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Enhancement of The Quality and Safety of Pastrami Using Fermented Milk Permeate

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PASTRAMI was manufactured from M. longissimus dorsi beef meat after marinating the meat in unfermented milk permeate T1 and fermented milk permeate with *Lb. paracasei* subsp. *paracasei* and *Lb. pentosus* T2 and T3, respectively and C, control treatment, was manufactured without being marinated. All treatments were stored at 5 ± 1°C for 60 days and analyzed at periods of (when fresh, 15, 30, 45 and 60 days). Peroxide values (POV), Thiobarbituric acid reactive substances (TBARS), free fatty acids (FFA), pH values, proteolysis, total viable count (TVC), total fungi, coliforms count, *Staphylococcus aureus* and lactic acid bacteria (LAB) and sensory analysis were determined. The results showed that C and T1 treatments had the highest values of POV, TBARS, FFA, pH and TVC. Whereas, T2 and T3 treatments recorded the highest values of proteolysis and LAB count. *Staphylococcus aureus* and coliforms were not detected in all treatments. While, some fungi appeared in C and T1 at the end of the storage period and disappeared in T2 and T3. Marinating of pastrami meat in fermented milk permeate significantly improved the sensory evaluation of pastrami. Finally, T2 and T3 had significantly higher quality improvement than C and T1.

Keywords: Pastrami, Milk permeate, *Lactobacillus paracasei, Lactobacillus pentosus,* Fermentation.

Introduction

Pastrami is a popular and traditional cured meat product which is produced from certain parts of beef carcasses, M. longissimus dorsi (Kaban, 2009). Pastrami is cured, dried, pressed and coated with paste containing (fenugreek, pepper, garlic, salt and water) and dried again (Aksu et al., 2005). Since the twelfth century, untill now Turkey produces pastrami which is considered the most popular dry-cured meat product in it (Gokalp et al., 2010). Although pastrami is a traditional Turkish meat product, it is produced in many parts of the world such as the Middle East, Middle Asia and some Mediterranean and European countries.

Toldra (1994) found that proteolysis has been observed during the dry-curing process in meat products. Muscle proteinases remain active during the ripening process in this type of meat product, and these enzymes are broken down into peptides. Ripening process of pastrami increases the amount of non-protein nitrogen (Toldra et al., 1993). The formation of aroma and tenderization in meat occur in pastrami during ripening. The tenderization is increased because of fragmentation of myofibrillar proteins during ripening of pastrami, (Price and Schweigert, 1987).

Many investigators stated that quality properties of dry-cured raw meat products like pastrami could be improved by using starter cultures (Aksu and Kaya 2001, Aksu and Kaya

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2002, a, b). Starter culture of Micrococcaceae (*Staphylococcus carnosus, S. xylosus,* and *Micrococcus varians*) can be used in meat products. These microorganisms have proteolytic and lipolytic activities, which cause increases in flavor compounds (Fadda et al., 1999). The starter culture consists of lactic acid bacteria LAB *(Lactobacillus plantarum, Lb. sakei, Lb. curvatus, Lb. pentosus)* and Micrococcaceae are commonly used in meat fermentation (Kröckel, 1995).

In recent years, the microbial stability and inhibition of pathogenic microorganisms in meat products can be achieved using protective cultures (Lücke, 2000). Schillinger et al. (1991) found that *Lactobacillus sakei* Lb 706 can be used as a protective culture in cured meat products.

The objective of the current study was to determine the effect of milk permeates fermented by *Lactobocillus paracasei* subsp. *paracasei* or *Lactobocillus pentosus* H4 as a protective cultures on the quality and safety of pastrami.

Materials and Methods

Materials

Milk permeate, a byproduct of ultrafiltration process of milk, was obtained from the dairy pilot plant, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. ***Lb. paracasei* subsp. *paracasei*** was obtained from the department of microbiology, Faculty of Agriculture, Fayoum University. The strain *Lb. pentosus H4* is identified on Genebank *http://www.ncbi.nlm.nih.gov/nuccore/JQ011466* under accession number (JQ011466). M. longissimus dorsi (beef meat part) was used for manufacturing of pastrami. Chemicals were obtained from Sigma and Merck companies and all chemicals used for this study were analytical grade (A.R.).

Methods

Preparation of milk permeate and marination process

Milk permeate that contains (5.94% TS, 4.80% lactose, 0.65% Ash and 0.27% protein) was used for marinating meat before manufacturing pastrami. The milk permeate was divided into three portions, one liter per each. The first one was used as it is, without fermentation. The second portion was inoculated with *Lb. paracasei* subsp. *paracasei* and the third was inoculated with *Lb. pentosus* H4 then the two portions were incubated at 32±2°C for 24 hours. The meat was divided into four portions, one kilogram per each. The first portion of meat was used without marination, as a control treatment. However the second portion of meat was marinated in unfermented milk permeate, the first portion of milk permeate, as T1 treatment. The third portion of meat was marinated in milk permeate fermented with *Lb. paracasei* subsp. *paracasei,* while, the fourth portion of meat was marinated in milk permeate fermented with *Lb. pentosus* H4, as T2 and T3 treatments respectively. The marination process of meat in milk permeates continued up to 24 hours at refrigerator temperature 5-7°C . before starting the manufacture of pastrami.

Manufacture of pastrami

Deboned beef hind-quarter including topside, silverside, knuckle and rump are the basic raw meat materials used for pastrami production. The meat is trimmed from fat, tendons and then tailored into pieces of one kg in weight. Each meat piece is stabbed with a knife in such a way that small opening is made, then the knife is forced forward and backward inside the meat to produce a long bottom. Meat is then dry salted using salt (200 g sodium chloride per kg meat). The salting operation is applied by wrapping every meat piece with salt, and left for one day. The salted meat is then washed in running water to remove excess salt and arranged of the meat cuts on the stage of metal press in layers with pieces of tissues in between. The arrangement on the pressing stage is done in a way that upon pressing the knife stabs are expected to be closed by the squeezing action of the press. The salted meat is pressed for 6-10 hours using a pressing power of 5kg/cm2 over a meat block of 70 cm high. During the pressing operation, the meat losses out about 1/3 of its original weight due to water exudation. After pressing, meat is hanged individually in an open place shade for a couple of hours to allow dripping and surface drying, it was then coated. The coat of pastrami is basically made from peeled garlic (10 %), fenugreek (6 %) and spices (Black pepper, hot pepper and common salt) (0.5 %). The dry mix of the coat is mixed with water to form a paste. The paste is adhesively applied over the surface of the cured meat and then hanged to dry in the open air for a couple of days according to Abdulatef *et al*. (2014). All treatments were stored at 5 ± 1°C for 60 days. All treated samples were then analyzed at different periods (fresh, 15, 30, 45 and 60 days)

Physicochemical analysis

Moisture, protein, fat, salt, ash content and pH value were determined according to the method

described by AOAC (2012).

Quality parameters of pastrami

Free fatty acids (FFA), Peroxide values (POV) and Thiobarbituric acid reactive substances (TBARS) were determined according to the method described by Pearson (1986).

Determination of proteolysis of native proteins of pastrami

The degree of proteolysis of pastrami was determined by the method described by (Adler- Nissen, 1979) using trinitrobenzen sulfonic acid (TNBS). Basically, this method is a spectrophotometric assay of the chromophore formed by the reaction of TNBS with primary amines. About 10g of minced pastrami samples were homogenized with 90 ml of borax buffer 2 % (w/v) of sodium dodecyl sulphate (SDS), 0.477 (w/v) Na2B4O7.10 H2O, pH 8.9 adjusted with HCl using a Stomacher lab-blender 400. The homogenate was transferred and shaken thoroughly in screwed capped 100 ml flask using a thermostatic water bath at 75° C for 15 min., then at 60° C for 2 hr. Thereafter, this solution was diluted to 50 and 100 times with borax buffer. Ten µl of the prepared sample or (0.25 – 4 mM Leucine / ml borax buffer) standard solutions were pipetted into the microtiter plate and the following reagents were added: 80 µl of phosphate buffer (0.2125 M Na2PO4 and 0.2125 M Na2HPO4) and 80 µl of 0.1 %. After the incubation period at 42°C for one hour, the absorbance at 405 nm was measured. The degree of proteolysis was determined using a standard curve, which was developed in the same manner with the unknown sample.

Microbiological analysis:

Total viable count (TVC) (on nutrient agar at 37°C for 2 days), total mold and yeast (on PDA at 32°C for 5 days), coliform bacteria group count (on macConkey agar at 37°C for 2 days), staphylococcus aureus (on mannitol salt agar at 32°C for 2 days) and Lactic acid bacteria (LAB) (on MRS agar at 32°C for 2 days) were determined according to the method described by APHA (1992).

Sensory analysis

Pastrami samples were judged by ten panelists of the staff members of the Food and Dairy Science Departments, Faculty of Agriculture, Fayoum University. The samples were scored for appearance, tenderness, flavor and overall eating according to the way demonstrated by Amerine et al. (1965).

Statistical analysis

All obtained data were subjected to the statistical analysis using SPSS version 19.0 software (SPSS. 1999. Statistical Package for Social Sciences. SPSS Inc., Chicago, IL, USA.), and Sigma plot 12.0 software programs.

Results and Discussion

Physicochemical analysis

The contents of moisture, protein, fat, salt and ash were determined in pastrami marinated in fermented and unfermented milk permeate and the results are shown in Table 1. The moisture content of all samples is in agreement with the Egyptian standard specifications (E.S.S) (1042 – 2005) that stated the moisture content doesn't exceed 60%. The obtained results are in harmony with those detected by Ceylan and Aksu (2011), who demonstrated that the moisture content for different types of pastrami ranged from 36.31 to 57.34%. The percentage of protein contents of all samples were high and ranged from 21.27 to 24.86 as shown in Table 1. Whereas the fat content was ranged from 3.20 to 4.21, also these findings coincide with E.S.S (1042 – 2005) that stated the fat % within limit of 5%.

TABLE 1. Physicochemical composition of pastrami marinated in fermented and unfermented milk permeate

Treatments	Moisture	Protein	Fat	Salt	Ash
C	50.04	23.90	3.92	8.52	10.73
T1	52.12	21.27	3.20	8.82	10.52
T2	52.23	22.04	4.21	9.11	11.69
T3	51.84	24.86	3.73	8.93	10.92

C: Pastrami control without milk permeate **T1:** Pastrami marinated in unfermented milk permeate
T2: Pastrami marinated in milk permeate fermented with *Lb. paracasei*
T3: Pastrami marinated in milk permeate fermented with *Lb. pentosus*

The sensory evaluation of pastrami is associated with salt % which affects the acceptability of pastrami by consumers. In this study, the salt % of all pastrami samples was ranged from 8.52 to 9.11%, whereas the E.S.S (1042 – 2005), not specified salt limit in pastrami. In Egypt, the pastrami is produced by drycuring method. In this method, the surface of meat is completely covered by salt for 24 hr. and then excess salt is washed by water. Therefore all samples were high in salt content. The current results ran with those published by Çakıcı, et al., (2015) who revealed that the salt % of different types of Turkish pastrami ranged from 6.32 to 8.49%.

Ash content % in pastrami samples is related to the salt ratio, therefore higher ash content was found in pastrami samples with the highest salt content. Concerning the percentage of ash, as shown in Table 1, ranged from 10.52-11.69%. These results are higher than those found by Çakıcı, et al. (2015) who denoted that the ash content in different types of Turkish pastrami

Quality parameters of pastrami

The curing of meat for manufacturing pastrami must be done very carefully, to avoid unwanted interactions such as lipid auto-oxidation, rancidity and pH changes. Lipid oxidation analysis in pastrami samples is important since compounds resulting during the process are related to undesirable sensory and biological effects. The primary oxidation products are peroxides, especially hydroperoxides, they are determined by peroxide value (POV). Figure 1 shows that weekly changes in the levels of POV in pastrami treatments marinated in fermented and unfermented milk permeate stored at 5˚C for 60 days. Pastrami treatment marinated in milk permeates fermented with *Lb. paracasei* (T2) and Pastrami treatment marinated in milk permeate fermented with *Lb. pentosus* (T3) had the lowest values of POV during the storage period and until the end period of storage. While pastrami treatment marinated in unfermented milk permeate (T1) and the control without permeate (C) have the highest values of POV at the end period of storage, such as finding coincides with that obtained by Talon et al. (2007) and Amanatidou et al., (2001) who found that LAB (*Lactobacillus sakei, Lactobacillus pentosus, Lactobacillus buchneri, Lactobacillus curvatus*, *Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus pentosum, Lactobacillus Brevis, Lactobacillus alimentarius, Carnobacterium maltaromaticum*) are responsible for inhibition of lipid oxidation.

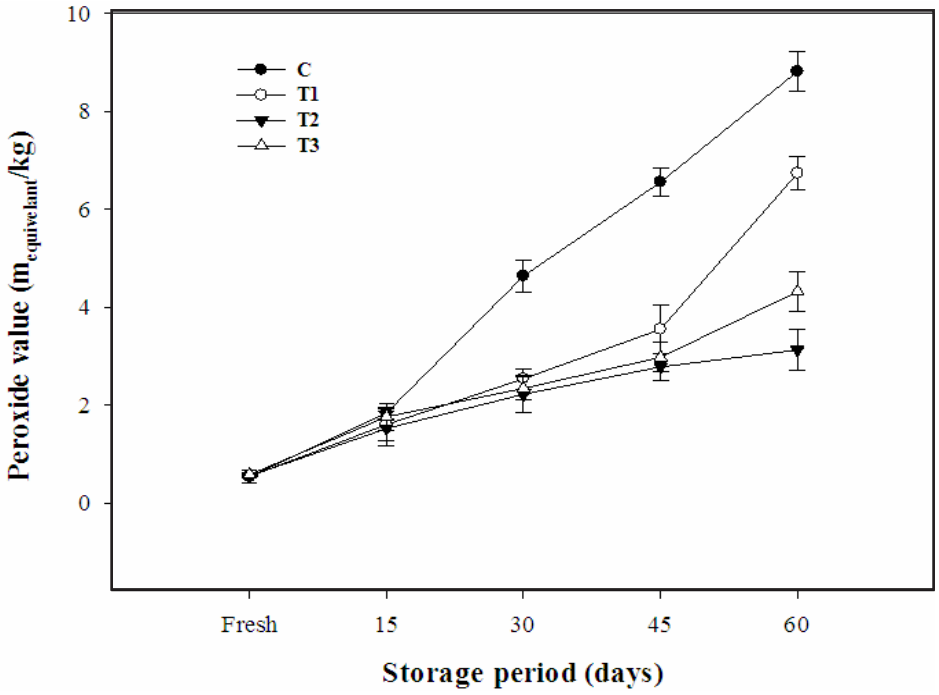

Fig. 1. Effect of fermentation on the peroxide value of pastrami marinated in fermented and unfermented milk permeate at different storage periods.

The Effect of fermentation on the ratio of Thiobarbituric acid reactive substances (TBARS) of pastrami marinated in fermented and unfermented milk permeate at different storage periods as shown in Fig. 2.The values of TBARS gradually increased during the storage period and the highest values at the end of the storage period were 4.37 and 5.41(mg malonaldhyde/kg) for treatments C and T1 respectively. While the lowest values of TBARS were 0.56 and 3.47 (mg malonaldhyde/kg) for T2 and T3, respectively, at the end of the storage period. Similar results were obtained by Zahra and Hedayat (2017), Talon, et al., (2007) and Amanatidou et al., (2001) who suggested that lactic acid bacteria (LAB) prevent lipid oxidation.

From the results in Fig. 3, it was cleared that the effect of fermentation on the acidity or free fatty acids (FFA) (as % oleic acid) of pastrami marinated in fermented and unfermented milk permeate at different storage periods, where, the acidity is defined as the percentage of free fatty acids liberated from fat by hydrolysis and may be a measure of hydrolytic rancidity. C and T1 showed higher values of FFA during the storage period and reach to the maximum levels by the end of the storage period, recording values of 7.43 and 6.87 % respectively. These results are, to some extent, in agreement with the data obtained by Ibrahim (2001) who demonstrated that tested pastrami samples had higher values of FFA during the curing period. Whereas, the lowest values of FFA were 3.64 and 4.32% for T2 and T3, respectively. These findings are more or less similar to those detected by Gao, (2014) who reported that FFA content was not significantly increased in fermented sausages.

The results in Fig. 4 showed the effect of fermentation on pH of pastrami marinated in fermented and unfermented milk permeate at different storage periods. C and T1 had the highest values of pH after 60 days of storage; the values were 5.8 and 5.8, respectively. While the lowest values of pH were recorded by T2 and T3 at the end of the storage period and were 5.2 and 5.5, respectively. The decrease in pH may be due to the lactic acid produced by *Lb. paracasei* and *Lb. pentosus* in T2 and T3 (Lorenzo et al., 2014 and Zahra & Hedayat 2017).

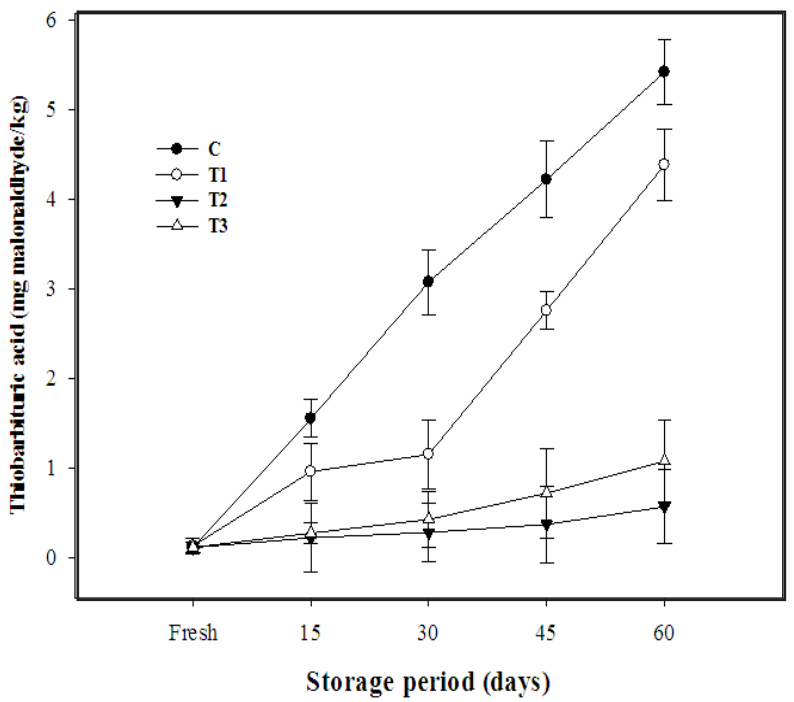

Fig. 2. Effect of fermentation on the ratio of thiobarbituric acid reactive substances of pastrami marinated in fermented and unfermented milk permeate at different storage periods.

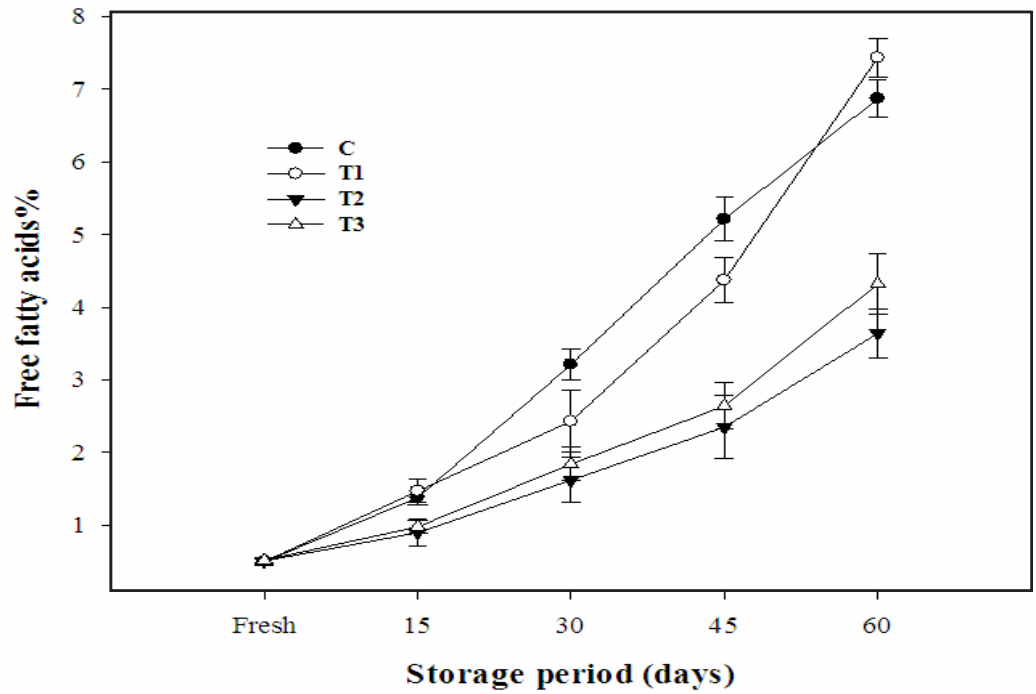

Fig. 3. Effect of fermentation on the free fatty acids% of pastrami marinated in fermented and unfermented milk permeate at different storage periods.

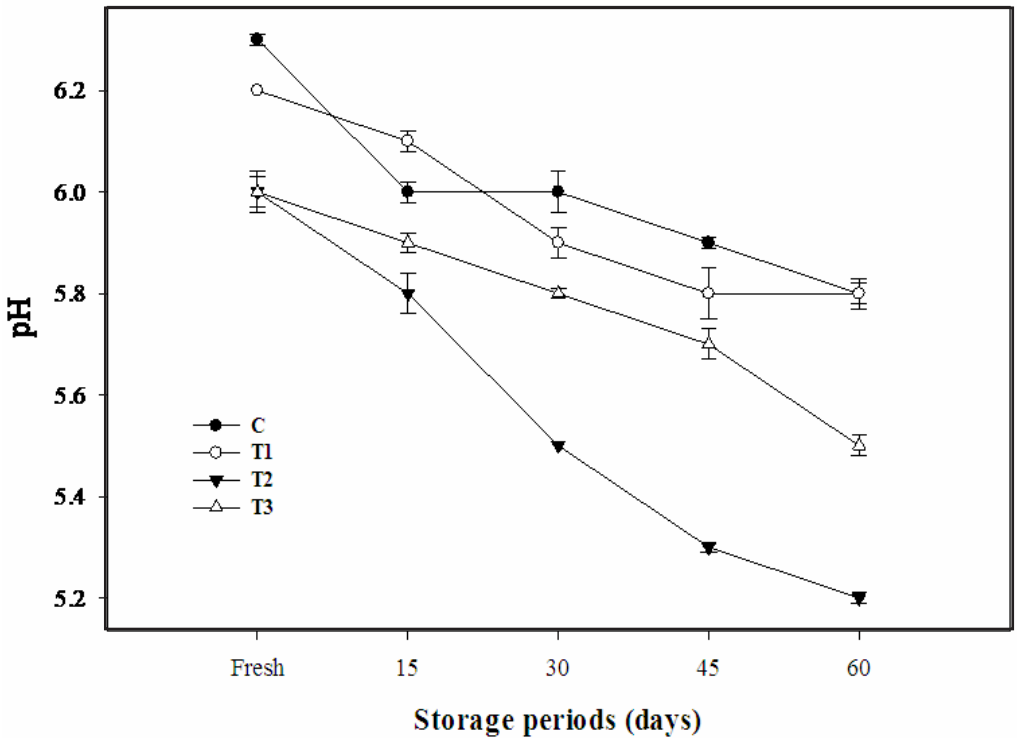

Fig. 4. Effect of fermentation on pH of pastrami marinated in fermented and unfermented milk permeate at different storage periods.

As shown in Fig. 5, T2 and T3 treatments had the highest values of native protein proteolysis throughout the storage period and reached at the end of storage period 4.22 and 2.65 mM leucine respectively. On the other hand, the results showed that unfermented C and T1 treatments had the lowest values of native protein proteolysis throughout the storage period and reached at the end of storage period 1.43 and 1.63 mM leucine respectively. Significant differences were noticed between (T2 & T3) and (C & T1) treatments because of fermentation process performed on T2 and T3 by *Lb. paracasei* and *Lb. pentosus* respectively. These lactic acid bacteria possess high proteolytic enzymatic system as proved by Ikram-ul-Haq and Mukhtar (2006); Osaana et al., (2007); Liu et al. (2010) and Abdulatef et al. (2014).

Microbiological analysis:

As illustrated in Fig. 6 total viable counts TVC of all treatments of pastrami significantly decreased during the first 15 days of the storage period and then gradually increased, except T2 (pastrami marinated in milk permeate fermented with *Lb. paracasei*) that significantly decreased after 30 days of storage and then it gradually increased. This result is due to the production of bacteriocin by *Lb. paracasei* as proved by several authors (Shehata et al., 2018, Tolinacki et al., 2010 and Vamanu & Vamanu, 2010). On the other hand, lactic acid bacteria LAB counts of all treatments significantly increased throughout the first 45 days of the storage period then started decreasing. Moreover, significant differences were observed between LAB counts of C (control) and T1 (pastrami marinated in unfermented milk permeate) and between C, and pastrami marinated in fermented milk permeate treatments (T2and T3). The highest LAB count was recorded by T2 and T3. However, C had the lowest LAB count. These significant results are due to the fermentation process performed on milk permeate before marinating the pastrami meat in it, and this led to spread LAB in pastrami treated with fermented milk permeate as occurred in T2 and T3. These results emphasized the results of pH degrees in Fig. 4, since T2 and T3 had the lowest pH degrees. It is worth to mention that *Staphylococcus aureus* and coliform bacteria group were not noticed in all pastrami treatments. While, some fungi was detected in C and T1 treatments at the end of the storage period and not detected in T2 and T3 treatments because of the bacteriocins (Slim et al., 2010 and Aqeela et al., 2015) produced by used LAB as mentioned above.

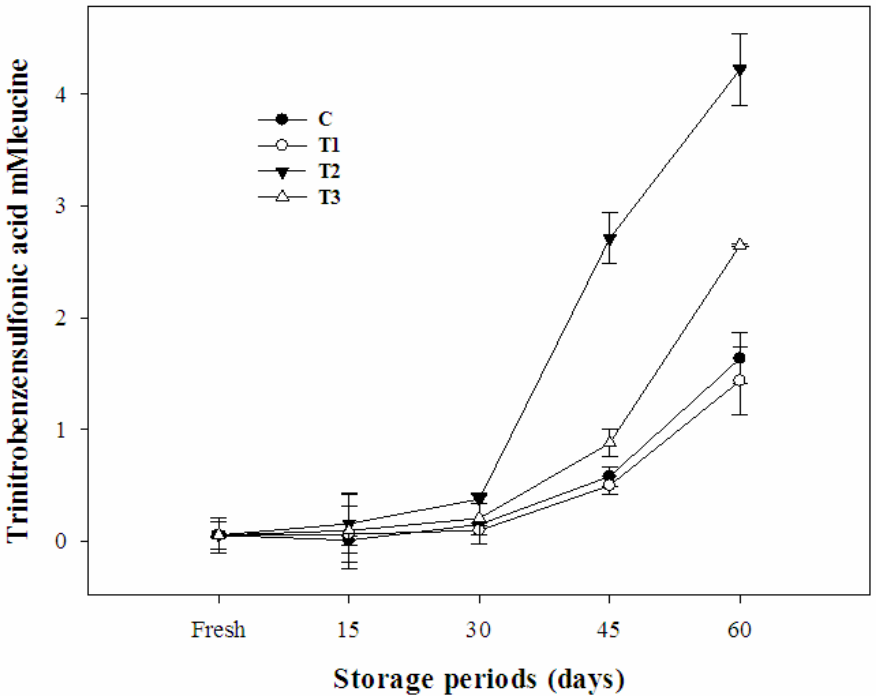

Fig 5. Effect of fermentation on the proteolysis of native protein of pastrami marinated in fermented and unfermented milk permeate at different storage periods.

Sensory analysis

As illustrated in Fig. 7 that there were significant ($p \leq 0.05$) scores between T3 and the other treatments (C, T1 and T2) in appearance and tenderness. Whereas, no significant scores found between all treatments in flavour and overall eating. However, the treatments marinated in milk permeate fermented with *Lb. paracasei* and *Lb. pentosus*, T2 and T3 respectively, had a light red colour compared to C and T1 treatments that they had an obvious red colour. This result is due to the high acidity developed in fermented treatments T2 and T3 as reflected by pH results that were previously mentioned in Fig. 4. This result is in agreement with (Hinkle 2010, Kim et al., 2015) who showed that the redness of the citric acid chicken breast groups was reduced compared to the control that undipped in citric acid. Moreover, marination of pastrami meat in fermented milk permeate significantly improved the tenderness of pastrami. As it is clear from Fig. 7, T3 significantly had the highest tenderness compared to the other treatments.

Conclusion

Depending on all the previous results we can conclude that the marination process of pastrami meat in fermented milk permeates actually enhanced the quality chemical analyses of pastrami, microbiological analysis and sensory evaluation of T2 and T3 treatments. The authors recommend marinating the meat in fermented milk permeate before manufacturing the pastrami.

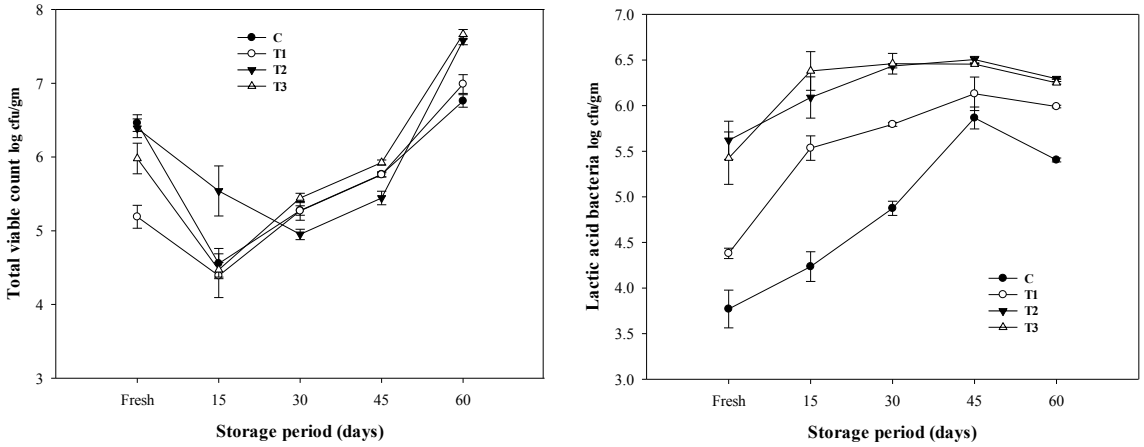

Fig. 6. Effect of fermentation on total viable and lactic acid bacteria counts of pastrami marinated in fermented and unfermented milk permeates at different storage periods.

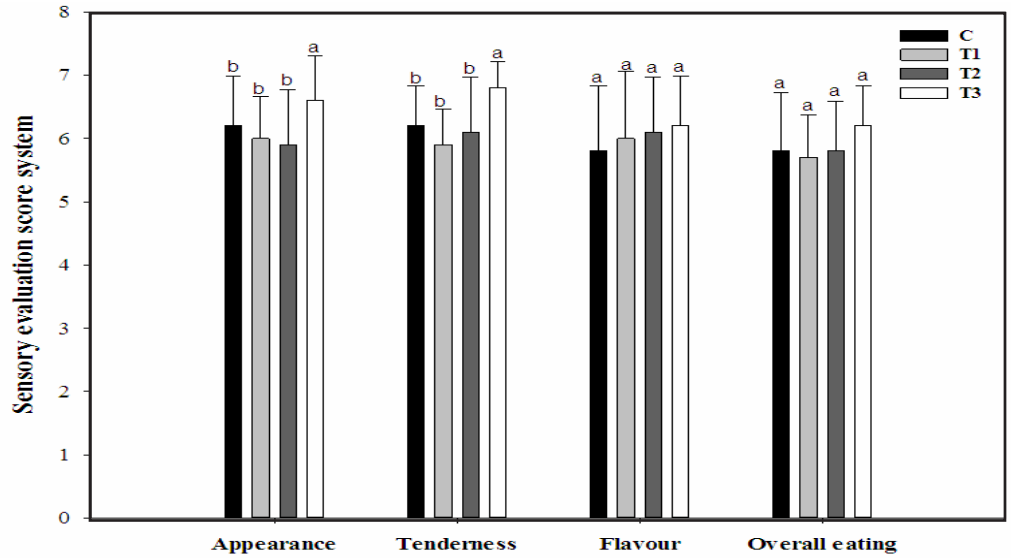

Fig. 7. Sensory analysis of pastrami marinated in fermented and unfermented milk permeate.

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تحسين جودة وأمان البسطرمة بإستخدام برمييت اللبن المتخمر

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تم تصنيع البسطرمة من اللحم البقري وقد تم تقسيمة الي ثلاثة أجزاء ليكون ثلاث معاملات المعاملة الأولى نقع اللحم في برمييت غير متخمر وسميت هذه المعاملة T1. المعاملة الثانية نقع جزء آخر من اللحم في برمييت متخمر بسلالة Lactobacillus paracasei subsp. paracasei وسميت هذه المعاملة T2 ، والمعاملة الثالثة هى نقع جزء آخر في برمييت متخمر بسلالة Lactobacillus pentosus وسميت هذه المعاملة T3. هذة المعاملات تم مقارنتها بالعينة الكنترول C التي صنعت بدون نقع اللحم في البرمييت. تم تخزين كل المعاملات على درجة حرارة 5 ± 1 °م لمدة 60 يوم حيث تم تحليل المعاملات على فترات (عندما كانت طازجة ، 15 ، 30 ، 45 ، 60 يوم). تم إجراء التحليلات التالية (البيروكسيد POV ، Thiobarbituric acid reactive substances (TBARS) ، النسبة المئوية للأحماض الدهنية الحرة pH ، FFA% ، التحلل البروتيني ، العد الكلي للبكتيريا ، الفطريات والخمائر ، مجموعة بكتيريا القولون ، الكشف عن الـ Staphylococcus aureus ، عد بكتيريا حامض اللاكتيك ، وأخيراً التقييم الحسي للمعاملات). أظهرت النتائج أن معاملات C ، T1 أخذت أعلى القيم في كلٍ من POV, TBARS, FFA%, pH and TVC بينما أخذت معاملات T3 ، T2 أعلى القيم في كلٍ من التحلل البروتيني وعد بكتيريا حامض اللاكتيك. ولم تكتشف كلٍ من بكتيريا القولون و الـ Staphylococcus aureus في كل المعاملات. إلا أن بعض الفطريات والخمائر ظهرت في بعض المعاملات C ، T1 أثناء المراحل الأخيرة من فترة التخزين وإختفت في المعاملات الأخرى T3 ، T2. لذلك حسن نقع اللحم في البرمييت المتخمر من الصفات الحسية للبسطرمة المصنعة منه. و أثبتت النتائج أن المعاملات ، T2 T3 كانت أعلى جودةً من T1 ، C.