# THE INFLUENCE OF INULIN ADDITION ON THE QUALITY OF FUNCTIONAL FERMENTED MILK

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ABSTRACT: Low fat set and stirred yogurt were made with 3% inulin and some probiotic strains (Bifidobacterium bifidum, Lactobacillus acidophilus, Lactobacillus rhamnosus and Lactobacillus reuteri). All samples were analyzed for acidity, rheologically, bacteriologically and organoleptically evaluated periodically when fresh and during the storage period at refrigerator temperature. Results indicated that acidity of all treatments (set and stirred yogurt) with 3% inulin were slightly higher than control when fresh and throughout the storage period. Samples with inulin had a higher viscosity throughout the storage period compared to controls. Addition of inulin to the low fat set yogurt increases the curd tension in the final product. The synersis of low fat set yogurt without inulin (control) was higher than the treatments with inulin when fresh and during the storage period.

Bacterial counts of all treatment samples with inulin were a higher than those without inulin and had the same trend of viability to reach a maximum after 5 days of the storage period and then decreased till the end of this period (10 days) at 5-7 °C. Set and stirred yogurt samples with inulin had a high scores compared to controls. All treatments with L. reuteri had a high scores compared to other treatment with other probiotic strains. The scores of all treatments slight decrease till the end of the storage period.

Key Words: Inulin, Low fat set yogurt, Low fat stirred yogurt, Probiotic bacteria.

## INTRODUCTION

During the last few years inulin has become a popular food ingredient due to its versatile functionality. Moreover, increased demand for healthy foods has resulted in a growing interest in the chicory-derived product. The fat substituting property of inulin is based on the products ability to stabilize water into a creamy structure, which has an excellent fat-like mouth-feel and which is alsmost taste-free (Blomsma, 1997).

Inulin is also considered to have functional properties such as the ability to act as a fat or sugar replacer (Tungland, 2000). Inulin plays a key role in enhancing the activity of probiotic bacteria (El-Nagar and Brennan, 2001).

Recent market studies have showing an increasing demand for functional foods containing live probiotic cultures (Mehanna *et al.*, 2002). Novel and new strains and also species have emerged and are likely to be included in

our diet. These included Lactobaclllus acidophilus, Lactobacillus rhamnosus, Lactobacillus reuterl and Bifidobacterium bifidum (Salminen and Saxelin, 1996). These probiotic bacteria may control of intestinal infections, anticarcinogenic activity and control of serum cholesterol levels (Kisla and Ünlütürk, 1998).

Probiotic bacteria are frequently used as the active ingredient in functional foods such as bio-yogurt, dietary adjuncts and health-related products (Brassart and Schiffrin, 1997), fermented milks as well as unfermented milks with culture added (Sanders *et al.*, 1996). Freshly consumed cheese, i.e. Tallaga cheese (El-Zayat and Osman, 2001), Kareish cheese (Osman, 2001), Domiati cheese (Effat, 2000) and Soft cheese (Mehanna *et al.*, 2002) represents another way to expand the probiotic product range.

One of the growing sectors within the food industry is the production with a reduced Calorie content. In general, consumers are becoming more conscious of the composition of food products and the food products must meet this demand by putting more effort into the development of new products with a lower energy content, e.g. by reducing the fat content.

The objective of this work is to examine the rheological, textural factors, survival rates of probiotic culture and sensory characteristics of low fat set and stirred yogurt mixes with 3% inulin when fresh and throughout the storage period at refrigerator temperature (5-7°C).

## MATERIALS AND METHODS

#### 1. Materials

#### 1.1. Inulin

Inulin was obtained from Sensus Food Ingredients, Netherlands. Chemical composition of it was 90 ( $\pm$  2.2)%.

#### 1.2. Bacterial Strains

Streptococcus thermophilus, Lactobacillus delbreuckii spp. bulgaricus and Bifidobacterium bifidum were obtained from Chr. Hansen's Lab., Denmark. Lactobacillus reuteri, B-14171 and Lactobacillus rhamnosus B-445 were donated by Dr. Nakamura (Northern Regional Research Laboratory, (NRRL), Illinois, USA).

## 1.3. Milk

Fresh whole buffalo's milk was obtained from the station of experimental farm, Faculty of Agriculture, Cairo University, Giza, Egypt. Skimmed using Alfa-Laval Cream separator and skimmed milk was standardized to 1% fat.

#### 2. Experimental Procedure

2.1. Preparation of Low Fat Set and Stirred Yogurt with Inulin

Fresh low fat (~1%) buffalo's milk heated to 85°C for 30 min., cooled to 42°C. Milk was inoculated with 3% of yogurt starter culture (*Lactobacillus* 

delbreuckii subspp. bulgaricus and Streptococcus salivarius subspp. thermophilus 1:1). Two types of yogurt (set and stirred) were manufactured with 1% probiotic cultures (*Bifidobacterium bifidum*, *Lactobacillus* acidophilus, *Lactobacillus rhamnosus* and *Lactobacillus reuteri*) and 3% inulin. Probiotic starter cultures and inulin were added immediately after adding of yogurt starter culture. Each type of yogurt (set and stirred) was divided into 5 batches:

(a) The first batch without inulin and probiotic cultures used as control.

(b) The second batch with B. bifidum.

(c) The third batch with L. acidophilus.

(d) The fourth batch with L. rhamnosus.

(e) The fifth batch with L. reuteri.

All treatments were incubated at 42°C. After complete coagulation, the yogurt samples stored at refrigerator temperature (5-7°C). All of set and stirred yogurt treatments were manufactured according to the method of Robinson and Tamime (1990).

# 2.2. Effect of Inulin on the Growth Rate of Probiotic Strains 2.2.1. Growth of Strains in Batch Cultures

Hundred ml of sterilized respective media for growth of each probiotic strains were prepared in Erlenmeyer flasks (250 ml in volume). These flasks were inoculated with 1 ml standard inoculum of respective strain and shaked (rotary shaker (160 rpm) for 24 hrs at 37°C). Samples (5 ml) were taken from the growing cultures periodically under aseptic condition to determine the bacterial growth rate by measuring the O.D. at 650 nm using a UV-VIS spectrophotometer (model 8452, Hewlett-Packard).

Growth rate and generation time were calculated from the experimental phase using the following equation according to Shin *et al.* (2000).

Ln	X L	١X。	
u –	t t	 >	
$dt = \frac{Ln2}{u}$	2		
where:	Ln	z	Napierian log
	u	Ŧ	growth rate (hr <sup>-1</sup> )
	X。	=	growth density at zero time
	Х	=	growth density after t time
	dt	=	doubling time (hr)
	1 <b>1</b>	-1 D	

### 3. Analytical Procedures

Samples stored at refrigerator temperature ( $5-7^{\circ}$ C). Set yogurt samples were taken when fresh (0 time), 3, 5, 7 and 10 days, while stirred yogurt were taken when fresh (one day), 3, 6, 9, 12 and 15 days. All treatments were

examined for pH, rheological, microbiological, sensory evaluations and microstructure.

#### 3.1. Titratable Acidity

Titrable acidity was determined according to International Dairy Federation IDF (1991).

### 3.2. Rheological Properties

## 3.2.1. Viscosity

Viscosity was measured by viscometer type RN at  $20^{\circ}\pm1^{\circ}$ C. Viscosity was calculated according to the following equation:

 $\eta = K \cdot M \cdot \alpha$  in fermented milk (set and stirred)

 $\eta$  = Dynamic viscosity (mpa.s) >

K = Constant (cp.skt)

**M** = speed factor, adjustable steps 1, 2, 4, 10 (which use No. 2).

#### 3.2.2. Curd Tension

The curd tension (g) of all low fat set and stirred samples were measured by the method of Chandrcasc Khara *et al.* (1957).

#### 3.2.3. Syneresis

Syneresis was determined by measuring the volume of separated whey (ml whey/ml yogurt). The amount of free whey collected after 30 min at room temperature  $(25\pm2^{\circ}C)$  was taken as the index of syneresis (Dannenberg and Kessler, 1988).

## 3.3. Bacteriological Analysis

• Lactic acid bacteria was enumerated according to Elliker et al. (1956).

- bifidobacteria *bifidum* was determined according to Blanchette *et al.* (1996) using modified MRS agar (Oxoid) supplemented with 0.05% L. cysteine-HCL (Merck, Germany). Plates were incubated at 37°C for 48 h. under anaerobic conditions (BBL Gas Pak, Becton Dickinson, Cockeysville MA, USA).
- Lactobacillus rhamnosus was counted on LC agar (Ramakanth and Nagendera, 1998). Plates were incubated anaerobically at 27°C for 72-96 h.
- Lactobacillus acidophilus was determined using Lactobacillus selective agar plus 0.2% Oxgall (LBSO) (Gilliland and Walker, 1990). Plates were incubated at 37°C for 4 days.
- Lactobacillus reuteri was enumerated on MRS-arabinose agar (Effat, 2000). MRS basal medium was prepared without dextrose, and 10 ml of membrane-filtered sterile solution of 10% L-arabinose was added per 90 ml of basal medium (10% final concentration) just before pouring the agar medium. Plates were incubated anaerobically at 37°C for 48 h.

#### 3.4. Sensory Evaluation

Low fat set and stirred yogurt were organoleptically scored when fresh and throughout the storage period at 5-7°C by 20 panelists of the experienced staff members of Dairy Department, National Research Center according to the International Dairy Federation IDF (1997) and British Standard Institutes (1986) as follow: acceptability flavour, appearance and texture.

## 3.5. Microstructure with Transmission Electron Microscopy

Yogurt samples for electron microscopy were taken from 3 cm below the surface of the fermented milk examined by Transmission Electron Microscopy (TEM) as described by Tamime et al. (1984, 1989 and 1991).

# **RESULTS AND DISCUSSION**

Effect of Inulin on the Growth Rate of Some Probiotic Strains

Data presented in Table (1) show that the growth rate of probiotic strains (*B. bifidum*, *L. acidophilus*, *L. rhamnosus* and *L. reuteri*) with addition of 3% inulin was higher than the control (without inulin). Besides the growth rate of *B. bifidum* was higher than other probiotic strains.

It could be noticed that the addition of inulin enhancing the growth rate of probiotic strains. Our results confirm the observations of Young (1998) and Van Loo et al. (1999) who reported that a prebiotic increase the numbers and/or activity of bifidobacteria and lactic acid bacteria in the human large intestine. In the same concern, Reberfroid et al. (1998) recorded that inulin stimulating the growth of lactic acid bacteria.

Table (1): Effect of 3%	inulin addition	on the growth	rate of some probiotic
strains.	•	•	•

Probiotic	Growth Rate (hr <sup>-1</sup> )			
Strains	Control	3% inulin		
B. bifidum	0.1369	0.20		
L. acidophilus	0.0969	0.16		
L. rhamnosus	0.117	0.1495		
L. reuteri	0.103	0.154		

# Low Fat Set and Stirred Yogurt Manufactured with 3% Inulin and Some Probiotic Strains

## I. Low Fat Set Yogurt

## I.1. Titratable Acidity

Data illustrated by Fig. (1) clearly show that the acidity of all treatments were slightly higher than the control when fresh (0 time) and throughout the storage period (10 days) except low fat set yogurt with *L. reuteri*, values of titratable acidity were similar to control. Acidity was slightly increase during the storage period in all treatments, this may be due to that the fermented milk cultures remained active. Increase in the acidity content during storage of yogurt were also reported by El-Shibiny *et ai.* (1979). Our results are in agreement with El-Nagar and Brennan (2001).

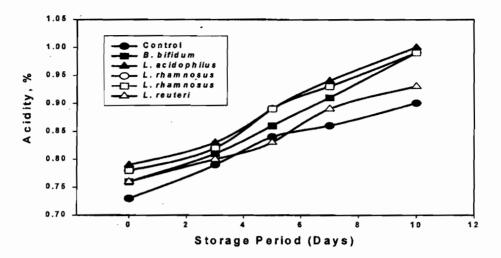


Fig. (1): Changes in the acidity of low fat set yogurt during storage at 5-7°C.

# I.2. Rheological Properties

## I.2.1. Consistency Index (Viscosity)

Effect of the inulin addition on the viscosity of low fat set yogurt during storage at 5-7°C are shown in Fig. (2).

The results indicated that low fat set yogurt with inulin had a higher viscosity when fresh and during the storage period compared to controls. The viscosity gradually increased throughout the storage period to reaching a maximum after 10 days, this means that the inulin improve the viscosity of yogurt. In this respect Adapa *et al.* (2000) recorded that addition of different exopolysaccharides to yogurt increased its viscosity. The improvement of viscosity may be due to the fact that the hydrocolloids has exceptional water binding capacity and ability to enhance viscosity (Wang *et al.*, 1998). Marshall and Rawoson (1999) suggested that it may not be the amount of polysaccharide which is important in affecting the viscosity, but the type of exopolysaccharides (EPS) and consequently the interaction of the polymer with the milk proteins during the fermentation.

Our results agree with Ohmes *et al.* (1998), Adapa *et al.* (2000) and El-Nagar and Brennan (2001) who reported that all yogurt treatments with inulin had a higher viscosity compared to control.

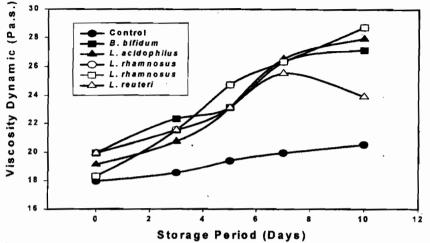


Fig. (2): Effect of inulin addition on the viscosity of low fat set yogurt during storage at 5-7°C.

## I.2.2. Curd Tension

Effect of inulin addition on the curd tension of low fat set yogurt are recorded in Table (2).

The results show that the addition of inulin during the manufacture of set yogurt increasing the curd tension in the final product. Hess *et al.* (1997) reported similar observations, they suggested that of the network prevented by exopolysaccharides associated with the casein matrix.

These results agree with those obtained by Dannenberg and Kessber (1988) who found that the increase in the total solids and acidity improves the texture of yogurt and increase the curd tension.

Probiotic	Low Fat Set Yoghurt		
Strains	Control	3% inulin	
B. bifidum	69.0	74.13	
L. acidophilus	68.13	75.13	
L. rhamnosus	69.25	75.29	
L. reuteri	68.13	74.89	

Table (2): Effect of inulin addition on the curd tension of low fat set yoghurt.

# I.2.3. Whey Synersis of Yogurt

Whey synersis of low fat set yogurt was affected by the addition of inulin used as shown in Table (3). It is obvious from these results that the synersis of the control (without inulin) was higher than the treatments with inulin which may be due to the low total solids. During the storage period at refrigerator temperature (5-7°C), the synersis gradually increased in all

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treatments till the end of this period (10 days). Experiments revealed that synersis decreased by increasing total solids especially polysaccharides. Similar results were reported by Omar and Abou El-Nour (1998), El-Nagar & Shenana (1998) and El-Nagar & Brennan (2001).

Table (3): Changes in the synersis ml/100g of low fat set yoghurt with 3% inulin during storage at 5-7°C.

Storage Period (Days)	Low Fat Set Yoghurt					
	1	2	3	4	5	
0	4.90	3.50	3.90	3.30	3.70	
3	5.20	3.60	4.10	3.70	4.0	
5	5.30	3.90	4.30	3.90	4.20	
7	5.70	4.10	4.50	4.25	4.40	
10	6.0	4.40	4.60	4.50	4.70	

(1) Control. (2) B. bifidum. (3) L. acidophilus. (4) L. rhamnosus. (5) L. reuteri.

#### **I.3. Lactic Acid Bacteria and Probiotic Counts**

Data presented in Fig. (3) show the lactic acid bacteria and probiotic counts in low fat set yogurt without and with 3% inulin when fresh and during the storage period (10 days) at 5-7°C.

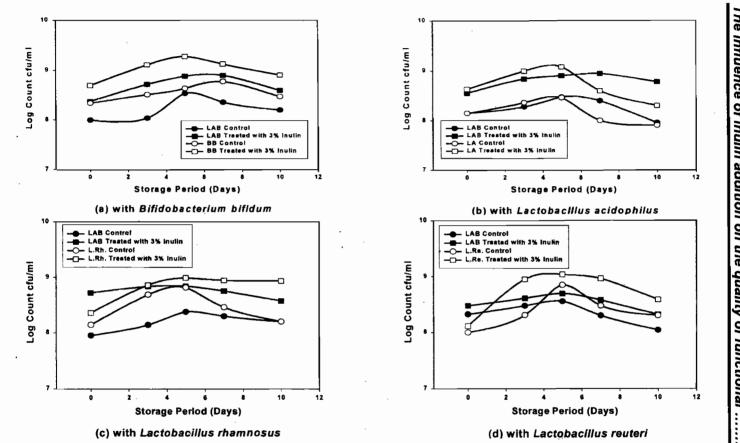
It could be noticed that log of lactic acid bacterial (LAB) counts of all treatments with inulin and probiotic strains had higher count compared to the controls (without inulin) when fresh till the end of the storage period. It could be due to the effect of inulin on the growth of lactic acid bacteria. Our results confirm the observations of Gibson and Roberfroid (1995) and Roberfroid *et al.* (1998) who reported that inulin stimulating the growth of lactic acid bacteria.

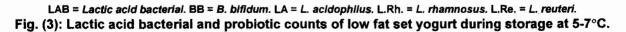
The counts of *B. bifidum* in yogurt with inulin (Fig. 3-a) was higher than the control (without inulin), the counts gradually increased during the first 5 days of the storage period and then decreased till the end of this period (10 days).

It is clear from (Fig. 3-b) that counts of *L. acldophilus* in yogurt with inulin was a higher than the control when fresh and throughout the storage period, *L. acidophilus* count gradually increased during the first 5 days of storage period and then decreased.

*L. rhamnosus* count in all treatments of low fat set yogurt (Fig. 3-c) increased throughout the first 5 days of the storage period and then decreased till the end of this period.

Finally, log of *L. reuteri* count (Fig. 3-d) gradually increased to reach a maximum after 5 days of the storage period and decreased till the end of this period. The reduction in number of probiotic strains was due to the sensitively of these bacteria to the acid produced during the storage period.



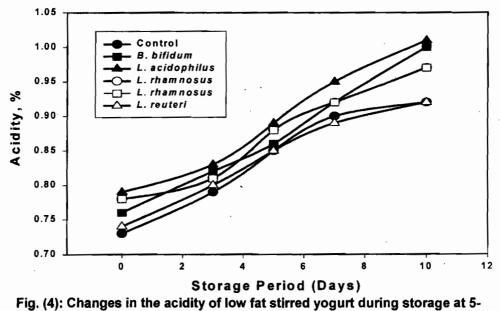


From the foregoing results, it could be noticed that inulin enhancing the growth of probiotic strains. Our results agree with Bouhnik *et al.* (1996), Menne *et al.* (1997) and Roberfroid *et al.* (1998).

## II. Low Fat Stirred Yogurt

#### II.1. Titratable Acidity

As shown in Fig. (4), the acidity of low fat stirred yogurt treatments with inulin were higher than those without inulin (control) except low fat stirred yogurt with *L. reuteri*, titratable acidity had the same values of the control. The acidity increased gradually throughout the storage period to reach a maximum to the end of this period. These results agree with El-Shibiny et al. (1979) and El-Nagar & Brennan (2001) who reported that acidity of yogurt increased during the storage period at 5-7°C.



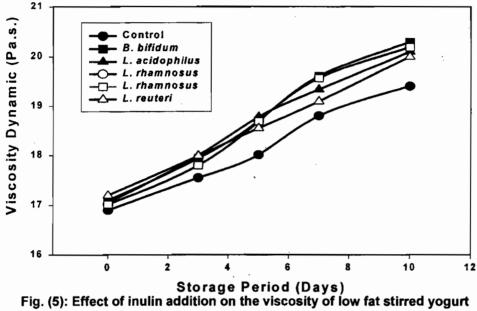
7°C.

## II.2. Consistency Index (Viscosity)

The viscosity of low fat stirred yogurt is shown in Fig. (5). It could be recommended that all treatments with 3% inulin had a high viscosity when fresh and during the storage period (10 days) at 5-7°C compared to the control. The results also indicated that the viscosity increased gradually throughout the storage period at 5-7°C. The increase in the viscosity in all

treatments with inulin can be explained through the fact the hydrocolloids has exceptional water binding capacity and ability to enhance viscosity (Wang et al., 1998).

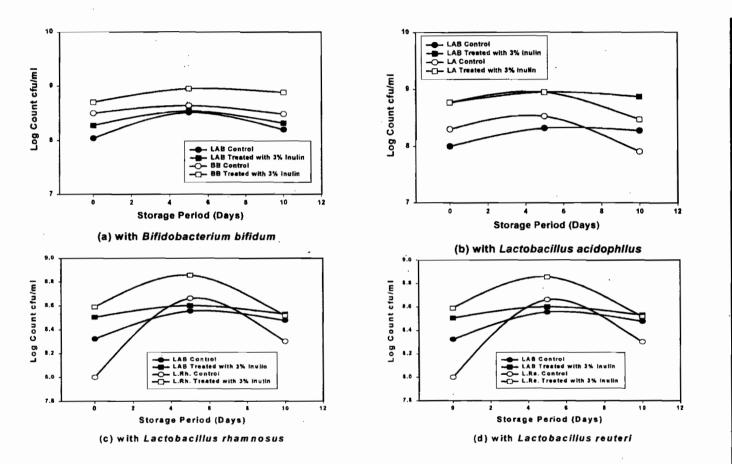
Our results are in line with the results reported by Wang et al. (1998) and El-Nagar & Brennan (2001).

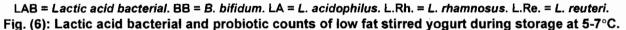


#### during storage at 5-7°C.

## **II.3. Lactic Acid Bacterial and Probiotic Counts**

Data presented in Fig. (6) illustrated that log of lactic acid bacterial counts of all treatments with inulin were higher than those without inulin and had the same trend of increasing to reach a maximum at the first 5 days of the storage and then decreased till the end of storage period (10 days) at  $5-7^{\circ}$ C. Roberfroid *et al.* (1998) reported that inulin stimulating the growth of lactic acid bacteria. Similar results were reported by Gibson and Roberfroid (1995), Roberfroid *et al.* (1998) and El-Nagar & Brennan (2001).





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The counts of probiotic strains (Fig. 7) (*B. bifidum, L. acidophilus, L. rhamnosus* and *L. reuteri*) in all stirred yogurt treatments with inulin were higher than those without inulin (control) when fresh and throughout the storage period at 5-7°C, that is may be due to the effect of inulin as prebiotic and stimulating the growth of this probiotic strains to have high viable number in all treatments with the addition of inulin.

Log count of probiotic strains counts increased gradually during the first 5 days of the storage period at 5-7°C and then decreased till the end of this period. This decrease may be due to the sensitively of micro-organisms to acid produced. Our results confirm the observation of Kebary & Kamaly (1991), Badwi & El-Sonbaty (1997), El-Nagar & Shenana (1998) and El-Ngar and Brennan (2001).

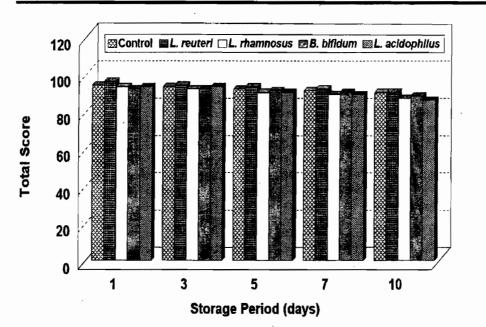
#### Sensory Evaluation of Set and Stirred Yogurt

The evaluation scores of low fat set and stirred yogurt with 3% inulin and different probiotic strains when fresh and during storage at  $5-7^{\circ}$ C are shown in Figs. (7) and (8).

As shown in Fig. (7) that all treatments of low fat set yogurt with inulin had total scores higher than control when fresh and during storage period, this may be attributed to the effect of the inulin on the viscosity and curd tension of set yogurt.

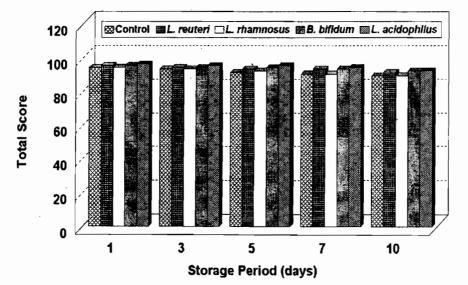
Results presented in Fig. (8) show the evaluation scores of low fat stirred yogurt with inulin. It could be noticed that the highest total scores was recorded for low fat stirred yogurt with *L. acidophilus*, followed by yogurt with *L. reuteri* and *B. bifidum* when fresh and during storage at  $5-7^{\circ}$ C.

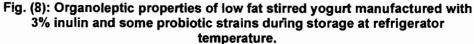
The results also indicated that low fat set and stirred yogurt with inulin had a high scores compared to control. Inulin plays a key role in enhancing the taste and the texture of all our set and stirred yogurt. The scores of all treatments slight decrease until the end of the storage period (10 days). This may be due to increasing the acldity which affect on the rheological properties. These results agree with Blomsma (1997) and El-Nagar & Brennan (2001).



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Fig. (7): Organoleptic properties of low fat set yogurt manufactured with 3% inulin and some probiotic strains during storage at refrigerator temperature.

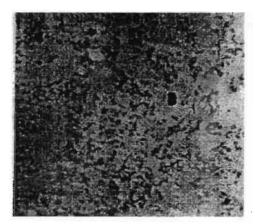




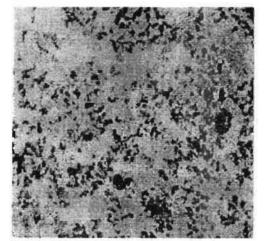
# Effect of Inulin on the Microstructure of Yogurt

Fig. (9) showed that the microstructure of low fat fermented milk with 3% inulin. It is clear that low fat had an effect in sturcture of protein matrix making network extracted to inulin molecules replacing in it. These results agree with Tamime *et al.* (1989 and 1991).

It can be concluded that enrichment of fermented milk with inulin improved texture, water-binding capacity, functionality, besides it can be used as thickening agents.



(a)



(b) Fig. (9): Effect of inulin addition on microstructure of low fat fermented milk. (a) control without inulin. (b) with 3% inulin.

#### CONCLUSIONS

From these results, it can be concluded that the counts of probiotic bacteria and organoleptic scores reached to the optimum throughout the storage period at refrigerator temperature. In the presence of a high growth rates of probiotic bacteria could be observed. Low fat set and stirred yogurt with inulin had a good quality of rheological properties (increased curd tension, increased viscosity and decreased syneresis) as reported in organoleptic properties.

Therefore, low fat set and stirred yogurt could be used a good source for delivering dietary fiber and probiotic bacteria to the consumer.

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تأثير إضافة الأنيولين على جودة الألبان المتخمرة الوظيفية نايره شاكر مهنا - جمال أحمد إبراهيم - ضياء عبد المنعم جاد الرب قسم الألبان -- المركز القومى للبحوث -- الدقى -- القاهرة

الملخص العربي

يهدف هذا البحث إلى استخدام الأتيولين في تصنيع بعض أنواع الألبان المتخمرة الوظيفية مثل الزبادي العادي والزبادي المخفوق مع إضافة بعض بادنات المدعمات الحيوية .

وقد صنع الربادي العدى والزبادي المخفوق باستخدام ٣% أنيولين وبعض بادنات المدعمات الحيوية مثل L. rhamnosus و L. acidophilus و B. bifidum .

تسم تحليل جميع العينات بالنسبة للحموضة ... الخواص الريولوجية ... الأعداد الميكروبية (بكتريا حامض اللكتيك ... بادئات المدعمات الحيوية).

تم التقييم حسياً بعد التصنيع مباشرة وكذلك خلال فترة الحفظ في الثلاجة (٥ – ٧م) . أوضحت النتائج حدوث زيادة طفيفة في الحموضة في العينات المضاف إليها ٣% أنبولين

فسي السزبادي - كذلك الزبادي المخفوق عن العينات الغير مضاف إليها أنيولين (كنترول). كما لوحظ أن هذه الزيادة في الحموضة تزداد تدريجياً حتى نهاية فترة الحفظ في الثلاجة.

إضافة الأتيولين إلى الزبادي العادي والمخفوق أدى إلى زيادة شدة الخثرة وتحسينها عن الأسواع الأخرى الغيير مضاف إليها. أما بالنسبة لافصال الشرش فقط لوحظ زيادة انفصال الشرش في الزبادي الغير مضاف إليه أنيولين (الكنترول) عن الزبادي المعامل بالأتيولين.

لوحيظ أن أعداد بكتريا حامض اللاكتيك كذلك بكتريا المدعمات الحيوية تزداد تدريجياً في عينات الربادي المصنع بالأتيولين عن الكنترول حتى اليوم الخامس من الحفظ على درجة حرارة الثلاجة ثم يقل العدد تدريجياً حتى نهاية مدة الحفظ. وذلك يرجع نتيجة تأثير الحموضة الناتجة. أوضحت النتائج أن جميع أنواع الزبادي المصنع بالأنيولين كانت أفضل جودة وحساً عن الكنترول – كما أن الزبادي العادي والمخفوق المصنع باستخدام L. reuteri كان أفضل حسياً من باقى الأنواع المصنعة بالأنواع الأخرى من بادئات المدعمات .

لقد أثبتت هذه الدراسة إمكانية استخدام الأليولين في تصنيع بعض أنواع الألبان المتخمرة الوظيفية حيث أنه يعمل على تشجيع وتحفيز بادئات المدعمات الحيوية على النمو والنشاط مما يؤدى إلى الفائدة الصحية والعلاجية لها.