

USE OF SESAME AND PROBIOTIC LACTOBACILLI IN MAKING NUTRACEUTICAL FERMENTED DAIRY PRODUCTS.

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ABSTRACT

Nutraceutical fermented milk product rich in polyunsaturated fatty acids and antioxidants was produced from buffaloes milk with *S. thermophilus* , *Lb. gasseri* or *Lb. rhamnosus* and sesame seeds. Treatments were made as follows: *S. thermophilus* and *Lb. gasseri* ; *S. thermophilus* and *Lb. rhamnosus* ; *S. thermophilus* ; *Lb. gasseri* and 2% sesame ; *S. thermophilus* ; *Lb. gasseri* and 4% sesame seeds ; *S. thermophilus* ; *Lb. rhamnosus* and 2% sesame seeds and *S. thermophilus* ; *Lb. rhamnosus* and 4% sesame seeds.

Chemical, microbiological and organoleptic properties were determined in fresh product and throughout 15 days of storage at 6 °C. The addition of sesame increased T.A, protein, fat, amino acids especially some essential amino acids and polyunsaturated fatty acids specially omega fatty acids. Streptococci and lactobacilli counts increased markedly by adding sesame. Mould and Yeast and enterobacteriaceae were not detected throughout 15 days of storage. Use of 2% sesame in the presence of both starter mixtures gave the best sensory scores. Therefore we recommend to use sesame in the production of fermented milk to produce a nutraceutical fermented product rich in omega fatty acids and some other prebiotics e.g.sesamine.

Keywords: Nutraceutical fermented milk , *Lb. gasseri*, *Lb. rhamnosus* , Sesame seeds , polyunsaturated fatty acids

INTRODUCTION

Probiotics are defined as selected viable microorganisms used as dietary supplements having potential for improving health of man or animal following ingestion (Gilliland 2001). Some lactobacilli are well known as beneficial bacteria for use as probiotics, and also have a worldwide industrial use as starter cultures in the manufacturing of some fermented milk products. There are a variety of potential health benefits described in the literature resulting from the consumption of products containing live cultures of probiotics. There are specific characteristics that one must consider in developing a product with the goal of delivering health benefits through the consumption of probiotics. Research has shown that among strains within a specific species of lactobacilli significant variation in the ability to provide one or all of the potential health benefits can be seen (Fortina *et al.* 1998; Janda and Abbott 2002; Buckley and Roberts 2006). Probiotic microorganisms should be at levels of at least 10⁶ cfu per gram or milliliter. It was reported that they increase body immunity, outgrow pathogens, improve mineral absorption, reduce blood serum cholesterol, prevent allergy development and inhibit cancer development (Pereira and Gibson 2002; Maiaama and Isolauri 1997; Wollowski *et al.* ,2001).

L. gasseri is characterized as an excellent probiotic strain (Fujiwara *et al.* ;2001), suitable as a starter for marking fermented milk. One of the important beneficial health effects attributed to *Lb. gasseri* is its ability to reduce blood serum cholesterol level and protecting from occurrence of mutagenesis of cells caused by food-borne mutagenesis like Trp-P1 (Usman and Hasono, 1999). Also, Atuma and Sato (2001) reported that fermented milk drink prepared with the two novel probiotic lactobacilli (*L.gasseri* and *L.casei*) improved human intestinal microflora. Also *L. gasseri* improved the properties of the product(shelf-life, flavour and physical properties(Kimura *et al.* 2003).

Sesame plays an important role in human nutrition. The importance of sesame (*Sesamum indicum*) as a source of edible oil rich in polyunsaturated fatty acids and high quality protein is continuously increasing. Sesamin is a major lignan constituent of sesame and possesses multiple functions such as antihypertensive, cholesterol lowering, lipid lowering and anti cancer activities. Most of the sesame seeds are used for oil extraction (Elleuch *et al.* 2007). The chemical composition of sesame shows that it is an important source for oil (50-60%), protein (18-25%)and fiber (11.8%) (Alpaslan *et al.* 2001, Kahyaoglu and Kaya 2006, Kamel-Eldin and Appelpist, 1994 and Yoshida 1994). Moreover, sesame is exceptionally rich in calcium, iron, magnesium, manganese and, copper and contains vitamin B1 and vitamin E(α , γ , δ tocopherols). Also it contains lignans which are phytoestrogens with antioxidant and anticancer properties. Also contains phytosterols, which reduce levels of blood cholesterol (Cheung *et al.* 2007).

The present work was undertaken to evaluate the applicability of nutraceutical fermented milk product made by using some lactobacilli and sesame seeds powder as well as following the acceptability of this product throughout 15 days of storage.

MATERIALS AND METHODS

Fresh raw buffalo's milk was obtained from the Industrial.Dairy Technology Unit, Animal Production Research Institute. *Sterptococcus thermophilus* was obtained from Chr. Hansen , Copenhagen,Denmark. *Lactobacillus gasseri* B-14160 (*Lb . gasseri*) and *Lactobacillus rhamnosus* B-445 (*L . rhamnosus*) were provided from the National Research Center , Egypt.White sesame seeds (50% fat and 20% protein) were obtained from the local market in Cairo.

Milk was standardized to 3 % fat, then divided into six equal portions. The first portion was heated at 90 °C for 15 minute, cooled to 37 °C inoculated with 1.5 % *S . thermophilus* and 1.5 % *Lb . gasseri*. To the second portion sesame seeds powder was added at rate of 2 %, heated at 90 °C for 15 min., cooled to 37 °C and inoculated as the first treatment. Sesame seed powder was added to the third portion at rate of 4 %, heated, cooled and inoculated as the second treatment. The 4th , 5th and 6th treatments were done as the first 3 treatments but *Lb. rhamnosus* was used instead of *Lb. gasseri*. The six treatments were poured into polystyrene containers and

incubated at 37 °C until coagulation, then kept under refrigeration at 6°C. Samples were taken from each treatment when fresh and after 3 ,7 ,10 ,and 15 days for chemical , microbiological as well as organoleptic properties determinations.

Chemical analysis:

pH was measured by using a digital pH meter model Hanna HT4817, Titratable acidity (T.A)according to(AOAC, 2007), Protein content according to ling (1963) and Fat content by Rose Gottlieb method (AOAC, 2007). Free fatty acids were determined in fresh samples and were extracted according to ISO, 14156 (2001), esterified by ISO 15884 (2002)and determined using gas chromatography- GC Hewlett-Packard 6890 by ISO 15885 (2002). Free amino acids were determined by using amino acid analyzer (Eppendorf- LC 3000) according to Block *et al* (1958).

Lb . gasseri and *Lb. rhamnosus* were counted by plating on MRS agar according to IDF (1995) *Str. thermophilus* was enumerated on M17 medium. Mould and yeast and enterobacteriaceae counts were determined according to the American Public Health Association (2004).

The sensory evaluation were assessed by members of the Dairy Department, Animal Production Research Institute . Samples were evaluated for flavour (50 points), body and texture (40 points) and appearance (10 points) according to Keating and Randwhite (1990).

RESULTS AND DISCUSSION

Figure (1) show the changes in titratable acidity of the different treatments. It was observed that the T.A increased in all treatments throughout the storage period Furthermore, the addition of sesame increased the T.A. It could be due to the addition of the sesame solids, that affect the total acidity of the product. Moreover the treatments using *Lb. rhamnosus* resulted in higher titratable acidity than the other treatments.

Fig (2) indicated the total solids of the fermented milk made from milk fortified with sesame seeds. It was observed that the two control treatments (C1 and C2) had the lowest T.S. when fresh and throughout the storage period. While treatments fortified with 2 and 4% sesame powder had a higher T.S, and the increase in T.S content was parallel to the ratio of sesame added. In all treatments the total solids increased gradually during storage that could be due to evaporation of moisture in the refrigerator.

Protein content of the fermented milk fortified with sesame was shown in fig (3). Addition of sesame increased the protein content and the fat content (data not showed), and it increased by increasing the sesame added. Sesame protein content is in the range of 18-25% (Murwan *et al.* 2008). Moreover, treatments manufactured with *L. rhamnosus* showed a slight increase in protein than the corresponding treatments with *L. gasseri* Furthermore , slight increase in protein content was noticed during 15 days storage.

Table 1 illustrates that the addition of sesame add nutritional value for the product, which is very important for many groups such as the athletes C1, 1.5% *S. thermophilus* + 1.5% *Lb. gasseri* (control 1), T1, 1.5% *S.*

thermophilus + 1.5% *Lb. gasseri* + 2% sesame seeds ,T2, 1.5% *S. thermophilus* + 1.5% *Lb. gasseri* + 4% sesame seeds , C2, 1.5% *S. thermophilus*+ 1.5% *Lb. rhamnusus* (control 2) . T3, 1.5% *S. thermophilus*+ 1.5% *Lb. rhamnusus*+2% sesame seeds ,T4, 1.5% *S. thermophilus*+ 1.5% *Lb. rhamnusus*+4% sesame seeds

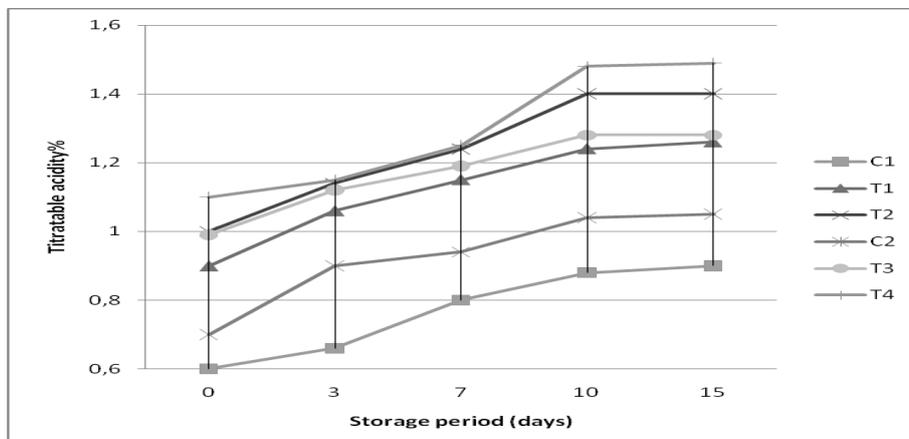


Fig.(1): Changes in titratable acidity of the fermented milk made by using different lactobacilli and sesame during storage at 6°C.

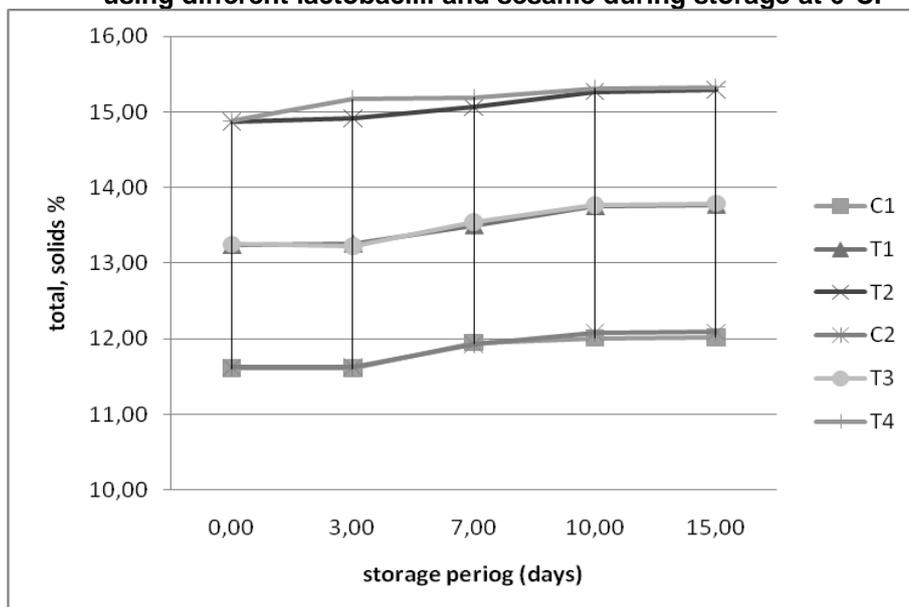


Fig.(2): Changes in total solids of the fermented milk made by using different lactobacilli and sesame during storage at 6°C. for abbreviation see Fig. (1)

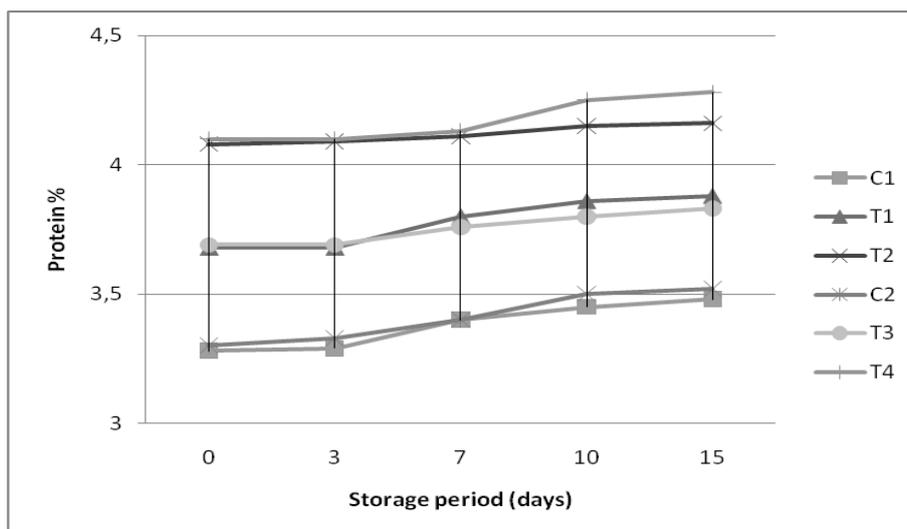


Fig (3): Changes in the protein content of the fermented milk made by using different lactobacilli and sesame during storage at 6°C. for abbreviation see Fig. (1)

Table (1): The amino acids content of the fresh fermented milk made by using different lactobacilli and sesame. (mg/g protein)+

Amino acids	C1	T1	C2	T3
Aspartic	1.72	2.51	1.77	2.9
Threonine *	0.71	1.51	0.6	1.76
Serine	1.72	2.27	1.73	2.2
Glutamic	6.14	7.12	6.2	7.39
Prolin	1.89	2.32	1.98	2.41
Glycine	0.4	1.17	0.46	1.15
Alanine	1.18	1.91	1.3	1.95
Valine *	1.85	2.66	1.82	2.6
Methionine *	0.52	1.08	0.56	1.04
Isoleucine *	0.53	1.83	0.51	1.55
Leucine *	2.70	3.62	2.93	3.75
Tyrosine	0.58	1.39	0.57	1.34
Phenylalanine *	0.56	1.73	0.52	1.68
Histidine *	1.73	2.42	1.77	2.1
Lysine *	1.64	2.35	1.47	2.27
Arginine	1.37	2.93	1.33	2.83
Total essential amino acids	10.24	17.2	10.18	16.75
Total amino acids	25.24	38.82	25.52	38.92

+for abbreviation see Fig. (1) * Essential amino acids

Table (2): The fatty acids content of the fresh fermented milk made by using different lactobacilli and sesame. (mg/g fat)

Fatty acid contents	C1	T1	C2	T3
Saturated Fatty acids%				
Caproic acid	0.15	0.3	0.15	0.32
Caprylic acid	0.83	0.87	0.95	1.21
Capric acid	4.48	2.15	4.63	2.18
Lauric acid	5.34	2.24	5.79	2.0
Myristic acid	15.46	10.07	15.43	10.06
Palmitic acid	36.9	30.09	36.88	30.3
Stearic acid	8.04	8.13	8.1	8.15
Total saturated	71.2	53.85	71.93	54.22
Unsaturated fatty acid				
Oleic acid	25.61	34.26	24.87	34.27
Linoleic acid	2.09	10.27	2.11	9.88
Linolenic acid (omega 6)	1.1	1.62	1.09	1.63
Total unsaturated	28.8	46	28.07	45.88

At the same time Table (2), it could be seen that the fatty acids content in fresh fermented milk samples, as they added more value for the products. It was observed that adding sesame increased the total unsaturated fatty acids in T1 and T3 than the corresponding control samples (C1 and C2), specially, oleic, linoleic acids and linolenic which are natural antioxidants (Bahkali, *et al.*1998; Murwan *et al.*,2008; Nzikou, *et al.*2009). On the other hand, addition of sesame lowered the total saturated fatty acids in T2 and T3 samples than the controls C1 and C2. Furthermore, palmitic, myristic and stearic showed the highest ratio between saturated fatty acids. Similar results were obtained by Nzikou *et al* (2009), who mentioned that the major saturated fatty acids in sesame seed oil were palmitic and stearic.

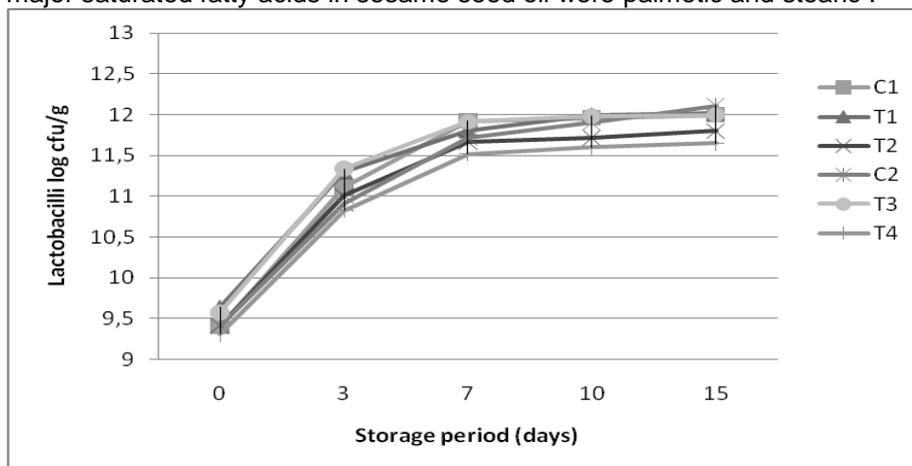


Fig (4): Changes in the lactobacilli counts in fermented milk made by using different lactobacilli and sesame during storage at 6°C. for abbreviation see Fig. (1)

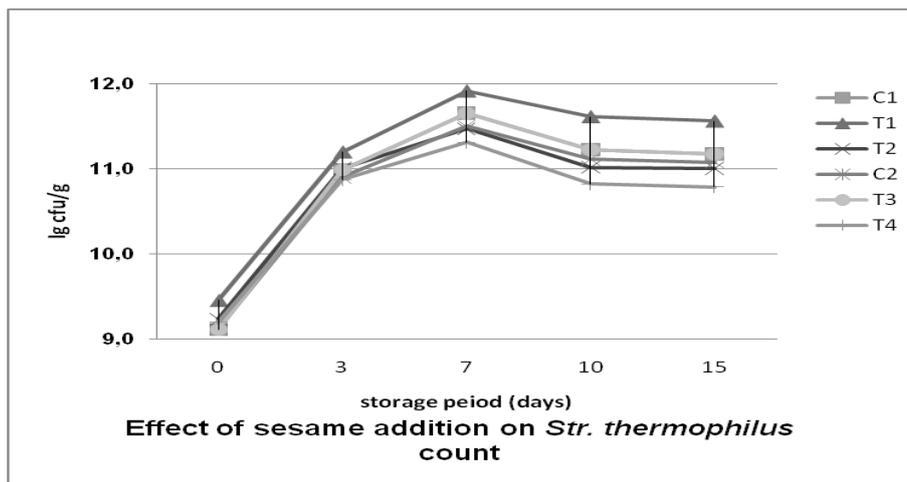


Fig (5): Changes in the *Str. thermophilus* counts in fermented milk made by using different lactobacilli and sesame during storage at 6°C.

for abbreviation see Fig. (1)

Fig(4) shows the effect of using sesame in the manufacture of fermented milk on the growth of *L. gasseri* and *L. rhamnosus* during storage. It was observed that T1 and T3 in which sesame were added at ratio 2% had the highest numbers of both *L. gasseri* and *L. rhamnosus* when fresh and during the storage period. On the other hand, Lactobacilli count in T2 and T4 were slightly lower than their corresponding controls (C1 and C2). Furthermore, it was clearly noticed that the count of both Lactobacilli strains highly increased after three days for about more than one log cycle and the number also increased till 15 days but in a lesser rate. Similar results were obtained by Rosenthal *et al.* (2001), who mentioned that the presence of sesame butter accelerated the growth rate of the starter during fermentation, but did not affect its survival during storage. The growth of *Str. thermophilus* (fig 5) also showed the same trend of the lactobacilli, but the number was slightly lower, and after 7 days the count decreased in all treatments. This could be due to the acidity, which affected streptococci more than Lactobacilli (Sharp, 1962). It could also be found that all treatments and the control were free from enterobacteriaceae and yeast and molds when fresh and along the storage period.

Table (3) shows the changes in the organoleptic properties of the fermented milk made with probiotic lactobacilli and sesame .It could be observed that the fermented milk product with 2% sesame and made with *L.gasseri* (T1) had the best flavoure during the first 7 days of storage followed by the corresponded product made with 2% sesame and *L. rhamnosus*. Also T1 and T3 were of the best body and texture throughout storage. Similar results were obtained by Abdel-Khalek *et al* (2004) who mentioned that set and stirred yoghurt like products manufactured with *Str. thermophilus* and *L. gasseri* had the best sensory evaluation. On the other hand, the control

samples C1 and C2 had flat flavour and weak body and texture when fresh due to their low acidity fig. (1). In all treatments the score of body and texture decreased by increasing the storage period and that was clear on T2 and T4 in which wheying off was observed, and the body becomes viscous in T4. These results were in agreement with Rosenahal *et al* (2001) who observed that sesame butter weakened the clot formed from milk, making it more prone to syneresis.

In conclusion we can recommend the use of sesame and the probiotic lactobacilli *L. gasseri* or *L. rhamnosus* to produce a new fermented dairy product rich in its nutritional value such as antioxidant , fiber , unsaturated fatty acids, minerals ...etc. and acceptable for the consumer.

Table (3): Sensory evaluation of the fermented milks made with probiotic lactobacilli and fortified with sesame.

Treatments	storage period(days)	Flavour 50	Body and Texture 35	Appearance 15	Total scores 100
C1*	fresh	42	31	12	85
	3	42	31	12	85
	7	43	31	12	86
	10	41	31	12	84
	15	37	29	12	78
T1	fresh	45	33	12	90
	3	45	32	12	90
	7	45	32	12	89
	10	41	30	12	83
	15	40	28	11	79
T2	fresh	42	33	12	86
	3	42	32	12	86
	7	41	31	12	84
	10	39	30	11	80
	15	33	27	10	70
C2	fresh	41	30	12	83
	3	42	30	12	84
	7	41	31	12	84
	10	41	31	12	84
	15	35	30	12	77
T3	fresh	43	32	12	87
	3	43	32	12	87
	7	40	31	12	83
	10	39	30	12	81
	15	38	29	12	79
T4	fresh	41	32	12	85
	3	41	31	12	84
	7	41	30	12	83
	10	38	29	11	78
	15	33	24	10	67

* for abbreviation see Fig. (1)

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استخدام السمسم وبعض المدعمات الحيوية من بكتريا حمض اللاكتيك العصوية لإنتاج منتج لبنى متخمّر عالي القيمة التغذوية

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في هذا البحث تم استخدام السمسم كمصدر غني بالأحماض الدهنية طويلة السلسلة عديدة عدم التشبع ومضادات الأكسدة وبعض المعادن والألياف الذائبة مع سلالاتين من بكتريا حمض اللاكتيك الهسوية وهما *L. gasseri* و *L. rhamnosus* في تصنيع منتج لبنى متخمّر من اللبن الجاموسى المعدل تركيبه وكانت المعاملات كالاتى: مقارنة ١ + 1.5% *S. thermophilus* ، 1.5% *Lb. gasseri* ، مقارنة ٢ ، 1.5% *Lb. gasseri* + 1.5% *S. thermophilus* ، معاملة ١ + 1.5% *S. thermophilus* ، معاملة ٢ نفس بادية معاملة ١ ولكن مع ٤% سمسم ، معاملة ٣ + 1.5% *S. thermophilus* ، معاملة ٤ نفس بادية معاملة ٣ ولكن مع ٤% سمسم . تم تخزين المنتج بالتلاجة على درجة حرارة ٦° م لمدة ١٥ يوم وتم خلالها اجراء التحليل الكيماوى والميكروبيولوجى وتقييم الصفات الحسية .

واظهرت نتائج التحليل الكيماوى ان التدعيم بالسمسم عمل على زيادة كل من الحموضة، المواد الصلبة الكلية، الدهن، البروتين، الاحماض الامينية وخاصة الاحماض الامينية الاساسية والاحماض الدهنية غير المشبعة خاصة حامض الاوليك و اوميغا٣ و اوميغا٦ وغيرها واظهرت نتائج التحليل الميكروبيولوجى ان بكتريا حامض اللاكتيك الكروية والعصوية زادت بوضوح فى العينات المحتوية على السمسم وكان المنتج خالى من الخمائر والأعفان والبكتريا المعوية طوال فترة التخزين

واظهر التحكيم الحسى ان المعاملات المضاف اليها ٢% سمسم مع *S. thermophilus* و *Lb. gasseri* كانت افضل المعاملات حيث حصلت على اعلى درجات التحكيم .

لذلك يمكن التوصية باستخدام السمسم بنسبة ٢% مع المدعمات الحيوية لإنتاج منتجات لبنية مرتفعة القيمة التغذوية لبعض الفئات الخاصة

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
كلية الزراعة – جامعة القاهرة

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