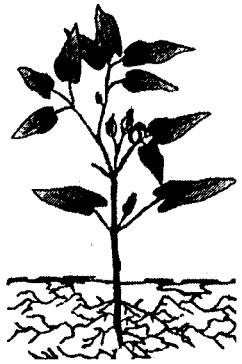

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TARHANA DOUGH AS A SOURCE OF PROBIOTICS IN PROCESSING OF FUNCTIONAL FERMENTED MEAT SAUSAGE (AS A HOME MADE TRIAL)

[26]

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ABSTRACT

Tarhana dough (which made from a wheat flour combined with barley whole meal in ratio (1:1) after 5 days of fermentation in wet form) as a function fermented food product was used in manufacturing of fermented sausage to add nutritional value as source of probiotic. Physicochemical parameters, lactic acid bacteria, enterobacteriaceae count and detection of some pathogenic bacteria were determined in fresh samples and during ripening at 25 °C for 35 days in different fermented sausage treatments (control treatment with *Lactobacillus plantarum* (LS), yoghourt treatment (YS), and tarhana dough treated with levels 25, 50 and 75 g /kg mix. TS1, TS2 and TS3, respectively). The respective contribution of tarhana dough in fermented sausage ripening was determined significantly decreasing both pH value and moisture content of samples with high concentration of tarhana dough. The puncture force and elasticity throughout the ripening period of this product were not intense according to tarhana adding except for puncture force value which was higher in the sample processed with 75 g tarhana /Kg mix. The study revealed that a desired increase in lactic acid bacteria count as well as reduction in enterobacteriaceae count and staphylococci detection can be achieved with increasing adding of tarhana dough in sausage mixture and when compared with samples without tarhana dough. *Salmonella* was not detected in any samples from the beginning of experiment and during 35 days of ripening. Adding of tarhana dough improved most sensory attributes of fermented sausage especially in the highest level of tarhana addition as indicated by panelists. Results suggest that tarhana dough could be successfully utilized for meat fermentation to produce safe, with good sensory attributes and highly nutritious synbiotic fermented sausage.

INTRODUCTION

Tarhana dough is generally prepared with wheat flour and yoghurt in the ratio of 1:1 or 2:1. The mixture is kneaded with vegetables and spices and then fermented with yoghurt bacteria and baker's yeast. Tarhana could be produced dry or wet and could be stored for 1-2 years under proper storage conditions. The soup prepared from dry or wet tarhana has a sourish taste and is popular as a breakfast and appetizer prior to main meals in Turkey. Also, dried nugget and biscuit forms of tarhana can be consumed as a snack food.

Tarhana's nutritional properties are a result of fermentation associated with yogurt bacteria and yeast. It is a good protein source because yogurt makes up for the limited amino acids (lysine) in wheat, which is a good source of minerals (Fe and Mn) and fiber (**Erbas *et al* 2005**). Tarhana is a functional food because of its prebiotic and physiological effects resulting from indigestible carbohydrate, vit.B, organic acid and free amino acid contents and as probiotic for its content of lactic acid bacteria. Tarhana dough is a good source of lactic

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acid bacteria (LAB), log number reach to 12.78, especially which is made from a wheat flour combined with barley whole meal in ratio (1:1) as found by **Elsheshetawy and yasin, (2008)**. This means that, the addition of barley whole meal to tarhana formula enhanced the LAB growth. Barley whole meal was utilized in tarhana production as a source of β-glucan (preblotic) as well as LAB (probiotic). Fermented cereal-based gruels have also been reported to improve the nutritional quality, protein digestibility and bioavailability of amino acids **(Svanberg and Lorri 1997 and Mugula *et al* 2002)**.

Fermented sausages are produced by fermenting and drying a raw meat batter containing sugar, seasoning / spices, and/or curing agents. The fermentation is conducted by natural microflora in the ingredients and/or by added starter cultures **(Hwang *et al* 2009)**. Dairy products such as fermented milk and yoghurt are often used as carriers for probiotic cultures, although recent attention has been directed to the use of different kinds of fermented sausages for the same purpose **(Klingberg *et al* 2005)**.

This study was carried out to utilize tarhana dough enhanced by β-glucan to enrich its nutritive value with various health promoting properties as a starter in ripening of dry fermented sausage (a trial to produce it in homes) with microbiological and physiochemical evaluation of its characteristics during ripening and organoleptically evaluation after ripening.

MATERIALS AND METHODS

1- Materials

Wheat flour (72% extraction), yoghurt, dried baker's yeast, tomato paste, green as well as red pepper, onion, lean meat, fat tissue, dry spices (black pepper, nut meg, all spices, red pepper, cloves, cinnamon ginger and cumin), shortening, natural casing and salt were purchased from local markets in Cairo. Glucose, sodium nitrite and sodium ascorbate, MRS agar (according to **DeMan *et al* 1960**), MacConkey agar, Bismuth sulphite agar media **(Difco and BBL Manual 2003)**. Barley (*Hordium vulgare L.)* whole meal used in this study was obtained from barley research section, Field Crops Research Institute, Agric. Research Center, Giza, Egypt. The starter culture *Lactobacillus plantarum* DSMZ 20174 was obtained from the Egyptian Microbial Culture Collection (EMCC), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

2- Preparation of tarhana dough

Tarhana samples were prepared according to the method of **Erkan *et al* (2006)** with omit of paprika to ingredients of tarhana. To prepare tarhana samples, onions 180 g, green and red peppers 75 g were chopped in a food processor (Toshiba). Tomato paste 112 g and salt 60 g were added and the mixture was well mixed. Yoghurt 600 g and baker's yeast 15 g were added to the mixture and blended until complete homogenization. Wheat flour and barley whole meal (1:1) 375 g were added and homogenate the mixture and kneaded in a steel saucepan with a spoon to constitute the tarhana dough. The dough was put into covered containers for fermentation at 30° C for 7 days. After fermentation, the tarhana dough was used to prepare fermented sausage.

3- Preparation of fermented sausage samples

Dry fermented sausage was prepared using the following formula: lean meat 68%, fat tissue12.8%, NaCl 2.3%, glucose 0.5%, water 15.5%, dry spices 0.82%, sodium nitrite 0.005%, sodium ascorbate 0.05% as well as starter culture. In control treatment (LS), the starter culture *Lactobacillus plantarum* DSMZ 20174 was grown in MRS broth at 30°C for 2 days then the culture was centrifuged and washed twice with distilled water and then was added to sausage material as active cultures aiming to 7 log cfu / g sausage. In yoghourt treatment (YS), yoghurt culture was added at a rate of 70g / kg mix according to **Ebeid, (2000)**. In tarhana dough treatments (TS), the starter culture was tarhana dough which was added to sausage mix with levels 25, 50 and 75 g /kg mix. The ingredients except starter culture were processed in a mincer until a homogenous distribution was reached, then inoculated with starter culture. The sausage mixtures were stuffed into natural casing. Sausage samples received a 1 min. dip in 0.1% sorbic acid solution to prevent any fungal growth on the surface during fermentation. After that, the sausage samples were ripened at ambient temp. (25°C) for 35 days.

4- Analytical methods

Physico-chemical analysis

Moisture content of dry fermented sausage samples was determined according to AOAC methods **(AOAC, 2007)**. pH was determined dur-

ing the fermentation and ripening of dry fermented sausage by blending sausage samples with distilled water (1:10) and the pH values of the suspensions were determined by pH meter (HANNA-Instrument, USA). Texture and elasticity of fermented sausage samples were measured by Instron Universal Testing Machine model 4302, (England) Load cell 100 N / 10 kN, compression 50% **(Philips *et al* 1988)**.

Microbiological analysis

At selected times during fermentation and ripening, duplicate samples (approximately 10 g) for each sausage sample at 0, 2, 7, 14, 28, and 35 days were aseptically transferred to sterile plastic bags and homogenised in a Stomacher machine (PBI, Milan, Italy) for 2 min with peptone water (Oxoid). Appropriate decimal dilutions of the samples were prepared using the same diluent and plated on MRS (Oxoid) agar and incubated at 30 °C for five days, to determine the lactic acid bacterial count. At the same time, fermented sausage samples were examined for, Enterobacteriaceae count, Salmonella and Staphylococci detection according to **Difco and BBL.Manual (2003).**

Sensory evaluation

The fermented sausage samples were sensory evaluated by a panel group of 10 members, randomly selected from the staff members, researchers and PhD students of the Food Science Dep., Fac. of Agric., Ain Shams University, Cairo, Egypt. As described by **Spaziani *et al* (2009)** with some modifications, a list of 12 attributes with definitions was used by the panel: 3 for appearance; (crust, fat/lean ratio, fat/lean demarcation); 2 for (texture; hardness, chewiness); 4 for (odor; pepper, sour, rancid, sweet) and 3 for (flavor; salty, sweet and mouldy). Each attribute was rated on a scale from one (absence of perception) to 10 (very intense perception). The sausage slices were 0.5 cm thick, cut with a knife and served at room temperature.

Statistical analysis

Experimental data were analyzed for variance (ANOVA), two ways and significant differences among the means were determined by Duncan's multiple – range test using the Statistical Analysis System (**SAS, 1996**) computer program.

RESULTS AND DISCUSSION

1- Physicochemical characterization

A- pH values

Data in **Table (1)** indicated that pH values of the fresh sausage were significantly ($p< 0.01$) higher than those during the ripening period at 25°C. The pH values of samples made from tarhana dough were significantly ($p< 0.01$) lower than control sample and the yoghurt treated samples from the beginning and during ripening period. This is due to the high number of LAB in these treatments and their activity **Elsheshetawy and Yasin, (2008).**

The data revealed that, there was a continuous decrease in pH values in all samples during 28 days of ripening period of fermented sausage reflecting the production of the bacterial metabolites, then exhibited a slight rise in pH values at 35 days of the storage at 25 °C. These results coincided with those of **Montet *et al* (2009).**

It was suggested that, the pH values increase in the later stages of the ripening period is related to the formation of peptides, amino acids and ammonia as a result of proteolysis as reported by **Montet *et al* (2009).**

B- Moisture %

The average initial values of moisture content were 55 - 62 % for the fresh sausages **(Table, 1).** The moisture % was significantly ($p< 0.01$) decreased during ripening period of sausages. The greater percentage of the moisture loss was appeared in both samples made with the tarhana dough (TS2, TS3). This loss of moisture % is attributed to the intense dehydration during ripening period and above that to the increase of salt content as discussed by **Lindqvist and Lindblad, (2009).**

C- Texture

Figs (1 & 2) show the changes in textural parameters including puncture force (N) and elasticity (N/cm) of dry fermented meat sausages from the beginning and during ripening period as affected by the addition of tarhana dough. These are considered positive attributes in sausage manufacturing:

Table 1. Changes of pH values and moisture content in different fermented sausage samples during ripening period at 25°C

Ripening period (days)	pH					Moisture %				
	LS	YS	TS1	TS2	TS3	LS	YS	TS1	TS2	TS3
0	6.1^{Aa}	5.9^{Ab}	5.6^{Ac}	5.3^{Ad}	5.06^{Ae}	58.0^{Ac}	62.1^{Aa}	60.03^{Ab}	55.7^{Ad}	56.3^{Ad}
2	5.8^{Ba}	5.5^{Bb}	5.4^{Bc}	5.1^{ABc}	4.9^{Bd}	55.8^{Bc}	60.9^{Ba}	58.9^{Bb}	54.4^{Bd}	54.7^{Bd}
7	5.4^{Ca}	5.0^{Cbc}	5.1^{Cb}	4.9^{BCc}	4.7^{Cd}	49.3^{Cc}	53.0^{Ca}	51.4^{Cb}	48.6^{Cd}	48.1^{Ce}
14	5.1^{Ea}	4.8^{Db}	4.8^{Db}	4.4^{Dc}	4.3^{Dc}	41.5^{Dc}	45.0^{Da}	43.6^{Db}	41.3^{Dd}	41.2^{Dd}
28	4.8^{Fa}	4.4^{Eb}	4.7^{Da}	4.4^{Db}	4.3^{Db}	31.2^{Ec}	33.5^{Ea}	32.4^{Eb}	30.1^{Ed}	30.4^{Ed}
35	5.2^{Da}	5.1^{Ca}	5.1^{Ca}	4.8^{Cb}	4.6^{Cc}	27.2^{Fb}	28.9^{Fa}	27.2^{Fb}	26.0^{Fc}	26.4^{Fc}

LS = fermented sausage with *Lactobacillus plantarum*
YS = fermented sausage with yoghurt
TS1= fermented sausage with tarhana dough (25g/kg mix.)
TS2= fermented sausage with tarhana dough (50g/kg mix.)
TS3= fermented sausage with tarhana dough (75g/kg mix.)

Means with the same superscript capital letter and small letter within the same columns and rows respectively are not significantly different ($p > 0.01$).

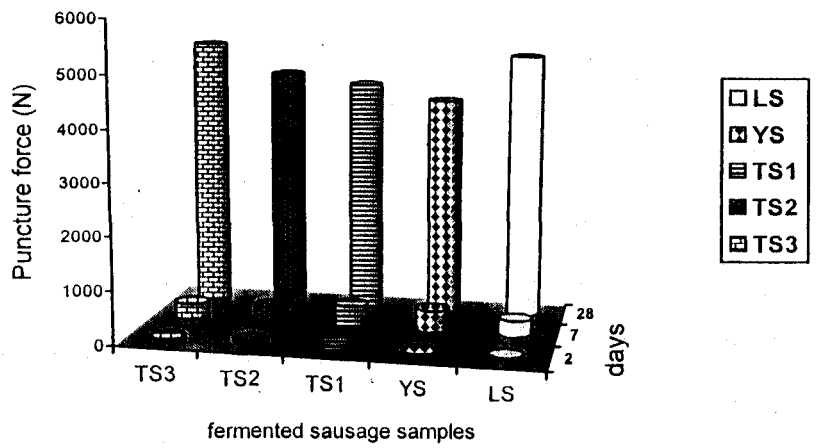

Fig. 1. Puncture force (N) of different fermented sausage samples during ripening period at ambient temperature.

LS = fermented sausage with *Lactobacillus plantarum*

YS = fermented sausage with yoghurt

TS1 = fermented sausage with tarhana dough (25g/kg mix.)

TS2= fermented sausage with tarhana dough (50g/kg mix.)

TS3= fermented sausage with tarhana dough (75g/kg mix.)

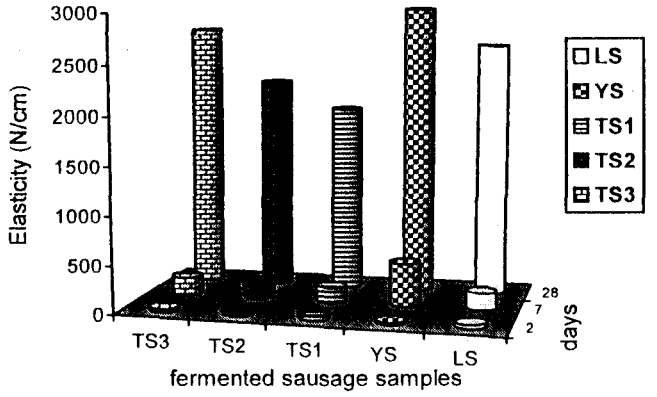

Fig. 2. Elasticity (N/cm) of different fermented sausage samples during ripening period at ambient temperature .

LS = fermented sausage with *Lactobacillus plantarum*

YS = fermented sausage with yoghurt as

TS1= fermented sausage with tarhana dough (25g/kg mix.)

TS2= fermented sausage with tarhana dough (50g/kg mix.)

TS3= fermented sausage with tarhana dough (75g/kg mix.)

1. Puncture force (N)

From **Fig. (1)** it could be found that, puncture force (N) was varied between different dry fermented meat sausages. The greater puncture force was achieved in sample which made by adding tarhana dough (75g / Kg mix). As ripening period increased the values of hardness were also increased. The greater puncture force values were appeared in TS3. It could be easily to arrange the samples descending based on puncture force values as: TS3, LS, TS2, TS1 and then YS, respectively. This is due to the fact that during the ripening of meat products shrinkage is proportional to the water loss and also to the increase in acid formation. These data are agreement with those of **Spaziani *et al* (2009).**

Generally, the major changes in fermented sausage structure take place during fermentation when the pH declines and the myofibrillar proteins aggregate to form a gel. After fermentation, ripening is a major factor affecting rheological properties (**Gonzalez – Fernandez *et al* 2006**).

2. Elasticity (N/cm)

From **Fig. (2)** it could be notice that, at the beginning of ripening period (2 days) at 25 °C. there were differences between elasticity values between different treatments. The sample treated with yoghurt had the lowest elasticity value than the other treatments. During the ripening of dry sausage, there was an increase in this criterion especially in YS treatment (which made with yoghurt) after 28 days of maturation period followed by TS3 (made with tarhana dough 75 g/ Kg mix). On contrast, the TS2, TS1 showed little increase in elasticity after the same period when compared to them.

Both hardness & elasticity may be considered positive characters affecting the fermented meat sausage sliceability and both pH & moisture content have been found to play significant role in texture of dry meat sausage as reported by **Herrero *et al* (2007)**.

2- Microbiological analysis

As known, fermented raw meat sausage, as prepared in laboratory, are subjected to relatively high drying temperature (> 24 °C) in order to enhance growth of the added lactic acid bacteria and decrease pH.

During the ripening of sausage, the lactic acid bacterial count was rapidly increased by almost 1 log cycle in all tarhana dough treatments as seen in **Table (2).** At the same time there were significant ($p< 0.01$) differences in the count of LAB in tarhana dough sausage from the beginning till the end of ripening period (8.8, 9.9 and 10.8 log number for TS1, TS2 and TS3, respectively). It is obvious that the viability of LAB significantly higher

Table 2. microbial profile (log cfu/ g) of different fermented sausage samples during ripening period at 25°C

Ripening period (days)	Log No. (cfu / g)																			
	LS				YS				TS1				TS2				TS3			
	LAB	En.	St.	S.	LAB	En.	St.	S.	LAB	En.	St.	S.	LAB	En.	St.	S.	LAB	En.	St.	S.
0	9.2^{Cd}	<2	<2	ND	9.6^{Bc}	<2	<1	ND	9.6^{Cc}	<2	<1	ND	9.9^{Cb}	<2	<1	ND	10.7^{Ca}	<2	<1	ND
2	9.8^{Ac}	<1	<1	ND	9.9^{Ac}	<1	<1	ND	10.6^{Bb}	<1	ND	ND	10.8^{ABc}	<1	ND	ND	11.8^{Aa}	ND	ND	ND
7	9.6^{Bd}	<1	ND	ND	9.9^{Ac}	<1	<1	ND	10.9^{Ab}	ND	ND	ND	10.7^{Bb}	ND	ND	ND	11.7^{Aa}	ND	ND	ND
14	9.5^{Bd}	ND	ND	ND	8.8^{Ce}	ND	ND	ND	9.7^{Cc}	ND	ND	ND	11.0^{Ab}	ND	ND	ND	11.4^{Ba}	ND	ND	ND
25	8.9^{Dd}	ND	ND	ND	8.6^{De}	ND	ND	ND	9.3^{Dc}	ND	ND	ND	9.7^{Cb}	ND	ND	ND	10.8^{Ca}	ND	ND	ND
35	8.7^{Dd}	ND	ND	ND	7.8^{Ec}	ND	ND	ND	8.8^{Ec}	ND	ND	ND	9.9^{Cb}	ND	ND	ND	10.8^{Ca}	ND	ND	ND

LS = fermented sausage with *Lactobacillus plantarum*

YS = fermented sausage with yoghurt

TS1= fermented sausage with tarhana dough (25g/kg mix.)

TS2= fermented sausage with tarhana dough (50g/kg mix.)

TS3= fermented sausage with tarhana dough (75g/kg mix.)

LAB= lactic acid bacteria. En. = enterobacteriaeace St. = Staphylococci . S.= Salmonella.

Means with the same superscript capital letter and small letter within the same columns and rows respectively are not significantly different ($p > 0.01$).

ND: not detected.

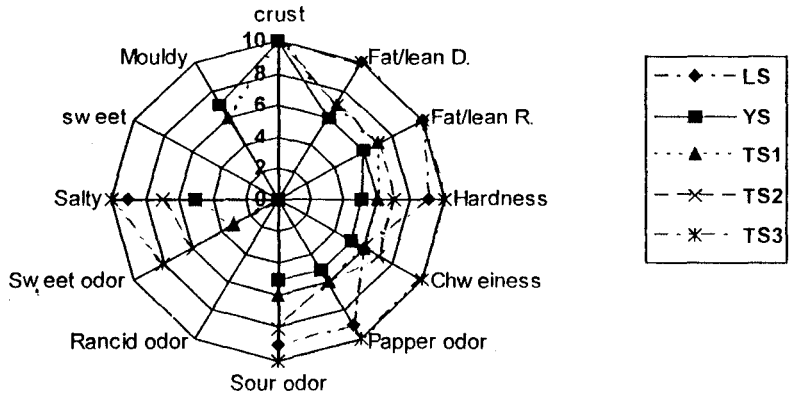

Fig. 3. Sensory profiles of different fermented meat sausage samples at the end of ripening period at ambient temperature

LS = fermented sausage with *Lactobacillus plantarum*

YS = fermented sausage with yoghurt

TS1= fermented sausage with tarhana dough (25g/kg mix.)

TS2= fermented sausage with tarhana dough (50g/kg mix.)

TS3= fermented sausage with tarhana dough (75g/kg mix.)

in TS3 sausage as compared with other sausage treatments. From **Table (2)** fermented sausage treated with 50 and 75 g of tarhana /kg of mixture gave the significantly higher values of LAB count when compared with LS and YS sausage.

Inactivation of pathogens during ripening period is a crucial step in the safe production of fermented sausage not undergoing heat-treatment. The metabolic substances produced by LAB have been found to possess bactericidal and bacteriostatic properties against spoilage and food poisoning microorganisms (**Daeschel, 1989; Klaenhanmer, 1988**).

Enterobacteriaceae count of all treatments was declined in accordance with decrease in pH and moisture % especially in TS3 as well as TS2 and TS1 which contain different levels of tarhana dough which do not have any Enterobacteriaceae after two and seven days of fermentation, respectively. The same trend was observed with pathogenic Staphylococci where dry fermented sausage with different levels of tarhana dough were not containing Staphylococci after two days when compared with LS and YS sausages. These results are agreement with **Riordan *et al* (1998)** as well as **Muthukumarasamy and Holley, (2007)** hence they referred to that the inactivation of pathogens increases with decreasing pH levels and increasing salt and nitrite levels. More precisely, **Shadbolt *et al* (1999)** observed that as the water activity was lowered, the magnitude of the first phase inactivation consistently increased. The variation of the count reduction for pathogens was contributed largely by the changes of pH value and moisture % is in general agreed with **Hwang *et al* (2009).** At the same time the ripening of dry fermented sausage at 25 °C resulted in faster inactivation of pathogens (**Sharma *et al* 2004 and Calicioglu *et al* 2001**) and this may be another explanation for rapid decrease in pathogens count. Salmonella was not detected in dry fermented sausage treatments from the beginning of the experiment till the end. That may be due to the efforts made to reduce contamination by selection good

raw material and improving hygiene and control procedures.

3- Sensory evaluation

Using the 12 descriptive attributes the sensory profiles of different fermented sausage samples (with different starter cultures and different levels of tarhana dough) at the end of the ripening period **(Fig. 3)** was produced. In agreement with its significant ($p< 0.01$) higher in LAB count and significant ($p< 0.01$) lower in pH and moisture % the TS3 treatment had the highest values in texture (hardness and chewiness), odor (pepper, sour and sweet) as well as flavor (salty). This may be due to the good enhancement of tarhana dough to dry fermented sausage at 75g /kg mix level in texture, odor and flavor. At the same time tarhana dough prevented mouldy flavor in TS2 and TS3 when compared with YS and TS1 (contain the lowest level of tarhana dough) and as a normal happens the mouldy flavor was not appeared in LS treatment. These results may be due to the preservative power of tarhana dough in TS3 which contained the highest count of LAB as well as lowest pH values and moisture%. There were no difference between LS (which made with *Lactobacillus plantarum* as a starter culture) and TS3 in all appearance characteristics but the TS3 treatment had the highest sensory scores in other attributes (texture, odor and flavor). This is due to the effect of flavor and composition of added tarhana dough **(Erkan *et al* 2006).**

Thus, it can recommend the use of tarhana dough to produce safe and highly nutritious dry fermented sausage with significant enhanced sensory characteristics.

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جامعة عين شمس ، القاهرة
مجلد(٥٤)، عدد (٢)، ٣٣٩-٣٤٨، ٢٠٠٩

إستخدام عجينة التارهانا كمصدر للميكروبات النافعة فى تصنيع سجق لحم متخمروظيفى (كمحاولة لتصنيعة فى المنزل)

[٢٦]

همت الششتاوى الششتاوى[١]- نسرين محمد نبيه يسن[١] - سلوى محمود أبو الفتوح[١]

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الموجـــز

تم إستخدام عجينة التارهانا (المصنوعة من خليط من دقيق القمح الأبيض مع مجروش الشعير بنسبة ١:١بعد فترة التخمير ٥ أيام على الصورة الرطبة) كمنتج وظيفى متخمر فى تصنيع السجق المتخمرالجاف حتى يضيف إليه قيمة تغذوية نظرالإنه مصدرللبكتريا النافعة (البروبيوتيك) . تم تقدير و تتبع التغيرات الفيزيوكيميائية و كذلك أعداد بكتريا حمض اللاكتيك وتقدير أعداد البكتريا المعوية مع الكشف عن بعض البكتريا الممرضه فى العينات الطازجة و خلال فترة التسوية عل ٢٥°م لمده ٣٥ يوم فى عينات السجق المتخمر المختلفة (الكنترول الملقحة بسلالة . *Lactobacillus plantarum* (LS) والملقحة بالزبادى (YS) والعينات المعاملة بعجينة التارهانا بنسب ٢٥ و ٥٠ و٧٥ جم/كجم مخلوط سجق والمسمى TS1 - TS2 - TS3 على التوالى). وقد لوحظ إنخفاض معنوى فى قيم كل من pH ونسبة الرطوبة فى العينات المحتوية على تركيزات مرتفعة من عجينة التارهانا.

ووجد أن قوة الإختراق والإستطالة خلال فترة التسوية للمنتج لم تتغير بإضافة عجينة التارهانا فيما عدا العينات المحتوية على ٧٥ جم عجينة التارهانا لكل كجم مخلوط.

كما تميزت العينات المحتوية على عجينة التارهانا بارتفاع أعداد بكتريا حمض اللاكتيك وإنخفاض فى أعداد البكتريا المعوية وعدم تواجد لبكتريا الستاف (staphylococci) مقارنة بالعينات الأخرى. وبالنسبة لبكتريا السالمونيلا *Salmonella* لم تتواجد فى كل العينات من بداية التجربة وكذلك خلال ٣٥ يوم من فترة التسوية . وقد حسنت إضافة عجينة التارهانا معظم الخصائص الحسية للسجق المتخمر وبصفة خاصة عينات TS3.

ومن مجمل النتائج يمكننا أن نستخدم بنجاح عجينة التارهانا فى تخميراللحوم لإنتاج سجق متخمر أمن و مرتفع القيمة الغذائية و مصدرلكل من الميكروبات النافعة (بكتريا حمض اللاكتيك) وكذلك البريبيوتيك (الراجع لوجود البيتا جلوكان فى تركيب عجينة التارهانا).

تحكيـم: أ.د نادية رفعت عبد الرحمن
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