The role of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB12 in improving some characteristics of fermented banana puree

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Abstract

Green banana puree was fermented using a combination of single and mixed probiotic cultures of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB12 by seven different treatments. All treatments showed statistically significant differences during the two fermentation periods (24 and 48 hours) in terms of the viability of probiotics, pH and sensory evaluation. It was found that the 48-hour period of fermentation is better than the 24-hour period in improving the quality and properties of fermented bananas. Treatment (Tre.5) which included a mixed probiotic culture prepared in a ratio of (50:50), obtained the highest viability of probiotics as reached 11.37 log CFU/g, an appropriate pH which reached 3.42, better sensory acceptance which reached 86% as compared to other treatments, and sufficient level of anti-bacterial activity against some species of pathogenic and undesirable bacteria.

Keywords: Green banana, probiotics, prebiotics, sensory evaluation, antibacterial activity

Introduction

Recently, consumers have increased interest in improving their health by consuming physiologically active foods called functional foods that provide positive health effects in addition to their traditional nutritional value (1; 18).

Bananas are edible fruit, rich in carbohydrates, dietary fiber, some vitamins, minerals and bioactive phytochemicals. Cavendish bananas are the main type of banana exporting countries (36). Probiotics defined as are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Addition of probiotics to food increases the nutritional and functional values of products by enhancing the quantity and availability of nutrients and bioactive compounds arising from microbial metabolism, including organic acids, conjugated linoleic acid and exopolysaccharides. These microorganisms can be supported by consuming certain ingredients that may confer a health benefit called prebiotics (39). There are many species of lactic acid bacteria (LAB) are known generally recognized as safe (GRAS) (25). The combination of probiotics and prebiotics is called "symbiotic", it causes a synergistic effect that stimulates the growth of probiotic strains belonging to the genus *Lactobacillus* and *Bifidobacterium* through the fermentation of prebiotics (39). The combination of probiotics and prebiotics improves the survival of the probiotics in the digestive tract as it enhances tolerate low pH, oxygen and temperature conditions (23).

Bananas are a common source of prebiotics that promote the growth of probiotics, as contain a sufficient amount of resistant starch, oligo fructose, inulin, and others (23).

Therefore, the study aimed to ferment banana puree with two strains of probiotics, *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB12, using seven treatments that different in terms of starter composition and determining the optimum treatment that provides a high count of probiotics, suitable pH and excellent sensory acceptance, as well as study the inhibitory activity of starter cultures against various pathogenic and undesirable bacteria.

Materials and Methods

Starters of probiotic cultures

Commercial lyophilized cultures of the probiotics *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* lactis BB12 (supplied by Chr-Hansen) were activated with deMan, Rogosa, and Sharpe (MRS) broth medium three times and then activated with skim milk three times until the initial counts reached 11.45 and 11.42 log CFU/ml for *Lb. acidophilus* LA-5 and *Bif. lactis* BB12 respectively.

Preparation of fermented banana puree

Green bananas were purchased from a local market in Baghdad, Iraq, and puree was produced from the pulp of the fruit. Banana fruits were treated with ethyl alcohol (70%) then were peeled, cut into small pieces and mixed using an electric mixer in order to obtain a homogeneous mixture. Then the puree was distributed into sterilized sealed glass cups (4). Banana puree (100 g) was inoculated with 10 ml of probiotic cultures and incubated at temperature 37 °C for 24 and 48 hours, with seven treatments as below:

Tre1: *Lb. acidophilus* LA-5.

Tre2: *Bif. lactis* BB12.

Tre3: *Lb. acidophilus & Bif. lactis* (10:90).

Tre4: *Lb. acidophilus & Bif. lactis* (25:75).

Tre5: *Lb. acidophilus & Bif. lactis* (50:50).

Tre6: *Lb. acidophilus & Bif. lactis* (75:25).

Tre7: *Lb. acidophilus* & *Bif. lactis* (90:10).

Probiotic bacteria enumeration

The viable cells of probiotics were enumerated by de Man, Rogosa and Sharpe (MRS) agar which prepared according to the manufacturer's instructions (Hi media). Ten grams of each fermented banana puree treatment were diluted in 100 ml of sterilized peptone water. One ml of every diluted treatment was poured on MRS agar. Incubation was conducted under anaerobic conditions at 37°C for 72 h. Results of enumeration were calculated as colony forming units/gram of sample (CFU/g). The enumeration of all the samples were conducted in triplicates.

pH measurement

The method mentioned in Chaudhary *et al*. (12) was followed by weighing 10 g of sample, adding 90 ml of deionized water to it and placing it in an electric mixer for 30 seconds to obtain a homogeneous mixture, after which the pH was measured directly using a pH-meter. The pH values of fermented banana puree treatments were measured by a pH meter which was previously calibrated using a buffer of pH 4 and pH 7. The measurement was done by dipping the pH meter electrode in 10 ml of the sample and waited until the number on the pH meter be stable.

Sensory evaluation of fermented banana puree

The form of sensory evaluation (table 2) was designed through monitoring many factors that affect banana puree treatments by professors and postgraduate students in the Department of Food Science - College of Agricultural Engineering Sciences- University of Baghdad.

Antibacterial activity of probiotic cultures

Disk Diffusion Agar method was used to determination of antibacterial activity of starter cultures (28). Sterile paper discs was treated with 250 µl of the supernatants probiotic starters. The target species were local isolates of *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus stearothermophilus* and *Streptococcus mutans* (supplied by Department of Food Science/ College of Agricultural Engineering Sciences/ University of Baghdad).

Statistical analysis

To detect the effect of different factors in study parameters. Statistical Analysis System- SAS (2012) program was used (10). Least significant difference – LSD test was used to significantly compare between the means in this study.

Results and Discussion

Probiotics viability in fermented banana puree

Figure (1) indicated that there were statistically significant differences in probiotic counts of the seven treatments of banana puree fermented with probiotic formulations during two periods of fermentation (24 & 48 hours). It was observed that there was an increase in the counts of probiotics after a 48-hour fermentation period, better than the

probiotics counts after a 24-hour fermentation period. The counts in the single treatments increased from 8.42 log CFU/ml after 24 hours to 9.34 log CFU/ml after 48 hours for treatment (Tre.1) which contained 10% *Bif. lactis*, and from 8.65 log CFU/ml after 24 hours to 9.78 log CFU/ml after 48 hours for treatment (Tre.2) which contained 10% *Lb. acidophilus*.

As for the treatments of mixed cultures, a better improvement was achieved in increasing the counts of probiotics in all treatments. Treatment (Tre.5) showed the highest counts of available probiotics in both periods as compared to the rest of the treatments. Counts of probiotics increased from 10.58 log CFU/ml after 24 hours to 11.37 log CFU/ml after 48 hours for the treatment (Tre.5) that included 10% *Lb. acidophilus* and *Bif. lactis* (50:50). Results are consistent with what Gallo *et al*. (20) (21) reported about statistically significant difference in the probiotics counts in banana puree that was fermented after 24 and 48 hours, as bacterial concentrations of 3.3 x 10^8 CFU/ml were obtained after 24 hours, and 8.7 x 10^8 CFU/ml after 48 hours. Also it was reported that the immature banana puree requires a longer fermentation time by *Lb. paracasei* as compared to the ripe banana puree, and this is reflected in the count of bacteria and the pH reduction of the banana puree. This is probably due to the difference in the nutritional composition of bananas, especially with regard to the content of soluble sugars and the accessibility of these bacteria to these substances.

The superiority of increasing counts in the 48-hour fermentation period may be attributed to a sufficient adaptation to qualify the probiotic starter towards the consumption of nutrients and the production of different metabolic products. Studies have indicated that the growth of *Bifidobacterium* species can be better in a formula containing *Lactobacillus* species, which leads to a proteolysis that supports growth (40). In a study on Cladode pulp fermentation using some strains of lactic acid bacteria, an increase in the total content of free amino acids was found (6). Also, bananas contain prebiotics and oligosaccharides that promote the growth of *Lactobacillus* (23).

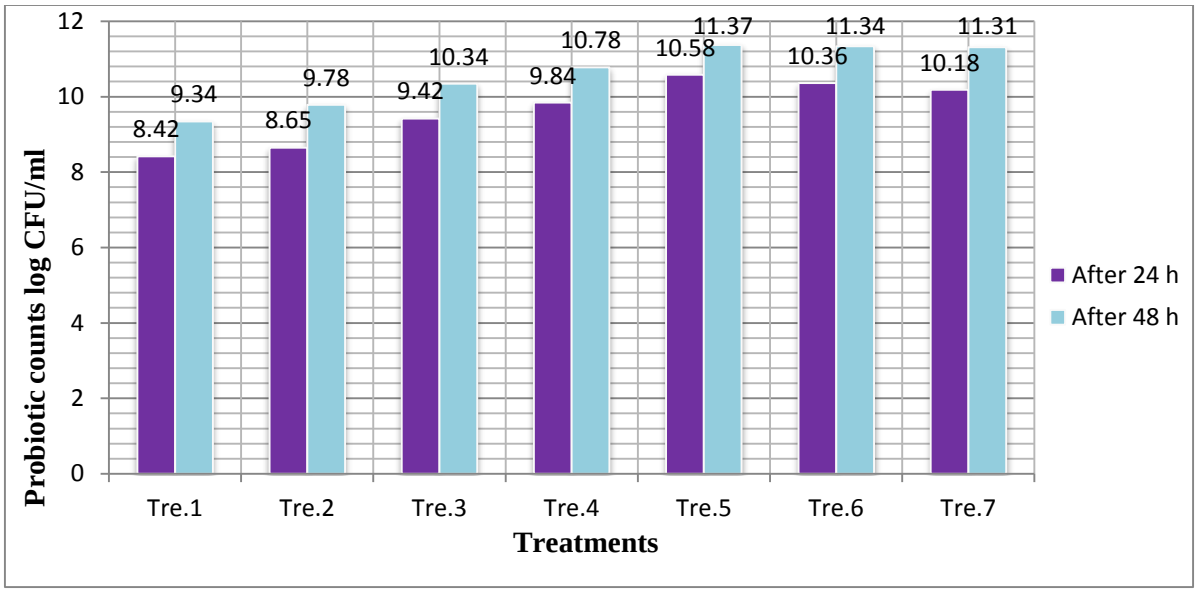

Figure 1. The viability of probiotics after fermentation for 24 and 48 h in fermented banana puree treatments

Guergoletto *et al*. (21) indicated that different vegetables, including bananas, can be sufficient substrates for the growth of lactic acid bacteria, especially species of *Lactobacillus* bacteria. They also reported a difference in the consumption of sugar content in the fermented juçara pulp according to the species of *Lactobacillus* bacteria, which makes the fermentation periods different and results in differences in the production of lactic acid. The ratio (1:1) of *Lb. acidophilus* and *Bifidobacterium* ssp. probably gives high counts of viable cells, as is the case when producing Acidophilus-Bifidus milk (40).

While Rekha and Vijayalakshmi (34) showed that fermentation of soybean milk after 24 hours led to the maximum growth of *Lb. acidophilus* B4496 (8.04 log CFU/ml), a marginal increase in the growth of these bacteria was observed with further incubation up to 48 hours (8.41 log CFU/ml). This is due to the highest activity of b-glucosidase enzyme when soybean milk was fermented for 48 hours with these bacteria. Also, *Lb. acidophilus* bacteria can produce exopolysaccharides (EPS) (11) as there components may form a protective layer on the surface of the bacteria, so they have a higher survival rate.

The population of mixed lactic acid bacteria (LAB) cultures (combination of five species including *Lb. acidophilus* and *Bif. bifidum*) in fermented banana juice peaked for 40 hours (10.47 log CFU/ml) as the viability of mixed cultures was higher than *Bif. bifidum* culture's because of the symbiotic relationship between these species (8). Regarding the green banana used in preparing the fermented puree in our study, we found that the count of bacteria increased significantly after the 48-hour fermentation period as compared to the fermentation period 24 hours.

pH values of fermented banana puree

Since the product is offered for human consumption, it is necessary for people to accept the taste of fermented banana puree. Therefore, the pH of all treatments was checked to see if the product is an extreme acidic, as people may not accept this product, especially when people are familiar with the natural product.

Table (1) indicated that there were statistically significant differences ($P \leq 0.05$) in the pH values of the seven treatments of banana puree fermented with probiotic formulations during two periods of fermentation (24 and 48 hours) which are caused by the difference in the amounts of lactic acid produced during the fermentation process and the interactions generated between the bacterial species during the fermentation. The pH in the single treatments decreased from 4.68 after 24 hours to 3.57 after 48 hours for treatment (Tre.1) which contained 10% *Lb. acidophilus* and from 4.76 after 24 hours to 3.68 after 48 hours for treatment (Tre.2) which contained 10% *Bif. lactis*.

The highest decrease in pH was recorded after 48 hours of fermentation in treatment (Tre.7) as included 10 *Lb. acidophilus* and *Bif. lactis* (90:10) which was 3.31, and the lowest decrease in pH was in treatment (Tre.3) included 10% *Lb. acidophilus and Bif. lactis* (10:90) which was 3.51. The decrease in pH was moderate in treatment (Tre.5) included 10% *Lb. acidophilus* and *Bif. lactis* (50:50) which was 3.42. A more decrease is observed in the treatments included high ratio of *Lb. acidophilus* as compared with *Bif. lactis* in the inoculum used in banana puree fermentation. This was evident in (Tre.6) and (Tre.7) treatments since *Lb. acidophilus* is an obligately homofermentative bacteria that produces lactic acid which is a single end product of the glycolysis of carbohydrates via Embden-Meyerhof (EM) pathway (32). *Bifidobacterium* produces lactic and acetic acid in a ratio of 3:2 by fermentation of glucose (2), this means an increase in the amount of lactic acid with an increase in the count of *Lb. acidophilus* which cause a higher acidity. Lactic acid is ten times more acidic than acetic acid because the pKa of lactic acid is less by 1 time than that of acetic acid (38).

Barba *et al*. (6) found that fermentation of Cladode pulp using starters containing *Lactobacillus* species at 30°C for 24 hours led to a decrease in pH from 4.3 to 3.98. The strains of *Lb. acidophilus* doubled the production of lactic acid by the incubation period 24–72 hours (31).

The fermentation period at 48 hours gives a greater decrease in pH as compared to the 24-hour fermentation period for all fermented banana puree treatments. This is supported by Reddy *et al*. (33) who reported the glucose concentration (%) in mango juice decreased to about 10% after 24-hours fermentation and then reached 7.2% after a 48-hour fermentation period. This suggests that sugar consumption and lactic acid metabolism was achieved in the fermentation period (30 - 48 hours) as the glucose concentration (%) was fixed after 48 hours of fermentation and further extension of the fermentation process (from 48 hours to 72 hours) did not give significant changes.

The decreased pH of the banana pulp puree are attributed to its prebiotic content, which in turn stimulates the metabolic activity of probiotics and the production of organic acids. In addition, production of lactic acid by starter culture of banana pulp puree treatments (*Lactobacillus acidophilus* and

Bifidobacterium lactis) should not be ignored during fermentation. The reason for increased acidity was reported as *Bifidobacterium* utilizes the complex polysaccharides and produces various organic acids (18).

Table 1. pH values of fermented banana puree treatments

Samples	pH values	
	After 24 h.	After 48 h.
Control (only banana pulp puree)	4.85	4.85
Tre.1: (10% *Lactobacillus acidophilus*)	4.68	3.57
Tre.2: (10% *Bifidobacterium lactis*)	4.76	3.68
Tre.3: *Lb. acidophilus* and *Bif. lactis* (10:90)	4.62	3.51
Tre.4: *Lb. acidophilus* and *Bif. lactis* (25:75)	4.58	3.49
Tre.5: *Lb. acidophilus* and *Bif. lactis* (50:50)	4.51	3.42
Tre.6: *Lb. acidophilus* and *Bif. lactis* (75:25)	4.47	3.38
Tre.7: *Lb. acidophilus* and *Bif. lactis* (90:10)	4.32	3.31
LSD value	0.492 *	0.755 *
* (P≤0.05).		

*** Statistically significant differences (P≤0.05)**

Chen *et al*. (13) support these findings as they reported that fermentation of papaya juice using *Lb. acidophilus* bacteria for 48 hours led to decreases the pH from 5.36 to 3.60 and decreases in the content of reducing sugar as a result of its consumption by the probiotic culture.

Results agree with what (6) reported on a recent study on the fermentation of green cactus fruit juice using probiotics including *Lactobacillus* species at 37°C for 48 hours, which reduced the pH from 5.4 to less than 4.5.

After we reached sufficient counts of probiotics and a good pH, we resorted to avoiding more incubation hours for more than 48 hours to avoid the accumulation of lactic acid causing a significant pH decrease and affects the physiology of bacteria and often causes a decrease in the LAB counts due by auto-acidification rather than the scarcity of nutrition. As the acidification of the cytoplasm and the failure of the proton stimulating force are the main reasons for the inhibition of LAB growth in fermentation (8).

Sensory evaluation of fermented banana puree

The characteristics of taste, odor, color and mouthfeel are among the factors that have been studied due to their contribution to the acceptance of products containing banana puree (30). The viscosity factor was also included in the sensory evaluation factors due to the banana puree has a complex rheological behavior in order to study the effect of fermentation to get a marked change in the rheological behavior of banana puree without using high temperature (17).

Results of sensory evaluation in Table (2) showed that there were significant statistical differences between the seven treatments of

fermented banana puree after a 24-hour fermentation period presented and after a 48-hour fermentation period.

Treatments (Tre.1) and (Tre.2) were similar in terms of taste at a regular level after a period of fermentation 24 hours. The improvement in taste occurred after a period of fermentation of 48 hours, since the species of *Bifidobacteria* spp appear generally, slow or limited growth as compared to the other lactic acid bacteria (7). As for the first treatment (Tre.1) that included a single culture of *Bif. lactis*, and for the second treatment (Tre.2) that included a single culture of *Lb. acidophilus*, as *Lb. acidophilus* doubles the production of lactic acid after a 48-hour fermentation period (31).

Bifidobacteria ferment glucose via a pathway called "bifidus" that leads to the production of 1 molecule of lactic acid and 1.5 molecules of acetic acid from the fermentation of one glucose molecule, while *Lb. acidophilus* produces lactic acid as a major product of glucose fermentation (2). The difference in the fermentation products of both species is reflected in the taste of the treatments resulting from mixed cultures in the treatments (Tre.3, Tre.4, Tre.5, Tre.6 and Tre.7).

The taste of the treatments that included mixed cultures was delicious because of lactic acid in the fermented product, which may give its delicious taste (9) with an amount of acetic acid that was within the acceptable limit in most of the treatments. It is well known that eating green bananas leaves a chalky aftertaste, which is the taste of resistant starch (26), but fermentation using *Lb. acidophilus* reduces the chalky flavor and that fermentation of food by *Lb. acidophilus* contributes to the unique taste, flavor and texture of food (5), taking into account that the last treatment (Tre.7) showed a slight decrease in the acceptance of the panelists, this may be due to the excessive growth of *Bifidobacterium*, which led to the development of acetic acid production giving the vinegar-like taste and smell (9). Yet, it was still accepted by the consumer.

Lactobacillus acidophilus culture is homofermentative, and therefore, has a high ability to metabolize the lactose present in milk into lactic acid (32). In addition to the possibility of *Lb. acidophilus* strains to change their fermentation profile from homofermentative to mixed acid fermentation depending on the composition of the media (9) . The results of treatments (Tre.3, Tre.4, Tre.5, Tre.6 and Tre.7) which contained mixed cultures of *Lb. acidophilus* and *Bif. lactis* are consistent with the study of Nguyen *et al*. (29) who found the nitrogen and carbon sources in pineapple juice are sufficient for LAB and resulted in very good molar ratios of lactic acid to acetic acid, providing a good taste to the fermented product, especially in the presence of lactic acid and a reasonable percentage of acetic acid. This was evident in the treatment (Tre.5), which included the use of a starter consisting of equal proportions of *Lb. acidophilus* and *Bif. lactis* (50:50). Also, *Lb. acidophilus* can produce exopolysaccharide (EPS) which it helps in improving taste of foods (37). Also, there is high content of carbohydrates in bananas that contains sugar and has sweetness (36).

Fermented banana was not heated, rather it was treated with alcohol (70%) since the vitamin C is easily oxidized at high temperatures (13). Therefore, the heating process was avoided to preserve the color

and the healthy benefits of the puree. Similar results were reported by Chen *et al*. (13) who mentioned that fermentation of papaya juice using *Lb. acidophilus* bacteria for 48 hours significantly decreased the content of reducing sugar as a result of its consumption by the probiotic culture. However, papaya fruit juices still contain a high percentage of reducing sugar, which preserved sufficient sweetness of the product. Davidson *et al*. (14) indicated that yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium* gives a milder flavor and aroma by balance in levels of organic compounds that improve the flavor.

Regarding the color characteristics, the color of all the fermented banana puree treatments was approved by the panelists, with significant differences due to the nature of probiotic starter combination used in each treatment. Because the pulp of bananas is rich in flavonoids (36), these compounds are responsible for the natural, desirable yellow color of bananas (19).

Lactobacillus and *Bifidobacterium* can metabolize the flavonoids during the fermentation process (15), all treatments maintained the level of color of the fermented banana puree that is acceptable to the consumer. However, there was a slight discrepancy in the color of the seven banana puree treatments mentioned in our study, and it was prominent in the treatment (Tre.1), which was represented by using a starter consisting of *Lb. acidophilus* bacteria. It may be due to the release of bioactive compounds (such as catechin, gallic acid, and quercetin) after fermentation with *Lactobacillus* strains resulting from the degradation and hydrolysis of phenolic compounds. Studies indicate a decrease in total flavonoid content (TFC), total tannin content (TNC), and total phenol content (TPC) after the fermentation process (1). However, changes in the color of product by fermentation do not necessarily represent a negative influence on consumer acceptance and preferences for foods.

Regarding the viscosity properties, the results of our study showed that treatments (Tre.1) and (Tre.2) were close in terms of viscosity at the normal level after a period of 24 hours fermentation, but the viscosity increased after the 48 hours fermentation period. Banana puree is described as a pseudoplastic fluid and a thixotropic fluid (17). Viscosity of all fermented banana puree treatments was well accepted by panelists after 24 and 48 hours of fermentation, with significant differences between treatments due to the nature of the starter probiotic formulation used in each treatment. It was noted that the viscosity of all treatments improved after a 48-hour fermentation period, especially the treatment (Tre.5), which received the highest evaluation by panelists as compared to the rest of the treatments. This may be attributed to the EPS produced by *Lactobacillus* bacteria (37).

Results showed acceptable degrees regarding the characteristics of mouthfeel for all treatments after 24 hours of fermentation, and this may be attributed to bananas containing resistant starch. Resistant starch is characterized by physicochemical properties particularly showed improved crispness and expansion as compared to products that included traditional insoluble fibers, beside that the resistant starch is characterized by low water holding capacity, and stability at high processing temperatures (35).

de Souza *et al*. (16) indicated that green bananas are rich in resistant starch (R.S) especially RS type 2, which is used as a fat substitute and improves the softness of cake batter. Fermentation for 48 hours led to an improvement in the mouthfeel in all treatments. This may be because the 48-hour fermentation period increased the count of probiotics (34). *Lactobacillus* can produce Exopolysaccharides (EPS) which have unique physical and chemical properties in the food industry such as viscosity, gel, thickener and emulsifier (37). Through the results of viable counts, pH values and sensory evaluation of fermented banana puree. we found that the fifth treatment (Tre.5) is the most appropriate for use in the various food applications, and the fermentation period 48 hour is the best according to the previous results, and achieving the maximum antimicrobial activity (25).

Table 2. Sensory evaluation of fermented banana puree treatments

Properties (Every factor: 20 scores)	Treatments							LSD value
	After a 24-hour fermentation period							
	Tre.1	Tre.2	Tre.3	Tre.4	Tre.5	Tre.6	Tre.7	
Taste	12	11	14	13	15	14	12	1.73 *
Odor	11	10	12	14	15	13	14	2.08 *
Color	12	15	14	13	15	15	14	1.89 *
Viscosity	13	12	11	12	14	13	14	1.66 *
Mouthfeel	11	10	12	13	15	14	13	1.72 *
Total 100%	59%	58%	63%	65%	74%	69%	67%	6.41 *
* (P≤0.05).								
Properties (Every factor: 20 scores)	Treatments							LSD value
	After a 48-hour fermentation period							
	Tre.1	Tre.2	Tre.3	Tre.4	Tre.5	Tre.6	Tre.7	
Taste	15	14	17	16	18	17	15	2.55 *
Odor	14	13	15	17	18	16	17	2.09 *
Color	14	16	16	15	17	17	16	1.87 *
Viscosity	15	14	13	14	16	15	16	1.79 *
Mouthfeel	13	12	14	15	17	16	15	2.33 *
Total 100%	71%	69%	75%	77%	86%	81%	79%	5.08 *
* (P≤0.05).								
Quality level based on scores								
1 to 4	5 to 8		9 to 12		13 to 16		17 to 20	
Awful	Bad		Regular		Good		Excellent	

* Statistically significant differences (P≤0.05)

Antibacterial activity of probiotics cultures

Table (3) showed the antibacterial activity of individual cultures of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium lactis* BB12 bacteria and their mixed culture against a group of gram-negative and positive bacteria. The inhibition zones were obtained by culture of *Bif. lactis* BB12 with diameters (13, 11, 14, 15, 12, 9) mm of species: *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus sterothermophillus*, and *Streptococcus mutans* respectively. It was noted that the inhibition by *Bif. lactis* BB12 was higher than the inhibition by *Lb. acidophilus*, because of the accumulation of acetic acid by *Bif. lactis* BB12, showing higher antibacterial activity as compared to lactic acid (25).

The inhibition zones were obtained by culture of *Lb. acidophilus* LA-5 with diameters (11, 13, 12, 10, 11, 8) mm of species: *Sallmonela typhimurm*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus sterothermophillus*, and *Streptococcus mutans* respectively. These activities result from the ability of *Lactobacillus acidophilus* to produce bacteriocins that may cause antimicrobial activity. Bacteriocins of *Lb. acidophilus* include acidophilucin A, lactacin B, lactacin F, acidocin A, acidocin B and others (5). The antibacterial activity of *Lb. acidophilus* can also attributed to the production of lactic acid, hydrogen peroxide, carbon dioxide, diacetyl, and exopolysaccharides (EPS) (11). Al-Mathkhury and Hasan (3) reported that *Lactobacillus* species was able to inhibit a variety of pathological microorganisms through different factors such as the production of bacteriocins, H_2O_2, and biosurfactants.

Hasan et al. (22) reported the inhibitory activity of *Lactobacillus acidophilus* supernatants against *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*. This activity is increased by the bacteriocin. *Lb. acidophilus* also has the ability to inhibit the biofilm formation of *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus mutans*, resulting from some anti-biofilm compounds such as exopolysaccharide and bio-surfactants (24).

The diameters of the inhibition zones were obtained by mixed culture of *Lactobacillus acidophilus* and *Bifidobacterium lactis* BB12 which reached (16, 17, 15, 17, 15, 11) mm of *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus sterothermophillus* and *Streptococcus mutans*, respectively. This result supported by Mathipa and Thantsha, (27) who indicated that a probiotic combination (or cocktail) of *Bifidobacterium* and *Lactobacillus* species leads to better inhibition of pathogens, especially *Escherichia coli* and *Staphylococcus aureus*.

Table 3. Antibacterial activity of probiotics cultures used in the study

The target species	Clear zone size (mm)		
	Lacobacillus acidophilus LA-5	*Bifidobacterium lactis* BB12	*Lacobacillus acidophilus* LA-5 +

			Bifidobacterium lactis BB12
Salmonella typhimurium	11	13	16
Escherichia coli	13	11	17
Staphylococcus aureus	12	14	15
Pseudomonas aeruginosa	10	15	17
Bacillus sterothermophillus	11	12	15
Streptococcus mutans	8	9	11
LSD value	2.57 *	2.41 *	2.37 *

* ($P \leq 0.05$).

* Statistically significant differences ($P \leq 0.05$), 3 discs (8 mm in diameter) per trail

Conflict of Interest

The authors have no conflict of interest.

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