THE BIO-PRESERVATION OF BUFFALO MEAT MANUFACTURED (PASTRAMA) BY USING *LACTOBACILLUS PLANTARUM* BACTERIA

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ABSTRACT

Bacterial starter *Lactobacillus plantarum* at concentration of 5% was added to the pastarma which was manufactured by using buffalo meat at ratio of 3 meat: 1fat. The fermentation process was done at 37 C° and relative humidity of 80-85% for 48 hours for each inoculated sample. After this process, spices and garlic were added, the pastrama was stuffed in natural covers. The pastrama was matured at 15-17 C° and relative humidity degree of 75-80% for 3 weeks. This study was carried out for following effect of the bio preservation by using *Lactobacillus plantarum* in the buffalo meat pastarma the microbial tests of the meat mixture showed that the total count of microorganisms, *coliform* bacteria, molds and yeasts count, the *Staphylococci aureus*, and the lactic acid bacteria count were 64×10^4, 68×10^2, 41×10^2, 27×10^2 and 62×10^2 CFU/ g respectively. The moisture, protein . fat and ash %, pH, free fatty acids ratio, cholesterol concentration, peroxide value and Thiobarbituric acid value in the buffalo meat were 73.04, 20-98, 2.81and 1.51%, 5.46, 0.09%, 105.22 mg 100gram-1 meat, 0.80 meq O2 kg-1 and 0.28 mg malonaldehyde kg-1 respectively. The total count of bacteria, the psychrophilic bacteria were for each, coliform bacteria count, the Staphylococci aurus count, lactic acid bacteria count and yeasts and molds count of pastrama mixture were recorded 57×104, 65×102, 68×10^2, 14×10^2, 56×10^2 and 37×10^2 CFU g-1 respectively. The moisture, protein, fat , and ash % were 61.50, 15.11, 20.93 and 1.66% respectively, pH, free fatty acid ratio, cholesterol concentration, non- protein nitrogen, peroxide value and Thiobarbituric acid value were 5.54, 0.12%, 135.60 mg 100g-1 meat, 1.91%, 0.62 meq O2 kg-1 and 0.66 mg malonaldehyde kg-1 respectively.

Key words: Bio-Preservation, Buffalo Meat, pastrama. Lactobacillus plantarum
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الحفظ الحيوي للبسطرمة المصنعة من لحم الجاموس باستعمال بكتيريا *Lactobacillus plantarum*

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المستخلص

تم إضافة بكتيريا البادئ اللاكتوباسلس بلانتارم بتركيز 5 %، الى البسطرمة المصنعة ا من لحم الجاموس وبنسبة 3 لحم: 1 دهن، واجريت عملية التخمير عند 37م ورطوبة نسبية 80– 85% ولمدة 48 ساعة للمعاملة الملقحة بالبكتيريا. بعد انتهاء عملية التخمير تم إضافة الثوم والتوابل وتم تعبئة البسطرمة في الاغلفة الطبيعية. نضجت البسطرمة في درجة حرارة– 15–17 م ورطوبة نسبية 75–80% لمدة 3 أسابيع. الغرض من البحث متابعة تأثير الحفظ الحيوي باستخدام ***Lactobacillus plantarum*** للبسطرمة المصنعة من لحم الجاموس ، أظهرت الاختبارات المايكروبية لخليط اللحم ان العدد الكلي للاحياء المجهرية ، عدد كل من بكتريا القولونية ، و الخمائر والاعفان ، المكورات العنقودية ايريس ، و بكتريا حامض اللبنيك كانت $64*10^4$ ، $68*10^2$ ، $41*10^2$ ، $27*10^2$ و $62*10^2$ وحدة مكونة للمستعمرة لكل غم (CFU/g) على التوالي.وكانت النسب المئوية للرطوبة ، البروتين ، الدهون و الرماد و قيمة الاس الهايدروجيني و نسبة الاحماض الدهنية الحرة و تركيز الكولوسترول و قيمة البيروكسيد و قيمة حامض الثايوباربتورك 73,04 ، 20,98 ، 2,81 ، 1,51 % ، 5,46 ، 0,09 % ، 105,22 ملغم/100 غم لحم ، 0,80 meq/kg ، 0,28 ملغم مالوندهايد على التوالي.بينما كانت قيم حساب عدد البكتريا الكلي ، البكتريا المحبة للبرودة ، بكتريا المكورات العنقودية ايريس ، بكتريا حامض اللبنيك و الخمائر والاعفان لخليط البسطرمة 57*104 ، 65*102 ، 68*102 ، 14*102 ، 56*102 و 37*102 وحدة مكونة للمستعمرة لكل غم (CFU/g) على التوالي. وأن النسب المئوية لكل من الرطوبة ، البروتين ، الدهن والرماد لخليط البسطرمةكانت 61,50 ، 15,11 ، 20,93 و1,66 % على التوالي و قيمة الاس الهايدروجيني ، نسبة الاحماض الدهنية الحرة ، تركيز الكولوسترول ،النايتروجين غير البروتيني ، قيمة البيروكسيد ، قيمة حامض الثايوباربتورك 5,54 ، 0,12 % ، 135,60 ملغم/100 كغم لحم ، 1,91 % ، 0,62 meq/kg و 0,66 ملغم مالونلدهايد/كغم على التوالي.

كلمات مفتاحية: الحفظ الحيوي ، لحم الجاموس ، الباسترما ، بكتريا lactobacillus plantarum
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INTRODUCTION

Fermentation is one of the old ways to keep food. It always leads to make food products safer to consumption as well as it enhances meat traits especially sensory characteristics. In years, fermentation of meat, demands on probiotics as food additives, to produce a group of functional foods that promote consumer health (11). The fermented sausage is a safe product for consumption. It stands safety against contamination which come from the growth of pathogenic or food spoilage microorganisms under unsuitable storage conditions. Usually the growth of these neighbors reduces pH (4.5-5) and that causes reduction of water activity(a_w). All of these factors prevent the growth of harmful bacteria, but at the same time the organisms used in conservation should be able to grow and have useful metabolic activities (5). This process encourages the growth of microorganisms that improve sensory and tactile characteristics and improve safety in addition to many other key qualities of the product (10). Due to the importance of these bacteria (*Lactobacillus plantarum*) and the possibility of using them in the fermentation and manufacture of fermented meat products, especially pastrama, which is one of the outstanding Iraqi products privacy, and also for the lack of studies and research on them, The idea of this study was the following: The effect of the use of bacteria (*Lactobacillus plantarum*) as a means of conserving factor of pastarma bacterium made of buffalo meat and evaluate up its effect in improving the qualitative and sensory characteristics and extend the shelf life.

MATERIALS AND METHODS

Method of manufacturing the pastrama:the manufacture of pastrama is as follows: mixture of the meat and fat were cut pure using an electric chopping machine , then add both table salt and sugar were ratio of 1.5 and 0.75% weight / weight added to the pure meat mixture and fat at and respectively, the activated bacteria were added homogenously to the chopped mixture at concentration of (2.5 & 5%) volume / weight of the bacteria ,the source of bacteria is local isolation incubated at 37 ° C for 48 hours ,Then the fresh spices and garlic were added to the chopped mixture by 0.5% & 1% weight / weight respectively The finely chopped pastrama mixture was then filled with natural casings, and sealed with cotton threads coated with wax .Adopted the method mentioned before (3) In the estimation of moisture, protein, fat and ash. The pH was estimated according to the method given in (6). The method in (3) was used in the estimation of peroxide. The method described by Antonopoulos and (2) was used in the estimation of non-protein nitrogen (NPN). The Pour-plate method (4) was used to estimate the total count of bacteria, psychrophilic bacteria, *coliform bacteria* count, molds and yeasts count, the (15) method was used to estimate *staphylococci aureus* and the lactic acid bacteria count. The method described by (13) was used to estimate the degree of visual sensory evaluation and to assess sensory scores. The data were analyzed according to a laboratory experiment applied with full random design (CRD). The averages are compared with the Duncan Multidimensional Test.

RESUITS AND DISCUSSION

Table 1 shows the chemical tests of the manufactured pastrama when adding *lactobacillus plantarum* with a concentration of 5%, the differed percentage of moisture in the manufactured pastrama model during the fermentation and period of maturation, the humidity was 54.63% after (48 hours) of fermentation but adding *Lb. plantarum* bacteria by 5%, The table above shows the decrease in the moisture percentage after fermentation (48 hours) and the period of maturation, the humidity ratio is directly proportioned to the concentration of the bacterial starter. The percentage of moisture in the third and final week of ripening was 5% when the bacteria were added by 5%

compared to the control treatment in which the percentage of moisture in the reduced to 40.85% in the third week of maturation the reason for is explained by the activity of *Lb. plantarum*, which reduces the moisture content and ferments the monosaccharides and the production of lactic acid, the reducing of pH, which reduced the protein's ability to carry water (1).

Table 1. Chemical tests of the manufactured Pastrama when adding *lactobacillus plantarum* bacteria at a concentration of 5%

Shelf life	*Humidity%*	*Protein%*	*Fat%*	*Ash%*
48 hours	**54.63 c**	**18.10 h**	**24.51 j**	**1.86 g**
Control	**0.00 l**	**0.00 i**	**0.00 l**	**0.00 i**
7 days	**49.42 f**	**20.78 e**	**26.45 g**	**1.92 f**
Control	**55.32 b**	**16.56 j**	**24.73 i**	**1.97 f**
14 days	**45.10 i**	**22.37 c**	**29.10 d**	**2.42 c**
Control	**53.00 d**	**16.34 k**	**27.20 f**	**2.06 e**
days 21	**40.85 k**	**24.16 a**	**30.54 a**	**3.33 a**
Control	**47.71 g**	**18.54 g**	**30.32 b**	**2.41 c**
Mean	**47.50 B**	**21.35 A**	**27.65 A**	**2.83 A**
	39.008 C	**12.86 C**	**20.56 C**	**1.61 C**

The averages that bear different letters are significantly different (0.05 & 0.01) among them (the capital letters are a major effect and the small letters are overlapping)

Table 1 also shows the percentage of protein in the manufactured pastrama model during the fermentation and maturation stages, The results of the above table show a rise in the protein percentage of the fermented bacterium form after 48 hours fermentation of which is directly proportional to the concentration of the used prefix compared with the control treatment, , The percentage of protein in the third week of maturation was 24.16% when adding bacteria by 5% compared to the treatment of control, where the proportion of protein was 18.54%, The reason for that bacterial manufactured pastrama is due to the low moisture after fermentation and ripening and to the activity of the starting bacteria, which leads to the concentration of protein and other compounds(14).Table 1 shows the percentage of fat in the manufactured pastrama form during fermentation and ripening stages, the results indicate that the percentage of fat in the processed pastrama form after the fermentation period (48 hours) and until the end of the ripening period increases. This increase is directly proportioned to the concentration of the used prefix bacteria, the percentage of fat in the third week of ripening was 30.54% compared to the control treatment in which the percentage of fat in the last week of maturation was 30.32%. The results showed a higher percentage of fat by increasing the period of ripening in the treatments added to the starting bacteria of the pastrama model, this is due to the low humidity after fermentation and ripening due to the activity of the initiator bacteria, which leads to the concentration of dry organic matter and thus increase the percentage of fat. Table 1 shows the percentage of ash in the manufactured pastrama model, it was noted the high percentage of ashes of the fermented pastrama model after fermentation for 48 hours and until

the end of the ripening period and in a relative proportion with the concentration of the used initiator bacteria, As the percentage of ash in the third week of ripening 3.33%, compared with the percentage of ash in the control treatment in the third week of maturation 2.41%, this way due to the low humidity after fermentation and ripening and to the activity of the initiator bacteria, which leads to the concentration of minerals and whech increased the percentage of ash.

Table 2. Chemical analysis of the manufactured pastrama when adding lactobacillus plantarum bacteria at a concentration of 5%

Shelf life	PH	Peroxide	Non-protein nitrogen NPN
48 hours	3.94 k	0.58 k	2.25 h
Control	0.00 l	0.00 l	0.00 i
7 days	4.14 i	0.70 i	2.42 g
Control	5.04 a	1.21 c	2.57 e
14 days	4.38 g	0.78 g	2.54 f
Control	4.84 C	2.11 b	2.68 d
21 days	4.66 d	0.94 e	2.96 b
Control	4.52 f	2.66 a	2.88 c
Mean	4.42 B	0.75 C	2.54 B
	3.60 C	1.49 A	2.03 C

The averages that bear different letters are significantly different (0.05 & 0.01) among them (the capital letters are a major effect and the letters are small overlapping) Table 2 shows that the pH after fermentation for 48 hours is inversely proportioned to the concentration of the used initiator bacteria, the pH value in the third week of ripening was 4.66 while the pH value of the control treatment in the third week of ripening was 4.52. The results showed a gradual increase in the pH values by the maturation period in the treatment which to the starting bacteria in the pastrama model, this is due to the ability of lactic acid bacteria in the production of proteolytic enzymes, the continuous decomposition of poly peptides and their transformation into peptides and then into free amino acids, thus releasing ammonia. The results indicated a gradual decrease in the pH values in the control treatment of the manufactured pastrama model by increasing the ripening period, this is due to the naturally occurring lactic acid bacteria in the meat the pH companied the starter bacteria (16). The results of Table 2 showed the increase in peroxide number the values of the of the fermented bacterium form after fermentation for 48 hours until the end of the ripening period in reverse proportion with the concentration of the starter bacteria. In the first week of maturation, the value of the peroxide number was 0.70 meq O_2 kg^{-1} Compared with the control treatment in the first week which was 1.21 meq O_2 kg^{-1}, The decrease in the number of peroxide in the treatment of added the *Lb. plantarum* bacteria was attributed to the role of the *Lb. plantarum* initiator in reducing the pH through the production of lactic acid and thereby reducing the effectiveness of lipid-lowering enzymes. Table 2 shows the percentage of non-protein nitrogen in the manufactured pastrama model, the percentage of non-protein nitrogen of the

fermented bacterium form after fermentation (48 hours) until the end of the ripening period, the ratio of non-protein nitrogen in the third week of ripening was 2.96% compared to control treatment with non-protein nitrogen ratio of 2.88%. The increase in non-protein nitrogen is due to the activity of proteolytic enzymes produced by the protein-destroying bacteria.

Table 3. Microbial analysis of pastrama manufactured when adding *lactobacillus plantarum* bacteria at a concentration of 5%

Shelf life	Total count of bacteria / g × 10^5	psychrophilic bacteria CFU / g × 10^1	coliform bacteria count CFU / g × 10^1	staphylococci aureus CFU / g × 10^1	lactic acid bacteria count CFU / g × 10^8	molds and yeasts count CFU / g × 10^1
48 hours	27.00 dc	8.0 b	7.5 d	14.0 e	35.0 a	8.0 dc
Control	0.00 g	0.00 f	0.00 f	0.00 g	0.00 g	0.00 d
7 days	29.0 c	4.0 d	5.0 e	8.5 f	27.4 c	4.0 dc
Control	24.00 d	15.5 b	16.0 c	22.5 c	18.5 d	25.0 a
14 days	7.0 f	0.00 f	0.00 f	0.00 g	17.0 ed	0.00 d
Control	33.5 b	24.0 a	20.5 b	28.0 b	26.0 c	22.5 ba
21 days	12.0 e	0.00 f	0.00 f	0.00 g	14.0 ef	0.00 d
Control	39.5 a	3.5 e	8.0 d	33.5 a	32.0 ba	11.5 bdc

The averages with different letters are significantly different (0.05 & 0.01) among them.Results Table 3 showed a clear decrease in the total number of bacteria after fermentation to the end of the maturation period, As the total count of bacteria reached 27×10^5 CFU g-1 Compared with control treatment in which the total bacterial count was reached 24×10^5 CFU g-1, while the total count of bacteria in the third week of maturation was 12×10^5 CFU g-1 Compared with control treatment in which the total count of bacteria increased 39×10^5 CFU g-1. The results of Table 3 show the count of psychrophilic bacteria, We see that the count of psychrophilic bacteria decreased after fermentation and the maturation period is advanced, It is observed in the second and third weeks of maturation that count was reduced of psychrophilic bacteria decreased to zero using the treatment in which the bacterial starter *Lb. Plantarum* compared to control treatment in which the number of bacteria after the third week of maturation reached 4×10^3 CFU g-1, This is due to the ability of lactic acid bacteria to produce antibodies such as hydrogen peroxide, bacteriocin and organic acid in the middle and make it unsuitable for growth, In addition, low degree of water activity and high fermentation temperature inhibits their growth. The results showed in Table 3 that there is a decrease in *coliform* bacteria count after the fermentation stage along to the end of the period of maturation, the count of *coliform* bacteria in the second and third weeks of the ripening period reached zero when adding the *Lb. Plantarum* compared to the control treatment in which the count of *coliform* bacteria in the third week of maturation was 8×10^3 CFU g-1, This was due to the ability of lactic acid bacteria to reduce the count of *coliform* bacteria to three

logarithmic cycles during fermentation and meat ripening (12). The results shown in Table 3 show a decrease in the count of *staphylococci aureus* during the fermentation phase and the maturation period, In the second and third weeks of maturation, the count of *staphylococci aureus* decreased to zero with the addition of *Lb. Plantarum* compared to the control treatment in which the count of *staphylococci aureus* 33.5×10^3 CFU g-1 In the third week of maturation. It was pointed out (7) That lactic acid bacteria inhibit the growth of *staphylococci aureus* by several factors, including reduction of pH and production of inhibitory compounds such as hydrogen peroxide and bacteriocin, As well as the consumption of nutrients necessary for growth such as vitamins, amino acids, sugars and minerals. The results of Table 3 show a decrease in the count of lactic acid bacteria, In the third week of maturation the count of lactic acid bacteria decreased 14×10^8 CFU g-1 when adding the *Lb. plantarum* bacteria initiator compared to control treatment, which increased the count of lactic acid bacteria to 32×10^4 CFU g-1. The reason for that was due to the ability of *Lb. Plantarum* in the produce of inhibitory substances for the growth of bacteria, including bacteria produced such as bacteria and this is what he found by (8). Results showed a significant decrease in the count of yeast and mold, the count of yeasts and mold in the second and third weeks of ripening reached zero at the addition of *Lb. Plantarum* compared to control treatment in which the count of yeast and molds reached the third week of maturation 15×10^3 CFU g-1. The reason for that was to produce the ability of lactic acid bacteria in the production of antibodies such as bacteriocins, hydrogen peroxide and which organic acid in the middle and make it inappropriate for the growth of yeast and mold, especially in the stage of maturation, as well as the decline of water activity that prevents growth.

. **Table 4. Sensory evaluation and sensory evaluation of the pastrama manufactured when adding lactobacillus plantarum bacteria at a concentration of 5%**

General shape	Virtual color	Flavor	Tenderness	Juiciness	Texture	Public acceptance
7.5	7.5	7.31	7.31	7.31	7.31	7.31

Table 4 shows the evaluation of the general shape of the finished pastrama in the last week of maturation, as it reached (7.5 very good) when adding the bacterial starter at a concentration of 5%, The results agreed with what he said (9) noting that the values of the overall shape did not differ when different types of broilers were used in sausage manufacturing compared with the control coefficients that obtained low values for the general form. While the value of virtual color (7.5 brown), Due to the deterioration of control factors during the period of ripening to slow the process of fermentation and retention Pastrama high pH and moisture content higher and thus decrease the available oxygen, resulting in the exposure of the color of myoglobin to the process of oxidation and converted to the dye dark Metmyoglobin. The results of studying the value of flavor (7.3) when adding the bacteria starter *Lb. plantarum* at a concentration of 5%, agreed with what was found by (19) The value of the flavor in the buffalo meat manufactured Pastrama and salted bacteria *Staph. Xylose* & *Staph.carnosus* 8.6 after the 28-day maturation period compared to the control treatment of 7.7. The results in Table 4 showed an increase in the values of tenderness in the cultures of the initiator *Lb. plantarum* the value of tenderness (7.5 was good). (17) pointed out That the process of ripening maintains a better coolness compared with the freezing process, with a value of 3.57 in the process of ripening compared to 2.75 in the process of freezing out of (5). While the value of amygdala (6.5

medium) was added when adding startir *Lb. Plantarum*. The results of the table show the appearance of the texture accepted in the treated treatment of the bacterial starter *Lb. Plantarum* with texture value (3.5 acceptable). Table 4 shows the degree of general acceptance in the manufactured pastrama model in the last week of maturation. The general tolerance score (7) was acceptable when adding the *Lb. Plantarum*. Startir Reference (18) mentioned That the process of fermentation at a temperature ranging from 30-37 m improves the general acceptance in the sausage product.

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