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RESEARCH ARTICLE

The Effect of Adding Probiotics on Microbial and Organoleptic Characteristics of Beef Burger Product

Asraa Yacoob Yousif*, Eftekhar Hassen Muhssen

Animal Production Department-Agriculture College-Basrah University-Iraq.

***Corresponding Author: Asraa Yacoob Yousif**

Abstract

The study examined the use of single and mixed probiotic isolates for the possibility of prolonging the preservation duration of meatballs in refrigeration. Chemical tests were conducted (free fatty acids, TBA and pH), sensory tests (color, flavor, juicy, tenderness and general acceptance), noting that the control sample was excluded from the experiment on the tenth day of conservation because the pH value exceeded the limit and according to the standard specifications. Samples of treatments with single and mixed probiotic isolates reached the allowable limits on the 11thday. A significant decrease in TBA and free fatty acid was observed in the single and mixed probiotic isolates compared with the control treatment during the preservation period. It was observed during the preservation period that the control sample was excluded from the experiment on the seventh day of preservation because the numbers of total bacteria and psychrophilic bacteria exceeded the permissible limits according to the standard specifications. The results of sensory evaluation showed a significant superiority of the probiotic isolates treated meat samples compared to the control sample, which improved all the sensory characteristics (color, flavor, juicy, tenderness and general acceptance). The highest of the sensory evaluation were in the mixed probiotic isolates treatments.

Keywords: *Probiotics, Organoleptic, Beef burger.*

Introduction

Meat is protein-rich food that can be damaged in a short time, with a high degree and has a short shelf life [1].Pollution is rapidly obtained by the pathogenic microorganisms found in animals before slaughter. There are several factors that lead to changes in the quality of meat while damage caused by microbial growth and its direct relation with meat quality [2].

Previously, because of the poor storage system led to the necessity of additives to meat to improve the storage process [3].Probiotics are commonly known as live microbes that provide health benefits to the consumer and are commonly used in commercial products, including *Lactobacillus* and *Bifidobacterium* where they have been developed and marketed in America and the Far East [4], the importance of bifid bacteria in digestion and digestive system for man encourages the use of these products [5].Also, its health benefits are providing the body with proteins, vitamins and activity against bacteria, treat liver damage, against cancer, stimulate the immune system and reduce levels of cholesterol [6], probiotics spread in many products such as yoghurt and milk [7, 8].It has been used for thousands of years to produce fermented food for its ability to produce changes in taste, flavor and texture so that to prevent disease-causing microorganisms such as a group of gram-positive bacteria that's pared widely in nature [3].

Probiotics kill bacteria that cause disease and spoilage such as Enterobacteria, strengthens the immune system and maintains the starter bacteria when adding it to yoghurt for more than 35 days [9].The study aimed to figure out the effect of the addition of probiotics on the preservation duration of refrigerated meat and testing the chemical, microbial and organoleptic characteristics.

Materials and Methods

Meat: was purchased from the local markets of the thigh area and keeping it in the refrigerator temperature of 4 °c for only one

day, the next day the process of cutting it completely from the flesh was done, after that the process of chopping by the electric chopper was done (diameter of the electric choppers to 4 mm).Fat: Bovine calf fat (around the kidneys) was purchased from the local markets and kept refrigerated at a temperature of 4 °c for only one day and chopped by the electric chopper.

Process of manufacturing and processing chopped meat

The meat was chopped by electric chopper and divided into three treatments

- 1000 g meat (Control treatment without addition).
- 1000 g meat + single probiotic isolate (10^9cfu/gm of *L. acidophilus*).
- 1000 g meat + mixed probiotic isolates10^9cfu/gm of *(L. rhamnosus*) (*L. plantarum*) (*L. salivarius*).

After that, a 100 g per burger disk was filled in polyethylene bags, vacuum-packed, closed well and kept refrigerated at a temperature of 4 ° C for different periods of time.

Chemical and Physical Tests of Burger Disks

Free Fatty Acid Percentage

Free fatty acids (FFA) were determined based on [10]. 3 g of finely chopped meat was weighted and added to 50 ml ethyl alcohol at a concentration of 98%, heated in a water bath until boiling; drops of the phenolphthalein indicator were added. The mixture was titrated with 0.1N potassium hydroxide solution until the solution turns to light pink and the percentage of free fatty acids was estimated by using the following equation:

$$Free\ Fatty\ Acid\% = \frac{Titration\ (A-B)\ x\ n\ x\ 282\ x\ 100}{1000\ x\ wt\ o\ sample\ gm}$$

A = Number of milliliters of KOH dissolved with a sample of fat or oil
B = the number of milliliters of KOH dissolved with the sample blank
282 = molecular weight of oleic acid

TBA Estimation Method

The technique was adopted by [11]. The TBA reagent was present from dissolving 0.2883 g of TBA in 90% acetic acid solution and to accelerate the solubility in a water bath using warmer heating and the volume then completed to 100 ml.10 g of sample is taken and mixed with 47.5 ml of distilled water and 2.5_3 ml of HCL solution is added (4N) to reduce PH to 1.5 and then 100 ml of distilled water is completed. Transfer the contents of the volumetric flask to a 250ml distillation flask and add 2 ml paraffin and one of the glass balls then connect the device and heat the heater as a thermal until you collect 50 ml of distilled liquid in 10 minutes.

Take 5 ml of distilled steam and mix with 5 ml of TBA reagent in a tightly sealed glass tube. The contents of the volumetric flask were then transferred to a 250ml distillation flask and added 2 ml paraffin oil and one glass pellet. Then, the device is connected and an electric heater is used to heat up the contents until 50 ml of distilled fluid is collected in 10 minutes.5 ml of distilled steam is taken and mixed with 5 ml of TBA reagent

in a tightly sealed glass tube. At the same time, a blank sample was placed with 5 ml of distilled water with 5 ml of reagent in a test tube. The tubes were then closed and then moved slightly and placed in a boiling water bath for 35 minutes. The tubes were then cooled for 10 minutes and the spectrophotometer was measured by a wavelength of 538 n m The TBA number is calculated from the following law:

TB ANO mg / kg meat) = 7.8 x Absorbance

PH Determination

The pH was calculated according to [12] using pH meter

Bacteriological Tests

Pour plate method was used in conducting the total count and psychrophilic bacteria numbers. Petri dished were incubated at 37 ° C for 24-48 h. for the total count. For

psychrophilic bacteria, Petri dishes were incubated in refrigerator at 4 ° C for 10 days.

Organoleptic Characteristics Test

It was carried out according to [13].

Statistical Analysis

The data were analyzed by using Complete Randomized Design (CRD) and using the SPSS program [14]. The averages were compared using the least significant difference of (LSD) at (P<0.05).

Results and Discussion

Table (1) shows the value of pH where we note from day zero that the value of pH was equal to all samples, which was 5.91. We note that the value of pH has recorded a significant change during the days of storage where we notice a significant increase in the value of pH at the level (P> 0.05) in control treatment compared to other treatments which wherein the seventh day of the storage of 6.93 in control treatment while in the treatments of single and mixed probiotics to 6.22 and 6.03, respectively, it noted that increasing storage period will increase the pH value because of the process of protein degradation to free amino acids and the release of ammonia that increase the basal state in the medium [15, 16].Similar results were mentioned by [17, 19].

Table 1: Effect of the use of Probiotics on the value of pH in refrigerated meat tablets

Days storage periods Transactions	0	2	5	7	9	10	11	Average
the control	5.91	6.03	6.45	6.93	7.33	_	_	6.53
Single probiotic	5.88	5.50	5.82	6.22	6.45	6.79	7.21	6.33
Mixed probiotics	5.82	5.61	5.81	6.03	6.23	6.70	7.15	6.25
Average	5.87	5.71	6.02	6.29	6.67	6.74	7.18	6.37

For periods L.S.D =0.14 treatment type L.S.D =0.13

Table (2) shows the impact of probiotics on the values of TBA during the cooling storage period. It is noticed from table (2) that there is a significant increase at (p<0.05) in control treatment compared to the other treatments. TBA value of control treatment reached to 2.53mg/kg and begins to damage on the seventh day of storage compared with the treatment of single and mixed probiotics which reached to 0.55 and 0.41 mg / kg, respectively. It was noticed that the treatment of single and mixed probiotics have continued to maintain the value of TBA within the acceptable limits, which reached at the tenth day in the single and mixed treatments (1.93 and 1.45) mg / kg respectively, this result shows that the single and mixed probiotics have led to a decrease in TBA value during cooling storage periods compared with the control treatment, which reached the maximum storage period which was seven days as the probiotics limited the growth of microorganisms that cause pollution and decay of meat because of the production of lipase enzyme that causes fat oxidation and this result is consistent with [21]who used lactic acid bacteria in the manufacture of sausage and led to lower TBA values during storage periods. These results are in contrast with [19, 21].

Table 2: Effect of the use of probiotics on TBA values of mg / kg. In refrigerated meat tablets (mg)

Days storage periods Transactions	0	2	5	7	9	10	11	Average
the control	0.15	1.53	2.03	2.53	_	_	_	1.89
Single probiotic	0.13	0.12	0.30	0.55	1.03	1.93	2.75	1.12
Mixed probiotics	0.11	0.19	0.28	0.41	0.73	1.45	2.65	0.95
Average	0.19	0.64	0.87	1.16	0.88	1.69	2.7	1.32

For periods L.S.D =0.043-treatmenttype L.S.D =0.032

Free Fatty Acids

Table (3) shows a significant decrease (P <0.05) in the ratio of free fatty acids in meat tablets which treated with probiotics compared to the control treatment. The control treatment was excluded on the seventh day as the ratio of free fatty acids has exceeded the allowable limits as it reached 1.52 while the meat tablets treated with probiotics maintained their survival within the acceptable limits to the tenth day. This may be due to the decrease in the

number of pathogenic bacteria in the tablets treated with both types of probiotics which discouraged the production of lipase enzyme which is produced by some types of pathogenic bacteria which work on the fat rancidity.

Table 3: Effect of the use of probiotics on the percentage of free fatty acids in refrigerated meat

Days storage periods Transactions	0	2	5	7	9	10	11	Average
the control	0.22	0.61	1.98	1.52	_	_	_	1.08
Single probiotic	0.20	0.38	0.59	0.74	0.83	0.92	1.53	0.74
Mixed probiotics	0.19	0.31	0.62	0.70	0.74	0.81	1.50	0.69
Average	0.20	0.43	0.58	0.98	0.78	0.86	1.51	0.83

For periods L.S.D = 0.17- For treatment type L.S.D = 0.12

Table (4) shows the total number of bacteria in meat tablets where the number of bacteria increased on the second day of storage in the control treatment at 63.67 × 10^4CFU/g compared with the single and mixed probiotics 31.67 × 10^4CFU/g, 25.03× 10^4CFU/g respectively. It was noticed that the total counts of bacteria in control treatment increased and exceeded the limits of the standards on the seventh day and therefore were excluded because they were unsuitable for human consumption due to pollution, while the single and mixed probiotics treatments preserved the frozen beef tablets for 10 days to keep the numbers of bacteria within the standard limits, where it was noticed that the superiority of the mixed probiotics on the rest of the treatments. It can, therefore, be used successfully to inhibit the growth of harmful bacteria and maintain the quality of frozen meat for the longest possible storage time [22].

Table 4: Effect of the use of probiotics on total bacterial numbers

Days storage periods Transactions	0	2	5	7	9	10	11	Average
the control	22.33 x10^4	63.67 x10^4	97.35 x10^5	1.9 x 10^7	_	_	_	46.31
Single probiotic	21.33	31.67 x10^4	45.93 x10^4	68.34 x10^4	73.33 x10^5	85.01 x10^5	18x10^7	53.71
Mixed probiotics	20.28	25.03 x10^4	40.65 x10^4	66.46 x10^4	70.20 x10^5	80.55 x10^5	12x10^7	49.14
Average	21.31	40.12	61.31	45.56	71.76	82.78	15	49.72

For periods L.S.D =15.12 - For treatment type L.S.D =19.20

Table (5) shows the number of psychrophilic bacteria, it was noticed that the number of psychrophilic bacteria in the control sample at the second day of storage increased and reached to 55.33×10^4cfu/g compared with the single and mixed probiotics treatments which reached to 25.02×10^4cfu/g, 28.37×10^4cfu/g respectively. It was noticed that the control treatment was excluded on the seventh day of storage, where the number of psychrophilic bacteria exceeded the standard specifications of the number of psychrophilic bacteria where it reached 11.01×10^4cfu/g, while the samples of the single and mixed probiotics treatments were kept within the limits of 7, 9 and 10 days of the storage period, their mean reached on day 10, 7.21×10^5 CFU/g, 72.37×10^5 CFU/g respectively. On the eleventh day, the number of psychrophilic bacteria in the samples of meat tablets exceeded the standard quality, so the probiotic worked on keeping the meat tablets in the refrigerator for ten days and this is due to the probiotics that produce antimicrobial products which inhibit the harmful bacteria [22].

Table 5: Effect of using probiotics on psychrophilic bacteria counts in refrigerated meat

Days storage periods Transactions	0	2	5	7	9	10	11	Average
the control	22.33 x10^4	63.67 x10^4	97.35 x10^5	1.9x10^7	_	_	_	46.31
Single probiotic	21.33	31.67	45.93	68.34	73.33	85.01	18 x10^7	53.71

		$x10^4$	$x10^4$	$x10^4$	$x10^5$	$x10^5$		
Mixed probiotics	20.28	25.03 $x10^4$	40.65 $x10^4$	66.46 $x10^4$	70.20 $x10^5$	80.55 $x10^5$	12 $x10^7$	49.14
Average	21.31	40.12	61.31	45.56	71.76	82.78	15	49.72

For periods L.S.D =18.22-For treatment type L.S.D =12.33

Table (6) shows a significant increase (P <0.05) for most of the sensory characteristics of the meat tablets treated with probiotics in both the single and mixed types compared to the control sample which was excluded on the seventh day of the sensory evaluation during the period of cold preservation, mixed probiotics meat tablets have been superior especially for flavor and general acceptance, highest increase for flavor and general acceptance was during the first five days of conservation. The reason may be due to the development of some desired flavors in the meat tablets due to the probiotics which give acid flavors that acceptable from the consumer. That is beside the decrease in the ratio of free fatty acids, the value of TBA and the number of total bacteria during the period of conservation compared to the control sample, which was reflected positively on the ratings of the sensory qualities of the probiotics meat tablets. These results are in contrast with [19].

Table 6: Effect of the use of probiotics on sensory properties in meatballs stored in the refrigerator

Transactions	Adjective Storage time	the color	Flavor	The coolness	Juicy	general admission	Average
the control	0	7.75	7.68	7.70	7.65	7.74	7.70
	2	7.70	7.53	7.57	7.52	7.57	7.57
	5	6.33	6.40	6.72	6.64	6.48	6.51
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
Average		7.26	7.20	7.33	7.27	7.26	7.26
Single probiotic	0	7.83	7.79	7.75	7.71	7.78	7.77
	2	7.92	7.88	7.78	7.74	7.90	7.84
	5	7.95	7.96	7.79	7.76	7.99	7.89
	7	7,80	7.72	7.50	7.43	7.70	7.63
	9	7.00	7.11	7.07	6.00	7.13	6.86
	10	6.22	6.68	6.62	6.56	6.60	6.53
Average		7.47	7.52	7.41	7.20	7.51	7.42
Mixed probiotics	0	7.92	7.83	7.79	7.75	7.80	7.81
	2	7.97	8.30	7.88	7.80	8.38	8.06
	5	7.99	8.48	7.89	7.87	8.50	8.14
	7	7.83	8.00	7.60	7.48	7.97	7.77
	9	7.10	7.44	7.38	7.26	7.50	7.33
	10	6.35	6.80	6.71	6.74	6.84	6.68
Average		7.52	7.80	7.54	7.48	7.83	7.63

For periods L.S.D == 0.26-For treatment types L.S.D =0.31

Conclusion

The addition of the probiotics led to the prolongation of meat conservation and improvement of physical, chemical, microbiological and sensory properties of probiotics meat tablets compared to control treatment by preventing the growth of harmful bacteria which lead to meat degradation during cold preservation.

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