



EFFECT OF SUBSTITUTION OF BUFFALOES' MILK BY LUPIN SEEDS EXTRACT ON THE QUALITY OF PROBIOTIC YOGHURT LIKE PRODUCTS

Ahmed A.M. Saker*, Neama M. El-Shafie and Kh.M. El-Zahar

Food Sci. Dept., Fac. Agric., Zagazig Univ., Egypt

ABSTRACT

The influence of substitution different levels of buffaloes' milk (4% fat) by lupin seeds extract (lupin like milk) at ratios of 10, 20, 30, 40 and 50% on the quality of probiotic yoghurt like products was evaluated. Mixture of active yoghurt starter containing *Streptococcus salivarius*, *thermophilus* EMCC 104 and *Lactobacillus delbruekii. bulgaricus* EMCC 1102 and *Bifidobacterium bifidum* DI 1:1 was added in the rate of 2% in the production of the probiotic like yoghurt. Resultant products were cooled, stored up to 14 days at $6\pm 1^{\circ}\text{C}$. Results indicated that substitution of buffaloes' milk by different levels of lupin extract increased both total protein content and titratable acidity while fat content decreased to be lower than control one. Also, formation of acetaldehyde decreased in all probiotic yoghurt like products during storage period to be lower than the control. In all probiotic like yoghurt treatment higher syneresis was observed in these treatments than the control probiotic yoghurt. On the other hand, the probiotic yoghurt like products made by substitution of buffaloes' milk with lupin extract showed lower lactic acid bacterial and lower probiotic bacterial counts than the control one. With regard to the sanitary quality, results of the microbiological analysis revealed the absence of coliform bacteria in fresh and during storage periods and little numbers of yeasts and moulds at the end of the storage period mould in all samples. With respect to the organoleptic quality, the control yoghurt had the highest scores, while increasing substitution ratio caused a decrease in organoleptic scores up to 50%. So, improving the organoleptic quality of these substituted treatments was carried out by mixing these substituted yoghurt samples after production with sucrose and strawberry flavour. Results indicated that these additives improved the score quality of substituted probiotic yoghurt treatments up to 30% substitution by lupin like milk and was accepted by the panelists.

Key words: Legumes, Lupin extract, lupin like milk, yogurt like product.

INTRODUCTION

The short supply of fresh milk besides the continuous rising of its price in many developing countries as well as the intolerance and allergic sensitivity of some people to milk proteins or lactose, have created a need for formulating some dairy analogous from vegetable sources. For a long time, soybean has been recognized as a rich source of low cost protein. However, its flavour was less acceptable owing to its beany flavour.

Recently, some varieties of non-dairy milk are distributed in some of the world's developed

markets as soy milk such as peanut milk and its dairy product analogous.

Kernels can be used to make soy like milk, lupin like milk is a healthy balanced plant based beverage, without the saturated fat which found in dairy milk. Lupin like milk can then be used to make excellent yoghurt (Camacho *et al.*, 1988 and Jimenez-Martinez *et al.*, 2003). "Lupin ice cream was became available in German shops (Anonymous, 2006).

Yoghurt is the most popular fermented dairy product in Egypt and worldwide owing to its

* Corresponding author: Tel. : +201003761182.

E-mail address: ah.sagr@yahoo.com

nutritive value and health effects (Rasic and Kurmann, 1978).

Production of probiotic fermented milks containing bifidobacteria increased because of their health beneficial effects (Crittenden, 1999). The health benefits of bifidobacteria include modulation of immune responses, modulation of intestinal microbiota, prevention of children diarrhoea, decrease constipation and antibacterial and anticarcinogenic activities (Gomes and Malcata, 1999 as well as Ouwehand and Salminen, 1999).

The present work was carried out to study the suitable conditions for obtaining lupin extract similar to the control buffaloes' milk in its contents of total solids. Substituting buffaloes' milk with different levels of lupin extract to obtain probiotic like yoghurt and evaluation of the different qualities of these products.

MATERIALS AND METHODS

Materials

Local variety of sweet lupin (*Lupinus albus*) was used in this investigation.

Buffaloes' Milk

Fresh whole buffaloes' milk was obtained from Dairy Technology Unit, Food Sci. Dept., Fac. Agric., Zagazig Univ.

Strawberry flavour

Kamena 610 Code No. 14720 (E122) Finest flavours & colours for food production. Made in Egypt N- of analysis of Ministry of health 13011.

Sugar

Commercial table sugar was obtained from local market and was added at a ratio of 5 and 10%.

Microbial cultures

Streptococcus salivarius. thermophilus EMCC 104 and *Lactobacillus delbruekii. bulgaricus* EMCC 1102 *Bifidobacterium bifidum* (D1) cultures were obtained from Chr. Hansen, Copenhagen, Denmark.

Methods

Extraction of lupin seeds

The seeds were extracted according to Jimenze-Martinez, *et al.* (2003), Lupin extract

(lupin like milk) was prepared by extracting lupin seeds in water at extract ratio (1:7) to obtain lupin like milk with total solids similar to that of control buffaloes' milk. The concentration of bitter flavour decreased through the debittering of the lupin seeds by soaking it in 0.5% NaHCO₃ solution at (93°C for 6 h) then cooled to room temperature.

Manufacture of a probiotic yoghurt and probiotic like yoghurt

Fresh buffaloes' milk was standardized to 4% fat and served to use in the preparation of a control of probiotic yoghurt and substituted by various ratios of lupin extract (lupin like milk) to manufacture probiotic like yoghurt.

Probiotic like yoghurt samples were made as follows:

Treatments T1, T2, T3, T4 and T5 were made by substituting the buffaloes' milk with 10, 20, 30, 40 and 50% of lupin extract, in respective order.

Both buffaloes' milk (control) and the five treatments were heated to 90°C for 15 min. then cooled to 42°C ±1°C, inoculated with 2% of mixed (1:1) of yoghurt starter lactic cultures and probiotic culture, packed in 100 ml plastic cups and incubated at 42°C until a uniform coagulation was obtained. The probiotic yoghurt samples from all batches were stored at 6±1°C and analysed after 1, 3, 7 and 14 days of storage. Experiments were carried out in triplicates.

Methods of Analysis

Chemical analyses

Seeds, fresh whole buffaloes' milk and experimental lupin like milk were analyzed for moisture, protein, fat and ash contents according to AOAC (2000), carbohydrates were calculated by subtracting the sum of moisture + protein + fat + ash from 100.

Chemical composition of yoghurt

Probiotic yoghurt samples were chemically analysed for total solids, fat, titratable acidity, and total protein as described in the AOAC (2000).

Acetaldehyde content

Acetaldehyde in yoghurt samples were determined as described by Lees and Jago (1969).

Rheological measurements (syneresis)

The released whey in yoghurt samples were measured according to the method of Aryana (2003). The quantity of whey collected from 100g of yoghurt in graduated cylinder after 2h of drainage at 20°C was used as an indication of syneresis.

Microbiological analysis

Total lactic acid bacterial counts in probiotic yoghurt samples were determined according to, APHA (1992). *Bifidobacterium bifidum* counts were determined according to Dave and Shah (1997).

Sanitary quality of probiotic yoghurt samples were examined by determination of

1. Coliform bacterial counts according to Fernandez (1988)
2. Total yeasts and moulds counts according to APHA (1992).

Sensory evaluation

Probiotic yoghurt samples were organoleptically examined after refrigerated storage (6±1°C) for 1, 3, 7 and 14 days according to Hunter and Muir, (1993).

Statistical Analysis

Statistical Analysis for the obtained data was carried out according to Clarke and Kempson (1997).

Experiments were repeated in triplicates and each analysis in duplicates and average results were tabulated.

RESULTS AND DISCUSSION

Chemical Composition of Ingredients Used in Making Probiotic Like Yoghurt

Table 1 shows the results of the chemical analysis of lupin seeds, buffaloes' milk and lupin milk. It can be observed that the lupin like milk has a higher protein content than buffaloes' milk. This is may be due to the fact that, during the debittering process, the carbohydrate, quinolizidinic alkaloids, tannin and other compounds were eliminated, thus allowing the relative proportion of protein to be increased

(Jimenez-Martinez *et al.* (2001). This is important because of the protein is one of the main component that contributes to the nutritional value of any food. On the other hand, the fat content was lower in the lupin like milk than the buffaloes' milk owing to the lower fat content of lupin seed (9.80%).

Chemical Composition of Probiotic Yoghurt Like Products

Table 2 shows that the moisture % of the control probiotic yoghurt was similar to moisture% of products made from buffaloes' milk substituted by different ratios of lupin milk.

This similarity in moisture % was due to the standardization of TS% of buffaloes' milk to be equal to TS% of lupin like milk. Moisture content of all treatments slightly decreased during the storage periods. Similar results were reported by Omar and Abou El-Nour (1998) and Jimenez-Martinez *et al.* (2003). The same table shows that increasing the rate of substitution of buffaloes' milk by lupin like milk, gradually increased the total protein in all treatments to be higher than the control probiotic yoghurt. On the other hand, the total protein of all treatments did not significantly changed throughout the storage period.

The obtained results in Table 2 also, illustrated that, the fat content of all treatments was lower than that of control probiotic yoghurt owing to the lower, fat content of lupin like milk which was used in substituting buffaloes' milk at different levels. Similar results were reported by Camacho (1989) for lupin like milk prepared from *L.albus-multolupa*.

The fat content of all treatments did not also significantly changed during the storage period. The acidity of probiotic like yoghurt was higher than that of buffaloes' milk yoghurt (control), Fig. (1). Slight differences were observed in the acidity of probiotic like yoghurts.

These results are in agreement with those reported by Lee *et al.* (1991) and Zedan *et al.* (2001).

Quality of probiotic like yoghurt

The property of this product was assessed by: determination of some flavour compounds *e.g.*

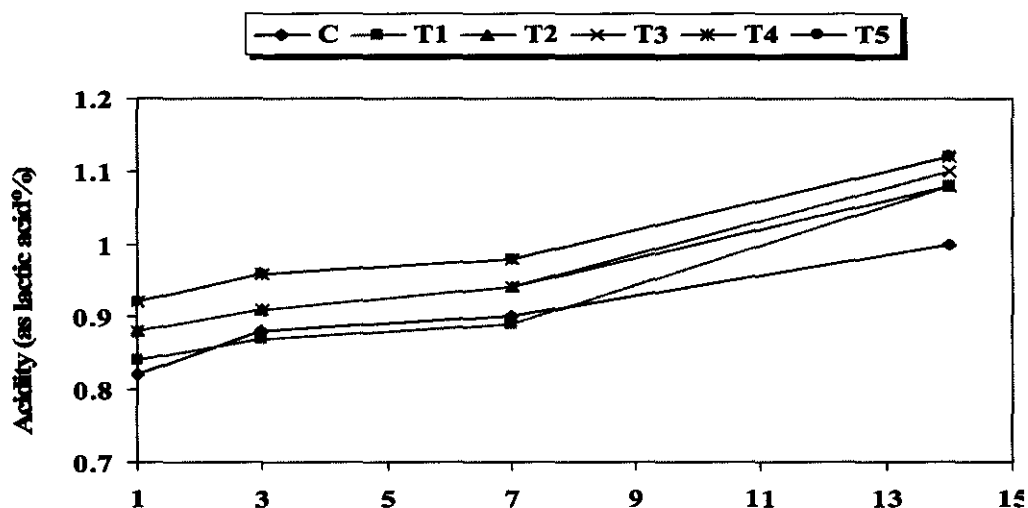
Table 1. Chemical composition of ingredients used in the manufacture of fermented milk like products

Sample	Moisture%	Protein%	Fat%	Carbohydrate%	Ash%
Lupin seed	9.00	36.00	9.80	35.00	3.00
Buffaloes' milk	87.20	3.80	4.00	4.60	0.80
Lupin extract (Lupin like milk) 1:7 in water	87.40	5.40	2.00	4.00	1.00

Table 2. Chemical composition of probiotic yoghurt like products

Treatments	Moisture%				Total protein%				Fat%			
	Storage period (days)				Storage period (days)				Storage period (days)			
	1	3	7	14	1	3	7	14	1	3	7	14
C	87.0	86.80	86.80	86.60	3.60	3.66	3.70	3.70	4.0	4.0	4.0	4.1
T1	87.2	87.00	86.80	86.50	3.80	3.80	3.90	4.00	3.8	3.8	3.7	3.7
T2	87.3	86.90	86.70	86.40	4.00	4.20	4.30	4.36	3.6	3.6	3.6	3.5
T3	87.3	86.80	86.80	86.20	4.20	4.30	4.40	4.60	3.4	3.4	3.4	3.3
T4	87.4	86.70	86.60	86.00	4.40	4.50	4.60	4.60	3.2	3.6	3.7	3.7
T5	87.4	86.50	86.40	85.90	4.60	4.70	4.80	4.94	3.0	3.0	2.92	2.9
L.S.D	1.38	1.27	1.21	1.32	0.18	0.21	0.20	0.15	0.31	0.28	0.28	0.25

- Each value in the table is the mean of three replicates. - C: control of probiotic yoghurt from buffaloes' milk 4% fat.
 - T1, T2, T3, T4 and T5: probiotic yoghurt like products made from buffaloes' milk substituted by lupin seeds extract at the ratio of 10, 20, 30, 40 and 50% respectively. - L.S.D.: Least significant difference ($P > 0.05$).

**Fig. 1. Effect of substitution of buffaloes' milk with lupin like milk on titratable acidity (as lactic acid%) of probiotic yoghurt like product, during storage at 6 ± 1 °C.**

acetaldehyde which have been reported as flavour contributors in yoghurt (Tamime and Deeth, 1980), and determination some rheological properties e.g. syneresis (the released whey in yoghurt treatment during storage periods, (Aryana, 2003).

It is evident from Fig. 2 that, substitution of buffaloes' milk by lupin like milk decreased acetaldehyde content of yoghurt like product to be lower than the control yoghurt. The lower concentration of acetaldehyde in yoghurt like products may be due to the lower fat content. Also, the concentration of acetaldehyde gradually decreased during the storage periods. This may be due to transformation of acetaldehyde to acetone. Similar results have been reported by Laye *et al.* (1993) and Atwa *et al.* (2008). Fig. 3 shows that, increasing the ratio of substitution of buffaloes' milk by lupin like milk in making probiotic like yoghurt increased the separation of whey. This may be due to gradually decrease in fat content of all treatments. The values of whey release gradually decreased during the storage period up to 7 days of storage, then increased in all treatments.

These results are in agreement with those reported by Kebary and Hussien (1999) and Atwa *et al.* (2008).

Bacteriological Quality of Probiotic Like Yoghurt

The bacteriological quality of probiotic like yoghurt samples were measured by the determination of lactic acid and probiotic bacterial counts which are illustrated in Figs. 4 and 5. Lactic acid bacterial count gradually increased during the storage period of control and samples with lupin like milk up to the third storage day, followed by decreasing of these counts in all treatments until 14 days of storage. The lactic acid counts in the control and lupin yoghurt were in agreement with those reported by Jimenez-Martinez *et al.* (2003).

The bifidobacterial count was lower than lactic acid bacterial counts in the probiotic like yoghurt treatments and decreased as the storage period advanced (Figs. 4 and 5). These results might be due to the gradual increase of the acidity percent of all treatments during the

storage period, (Prasad *et al.*, 1998 and Masco *et al.*, 2004).

Sanitary Quality of Probiotic Like Yoghurt

The microbiological analysis indicated the absence of coliform bacteria in the control and all treatments of probiotic like yoghurt. However, Table 3 shows the absence of moulds and yeasts in the first day old samples of all treatments, while at the three days of storage period, small numbers of these microorganisms were observed in all treatments and increased up to the end of the storage period (14 days). These results indicated that the sanitary conditions in the laboratory were adequate to obtain a good product. Similar results have been reported by Jimenez-Martinez *et al.* (2003). These results could be attributed to the fermentation process of the yoghurt (Low pH and possible production of bacteriocins) which also might help to keep the product in good sanitary conditions by inhibiting the growth of contaminant microorganisms (Fernandez, 1988).

Sensory Evaluation

Scores of organoleptic properties of probiotic yoghurt like products of all treatments are shown in Tables 4 and 5. It is evident from these results that, increasing the substitution ratio of buffaloes' milk with lupin extract reduced the scores for the organoleptic properties of the resultant plain products, while, fortification of the yoghurt like products of different substitution levels with sugar and strawberry flavour improved the organoleptic properties. Good acceptable quality of yoghurt like products were obtained when 10% sugar and strawberry flavour were added. Treatment of 30% substitution with lupin extract and fortified with 10% sugar and strawberry juice was comparable with the control probiotic yoghurt. All the scores of all probiotic yoghurt treatments gradually decreased up to the end of storage period. These results were in agreement with those reported by Buono *et al.* (1990), Jimenez-Martinez *et al.* (2003) and Atwa *et al.* (2008).

Conclusion

It could be concluded that substitution up to 30% of buffaloes' milk with lupin like milk and fortification with sucrose and strawberry juice produced probiotic like yoghurt of improved organoleptic quality.

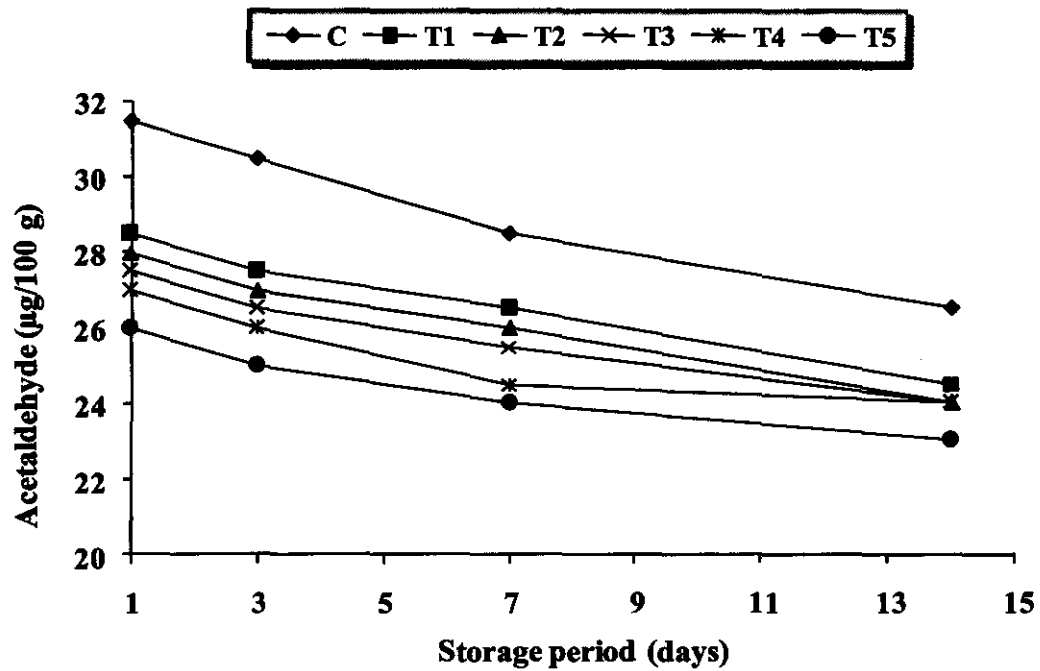


Fig. 2. Effect of substitution of buffaloes' milk with lupin like milk on acetaldehyde content of probiotic yoghurt like product, during storage at 6 ± 1 °C

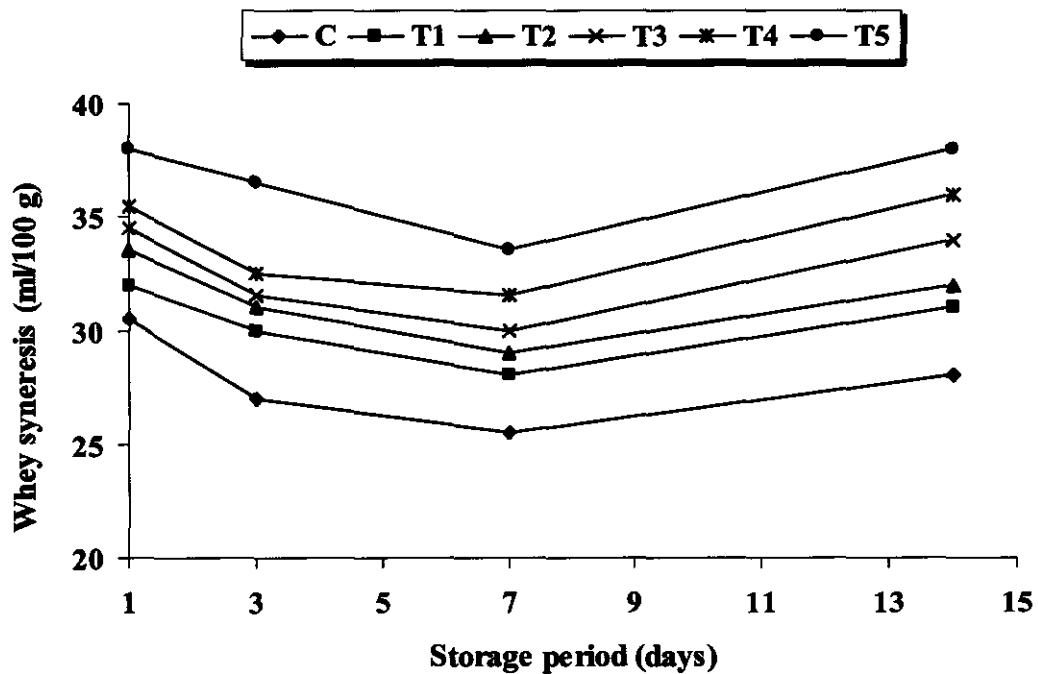


Fig. 3. Effect of substitution of buffaloes' milk with lupin milk on whey syneresis of probiotic yoghurt like product, during storage at 6 ± 1 °C

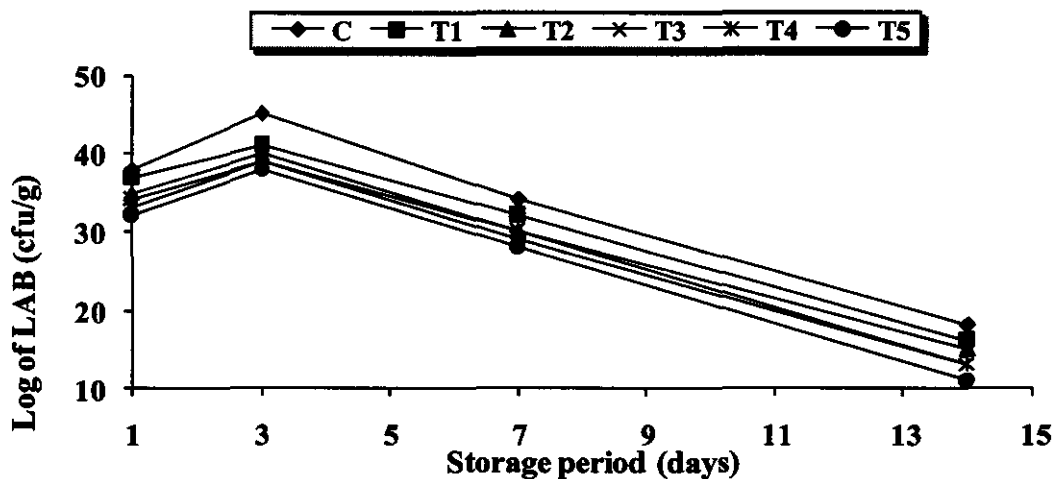


Fig. 4. Effect of substitution of buffaloes' milk with lupin like milk on total lactic acid bacteria (LAB) counts of probiotic yoghurt like product, during storage at 6 ± 1 °C

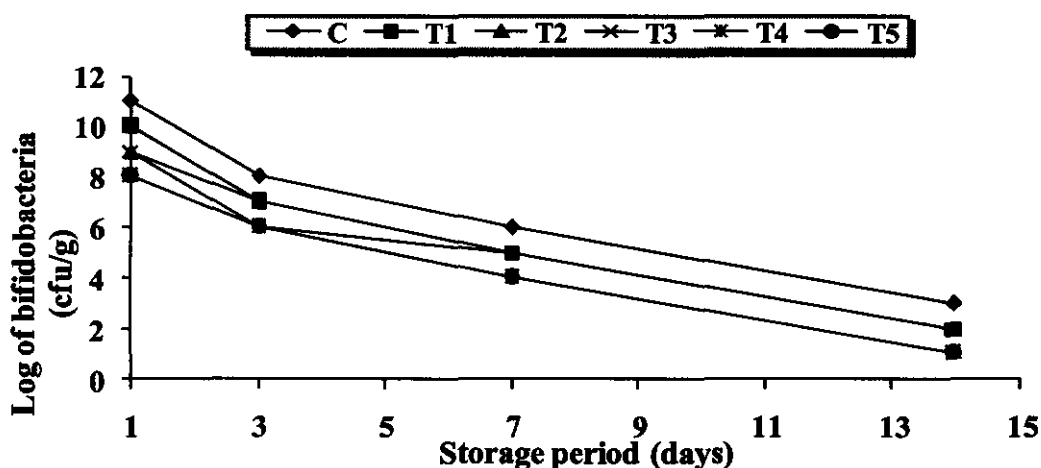


Fig. 5. Effect of substitution of buffaloes' milk with lupin like milk on bifidobacteria counts of probiotic yoghurt like product, during storage at 6 ± 1 °C

Table 3. Yeasts and moulds counts of probiotic like yoghurt during storage period at (6 ± 1 °C)

Treatments	Storage period (days)			
	1	3	7	14
C	-	1	2	4
T1	-	1	2	4
T2	-	2	3	5
T3	-	1	2	4
T4	-	2	3	4
T5	-	1	2	5
L.S.D	-	0.02	0.04	0.42

- Each value in the table is the mean of three replicates. -C: control of probiotic yoghurt from buffaloes' milk 4% fat.
 - T1, T2, T3, T4 and T5: probiotic yoghurt like products made from buffaloes' milk substituted by lupin seeds extract at the ratio of 10, 20, 30, 40 and 50% respectively. - L.S.D.: Least significant difference ($P < 0.05$).

Table 4. Sensory evaluation of probiotic yoghurt like product made from buffaloes' milk substituted by lupin like milk during storage at (6±1°C)

Treatments	Flavour (45)				Body & Texture (35)				Appearance (10)				Acidity (10)				Total score (100)			
	Storage period (days)				Storage period (days)				Storage period (days)				Storage period (days)				Storage period (days)			
	1	3	7	14	1	3	7	14	1	3	7	14	1	3	7	14	1	3	7	14
C	42.7	42.9	41.8	41.2	34.3	33.3	32.7	31.5	9.0	8.7	8.7	8.3	8.7	8.3	7.0	7.0	95.00	93.5	90.1	88.0
T1	42.4	42.9	42.1	41.0	34.0	32.7	32.0	31.7	8.5	8.3	8.0	7.8	8.3	7.7	7.0	6.7	93.20	91.6	89.1	86.8
T2	40.9	41.6	40.5	38.0	33.3	32.3	32.0	30.8	8.5	8.3	8.0	7.3	8.0	7.3	6.7	6.7	91.10	89.6	87.2	85.1
T3	40.7	40.7	40.0	38.0	33.3	32.8	31.4	31.5	8.5	8.0	7.7	6.7	8.0	7.3	6.7	6.3	90.20	88.8	85.4	82.8
T4	40.1	40.0	40.0	38.0	32.3	31.0	28.3	28.3	7.7	7.3	7.3	6.9	8.0	7.3	6.7	6.3	87.40	85.6	82.4	79.5
T5	39.6	40.0	39.3	38.6	31.7	30.7	28.7	26.7	7.3	7.2	7.1	6.0	7.3	6.0	6.0	5.7	86.20	84.2	80.8	76.6
L.S.D	1.22	1.39	2.91	2.58	1.19	1.25	1.25	0.83	0.84	0.94	0.83	0.94	0.73	1.39	1.03	1.19	2.58	3.82	5.20	4.63

- Each value in the table is the mean of three replicates. - C: control of probiotic yoghurt from buffalo's milk 4% fat.

- T1, T2, T3, T4 and T5: probiotic yoghurt like products made from buffalo's milk substituted by lupin seeds extract at the ratio of 10, 20, 30, 40 and 50% respectively. - L.S.D.: Least significant difference (P< 0.05).

Table 5. Effect of using table sugar and strawberry flavour on the organoleptic scores of probiotic like yoghurt during storage at (6±1°C) for 14 days

Treatment	Flavour (45)						Body & Texture (35)						Appearance (10)						Acidity (10)						Total score (100)					
	Storage period (days)						Storage period (days)						Storage period (days)						Storage period (days)						Storage period (days)					
	1		7		14		1		7		14		1		7		14		1		7		14		1		7		14	
	Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar		Sugar	
5%		10%		5%		5%		10%		5%		5%		10%		5%		5%		10%		5%		5%		10%		5%		
C	42.7	41.8	41.2	34.3	32.7	31.5	9.0	8.7	8.3	8.7	7.0	7.0	95.00	90.1	88.0															
C1	44.3	43.7	43.8	43.5	43.5	33.3	33.8	33.2	33.0	33.2	9.7	9.7	9.0	9.5	9.0	9.0	8.6	8.7	7.3	8.3	6.7	7.0	95.8	96.5	93.6	94.8	90.5	92.0		
T1	42.7	43.5	42.1	43.7	41.8	43.0	32.5	33.8	32.3	33.3	32.1	33.5	9.0	9.0	8.3	8.7	8.3	8.7	8.7	8.0	8.0	7.3	7.0	94.5	96.0	91.4	93.6	89.2	91.8	
T2	42.3	43.0	41.7	42.7	41.5	42.7	32.5	33.8	32.3	33.1	31.4	33.5	9.0	9.0	8.7	9.0	8.3	9.0	8.3	9.0	8.0	8.3	7.0	7.8	92.2	95.8	89.6	92.8	88.2	91.8
T3	42.6	43.5	40.8	42.1	40.7	38.7	32.7	33.7	31.6	32.7	32.0	31.7	8.5	9.7	8.0	9.0	8.3	8.3	8.0	9.0	8.0	8.0	7.0	7.0	92.0	95.4	89.4	91.8	88.0	90.6
T4	41.3	42.2	40.6	41.0	40.5	38.6	32.3	33.0	31.0	33.3	31.5	31.5	8.3	8.0	8.3	9.0	8.2	8.3	7.0	8.3	6.3	7.0	6.3	6.0	88.6	91.8	86.4	90.0	85.2	89.2
T5	41.2	42.0	40.5	40.4	39.2	38.2	31.3	33.0	30.0	31.3	31.2	31.0	8.3	8.5	8.3	8.0	8.0	8.0	7.3	7.0	7.0	6.0	6.0	87.2	90.0	85.0	85.0	84.0	86.2	
L.S.D	1.11	1.03	1.19	1.11	1.51	1.51	1.26	1.93	1.26	0.84	1.26	0.84	1.51	0.59	1.51	0.42	0.84	0.84	0.84	2.31	0.94	0.59	0.73	0.73	1.28	1.93	3.56	3.56	1.51	2.81

- Each value in the table is the mean of three replicates. - C: control of probiotic yoghurt from buffalo's milk 4% fat.

- T1, T2, T3, T4 and T5: probiotic yoghurt like products made from buffaloes' milk substituted by lupin seeds extract at the ratio of 10, 20, 30, 40 and 50% respectively. - L.S.D.: Least significant difference (P< 0.05).

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تأثير استبدال اللبن الجاموسى بمستخلص بذور الترمس على جودة شبيهه اليوجورت الحيوى

أحمد عبدالحليم متولى صقر- نعمة محمد الشافعى- خالد مغاورى الزهار

قسم علوم الأغذية - كلية الزراعة - جامعة الزقازيق - مصر

تم دراسة تأثير استبدال نسب مختلفة من اللبن الجاموسى بمستخلص بذور الترمس على جودة المنتج المتخمر شبيهه اليوجورت الحيوى حيث تم استبدال نسب من اللبن الجاموسى (٤% دهن) بمعدلات (١٠، ٢٠، ٣٠، ٤٠ و ٥٠%) من مستخلص الترمس المماثل للبن الجاموس فى محتواه من الجوامد الكلية. وتم إضافة بادئ اليوجورت والبائى الحيوى (١:١) بمعدل ٢% لكل معاملات شبيهه اليوجورت وعينة المقارنة المصنع من اللبن (الجاموسى ٤% دهن). ولقد أوضحت النتائج أن استبدال اللبن الجاموسى بمستخلص بذور الترمس أثرت على التركيب الكيماوى لمعاملات اليوجورت فقد زادت الحموضة الكلية وكذلك محتوى البروتين وانخفض محتوى الدهن وكان ذلك مرتبطا بزيادة نسبة الاستبدال بمستخلص الترمس، كما أدى أيضا إلى انخفاض محتوى الأستالدهيد عن الكنترول. وأيضا وجد أن عملية الاستبدال لليوجورت لم تحسن الخواص الريولوجية للمنتج الشبيهه باليوجورت الحيوى حيث أدت إلى زيادة انفصال الشرش من هذه المعاملات أعلى من عينة المقارنة. أما محتوى المعاملات الشبيهه باليوجورت الحيوى من بكتريا حامض اللاكتيك فقد نقص عن الكنترول أما محتوى تلك المعاملات من البكتريا الحيوية فكان أقل من الكنترول وهى طازجة وخلال فترات التخزين بسبب ارتفاع الحموضة الكلية فى هذه المعاملات نتيجة زيادة نشاط بكتريا حامض اللاكتيك بها. أما عن الجودة الصحية لهذه المعاملات فكانت مرتفعة بسبب انعدام نمو بكتريا القولون، وبداية نمو أعداد قليلة من الخمائر والفطريات بعد ٣ أيام من التخزين وكان ذلك فى عينة المقارنة وكل المعاملات. وبالنسبة للجودة الحسية لمعاملات شبيهه اليوجورت الحيوى فكان أعلاها فى عينة المقارنة وقلت بزيادة نسبة الاستبدال فى باقى المعاملات. وقد أدى إضافة نكهة الفراولة و ١٠% سكر لتلك المعاملات إلى زيادة الجودة الحسية لمعاملات شبيهه اليوجورت الحيوى وكانت أفضل المعاملات عند نسبة استبدال ٣٠% بمستخلص الترمس مع إضافة ١٠% سكر ونكهة الفراولة.

المحكمون:

١- أستاذ الألبان رئيس قسم علوم الأغذية- كلية الزراعة - جامعة الزقازيق.
٢- أستاذ الألبان - كلية التكنولوجيا والتنمية - جامعة الزقازيق.

١- أ.د. أحمد علاء الدين النشوى
٢- أ.د. عبدالنبي عبدالغنى فرج