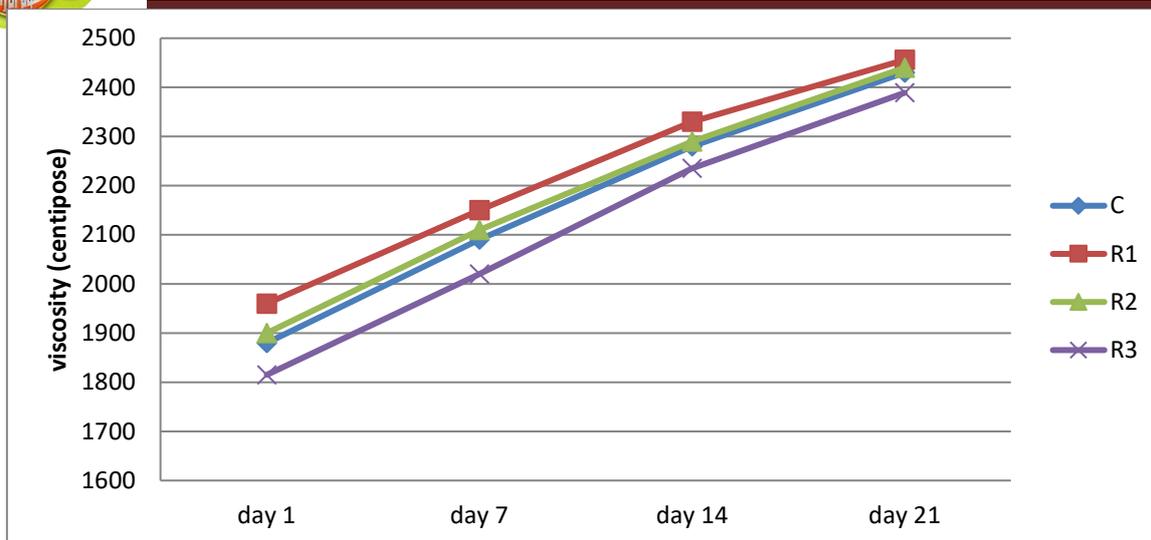


Figure (3): Viscosity values of yogurt prepared with different concentrations of aqueous basil extract (R) and stored for 21 days at $(5 \pm 1)^\circ\text{C}$.

Syneresis

Table (1) demonstrates the syneresis values of yogurt after a day of manufacturing, the control was 3.84 ml, while the syneresis values for the R1, R2 and R3 treatments were 3.76, 3.79 and 3.90 ml, respectively. The results showed that treatments R1 and R2 had the lowest syneresis, and this indicates that the addition of basil extract improved the syneresis characteristic of yogurt. The reason may be due to the role of some compounds in increasing the accumulation of milk proteins, and the retention of water inside them, while the R3 treatment was the higher in the syneresis, this may be due to the weakening of the protein network as a result of reducing the ability of the protein network gel to retain the aqueous phase, and then obtaining an unstable protein network that allows water permeability [25]. After 21 days of storage, the syneresis levels decreased from the first day for all treatments, as the control was 2.66 ml, and the R1, R2 and R3 treatments were 2.57, 2.61 and 2.70 ml, respectively, the decrease in syneresis in all treatments during storage is attributed to an increase in the affinities between milk proteins [18]. The results of the statistical analysis showed that there were significant differences ($P < 0.05$) between the levels of syneresis for the control, and R1, R2 and R3 treatments throughout the storage period.

Table (1): syneresis and hardness values of different yogurt treatments, immediately after manufacturing and during storage at $(5 \pm 1)^\circ\text{C}$ for 21 days.

Treatment	Storage period (day)	Syneresis ml/50 ml	Hardness /g
C	1 day	3.84	89.7
	7 day	3.79	91.4
	14 day	3.68	102.6

	21 day	2.66	110.4
R1	1 day	3.76	93.5
	7 day	3.70	95.7
	14 day	3.58	107.3
	21 day	2.57	114.5
R2	1 day	3.79	91.8
	7 day	3.74	94.5
	14 day	3.62	104.7
	21 day	2.61	110.7
R3	1 day	3.90	83.9
	7 day	3.85	85.6
	14 day	3.74	96.7
	21 day	2.70	102.8
LSD	---	0.836 *	14.37 *
*(P≤0.05).			

C: control , R1 : 1% basil extract , R2 : 3 % basil extract , R3: 5% basil extract .

Hardness

Table (1) Shows the hardness values of yogurt after a day of manufacture for R treatment; The control was 89.7 g, and for R1, R2 and R3 treatments it was 93.5, 91.8 and 83.9 g, respectively. The above results showed that adding aqueous basil extract improved the hardness of yogurt, especially R1 treatment, as for the high concentration of basil extract in the R3 treatment, it led to a lower level of hardness compared to the control. After 21 days of storage, the hardness values increased for all treatments, reaching 110.4 g for the control, and for R1, R2 and R3 treatments were 114.5, 110.7 and 102.8 g, respectively, these results are consistent with Mustafa and Albadawi, [26], where they indicated that the hardness values of yogurt for the control immediately after processing increased from 71 g to 110 g at the end of the 21-day storage period. The reason for the high hardness during the storage period may be attributed to the effectiveness of the starter bacteria that reduce the pH of the yogurt, which leads to a higher hardness, and then an increase in its viscosity, and this is consistent with Walstra et al.,[27]. Mousavi et al.,[28] indicated that the hardness of yogurt depends on the nature of the additive contents, the starter bacteria level, and the incubation time, they stated that the level of starter bacteria can increase the hardness of the yogurt. The

results of the statistical analysis showed that there were significant differences ($P < 0.05$) in the hardness values of yogurt after a day of processing and after storage for 21 days between the control, and R1, R2 and R3 treatments .

Microbiological tests of Yogurt

Table (2) indications in numbers of the starter bacteria, coliform bacteria, and *Staphylococcus aureus* bacteria in yogurt supplemented with aqueous basil extract, immediately after manufacturing and during storage at a temperature of $(5 \pm 1) ^\circ\text{C}$ for 21 days. Results of this study showed that there were significant differences in the numbers of the starter bacteria immediately after manufacturing and for all treatments. the values for control was 67×10^7 CFU/ml , and this result is close to what was found by Sadiq [29] for yogurt amounting to 69×10^7 CFU/ml , it is within the limits set by the International Organization (WHO/FAO,1997;IDF/ FIL, 1997), which requires that the number of live cells of the starter bacteria should not be less than 10^7 CFU/ml. While the total number of starter bacteria for R1, R2, and R3 treatments were 62×10^7 , 65×10^7 and 68×10^7 CFU/ml, respectively. As for during storage for 21 days, a gradual decrease in the number's starter bacteria was observed for all treatments, where was for control 17×10^7 CFU/ml, and for R1, R2, R3 treatments were 19×10^7 , 20×10^7 , and 21×10^7 CFU/ml, respectively. The reason for this decrease is most likely due to the development of acidity during storage. These results are consistent with Joung et al., [30] who indicated that this decrease during the storage period could be due to the accumulation of lactic acid by the starter bacteria, which leads to a decrease in pH and an increase of acidity. We found that the number of bacteria increases with the increase in the concentration of plant extracts, the reason may be attributed to the phenolic compounds found in herbal extracts that play a stimulating role and promote the growth of yogurt starter bacteria [31] and probiotic bacteria [32]. This indicates that the addition of these extracts improves the proteolytic activity of the starter bacteria in the yogurt as it does not affect this bacterium. The results of the statistical analysis of yogurt treatments showed that there were significant differences ($P < 0.05$) between the total number of starter bacteria of the control and treatments that supplemented with aqueous basil extract. The R3 treatment recorded the highest number of starter bacteria compared to the other samples. These results are in agreement with what Tomar et al. [33] found, which showed that there were significant differences between the total number of starter bacteria for the control treatment and the treatments to which alcoholic basil extract was added. Also, these results are consistent with Ghaleh Mosiyani et al., [34], who showed there were significant differences between the total number of starter bacteria for the control treatment and the treatments to which aqueous extract of the plant (basil and thyme) were added to yoghurt supplemented with probiotics (*Lactobacillus paracasei* ssp. paracasei), they found that with an increase in the amount of aqueous extract of the plant (basil and thyme) in the yogurt significantly increased the survival of probiotic bacteria compared to the control during the storage period of 21 days. As for the numbers of coliform bacteria, we note from Table (2). the results were close for all treatments where no numbers appeared indicating contamination immediately after manufacturing and during storage for a period of 21 days for all treatments. This may

be due to the high pasteurization temperature, good manufacturing conditions, and good production. This is consistent with Nawar et al., [35], who did not observe any growth of coliform bacteria in all treatments of yogurt, they pointed out that the reason for this is due to good manufacturing and production conditions, and adherence to the necessary health conditions during manufacturing and storage. As we notice from Table (2). the Total number of *Staphylococcus aureus*, the results were also close for all treatments during the first days, as no numbers appeared to indicate contamination. But during the storage period, the control recorded weak growth on day 7 and 14, while R1 and R2 treatments recorded weak growth on day 7, and did not observe any growth of *Staphylococcus aureus* was recorded for R3 treatment. This is consistent with Mohammed, [36] who studied the effectiveness of basil extract as an antidote to some pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus*, and he found that higher concentrations show greater effectiveness on these bacteria compared to lower concentrations. This may be due to the nature of the main components present in the aqueous basil extract, the characteristics of these compounds, and the degree of their polarity [37].

Table (2): Microbiological tests for different yogurt treatments, immediately after manufacturing and during storage at $(5 \pm 1) ^\circ\text{C}$ for 21 days.

Treatments	Shelf life of storage (day)	starter bacteria (CFU /ml)	<i>Staphylococcus aureus</i> (CFU /ml)	<i>E.Coli</i> (CFU /ml)
C	1 day	67×10^7	0	-
	7 day	79×10^7	3	-
	14 day	43×10^7	1	-
	21 day	17×10^7	0	-
R1	1 day	62×10^7	0	-
	7 day	77×10^7	3	-

	14 day	44×10^7	0	-
	21 day	19×10^7	0	-
R2	1 day	65×10^7	0	-
	7 day	80×10^7	1	-
	14 day	43×10^7	0	-
	21 day	20×10^7	0	-
R3	1 day	68×10^7	0	-
	7 day	81×10^7	0	-
	14 day	45×10^7	0	-
	21 day	21×10^7	0	-
LSD	---	31.08 *	NS	NS

•Reading is an average of three repetitions.

Sensory evaluation

The results of sensory evaluation of the treatments of yogurt prepared by adding different concentrations of aqueous basil extract showed a difference in taste, flavor, texture, and the degree of total acceptance of all treatments. Table (3). shows the total sensory scores granted by the assessors to the yogurt immediately after manufacturing at a temperature of 5 ± 1 °C. The sensory assessment score given to the control immediately after manufacturing was 92.3, and for R1, R2 and R3 treatments, were 91.50, 94.6 and 93.50, respectively. As for the total sensory evaluation scores granted to the treatments during 21 days of refrigerated storage at (5 ± 1) °C, it was 86.5 for the control, and R1, R2 and R3 treatments, were 86.4, 90.10 and 89.00, respectively, of the evaluation scores above, R2 and R3 treatments got the highest evaluation scores 90.10 and 89.00, respectively, followed by the control and the R1 treatment which were close throughout the storage periods. As the high concentration of added basil extract obtained higher degrees compared to the control. It is noticeable from the results that the R2 treatment outperformed in all the sensory evaluation characteristics of taste, flavor and acidity, which the evaluators described as more receptive. This is consistent with Gurkan and Hayaloglu [38], they indicated that the addition of basil improved the sensory qualities of yogurt, they mentioned that yogurt with basil extract was more preferred than yogurt with basil powder added. As for the low concentration, there were no significant differences on the sensory characteristics compared to the control because the smell, taste and flavor of basil was not clarity in the R1 treatment with concentration 1%, So the reduced the degree of sensory evaluation, and it was close to the control.

Table (3): The sensory evaluation of different yogurt treatments, immediately after manufacturing and during storage at $(5 \pm 1) ^\circ\text{C}$ for 21 days.

Treatment	Storage period (day)	Flavor 45°	Texture 35°	Acidity 10°	Appearance 10°	Total 100°
C	1 day	.041	3.23	9	10	92.3
	7 day	40.25	31.4	9	9	89.65
	14 day	40.0	31.0	9	9	89.00
	21 day	39.7	30.8	8	8	86.50
R1	1 day	41.0	32.5	9	9	91.50
	7 day	40.5	32.10	8	9	89.60
	14 day	40.1	31.9	8	9	89.10
	21 day	39.5	31.0	8	8	86.40
R2	1 day	042.	33.6	10	9	94.6
	7 day	41.7	32.9	9	9	92.60
	14 day	5.14	32.7	9	9	92.20
	21 day	40.6	32.5	8	9	90.10
R3	1 day	42.5	33.0	9	9	93.50
	7 day	41.6	32.8	9	9	92.40
	14 day	41.2	32.6	9	9	91.80
	21 day	40.0	32.0	9	8	89.00
LSD	---	2.94 *	1.91 *	1.67 *	1.85 *	5.62 *
.(P≤0.05) *						

C: control , R1 : 1% basil extract , R2 : 3 % basil extract , R3: 5% basil extract .

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