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MANUFACTURE OF PROBIOTIC YOGHURT BY INCORPORATION LACTOBACILLUS RHAMNOSUS GR-1

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ABSTRACT: *Five batches of yoghurt were made to study the influence of incorporating Lactobacillus rhamnosus GR-1 on quality of yoghurt. One of them made without Lb. rhamnosus GR-1 served as control (c), while four treatments made with adding Lb. rhamnosus GR-1 at the ratio of 1, 2, 3 and 4% (T_1, T_2, T_3 and T_4), respectively. All yoghurt treatments were sampled at 1, 3, 6, 9 and 12 days and analyzed for chemical, rheological, bacteriological properties and sensory evaluation. Adding of Lb. rhamnosus GR-1 did not affect the chemical composition (TS, TP, F, ash and lactose) at the first day of storage period. Lactose content decreased during storage period and the titratable acidity increased slightly with increasing the rate of adding Lb. rhamnosus GR-1, while pH values followed an opposite trends of those of titratable acidity as affected by adding Lb. rhamnosus GR-1 and storage period. Results indicated that adding of Lb. rhamnosus GR-1 did not affect acetaldehyde content and syneresis. Survival of Lb. rhamnosus GR-1 did not changed during storage period. Yoghurt treatment that made by incorporating 4% of Lb. rhamnosus GR-1 was the most acceptable yoghurt treatments and gained the highest scores of organoleptic properties.*

Key words: *Yoghurt, Lactobacillus rhamnosus GR-1, acetaldehyde.*

INTRODUCTION

Yoghurt is the most popular fermented milk produced in Egypt and worldwide. Its consumption in Egypt has been increased tremendously. The value of yoghurt in human nutrition is based, not only on the nutritive value of the milk from which it is made and increased digestibility, but also on the beneficial effect of intestinal microflora, prophylactic and healing effects (Rasic and Kurman, 1978; Buttriss, 1997). Many health benefits have been attributed to yoghurt such as improved lactose tolerance, protection against gastrointestinal infections, effective treatment for specific types of diarrhea, relief of constipation, improved immunity and cholesterol reduction concentration (Tvede, 1996 and Buttriss, 1997).

Probiotic bacteria are recognized currently as those promoting the health of human by exhibiting autigomitric effects towards enteropathogenic bacteria, reducing the risk of diarrhea, enhancing immune function, reducing cholesterol levels, reducing the risk of eczema, relieving lactose intolerance symptoms, synthesizing vitamins and exhibiting antitumonigenic activity (Kebary, 1995, Ibrahim *et al.*, 2005 and Hussein *et al.*, 2006).

It has been speculated that minimal number of viable cells of probiotic bacteria should be more than 10^5/g to achieve the therapeutic effects (Samona and Robinson, 1991; Lee *et al.*, 1996). Some researchers suggest that multiple species or high numbers of probiotic organisms need to be administered to achieve colonization, as shown in the treatment of pouchitis (Reid *et al.*, 2003). One of the most important properties of probiotic bacteria is their ability to survive passage through the gastrointestinal tract and persist for a sufficient time in the gut so they can provide beneficial health effects (Huang and Adams, 2004).

Although various strains of lactic acid bacteria have been described as probiotic, relatively few meet the standards of the united Nations of having clinical trial documentation, and many are too sensitive to intense acidity and presence of bile salts in the human gastrointestinal tract, so they die on route to the gut (Kim and Gilliland, 1984).

Various strains of lactic bacteria are considered probiotics. Two of the most documented probiotic strains, lactobacillus reuteri (Formerly fermentum) RC-14 and *Lactobacillus rhamnosus* GR-1 can colonize the intestine and vagina and reduce recurrences of bacterial raginosis, yeast vaginitis and urinary tract infections (Reid and Burton, 2002; Cadieux *et al.*, 2002; Reid, Burton, Hammond & Bruce, 2004 and Sharareh *et al.*, 2009).

MATERIALS AND METHODS

Materials:

Active *Streptococcus thermophilus* ENCC 1043 and *Lactobacillus delbruechii* subsp. *bulgaricus* EMCC 1102 were obtained from the Egyptian microbial culture collection (EMCC) at Cairo Microbiological Resources Center (Cairo Mircen), Faculty of Agriculture, Ain Shams University. While *Lactobacillus rhamnosus* GR-1 was obtained from Urex Biotech. Inc., London, Ontario, Canada. These strains were activated individually by three successive transfers in sterile 10% reconstituted non-fat dry milk.

Manufacture of yoghurt:

Fresh buffalo milk, was obtained from Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Milk was standardized to (3% fat) and heated to 90°C for 10 min., then cooled to 40°C and divided into five batches. Control yoghurt treatment (C) was made by adding 2% of normal starter (50% *Str. thermophilus* + 50% *Lb. delbruechil* subsp. *bulgaricus*) while the other four treatments were made as explained in control treatment except that *Lactobacillus rhamnosus* GR-1 was added at the rate of 1, 2, 3 and 4% (T_1, T_2, T_3 and T_4), respectively. The inoculated batches were packed in plastic cups and incubated at 42°C for 2 – 3 hr. until complete coagulation. All batches were stored at 6 ± 1°C for 12 days and sampled for analysis after 1, 3, 6, 9 and 12 days. This experiment was triplicated.

Method of analysis:

1. Chemical analysis:

Yoghurt treatments were analyzed for total solids (T.S%) fat (%), total protein (%), ash (%), titratable acidity (%) and pH value according to the methods of A.O.A.C (1995), lactose content was calculated by difference. The acetaldehyde content was measured according to the method described by Bradly *et al.* (1992).

2. Rheological properties:

Syneresis which is considered the most important rheological properties of the yoghurt was measured according to the method described by Dannenberg and Kessler (1988).

3. *Lactobacillus rhamnosus* GR-1 count:

Lactobacillus rhamnosus GR- 1 was enumerated using MRs pH 6.2 plus vancomycin (10 µ g/ml), and incubated under anaerobic conditions (Anaero Gen; Oxoid Ltd) at 37°C for 72 h (Lankaputhra and Shah, 1996; Tharmaraj and Shah, 2003).

4. Sensory evaluation:

Flavour, appearance, body and texture were organoleptically evaluated by well trained ten panelists of the staff members of Dairy Technology Department, Food Technology Research Institute, Agric. Res. Center. Results were recorded in a score sheet described by Nelson and Trout (1981).

5. Statistical analysis:

Statistical analysis were carried out by Spssio (SPSS, Chicago, Ill) program for windows. The level of statistical significance was set at $p < 0.05$ as reported by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical composition of probiotic yoghurt as affected by using different ratios of *Lactobacillus rhamnosus* GR-1 is presented in Table (1). Total solid (TS), total protein (TP), fat, lactose and ash of all treatments were not significantly different at the first day of storage period (Table 1). Lactose content of all yoghurt treatments decreased slightly during storage period ($p \leq 0.05$), which was mainly attributed to the metabolic activity of the starter culture and probiotic organisms. Similar observations were reported by Dirar (1993), Cunha *et al.* (2002), Venturoso *et al.* (2007) and Fanti *et al.* (2008), while TS, TP, fat and ash content did not change significantly throughout storage period (Table 1). These results are in agreement with those reported by El-Etriby *et al.* (1997), Badawi *et al.* (2008), Abd El-Rahman and Salama (2008), Hamed *et al.* (2010).

Table (1). Effect of adding *Lactobacillus rhamnosus* GR-1 on chemical composition of fresh and stored probiotic yoghurt.

Yoghurt treatments	Total solid (%)		Total protein (%)		Lactose (%)		Fat (%)		Ash (%)	
	1 day	12 day	1 day	12 day	1 day	12 day	1 day	12 day	1 day	12 day
C	14.06^{Aa}	14.21^{Aa}	4.39^{Aa}	4.42^{Ba}	5.34^{Aa}	5.28^{Ab}	3.5^{Aa}	3.7^{Aa}	0.83^{Aa}	0.91^{Aa}
T_1	14.05^{Aa}	14.13^{Aa}	4.39^{Aa}	4.44^{Ba}	5.34^{Aa}	5.27^{Ab}	3.5^{Aa}	3.7^{Aa}	0.82^{Aa}	0.92^{Aa}
T_2	14.04^{Aa}	14.09^{Ba}	4.40^{Aa}	4.52^{Aa}	5.33^{Aa}	5.08^{Bb}	3.5^{Aa}	3.8^{Aa}	0.81^{Aa}	0.94^{Aa}
T_3	14.06^{Aa}	14.16^{Ba}	4.41^{Aa}	4.55^{Aa}	5.32^{Aa}	4.92^{BC}	3.5^{Aa}	3.7^{Aa}	0.83^{Aa}	0.97^{Aa}
T_4	14.07^{Aa}	14.10^{Ca}	4.41^{Aa}	4.59^{Aa}	5.32^{Aa}	4.76^{Cb}	3.5^{Aa}	3.8^{Aa}	0.84^{Aa}	0.94^{Aa}

Different capital letters in the same column means the treatments are significantly different from each other, while the small letters in the same row means the treatments are significantly different from each other.
C: Yoghurt made by normal starter (control).
T_1: Yoghurt made by normal starter and 1% *Lactobacillus rhamnosus* GR-1.
T_2: Yoghurt made by normal starter and 2% *Lactobacillus rhamnosus* GR-1.
T_3: Yoghurt made by normal starter and 3% *Lactobacillus rhamnosus* GR-1.
T_4: Yoghurt made by normal starter and 4% *Lactobacillus rhamnosus* GR-1.

Acidity and pH most probiotic bacteria grow slowly in milk and the rate of acid production is usually too slow to support an adequate fermentation process in yoghurt (Shah, 2000).

Slight differences were noticed among yoghurt treatments in titratable acidity (Table 2) (Shah, 2000). On the other hand, titratable acidity of all yoghurt treatment increased significantly ($p \leq 0.05$) as storage period progressed (Table 2). The value of pH as affected by the rate of adding *Lactobacillus rhamnosus* GR-1 to yoghurt and storage period followed opposite trends of those of titratable acidity (Table 2). Similar results are reported by Abd El-Salam *et al.* (1996), Badawi and El-Sonbaty (1997), Hassan *et al.* (1999), Harby and El-Sabie (2001) and Zedan *et al.* (2001).

Table (2). Effect of adding *Lactobacillus rhamnosus* GR-1 to yoghurt on acidity and pH of fresh and stored probiotic yoghurt.

Yoghurt treatments *	Titratable acidity					pH value				
	Storage period (days)					Storage period (days)				
	1	3	6	9	12	1	3	6	9	12
C	0.99^{Ad}	1.07^{Ac}	1.16^{Ab}	1.23^{Aab}	1.24^{Aa}	4.6^{Aa}	4.41^{Ab}	4.26^{Ac}	4.12^{Ad}	4.07^{Ad}
T_1	0.98^{Ad}	1.08^{Ac}	1.18^{Ab}	1.25^{Aab}	1.26^{Aa}	4.61^{Aa}	4.40^{Ab}	4.21^{ABc}	4.09^{Ad}	4.05^{Ad}
T_2	1.00^{Ad}	1.11^{ABc}	1.19^{Ab}	1.24^{ABab}	1.27^{Aa}	4.58^{Aa}	4.39^{Ab}	4.18^{Ac}	4.06^{ABd}	4.04^{ABd}
T_3	1.01^{Ad}	1.12^{Ac}	1.20^{Ab}	1.27^{Aa}	1.27^{Aa}	4.57^{ABa}	4.36^{Ab}	4.19^{Ac}	4.03^{Ad}	4.02^{Ac}
T_4	1.02^{Ad}	1.14^{Ac}	1.21^{Ab}	1.28^{Aa}	1.28^{Aa}	4.54^{Aa}	4.36^{Ab}	4.20^{Ac}	4.02^{Ac}	4.00^{Ac}

* See Table (1).

Syneresis values of all yoghurt batches were not significantly ($p > 0.05$) different. Whey syneresis decreased during the first six days in all yoghurt treatments, then increased up to the end of storage period (12 days). Data in Table (3) revealed that whey syneresis increased slightly ($p > 0.05$) by adding *Lactobacillus rhamnosus* GR-1 and this increase was proportional to the rate added. The increase in syneresis might be due to rate of acid production during fermentation process (Sharaeh Hekmat and Gregor Reid, 2006).

The acetaldehyde of probiotic yoghurt as affected by using different levels of *Lb. rhamnosus* GR-1 is shown in Table (4). Results indicated that there were no significant differences among yoghurt treatment in acetaldehyde content, that mean that *Lb. rhamnosus* GR-1 cannot produce acetaldehyde, but *Lb. bulgaricus* produce acetaldehyde which is the most important aroma compound in yoghurt (Marshal and Arbuckle, 1996). In general, the levels of acetaldehyde of all treatments including control. At the end of storage period, the acetaldehyde content was decreased in all yoghurt treatments, this decrease may be attributed to decrease in carbonic compounds in yoghurt by the end of the storage period (Tamime and Robinson, 1997).

Table (3). Effect of adding *Lactobacillus rhamnosus* GR-1 on whey syneresis (ml/100 ml) of fresh and stored probiotic yoghurt.

Yoghurt treatments*	Whey syneresis				
	Storage period (days)				
	1	3	6	9	12
C	41.0^{Aa}	38.0^{ABb}	36.0^{Ac}	36.1^{ABc}	37.7^{ABb}
T_1	40.6^{Aa}	38.3^{ABb}	36.2^{Ac}	36.4^{Bc}	37.8^{ABb}
T_2	41.0^{Aa}	38.5^{Ab}	36.5^{Ac}	36.9^{Ac}	37.3^{Ab}
T_3	40.7^{Aa}	38.8^{Ab}	36.8^{Ac}	37.2^{Abc}	37.9^{Ab}
T_4	40.9^{Aa}	39.0^{Ab}	37.2^{Ac}	37.5^{Ac}	37.9^{Ab}

* See Table (1).

Table (4). Effect of adding *Lactobacillus rhamnosus* GR-1 on acytaldhyde content of probiotic yoghurt (ml / 100 g) of fresh and stored probiotic yoghurt.

Yoghurt treatments*	Acytaldhyde				
	Storage period (days)				
	1	3	6	9	12
C	35.6^{Ac}	38.6^{Aa}	37.1^{Ab}	33.0^{Ad}	24.5^{Ae}
T_1	35.5^{Ac}	38.7^{Aa}	37.2^{Ab}	32.8^{ABd}	24.31^{Ae}
T_2	35.7^{Ac}	38.5^{Aa}	37.0^{Ab}	33.1^{Ad}	24.5^{Ae}
T_3	35.7^{Ac}	38.6^{Aa}	36.9^{Ab}	33.2^{Ad}	24.4^{Ae}
T_4	35.6^{Ac}	38.7^{Aa}	38.7^{Ab}	33.0^{Ad}	24.3^{Ae}

* See Table (1).

Lb. rhamnosus GR-1 count of *Lb. rhamnosus* GR-1 in all yoghurt treatments are presented in (Table 5). Counts of *Lb. rhamnosus* GR-1 increased significantly ($p \leq 0.05$) with increased the rate of adding *Lb. rhamnosus* GR-1 T_4 (4% *Lb. rhamnosus* GR-1) exhibited the highest contents while T_1 (1% *Lb. rhamnosus* GR-1) contained the lowest contents of *Lb. rhamnosus* GR-1 (Table 5). Probiotic yoghurt treatments even after storage for 12 days contained higher counts of *Lb. rhamnosus* GR-1 than the count should be present to achieve the therapiotic effect of probiotic bacteria (Adhikari *et al.*, 2000 and Sharreh *et al.*, 2009).

Table (5). Counts of *Lactobacillus rhamnosus* GR-1 of fresh and stored probiotic yoghurt.

Yoghurt treatments*	Probiotic yoghurt				
	Storage period (days)				
	1	3	6	9	12
C	ND	ND	ND	ND	ND
T_1	$23^{Da} \times 10^6$	$22^{Da} \times 10^6$	$21^{Da} \times 10^6$	$20^{Da} \times 10^6$	$20^{Da} \times 10^6$
T_2	$35^{Ca} \times 10^6$	$33^{Ca} \times 10^6$	$33^{Ca} \times 10^6$	$32^{Ca} \times 10^6$	$32^{Ca} \times 10^6$
T_3	$44^{Ba} \times 10^6$	$43^{Ba} \times 10^6$	$42^{Ba} \times 10^6$	$42^{Ba} \times 10^6$	$41^{Ba} \times 10^6$
T_4	$53^{Aa} \times 10^6$	$52^{Aa} \times 10^6$	$51^{Aa} \times 10^6$	$50^{Aa} \times 10^6$	$50^{Aa} \times 10^6$

* See Table (1).
ND: Not detected.

Scores of organoleptic properties (flavor body & texture, appearance, acidity and total score did not affected by adding of *Lb. rhamnosus* GR-1 to yoghurt (Table 6). *Lb. rhamnosus* GR-1 did not inhibit the standard yoghurt culture overtly contribute to acid production from conversion of lactose to lactic acid (Batish *et al.*, 1997) from Table (6), it could be also seen that, the scores of organoleptic properties of all yoghurt treatments decreased gradually (significant at $p \leq 0.05$) during storage period. Similar trends were obtained by Abd El-Rhman and Salama (2008).

Table (6). Organoleptic properties of probiotic yoghurt made by *Lactobacillus rhamnosus* GR-1 of fresh and stored probiotic yoghurt.

Yoghurt treatments*	Flavour (45)					Body & texture (30)					Appearance (15)					Acidity (10)					Total score				
	Storage period (day)					Storage period (day)					Storage period (day)					Storage period (day)					Storage period (day)				
	1	3	6	9	12	1	3	6	9	12	1	3	6	9	12	1	3	6	9	12	1	3	6	9	12
C	44^{Aa}	43^{Aa}	41^{Ab}	40^{Ab}	39^{Ab}	27^{Aa}	26^{Aa}	25^{Aa}	25^{Ab}	24^{Ab}	13^{Aa}	14^{Aa}	12^{Ba}	11^{Ab}	10^{Ab}	9^{Aa}	9^{Aa}	8^{Aa}	8^{Aa}	8^{Aa}	93^{Aa}	92^{Aa}	87^{Ab}	84^{Ac}	81^{Ad}
T_1	44^{Aa}	43^{Aa}	40^{Ab}	40^{Ab}	39^{Aab}	27^{Aa}	26^{Aa}	25^{Aa}	25^{Ab}	24^{Ab}	13^{Aa}	14^{Aa}	12^{Ba}	11^{Ab}	10^{Ab}	9^{Aa}	9^{Aa}	8^{Aa}	8^{Aa}	8^{Aa}	93^{Aa}	90^{Bb}	86^{Ac}	84^{Ad}	81^{Ae}
T_2	44^{Aa}	42^{Aa}	41^{Aa}	39^{Ab}	39^{Aab}	27^{Aa}	26^{Aa}	25^{Ab}	25^{Ab}	24^{Ab}	14^{Aa}	14^{Aa}	12^{Ba}	11^{Ab}	10^{Ab}	9^{Aa}	9^{Aa}	8^{Aa}	8^{Aa}	8^{Aa}	94^{Aa}	91^{Bb}	86^{Ac}	83^{Ac}	81^{Ad}
T_3	44^{Aa}	42^{Aa}	40^{Aa}	39^{Ab}	39^{Aab}	28^{Aa}	27^{Aa}	24^{Ab}	24^{Ab}	23^{Ab}	14^{Aa}	14^{Aa}	13^{Aa}	12^{Aa}	10^{Ab}	9^{Aa}	8^{Aa}	8^{Aa}	8^{Aa}	7^{Bb}	95^{Aa}	91^{Bb}	86^{Ac}	84^{Ac}	79^{Bd}
T_4	44^{Aa}	43^{Aa}	41^{Ab}	39^{Ab}	38^{Abc}	28^{Aa}	27^{Aa}	23^{Ab}	23^{Ab}	22^{ABc}	14^{Aa}	14^{Aa}	14^{Aa}	11^{Ab}	9^{Ab}	9^{Aa}	8^{Aa}	8^{Aa}	7^{Bb}	7^{Bb}	95^{Aa}	93^{Aa}	88^{Ab}	84^{Ac}	80^{Ad}

* See Table (1).

It could be generally concluded from these results that, addition of *Lb. rhamnosus* GR-1 did not affect significantly successful and the chemical composition of yoghurt treatments. T_4 was the most acceptable yoghurt treatments counts of *Lb. rhamnosus* GR-1 increased by increasing the rate of adding *Lb. rhamnosus* GR-1. Yoghurt treatments even after 12 days of storage contain high counts of *Lb. rhamnosus* GR-1 than the counts should be present to achieve the health benefits.

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تصنيع الزبادى الحيوى بإضافة *Lactobacillus rhamnosus* GR-1

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الملخص العربى :

تم فى هذا البحث تصنيع ٥ معاملات من اليوجورت من اللبن الجاموسى بعد تعديله إلى ٣% دهن والمعاملات هى C ، T_1 ، T_2 ، T_3 ، T_4 ، وقد أضيف لها بكتريا *Lactobacillus rhamnosus* GR-1 الحيوية بنسب صفر ، ١ ، ٢ ، ٣ ، ٤% من حجم اللبن على التوالى . وقد تم أخذ عينات من كل المعاملات عند الأيام ١، ٣ ، ٦ ، ٩ ، ١٢ يوم وذلك لإجراء التحليلات الريولوجية والكيماوية والبكتريولوجية والحسية. ولقد أوضحت النتائج المتحصل عليها بعد تحليلها إحصائياً ما يلى :

١- لم تتأثر قيم كلاً من الجامد الكلية والبروتين الكلى والدهن ، اللاكتوز والرماد عند عمر ١ يوم بإضافة بكتريا *Lactobacillus rhamnosus* GR-1 بينما انخفضت قيم اللاكتوز خلال فترة التخزين .

٢- زادت قيم الدهن والرماد والبروتين الكلى بنسبة طفيفة غير معنوية أثناء عملية التخزين .

٣- زادت الحموضة بنسب طفيفة مع زيادة نسب الإضافة بينما التقدم فى عمر التخزين أدى إلى زيادة الحموضة زيادة معنوية وهذا أيضاً يُقابله نقص فى الـ pH بنفس النسب تقريباً.

٤- انخفضت قيم انفصال الشرش فى كل المعاملات حتى عمر ٦ أيام ثم بدأت فى الزيادة مرة أخرى أثناء التخزين ، وكلما زادت نسبة الإضافة زادت نسبة انفصال الشرش بنسب بسيطة .

٥- لم تتأثر قيم الأسيتالدهيد بإضافة *Lactobacillus rhamnosus* GR-1 فى المنتج الطازج ولكن انخفض بصورة معنوية أثناء التخزين .

٦- ازدادت أعداد *Lactobacillus rhamnosus* GR-1 بزيادة نسبة إضافتها وكانت أعدادها شبه ثابتة أثناء التخزين .

٧- أدى إضافة *Lactobacillus rhamnosus* GR-1 إلى تحسين فى القوام والطعم والمظهر والحموضة والقيمة الكلية للتحكيم .

٨- أدى إضافة *Lactobacillus rhamnosus* GR-1 بنسبة ٤% إلى إنتاج يوجورت يتشابه تماماً مع الكنترول ويتميز هذا اليوجورت باحتوائه على نسبة عالية من البكتريا الحيوية *Lactobacillus rhamnosus* GR-1 طوال فترة التخزين .