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PRODUCTION OF FERMENTED SAUSAGE FROM OLD BUFFALO MEAT USING TRANSGLUTAMINASE

BY

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ABSTRACT

Sausage was produced from old buffalo meat using starter culture, transglutaminase and a mixture of them. The sausage samples were stored at 4°C for four weeks.

The total viable aerobic bacterial count was higher in non fermented sausage than in fermented sausage. In contrast the lacticacid bacterial count was higher in fermented sausage than in the non fermented sausage and gave higher shelf-life for the product. At the same time the yeast and mold count were lower in the fermented product. All samples were free from *Salmonella*.

The addition of transglutaminase, starter culture or a mixture of them increased the protein content of sausage. Moisture content of all samples was slightly decreased with increasing of storage period. Sausage containing mixture of transglutaminase and starter culture had a lower percentage of moisture reduction. The values of TVN, TBA, TVFA and acidity increased with increasing storage period. The development of these parameters in the fermented sausage contained transglutaminase was lower than the other samples. In conclusion it could be recommended that, the use of transglutaminase and starter culture to produce restructured old buffalo fermented sausage with adequate binding capacity. This might to enhance the market value of old buffalo meat and introduce a new product in the meat market.

INTRODUCTION

Fermented meat products are those that are deliberately incubated, or "aged" during their processing cycle to allow for sufficient microbial activity to alter the product characteristics. Although microorganisms generally are considered the adversary in the processing of meat, fermented products rely upon controlled microbial activity of specific type of bacteria, molds and sometimes yeasts. Some manufactures still depend upon naturally occurring microflora to obtain the desired results. However, most fermented meat processors, particularly in United States, now employ specific starter cultures to assure the numerical dominance of the desired microorganisms at the start of the processing cycle. Lucke (1983) reported that, fermented sausage in Germany usually contains pork and beef in approximately equal amounts, while Hungarian and Italian salamis

are manufactured from pork only. Obviously, eating habits, religious traditions and meat cost determine the type of used meat. From a technologist's point of view, the suitability of meat to be processed as fermented sausage depends on its final pH, its water holding capacity and on the desired intensity of the curing color. Leistner and Dresel (1986) working on Chinese raw dry sausage reported that ranges for composition, were as follows: pH 5.6-6.3, a_w 0.57-0.87; NaCl 2.5-10.9% $NaNO_3$ 1-21.5 ppm and KNO_3 16-113.5 ppm. However, the cfu/g of total viable aerobic count was 10^4-10^7, lactic acid bacteria 10^3-10^6, Enterobacteriaceae $< 10^2$ and *Staphylococcus aureus* $<10^2$ in all samples.

Amich (1988) investigated the effect of addition of starter cultures and sugars on development of bacterial flora, pH and lactic acid throughout fermentation and curing of sausages prepared from good quality pork.

Graciela *et al.* (1988) evaluated the use of *Lactobacillus plantarum* and *Micrococcus varians* in the ripening of fermented suasage.

Gossling (1990) reported on the enzymatic ripening of raw sausage and results were given of ripening tests on raw sausage made either with a traditional starter culture (*Podiococcus* and *Micrococcus* spp) and 1% lactase maltodextrin mixture or with and enzyme complex extracted from *Lactobacillus plantarum* and 3% glucose.

Transglutaminase (TGase) catalyzes an acyl transfer reaction between a γ-carboxyamide of peptide or protein bound glutamine and a primary amine. When TGase acts on protein molecules, ε-(γ-glutamyl) lysine cross links are formed. Many studies have been carried out to use this unique enzyme reaction, cross linking between protein molecules, to change rheological properties of food proteins (Kuraishi, *et al.*, 1997). Various methods of restructuring and extending low-value cuts and trimmings have been developed to improve appearance and textural properties and enhance market value.

The aim of this work was to develop a useful method for restructuring of fermented sausage produced from old buffalo meat using transglutaminase, which bind meat pieces, and a mixture of starter culture containing *Lactobacillus* and *Staphylococcus* sp. to be more acceptable for the consumers.

MATERIALS AND METHODS

Materials:

- A mixture of *Lactobacillus* sp. and *Staphylococcus* sp. produced by Gewerzmueller, GmbH, Stuttgart, Germany under the commercial name B1TEC LS 25 was used as starter culture.
- Old buffalo meat from round cuts were obtained from local market.
- Dried transglutaminase (TG) produced by Ajinomots, Tokyo, Japane.
- Mutton fat and spices were purchased from the local market.

Methods

Preparation of fermented suasage:

Raw sausage was prepared according to the method described by Shehata (1989) with minor modification. The ingredient of sausage mixtures were 66.00% Old buffalo meat, 12.44% Fat tissue, 2.25% Sodium chloride, 15.00% Water (as ice), 2.50% Starch, 0.80% Spices mixture, 0.5% Sodium perophosphate and 0.5% Sodium nitrate. Spices mixture contains 30.89% black pepper, 15.43% nutmeg, 15.43% all spice, 7.65% red pepper, 7.05% cloves, 7.65% cinnamon, 7.65% ginger and 7.65% mustard. Meat was cut into small pieces and minced using electric grinder. Fat tissues were also minced then mixed with the minced meat. Spices were milled and weighed while salt was dissolved in a small proportion of water and the previous ingredients were added to the mixture. Sausage sample was divided into four portions, the first was used as control (sample), the second was mixed with 0.1% transglutaminase enzyme solution (sample 2) the third was mixed with starter culture mixture (0.5 g/1 kg), culture mixture (0.5 g/1 kg), (sample 3) and the fourth mixed with starter culture mixture (0.5 g/1 kg) and 0.1% enzyme solution (sample 4). Afterwards, the sausage mixture was ground to give a uniform distribution of the ingredients and stuffed into mutton casings and incubated at 30°C for 36 hours and then stored at 4°C up to four weeks.

Microbiological assays:

The total viable aerobic bacterial count as recommended by Vanderzant and Splittstoesser (1992) was carried out using nutrient agar (Merck). MRS agar from (Merck), which is reccomended by DeMan *et al.* (1960) was used to determine lactic acid bacteria count.

The coliform count was determined using violet red bile (VRB) agar (Merck) as mentioned by Davis (1981). Baird-Parker agar (Merck) was used for the count of *Staphylococci* as recommended by Vanderzant and Splittstoesser (1992).

The pre-enrichment of *Salmonella* was carried out using peptone water, while tetrathionate enrichment broth (Merck) was used for the selective enrichment of the cells. The isolation of the *Salmonella* cells was then carried out using bismuth sulfite agar, which is recommended by Vanderzant and Splittstoesser (1992). Glucose yeast extract oxytetracycline agar was used for the yeast and molds count according to Vanderzant and Splittstoesser (1992).

All media other than VRB and bismuth sulfite agar were sterilized at 121°C for 20 min.

Analytical methods:

* Chemical analysis of moisture, fat, protein and ash contents were determined according to AOAC (1995) and nitrogen free extract (NFE) was calculated by difference.

* Determination of total volatile nitrogen (TVN) was performed according to the method of Harold *et al.* (1987). The results were calculated as mg. TVN/100 g dry basis.
* Thiobarbituric acid (TBA) value was determined colorimetrically as the method mentioned by Harold *et al.* (1987).
* Total volatile fatty acids (TVFA) were determined by using direct distillation as described by AOAC (1995). The results were calculated as ml of NaOH 0.1N/100 g dry basis.

Physical properties:

* Titratable acidity was measured according to the method described by Cunningham and Bowers (1977).
* Water holding capacity (WHC) and plasticity as indication for tenderness were measured by the filter press method of Volovinskovia and Merkoolova (1958).
* Cooking methods: Each of the sausage samples were divided into batches, the first cooked by deep frying at 149°C until turned to browning color (Baker *et al.* 1984). The second one was cooked for 15 min in boilling water.
* Sensory characteristics of sausage samples were evaluated according to Larmond (1970).

RESULTS AND DISCUSSION

The sample of sausage without enzyme or starter (sample No.1) as well as the sample of sausage with enzyme only (sample No. 2) was microbiologically followed up after two weeks because *Staphylococcus aureus* was detected and their sensory properties were unacceptable.

After preparation and during the four week storage all samples were found free of *Salmonella* and coliform bacteria. Initial viable count of raw meat which used for the preparation of sausage at zero time, was examined.

Figure 1 shows the total viable aerobic count (TVC) in the fermented sausage samples. The samples which were prepared without starter show higher TVC at earlier storage time than samples No. 3 and 4 prepared using starter culture. This is probably the reason of sensory rejection of these samples. However, the samples No. 3 and 4 which were prepared with starter culture show little TVC changes during the first three weeks. Therefore, these samples were acceptable till the end of storage period. The TVC ranges were similar to that obtained in Spanish fermented sausage by Dolazo *et al.* (1999).

The development of lactic acid bacteria during storage of old buffalo sausage was illustrated in Figure 2. The lactic acid bacterial count in all samples but the increase in the fermented sausage was higher. This means that lactic acid bacteria were metabolically active as described by Leistner (1995) and Mukherjee *et al.* (2006) who mentioned that lactic acid bacterial count increased within the fermentation. The lactic acid bacterial count was slightly decreased during storage, because they grow in nests within the sausage matrix and were in keen competition for nutrients and impair each other by their metabolic products. This is in agreement with Leistner (1995) who stated that lactobacilli in their nests have degenerated and many have died at the end of the ripening process.

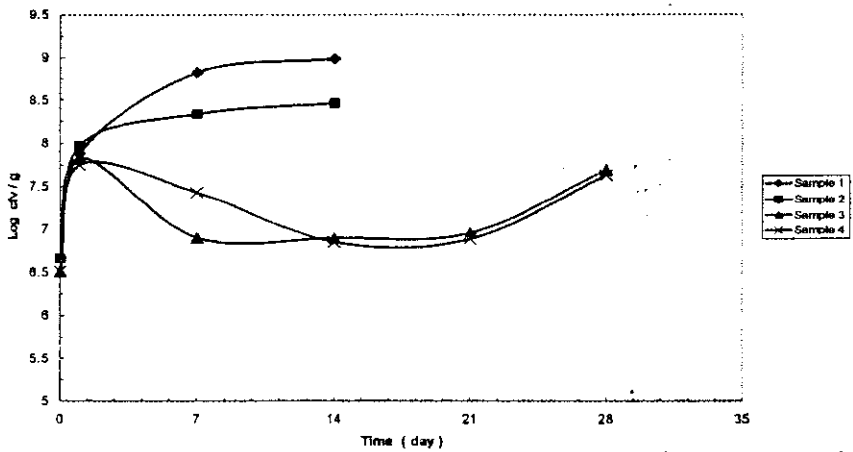

Figure (1):- Total viable aerobic bacterial count in the old buffalo sausage during storage at 4°C. Samples 1 and 2 were rejected after two weeks.

- Count of meat sample = 6.80 log cfu/g.
- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.

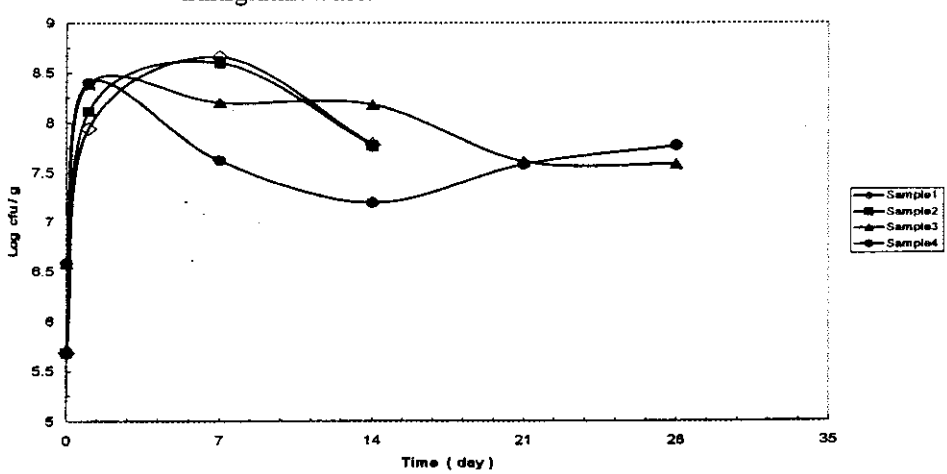

Figure (2): Lactic acid bacterial count in the old buffalo sausage during storage at 4°C. Samples 1 and 2 were rejected after two weeks.

- Count of row meat sample = 5.99 log cfu/g.
- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.

Data in Figure 3 show the changes in *Micrococci* and *Staphylococci* count in the old buffalo sausage during storage. The count of samples No. 1 and 2 was rapidly increased at the first 24h of fermentation period from $9.1x10^5$ to $6.2x10^7$ and $2.7x10^8$, respectively and slightly increased during the first two weeks of storage. After that *Staphylococci aureus* were detected in these samples and they were rejected. Meanwhile, the samples No. 3 and 4 prepared with starter culture had a higher *Micrococci* and *Staphylococci* counts ($2.6x10^7$) than samples No. 1 and 2. The counts increased also slightly during the frist two weeks of storage and then slightly decreased until the end of storage due to the competition of the microorganisms as mentioned by Dolazo *et al.* (1999).

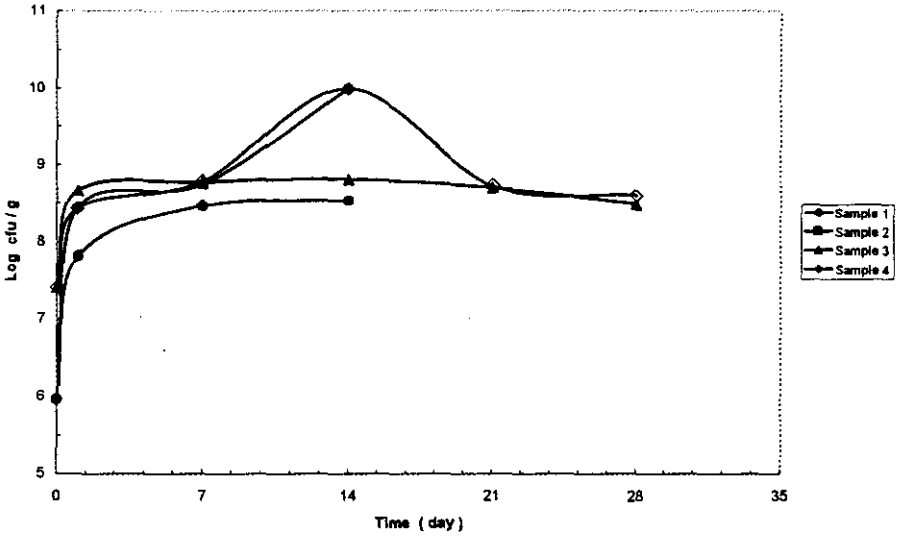

Figure (3):- *Staphylococci* and *Micrococci* count in the old buffalo sausage during storage at 4°C. Samples 1 and 2 were rejected after two weeks.

- Count of row meat sample = 5.7 log cfu/g.
- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.

Figure 4 shows the yeast and molds count during storage of old buffalo sausage. During the first week of storage the yeast and molds count were increased in all samples from $1.3 - 2.5x10^4$ at zero time to $0.5 - 2.4x10^7$. During the next storage period the counts were gradually decreased.

The chemical composition of the old buffalo sausage was determined and given in Table (1). The addition of enzyme and starter increased the protein content of the sausage.

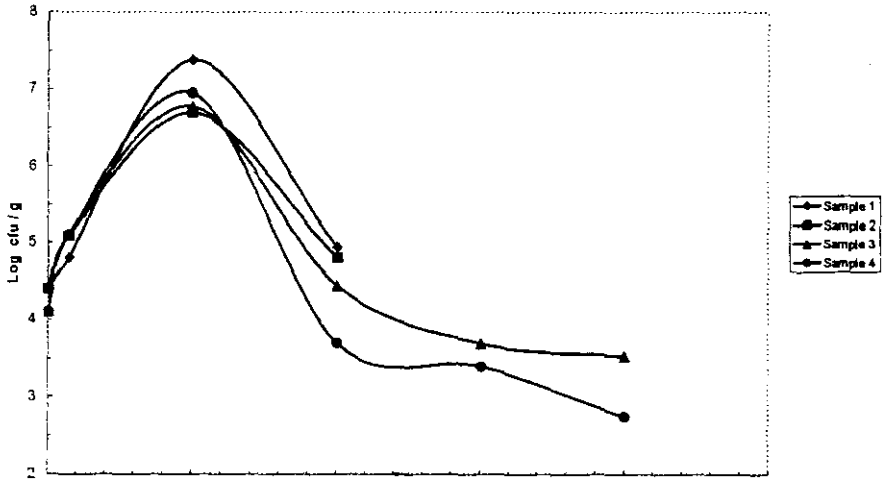

Figure (4): Yeast and molds count in the old buffalo sausage during storage at 4°C. Samples 1 and 2 were rejected after two weeks.

- Count of row meat sample = 4.63 log cfu/g.
- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.

Table (1):- Chemical composition of fermented sausage on dry basis.

Samples	Component percentage on dry basis			
	Protein	Ash	Fat	NFE
1	32.87	9.30	41.29	16.54
2	33.34	9.01	40.70	16.95
3	33.54	9.47	40.21	16.78
4	37.19	9.10	40.55	16.62

- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.
- NFE: Nitrogen free extract.

The moisture content during storage is given in Table (2). Results show that moisture content of all samples was decreased at the end of storage. The decrease of moisture content during progression of cold storage is due to the evaporation of water. Initially, moisture content of all samples was similar, however, during storage after one week, a graduall decrease of moisture content was observed. The sausage containing mixture of starter culture and transglutaminase had a lower reduction percentage than the other samples. Whereas, sausage containing only transglutaminase had a higher reduction

percentage of moisture content. A lower moisture reduction percentage of sausage containing starter culture and transglutaminase may be due to the higher content of protein in this sample as well as the effect of this enzyme on its structure by denaturation and restructure of protein. Miller, *et al.* (1968) reported that, when the ability of the protein to bind moisture decreases, the moisture loss increases. These findings are in agreement with our results.

Table (2): Effect of cold storage at 4°C on moisture content of the old buffalo sausages.

Storage period (week)	Samples							
	1		2		3		4	
	Moisture content	Decreasing %	Moisture content	Decreasing %	Moisture content	Decreasing %	Moisture content	Decreasing %
Zero	47.69	0.00	48.08	0.00	48.84	0.00	48.28	0.00
1	46.74	1.99	47.64	0.92	47.47	2.81	47.91	0.77
2	46.47	2.59	47.21	1.81	46.43	4.46	47.43	1.76
3	rejected		rejected		46.32	4.46	47.43	1.76
4	rejected		rejected		46.13	4.85	47.29	2.05

- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.

Total volatile nitrogen (TVN) used as an indication of protein degradation during storage (Foda *et al.*, 1984). Data in Table (3) show that the amount of TVN of all samples at zero time was slightly different. Continuous increase of TVN during storage of fermented sausage was noticed in all samples. Results also revealed that the sausage samples containing enzyme had a higher increase in TVN after two weeks of storage followed by the control sample than samples contained starter culture or mixture of starter culture and enzyme. The increase in TVN during storage period of sausage may be attributed to the breakdown of nitrogenous substances by microbial activity. These results are similar to that of Abd-El-Salam (1978) and El-Dashlouty (1978) who found that TVN content increased by prolonging the cold storage period of sausage. The effect of cold storage at 4°C and storage period on the TBA value can be followed up in table (3). This test is widely used for muscle foods. It can be seen that TBA values increased as a function of storage period indicating the oxidation of some fat during storage. This trend is similar to TVN trend. The elevation increase of TBA may be due to the formation of lipid peroxides by non enzymatic oxidation and/or by lipoxygenase enzyme which perform further metabolization to carbonyl compounds and fatty acids affecting flavor and formation of toxic substances (Cerise *et al.*, 1973). Total volatile fatty acids (TVFA) were formed during lipolysis of fats and oxidation of peroxides and carbonyl compounds. The TVFA values had a similar trend as of TVN and TBA (Table 3).

Table (3): Quality parameters of old buffalo sausage as affected by storage period at 4°C.

Sample No.		Storage period (week)				
		Zero	1	2	3	4
1	TVN	90.53	127.56	515.33	rejected	rejected
	TBA	0.071	0.138	0.370	rejected	rejected
	TVFA	27.38	39.96	63.70	rejected	rejected
2	TVN	93.18	149.40	631.75	rejected	rejected
	TBA	0.063	0.140	0.349	rejected	rejected
	TVFA	45.88	60.68	82.88	rejected	rejected
3	TVN	92.54	131.30	142.20	159.16	428.38
	TBA	0.062	0.081	0.148	0.205	0.260
	TVFA	51.80	56.98	60.68	103.60	147.26
4	TVN	87.57	132.41	141.50	150.77	160.53
	TBA	0.066	0.088	0.153	0.196	0.223
	TVFA	54.02	62.90	65.86	105.82	146.04

- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.

Data in Table (4) show the effect of cold storage on the acidity of sausage samples. It can be noticed that acidity increased by increasing the storage period. This increase may be due to the production of lactic acid by starter culture or meat flora in samples without starter. This caused the continuous increase of acidity during storage. WHC and plasticity were increased after one week of storage and decreased after two weeks. This decreases possibly due to the restructure and/or aggregation, or to the biochemical changes associated with cooling of meat products as mentioned by Fox *et al.* (1990).

Table (4): Changes of acidity, water holding capacity and plasticity of old buffalo sausage during storage at 4°C.

Sample No.	Parameter	Storage period (week)				
		Zero	1	2	3	4
1	Acidity	0.574	0.610	0.724	rejected	rejected
	WHC cm^2/0.3 g sample	7.96	11.49	8.76	rejected	rejected
	Plasticity cm^2/0.3 g sample	3.56	3.87	2.87	rejected	rejected
2	Acidity	0.625	0.668	0.710	rejected	rejected
	WHC cm^2/0.3 g sample	13.25	9.48	9.96	rejected	rejected
	Plasticity cm^2/0.3 g sample	3.41	4.39	2.42	rejected	rejected
3	Acidity	0.635	0.714	0.747	0.792	0.853
	WHC cm^2/0.3 g sample	10.01	8.45	7.63	7.86	7.72
	Plasticity cm^2/0.3 g sample	2.97	3.49	2.56	2.04	1.84
4	Acidity	0.580	0.768	0.811	0.840	0.912
	WHC cm^2/0.3 g sample	6.75	8.82	8.60	8.53	8.10
	Plasticity cm^2/0.3 g sample	2.58	3.38	2.14	2.28	2.07

- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase..

Sensory evaluation:

Table (5) shows the means values of aroma taste, color, texture, acceptability and overall acceptability. Data show that samples prepared using starter culture or mixture of it with transglutaminase has higher scores of all sensory properties studied than the other two samples. Generally, sausage prepared with the mixture of starter culture and transglutaminase was preferred.

Table (5): Sensory evaluation (means of panelists) of the cooked and fried old buffalo sausage.

Sample	Aroma		Taste		Color		Texture		Acceptability		Overall	
No.	Cooked	Fried	Cooked	Fried	Cooked	Fried	Cooked	Fried	Cooked	Fried	Cooked	Fried
1	6.6	6.8	5.8	6.0	6.0	6.8	6.4	7.2	6.8	6.4	6.32	6.64
2	5.2	6.4	6.4	6.0	6.2	5.4	6.6	6.2	5.8	6.2	6.04	6.04
3	6.5	7.0	6.2	7.4	6.4	5.6	7.2	6.8	6.0	6.8	6.46	6.72
4	7.4	7.2	7.0	7.4	8.0	8.2	7.8	8.2	8.0	8.4	7.64	7.88

- Sample 1: Control (without starter and enzyme).
- Sample 2: Sausage with transglutaminase.
- Sample 3: Sausage with starter.
- Sample 4: Sausage with a mixture of starter cultures and transglutaminase.

In conclusion it is possible to recommend the use of transglutaminase and starter culture to produce restructured old buffalo fermented sausage to enhance the market value of old buffalo meat and introduce a new product in the meat market.

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انتاج سجق متخمر من لحم الجاموس العجوز باستخدام انزيم الترنس جلوتاميناز

جمال عبدالتواب الشطانوفي

قسم علوم الاغذية – كلية الزراعة – جامعة عين شمس – القاهرة – مصر

تم انتاج السجق المتخمر من باستخدام مزرعة باديء وانزيم الترنس جلوتاميناز وكذلك باستخدام خليط منهما، تم تخزين عينات السجق لمدة أربعة أسابيع علي درجة حرارة ٤ °م.

العدد الكلي للبكتريا الهوائية كان اعلي في السجق الغير متخمر عن السجق المتخمر، وعلي العكس فان عدد بكتريا حمض اللاكتيك كان اعلي في السجق المتخمر

كما اعطي مدة حفظ اطول للمنتج، وفي نفس الوقت فان عدد خلايا الخمائر والفطريات كانت اقل في المنتج المتخمر، جميع العينات كانت خالية من السالمونيلا.

اضافة انزيم الترانس جلوتاميناز ومزرعة البادىء أو خليط منهما أدي الي زيادة طفيفة في محتوي البروتين في السجق. انخفض المحتوي الرطوبي لجميع العينات بدرجة بسيطة بزيادة مدة التخزين، وكان نسبة انخفاض الرطوبة اقل في حالة السجق المحتوي علي خليط من الترانس جلوتاميناز ومزرعة البادىء. وحدث زيادة لمعدلات النتروجين الكلي المتطاير (TVN) والـ Thiobarbituric acid (TBA) والاحماض الدهنية المتطايرة الكلية (TVFA) بزيادة مدة التخزين وكان تطور هذه العوامل في السجق المتخمر المحتوي علي الترنس جلوتاميناز اقل من العينات الاخري.

وفي النهاية فنحن نوصي باستخدام انزيم الترانس جلوتاميناز ومزرعة باديء لانتاج سجق متخمر من لحم الجاموس العجوز معدل التركيب ذو قدرة ربط ملائمة وذلك لتحسين القيمة التسويقية للحم الجاموس العجوز، ولتقديم منتج جديد في سوق اللحم.