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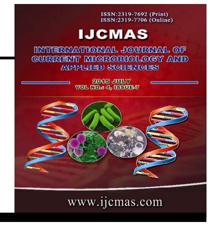

Original Research Article

Quality of Probiotic UF Domiati Cheese made by *Lactobacillus rhamnosus*

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A B S T R A C T

Keywords

UF cheese, Domiati cheese, Probiotic, *Lactobacillus rhamnosus*

Six UF Domiati cheese treatments were made to study the effect of incorporating *Lactobacillus rhamnosus* on the quality of cheese. Control cheese was made without adding starter, while the other five treatments were made by adding 0.25, 0.50, 0.75, 1.0 and 1.25% *Lactobacillus rhamnosus.* Incorporating of *Lactobacillus rhamnosus* caused a significant increase in fat, total nitrogen, salt, soluble nitrogen content, total volatile fatty acids, shilovich number, while decreased moisture content. Adding *Lactobacillus rhamnosus* up to 0.75% improved the organoleptic properties of the resultant cheese. Fat, total nitrogen, salt, ash, soluble nitrogen content, total volatile fatty acids, shilovich number and scores of organoleptic properties increased as pickling period proceeded, while moisture content and total nitrogen content decreased.

Introduction

Domiati cheese is the most popular soft white pickled cheese in Egypt (Abou-Donia *et al.*, 1986). It is consumed fresh or after 3-6 months of ripening period in pickling solution. The salt concentrations used in Domiati cheese manufacture are affected by some factors such as milk type, ripening time and season. There has been growing interest in using probiotic microorganisms as dietary adjuncts in food.

Probiotic bacteria are applied to balance disturbed intestinal microflora and important in the treatment of a wide range of human disorders including lactose intolerance, diarrhea, food allergies, intestinal infection, constipation gastroenteritis, hepatic, flatulence, colitis, gastric acidity, osteoporosis, high blood cholesterol and cancer (Salminen *et al.*, 1998; Fooks *et al.*, 1999; Lee *et al.*, 1999; Roberfroid, 2000; Pitino *et al.*, 2012).

The most commonly organisms used as probiotic belong to bifidobacteria, *Lactobacillus* and some of *Enterococcus* ssp. (Fokks *et al.*, 1999).

Foods containing probiotic is consider as "functional foods' which are described as food claimed to have positive effects on health (Saxelin *et al.*, 2003). Because of their health benefits, probiotic bacteria are increasingly incorporated into dairy products, such as fermented milks; milk beverages; cheese and baby foods. The suggested concentration for probiotic bacteria to provide health benefits is ≥ 10^6 cfu/gm of a product (Rybka and Kailasapathy, 1995).

Probiotic bacteria in cheese especially lactobacilli possess several peptidases, which can hydrolyze peptides to oligopeptides and amino acid and induce change in flavor, body and texture and, consequently, in sensory properties of the cheese (Shihata and Shah, 2000; Soufa and Saad, 2009; Santillo and Albanzia, 2008).

Ultra filtered soft cheese prepared from full concentration retentate has gained popularity in Egypt during the last two decades.

Therefore the objectives of this study were to study the possibility of making a good quality probiotic soft cheese using *Lactobacillus rhamnosus* and monitor the changes of cheese quality during pickling period.

Materials and Methods

Bacteria strains and propagation

Active *Lactobacillus rhamnosus* ATCC7460 were obtained from the Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Center (Cairo Mircen), Faculty of Agriculture, Ain Shams University. These strains were activated individually by three successive transfers in sterile 10% reconstituted non-fat dry milk.

Preparation of Retentate

Fifty liters of buffalo's milk (standardized to 3%fat) were ultrafiltrated until volume of retentate reached 12L (x=4.167 Concentration 23%, pH 6.7, Temperature 45°C, Pressure 60–65).

Cheese making

The retentate heated to 45C (72.64% Moisture, 11.5% fat and 15.5% protein), 5% salt and rennet powder 1.25gm were added and divided to six batches. Control cheese treatment (C) was made without starter and the other five treatments were made by adding 0.25% *Lactobacillus rhamnosus* (T1); 0.50% *Lactobacillus rhamnosus* (T2); 0.75% *Lactobacillus rhamnosus*; (T3), 1.0% *Lactobacillus rhamnosus* (T4) and 1.25% *Lactobacillus rhamnosus* (T5) and respectively. Domiati cheese treatments were made according to Abdel-Salam *et al.* (1981).

The resultant cheese was pickled for two months. All cheese treatments were sampled and analyzed when fresh and at 2 week, 4 week, 6 week and 8 weeks for chemical, microbiological and sensory properties. The whole experiment was duplicated.

Chemical analysis

Cheese samples were analyzed for moisture, salt, fat, ash according to AOAC (2000). Titratable acidity, pH, water soluble nitrogen (WSN), and total nitrogen (TN) were determined as described by Ling (1963). Total volatile fatty acid (TVFA) was estimated by the method of Kosikowski (1966). Shilovich ripening index was determined according to Abdel-Tawab and Hofi (1966).

Microbiological analysis

Total viable bacterial counts were enumerated on standard plate count agar (Marth, 1978). Mould and yeast, Coliform bacteria, libolytic and proteolytic bacterial counts were determined according to American Public Health Association (APHA, 1994) while MRS agar medium was used to enumerate *Lb. rhamnosus* (DeMan *et al.*, 1960).

Sensory evaluation

Cheese samples were evaluated for appearance, flavor and body & texture according to the scoring sheet of El-Shafei *et al.* (1995) by the staff members of Dairy Science Dept., Food Technology Institute, Agriculture Research Center, Giza, Egypt.

Statistical analysis

2X3 factorial design was used to analyze all the data and the student Newman Keuls' test was used to make the multiple comparisons (Steel and Torrie, 1980), using Costat program, significant differences were determined at p ≤0.05.

Results and Discussion

Moisture: Moisture content of all cheese treatments are presented in table 1. There were significant differences among cheese treatments which mean there was significant effect of incorporating *Lactobacillus rhamnosus* during cheese manufacter. On the other hand moisture content of all cheese treatments decreased significantly (P≤0.05) as pickling period proceeded (Tables 1 and 5). This decrease could be attributed to the contraction of curd as a result of developed acidity during pickling period, which helps to expel the whey from the curd. These results are in an agreement with these reported by Khalif *et al.* (2010), Badawi and Kebary (1996). The lower moisture content of treatments with high concentration of starters is due to the higher acidity development attained due to starter growth. The higher acidity in the milk reduces the coagulation time and decreases the moisture content (El-Abd *et al.*, 2003; El-Zayat and Osman, 2001).

Total Nitrogen (TN%): Total Nitrogen in UF cheese treatments with different concentration of *Lactobacillus rhamnosus* are presented in table 1. These results indicated that TN content of treatments increased as the pickling period advanced and increased by increasing the *Lactobacillus rhamnosus* level due to the decrease in the moisture content in cheese and increasing the bacterial counts. Similar trends were obtained by Fareg *et al.* (1988) and Hussein (1985).

Fat content: Fat content in UF cheese with different concentration of starter and without *Lactobacillus rhamnosus* (control) are shown in table 1. These results indicated that fat content in all treatments increased throughout the pickling period and increased by increasing the level of *Lactobacillus rhamnosus* due to the decrease in the moisture content in cheese.

Ash and salt: Ash and salt content of all UF cheese treatments are presented in table 2. These results indicated that ash and salt content in all treatments increased the pickling period advanced and increased with the high concentration of *Lactobacillus rhamnosus* due to the decrease in the moisture content in cheese.

Titratable acidity (TA %): Changes in titratable acidity of cheese made from retentate with different concentration of *Lactobacillus rhamnosus* are shown in table 2. The results showed that titratable acidity of fresh UF-cheese samples were 0.2, 0.3, 0.35, 0.45, 0.5 and 0.59% for control, T1, T2, T3, T4 and T5 cheeses respectively. The corresponding values of acidity after 8weeks of pickling were o.8, 1.3, 1.6, 1.9, 2.2 and 2.6% in the same order. All cheese treatments exhibited higher acidity values than the control cheese. There were slight differences among fresh cheese treatments but the significant difference started after

6weeks. This increase in acidity could be due to the conversion of residual lactose in cheese by *Lactobacillus rhamnosus*. Similar trends were obtained by Farag *et al.* (1988) and Hefny (1975). The significant differences among cheese treatment in acidity might be attributed to the growth rate of added *Lactobacillus rhamnosus* and ability to ferment lactose during pickling period. These results are in agreement with those obtained by El-Abd *et al.* (2003). El-Zayat and Osman (2001) and Mehanna *et al.*, (2002a), who found that the use of different starter cultures in Domiati cheese manufacture increased the acidity development in the product.

Ripening indices

Soluble nitrogen, total volatile fatty acids and Shilovich number had approximately similar trends. Ripening indices increased significantly as pickling period advanced in all cheese treatments. These results were in accordance with those reported by Ezzat (1990). Farag *et al.* (1988), Hussein (1985) and Kebary *et al.* (1991). Cheese treatment without *Lactobacillus rhamnosus* (control cheese) had the lowest values of ripening indices and was significantly ($P<0.05$) different from all cheese treatments. Ripening indices increased markedly as the amount of added *Lactobacillus rhamnosus* increased. There was a positive correlation between the amount of *Lactobacillus rhamnosus* and the values of ripening indices. These results could be attributed to the higher proteolytic and lipolytic activities which are important in developing the texture and flavor of cheese (Crow *et al.*, 1994; Haandrikman *et al.*, 1994 and Ten *et al.*, 1993).

Soluble Nitrogen (SN %): Table 3 shown the soluble nitrogen of UF cheese treatments during the pickling period. The rate of accumulation of SN increased in all cheese treatments in the pickling period proceeded. This was attributed to the rate of proteolysis throughout the storage period.

SN content in fresh cheese samples were 0.039, 0.091, 0.92, 0.109, 0.127 and 0.135% for control, T1, T2, T3, T4 and T5 cheese treatments, respectively. These results indicated that there was positive correlation between the level of adding the starter and SN content, which means soluble nitrogen content increased by increasing the level of adding *Lactobacillus rhamnosus.* This increase could be due to the activity of proteinases and peptidases released from *Lactobacillus rhamnosus* which resulted in higher proteolysis in cheese.

Total volatile fatty acids content (TVFA)

Results in table 3 included the TVFA in both of UF cheese (control) and cheese with different concentration from starter during the pickling period. Obtained results indicated that the rate of accumulation TVFA increased in all cheese treatments during the pickling period. From these data, it could be noticed that among treatments, T5 contained the highest value of TVFA while control cheese had the lowest. This increase in TVFA could be due to the lipolytic activity of *Lactobacillus rhamnosus*.

Shilovich number: Shilovich number increased significantly ($P\leq0.05$) by increasing the rate of adding *Lactobacillus rhamnosus* (Tables 3, 5). There was positive correlation between the rate of adding *Lactobacillus rhamnosus* and the values of shilovich number. This results are in agreement with those reported by Chen *et al.* (2009) and Mehanna *et al.* (2002b). These results could be attributed to the presence of proteolytic and lipolytic system of *Lb. rhamnosus* (Bergamini *et al.,* 2009). Also shilovich number increased significantly throughout the ripening period. These results are in agreement with these reported by Fayed *et al.* (2006).

Microbiological Examination

Coliforms, aerobic spore forming bacterial and yeast & mould: These bacteria did not detect in all cheese treatments either when fresh or during the pickling period. This might be due to high hygienic condition during making cheese and pickling period and the development in the acidity in cheese when fresh and during the pickling period.

***Lactobacillus rhamnosus* count:** Table 4 illustrates the changes in *Lactobacillus rhamnosus* counts of UF cheese during the pickling period. *Lactobacillus rhamnosus* count gradually increased till the first 4week of pickling period then decreased slightly at the end of this period, Similar results were reported by Abdeen (2000), who reported that during the storage of cheese, the total bacterial counts slightly increased during the first period of storage, and then gradually decreased till the end of the storage period this could be attributed to the development of the acidity in cheese. Even after pickling period for 8 weeks the counts of *Lactobacillus rhamnosus* in all cheese treatments were higher than the counts should be present in the product to achieve the health benefits of probiotics bacteria. Results indicated that *Lactobacillus rhamnosus* counts in control was less than those of all cheese treatments. This might be attributed to the different concentration of starter added in cheese treatments.

Proteolytic and lipolytic bacterial count had similar growth trends. They reached the maximum growth after 4week of pickling period then decreased gradually up to the end of pickling period. This decreases the probably due to the acidity development. Addition of *Lactobacillus rhamnosus* to cheese increased total proteolytic and lipolytic bacterial counts of the resultant cheese than the control cheese without starter.

Sensory evaluation: Results in table 5 show that general acceptability of cheese as total Scores and indicated that T3 gained the highest acceptability among all fresh cheese samples. On the other hand, the control cheese sample gained the lowest score being significantly ($p<0.05$), different from those cheese treatments with different concentration of starter. Storage has affected the total acceptability of cheese properties.

In all cases the acceptability increased during the early stage of the storage and by extending the pickling period. The improvements were slow in control cheese treatment while it was faster in the treatment with increasing the starter concentration. Stored cheese samples of T3 gained the highest score at the pickling period. This increase probably due to the increase of protein degradation and fat hydrolysis, which could be proved by increasing of all ripening indices throughout the pickling period (Farag *et al.,* 1988 and Kebary *et al.,* 1991). The maximum scores at 6wk then decreased as pickling period proceeded which might be due to the excessive proteolysis and lypolysis. It be could be concluded that for the fast UF cheeses consumption, addition of 0.75% *Lactobacillus rhamnosus* as starter culture can be recommended to accelerate cheese ripening and shorten the ripening period and it increased the amount of soluble nitrogen coefficient, TVFA, and organoleptic scores of the resultant cheese.

Table.1 Moisture, Fat and total nitrogen (TN) content of UF cheese as affected by probiotic bacteria and storage period

Cheese Treatment	**Storage Period (weeks)**					**Storage Period (weeks)**					**Storage Period (weeks)**				
	0	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**
	Moisture (%)					**Fat (%)**					**TN (%)**				
C*	62.04	61.39	60.54	60.2	59.3	9.4	9.45	9.55	9.6	9.7	1.4	1.5	1.56	1.6	1.63
T1	61.23	61.02	60.3	60	59	9.45	9.5	9.6	9.65	9.73	1.48	1.52	1.57	1.611	1.65
T2	60.66	60.64	59.94	59.8	58.7	9.5	9.55	9.63	9.7	9.8	1.49	1.54	1.58	1.65	1.69
T3	60.5	60.48	59.5	59.5	58.4	9.6	9.7	9.8	9.9	10	1.52	1.59	1.63	1.72	1.75
T4	60.25	60.21	59.48	59.44	58.3	9.75	9.8	9.9	10	10.2	1.58	1.65	1.69	1.73	1.77
T5	60.06	60	59.37	59.3	58.2	9.9	10	10.2	10.3	10.4	1.6	1.71	1.73	1.8	1.85

C*: Control cheese made without adding. *Lb. rhamnosus*
T1: Cheese made with 0.25% *Lb. rhamnosus*
T2: Cheese made with 0.50% *Lb. rhamnosus*
T3: Cheese made with 0.75% *Lb. rhamnosus*
T4: Cheese made with 1% *Lb. rhamnosus.*
T5: Cheese made with 1.25% *Lb. rhamnosus*

Table.2 Salt, titratable acidity and Ash content of UF cheese as affected by probiotic bacteria and storage period

Cheese Treatment	**Storage Period (weeks)**					**Storage Period (weeks)**					**Storage Period (weeks)**				
	0	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**
	Salt (%)					**Titratable acidity (%)**					**Ash (%)**				
C*	2.036	2.106	2.2	2.789	2.872	0.2	0.3	0.5	0.7	0.8	4.86	4.998	5.232	5.317	5.437
T1	2.106	2.136	2.638	2.895	2.954	0.3	0.4	0.7	0.9	1.3	4.911	5.165	5.251	5.379	5.599
T2	2.141	2.192	2.79	2.907	2.966	0.35	0.45	0.75	1.0	1.6	4.912	5.187	5.287	5.388	5.711
T3	2.145	2.206	2.87	2.92	2.982	0.45	0.5	0.8	1.3	1.9	4.972	5.206	5.347	5.527	5.723
T4	2.148	2.41	2.88	2.941	2.989	0.5	0.6	0.9	1.5	2.2	5.085	5.233	5.36	5.561	5.806
T5	2.156	2.63	2.89	2.954	2.989	0.59	0.7	1.0	1.7	2.6	5.161	5.32	5.37	5.7	5.834

See table (1)

Table.3 Effect of brobiotic bacteria on UF cheese ripening indices

Cheese Treatment	Storage Period (weeks)					Storage Period (weeks)					Storage Period (weeks)				
	0	2	4	6	8	0	2	4	6	8	0	2	4	6	8
	SN (%)					TVFA (ml 0.1 NaOH/100g)					Shilovich Number				
C*	o.o39	0.098	0.112	0.116	0.2	10	13	17	21	25	10	12	15	16	17
T1	0.091	0.104	0.119	0.139	0.21	10.5	15	19	26	31	11	14	19	24	25
T2	0.092	0.114	0.13	0.141	0.25	10.6	16	21	27	33	11	17	23	28	30
T3	0.109	0.117	0.138	0.144	0.26	10.8	18	24	30	37	12	18	25	30	31
T4	0.127	0.139	0.203	0.24	0.46	10.9	19	25	32	39	12	20	26	32	33
T5	0.135	0.146	0.36	0.374	0.5	11	20	27	35	42	13	20	26	33	33

Table.4 Changes in microorganisms counts of cheese during pickling period (week)

Properties	Treatments*	Storage Period (weeks)				
		Fresh	2w	4w	6w	8w
Total bacterial count (c.f.u./gm)	Control	11x10^{4}	25x10^{6}	17x10^{7}	35x10^{8}	9x10^{9}
	T1	7x10^{5}	15x10^{7}	56x10^{7}	97x10^{8}	52x10^{9}
	T2	13x10^{6}	29x10^{7}	63x10^{8}	21x10^{9}	81x10^{9}
	T3	31x10^{7}	43x10^{7}	71x10^{8}	23x10^{9}	89x10^{9}
	T4	42x10^{7}	92x10^{7}	92x10^{8}	82x10^{9}	97x10^{9}
	T5	6x10^{8}	12x10^{8}	95x10^{8}	92x10^{9}	99x10^{9}
Lactobacillus rhamnosus	Control	6x10^{1}	11x10^{2}	21x10^{2}	4x10^{3}	7x10^{3}
	T1	8x10^{8}	65x10^{9}	21x10^{10}	43x10^{8}	8x10^{6}
	T2	3x10^{9}	31x10^{9}	24x10^{10}	46x10^{8}	9x10^{7}
	T3	4x10^{9}	71x10^{9}	33x10^{10}	66x10^{8}	15x10^{7}
	T4	7x10^{10}	84x10^{10}	16x10^{11}	82x10^{9}	21x10^{7}
	T5	9x10^{10}	33x10^{11}	41x10^{11}	61x10^{10}	32x10^{8}
Pro. bacteria	Control	15x10^{1}	2x10^{2}	32x10^{3}	53x10^{2}	9x10^{2}
	T1	33x10^{1}	5x10^{2}	38x10^{3}	74x10^{2}	23x10^{2}
	T2	46x10^{1}	11x10^{2}	65x10^{3}	8x10^{3}	34x10^{2}
	T3	55x10^{1}	24x10^{2}	71x10^{4}	23x10^{3}	19x10^{2}
	T4	76x10^{1}	33x10^{2}	79x10^{4}	45x10^{3}	26x10^{2}
	T5	81x10^{1}	54x10^{2}	88x10^{4}	76x10^{3}	43x10^{2}
Lip. Bacteria	Control	44x10^{1}	16x10^{2}	34x10^{3}	46x10^{2}	16x10^{2}
	T1	53x10^{1}	42x10^{2}	89x10^{3}	9x10^{3}	45x10^{2}
	T2	14x10^{2}	46x10^{2}	97x10^{4}	2x10^{4}	9x10^{2}
	T3	37x10^{2}	78x10^{2}	12x10^{4}	86x10^{3}	14x10^{2}
	T4	46x10^{2}	92x10^{2}	34x10^{4}	89x10^{3}	11x10^{3}
	T5	57x10^{2}	12x10^{2}	78x10^{4}	9x10^{4}	19x10^{3}
Yeast & Mould	Control	N.D	N.D	N.D	16x10^{2}	88x10^{2}
	T1	N.D	N.D	N.D	45x10^{2}	93x10^{2}
	T2	N.D	N.D	N.D	63x10^{2}	42x10^{3}
	T3	N.D	N.D	N.D	87x10^{2}	76x10^{3}
	T4	N.D	N.D	N.D	89x10^{2}	79x10^{3}
	T5	N.D	N.D	N.D	17x10^{3}	24x10^{4}

Table.5 Effect of incorporating probiotic bacteria on sensory evaluation of the UF cheese

Cheese Treatment	Storage Period (weeks)					Storage Period (weeks)					Storage Period (weeks)					Storage Period (weeks)				
	0	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**	**0**	**2**	**4**	**6**	**8**
	Flavour (50)					**Body and Texture (40)**					**Appearance (10)**					**Total score (100)**				
C*	35.00	40.67	41.00	41.67	39.34	33.25	35.34	34.67	34.34	33	7.75	8.67	8.00	8.00	7.00	76.00	84.68	83.67	84.01	79.34
T1	32.00	41.33	44.00	39.35	38.67	28.75	36.0	33.67	34.34	34	6.75	8.67	8.67	834	8.00	67.50	86.00	86.34	82.03	80.67
T2	39.75	42.00	43.34	46.00	45.00	27.00	35.67	35.66	38.67	38	8.50	8.67	8.67	8.67	8.34	75.25	86.34	87.67	93.34	91.34
T3	40.00	43.67	44.00	47.34	46.00	35.00	37.67	36.00	38.00	38	8.25	8.34	8.00	9.00	8.00	83.25	89.68	88.00	94.34	92.00
T4	38.75	43.00	37.67	41.00	43.00	33.00	37.34	32.67	31.00	29	8.00	8.34	8.67	7.50	7.00	79.75	88.68	79.01	79.50	79.00
T5	34.50	42.34	37.34	40.00	41.00	31.25	36.67	31.00	30.00	28	7.50	8.34	8.00	6.00	6.00	73.25	87.35	76.34	76.00	75.00

See table (1)

Table.6 Statistical analysis of properties of UF cheese as affected by probiotic bacteria

Cheese Properties	Effect of treatments		Effect of ripening per.(week)	
	Mean Squares	Multiple Comparisons	Mean Squares	Multiple Comparisons
		C T1 T2 T3 T4 T5		0 2 4 6 8
Chemical Properties:-				
Moisture (%)	10.347*	A A AB AB BC C	40.159*	A A B B C
Fat (%)	17.920*	A A A AB AB AB	18.350*	B B AB AB A
Total nitrogen (%)	0.2081*	C B B AB AB A	0.5198*	C B B AB A
Salt content (%)	0.065*	B AB AB AB AB A	0.7457*	C BC B AB A
Acidity	0.007*	BC B B AB AB A	0.0135*	E D C B A
Ash content (%)	0.157*	B B AB AB A A	0.905*	C B B AB A
SN (%)	0.057*	B B AB AB A A	0.1369*	C B AB AB A
TVFA (ml 0.1N NaOH/ 100gm)	4.559*	C B AB AB A A	52.40*	C CB B AB A
Shilovich number	54.00*	C B AB AB A A	296.25*	B AB AB A A
Organoleptic:-				
Flavour	56.216*	C C A A B C	127.645*	D B C A B
Body and texture	25.375*	B B A A B C	81.745*	E A D B C
Appearance	1.876*	A A A AB AB B	2.698*	CB B BA A A
Total score	10.5*	C DC CB BA A AB	5.33*	CB B BA A AB

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