Impact of Some Probiotic Bacteria on Flavor and Quality of Novel Cheese Slurry

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Abstract

Five probiotic strains include ***Lactococcus lactis* IS9, *Bifidobacterium breve* ISO8**, ***Lactobacillus rhamnosus* ISO7, *Lactobacillus plantarum* E and *Lactobacillus plantarum* ATCC14917** were studied to be used as adjunct cultures in cheese-making experiments (cheese slurry). The probiotic strains were assessed for their viability, their resistance to NaCl concentrations and their ability to develop pH and acidity. All the tested strains were used in making cheese slurry. Proteolysis and lipolysis of the cheese slurry were improved in which the proteolytic and lipolytic bacterial counts of fresh cheese slurry reached its highest counts at the end of incubation for 7 days at 40ºC. All the prepared cheese slurry had good acceptable properties during the sensory evaluation. The amino and fatty acids profiles demonstrated that the tested probiotic bacteria could be useful for making cheese slurry. Cheese slurry can be an effective vehicle for delivery of some probiotic organisms with good proteolytic and lipolytic properties to wide variety of cheese types.

Keywords: Probiotic; cheese slurry; proteolysis, fatty acids.

Introduction

Milk products containing probiotic bacteria are becoming popular due to their health promoting properties. According to Food and Agriculture Organization (FAO) of United Nations and World Health Organization (WHO) requirements, fermented milks should contain at least 10^6 cfu probiotics. As indicated by **Stefanovic *et al,* (2018),** cheese flavor is recognized as a balanced blend of fatty acids, organic acids, amino acids, carbonyl compounds, esters, alcohols, and sulfur compounds.

The development of cheese flavor is strongly dependent upon the starter microorganism utilized (**Fitzsimons *et al.*, 1999; Gobbetti *et al.*, 2015 and Stefanovic *et al.*, 2018)** and appears to be under the influence of biological control of interrelated pathways. Slurry process is supposed to be one of the important and suitable procedures for the acceleration of cheese ripening where it considered as a good source of enzyme, small nitrogenous components, and free fatty acids **(Abdel-Baky *et al.*, 1982).** The cheese-slurry system is applicable to a wide variety of cheese types.

Harper and Krisoffersen (1970) evaluated a cheese slurry system which permits cheese flavor development in a few days' time, in respect to its efficacy in studying cheese ripening and as a means of studying biochemical changes during the ripening process. The cheese slurry systems replicated the ripening processes in natural cheeses. Flavor development in cheese appears to be under specific biochemical control in which glutathione plays a multiple role in respect to the cheese slurry system including the disassociation of peptides, making them more available for proteolytic attack, protection of enzyme groups, and feedback control in respect to diacetyl and acetaldehyde formation, suggesting a number of operative feedback systems. Lipid, protein and carbohydrate fermentation are interrelated, and the direction of the fermentation can be influenced by the compositional balance of the milk constituents **Hofi *et al.* (1991)**. Added pre-ripened full flavor-slurry either to cheese milk before adding the starter, at a level of 1%, or to the curd particles before hooping at a level of 1% or 2% of cheese milk weight. Cheese slurry improved the quality and the flavor development, protein degradation and fat hydrolysis of resultant cheese. Cheese with 2% slurry had the same acceptable full aged properties after 45 days against 120 days for control cheese.

This study aimed to use some probiotic bacterial strains and investigate their impact to develop cheese slurry system to be applicable to wide variety of cheese types.

Materials and Methods

Materials

Raw Milk: Fresh mixed Cow's and Buffaloe's milk (1:1) were obtained from the herds of Moshtohor, Fac., of Agric., Benha Univ.

Starter cultures: Freeze dried starter cultures include: ***Lactococcus lactis* IS9**, ***Bifidobacterium breve* ISO8**, ***Lactobacillus rhamnosus* ISO7**, and ***Lactobacillus plantarum* E** were obtained friendly from Institute of Microbiology, Federal Research Center for Nutrition and Food, Kiel, Germany through personal communication with Dr. El-Sayed Ismail Dairy Sci., Dept., Fac., of Agric., Moshtohor Benha Univ., Egypt. ***Lactobacillus plantarum* ATCC14917 (16)** was obtained from National Research center, Giza, Egypt. Yoghurt starter culture consisting of ***Streptococcus thermophilus*** and ***Lactobacillus delbrueckii*** subsp**. *bulgaricus*** (1:1) was obtained from Chr. Hansen's Laboratories, Copenhagen, Denmark.

Rennet: A liquid rennet was used for making the cheese which was prepared in the Dairy Sci., Dept., Fac., of Agric., Moshtohor and used for soft white cheese making.
Salt: A commercial pure fine grade table salt was obtained from El-Naser Company, Egypt.
Potassium sorbate: Potassium sorbate was obtained from El-Gomhoria Co., Cairo Egypt.

Methods

Growth measurement of the bacterial cultures: 1% of overnight bacterial culture was inoculated in MRS broth for ***Lactobacillus*** sp., Cystain-MRS for ***Bifidobacterium breve*** and M17 for ***Streptococcus.*** All bacterial cultures were incubated at 37ºC in water bath and the growth was measured by following the optical density (O.D.) at 620 nm using a photometer NANOCOLOR 500D (Macherey-Nagel, Dürem, Germany, (**Ismail 2007**).

Preparation of Slurries and fresh curd:

Cheese curd was prepared from cow's milk by **Hofi *et al.* (1973).** Slurries were prepared according to **Abd-El-Hamid *et al.* (1991).** The first part was used as a (Control) inoculated with 1% of yoghurt starter culture (***Streptococcus thermophilus*** and ***Lactobacillus delbrueckii*** subsp**. *bulgaricus***). The other 5 parts were as follows: T1: inoculated with 1% of yoghurt starter + ***Lactococcus lactis*** IS9 (1:1). T2: inoculated with 1% of yoghurt starter + ***Bifidobacterium breve*** ISO8 (1:1). T3: inoculated with 1% of yoghurt starter + ***Lactobacillus rhamnosus*** **ISO7** (1:1). T4: inoculated with 1% of yoghurt starter **+ *Lactobacillus plantarum*** E (1:1). T5: inoculated with 1% of yoghurt starter + ***Lactobacillus plantarum*** ATCC14917 (1:1). All the treatments were incubated at 40˚C for 7 days. All cheese-slurry samples were analyzed when fresh and after 7 days for organoleptic properties, chemical analysis, total, proteolytic and lipolytic bacterial counts, free fatty acids, and free amino acids.

Chemical analysis:

Total Solids, Ash, Titratable Acidity, pH values, and **Fat** were determined according to the methodology mentioned in **IDF (1987), AOAC (2012), IDF (1996)** and BSI **(1989),** respectively**.**
Carbohydrate Content (CHO) of cheese-slurry samples were calculated by differences as follows: CHO= TS - (Protein + Fat + Ash)
Total nitrogen (T.N) and soluble nitrogen (S.N) were determined according to **AOAC (2012).**
Analysis of Fatty Acids Composition by GC: Fatty acids esters of cheese-slurry were prepared according to **ISO 12966-2 (2011),** then fatty acids fractions were analyzed using gas chromatograph (Agilent GC 6890A).

Determination of Total Amino Acids (TAA): Determination of free amino acids of cheese-slurry as described by **AOAC (2016).**

Microbiological examination:
Total Bacterial Count: The total bacterial counts of cheese-slurry were determined according to the American Public Health Association **(APHA, 2004).**
Proteolytic and Lipolytic Bacterial Counts: of cheese-slurry were counted according to **IDF (1991).**

Sensory Evaluation: The organoleptic evaluations were done by 10 experienced food scientists of Dairy Sci., Dept., Fac., of Agric., Moshtohor, Benha Univ. Samples of cheese slurry were sensory evaluated according to the scheme described by **IDF (1997).**

Statistical Analysis: Statistical analysis of the obtained data was performed according to the user's guide given by **SAS** (1990).

Results and Discussion

Effect of NaCl concentration on the growth of some probiotic strains

Figs.1(a-d) Illustrates the growth rate of ***Lactococcus lactis*** IS9, ***Bifidobacterium breve*** ISO8, ***Lactobacillus rhamnosus*** ISO7, ***Lactobacillus plantarum*** E and ***Lactobacillus plantarum*** ATCC14917(16) in MRS broth containing different concentrations of NaCl (0, 2, 4, and 6 %). The obtained results in (**Figure 1a**) revealed that the growth rate for all experimental strains were increased with different rates in the absence of salt (0%NaCl), all over the incubation time up to 8 hours. The growth rate was then decreased with more different rates by increasing the salt concentration and that decrease was proportional with the amount of sodium chloride. In general, the present results revealed that the control had always the higher growth rate followed by 2 and 4% NaCl for all tested strains. Whereas the 6% NaCl recorded the lowest growth rate of all tested strains which suggests inhibition impact of different NaCl concentrations. Such decrease could be attributed to the increase of osmolality of medium because of salt content. These results agreed with that obtained by **EL-Wahsh (2013).** ***Bifidobacterium breve*** ISO8 was the more tolerant recorded the highest growth rate all over the different treatments whereas, ***Lactococcus lactis*** was the lowest salt tolerant, recorded the lowest growth rate (O.D). The wide rate differences for growth rate of tested strains were recorded at 2% followed by 6% NaCl (**Figure 1b and c**) which confirmed by **EL-Alfy *et al* (2018)** they studied the effect of different sodium chloride concentrations on the viability of nine strains of probiotic bacteria, and they found that the counts of all tested strains were decreased with increasing salt concentration up to 4%. These results will be useful during using these probiotic strains in cheese making.

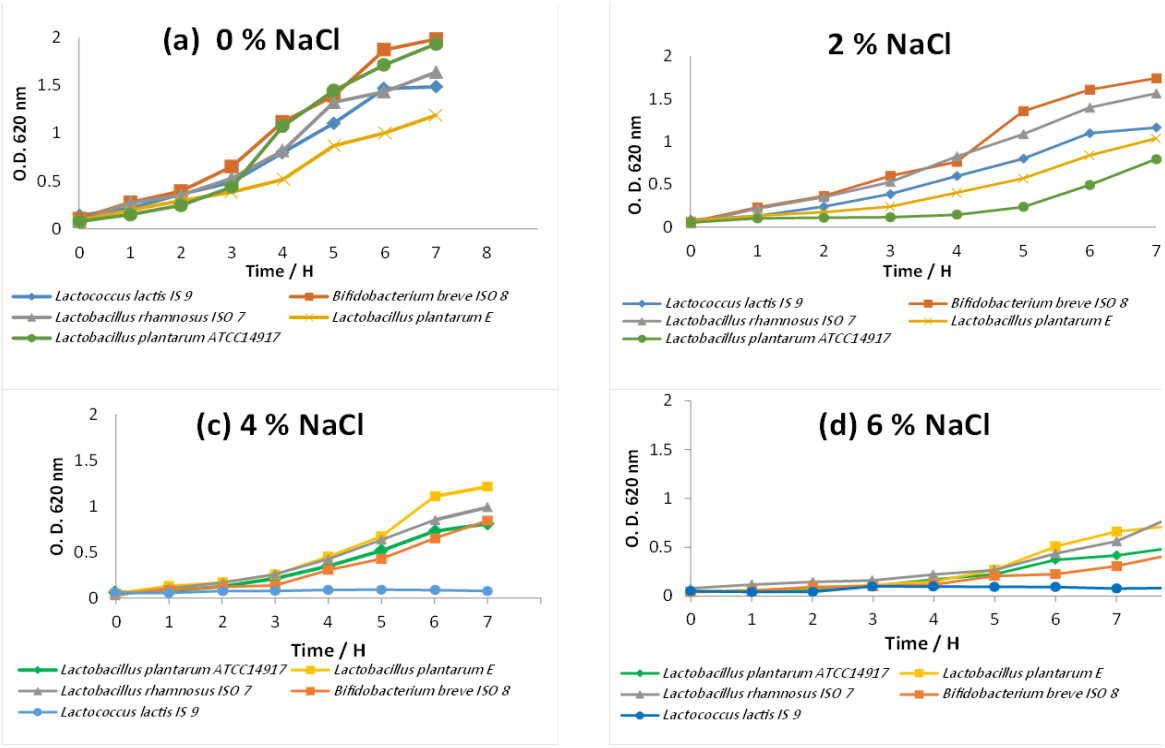


Figure 1. (a-d): Effect of NaCl concentrations on the growth of some probiotic bacteria

Acidity (%) and pH development:

Development of acidity and the change of pH produced by the tested probiotic strains of the slurry during incubation are presented in **Figure 2**. Acidity development nearly still occurred within the first 2 hours followed by slightly increase with the same rate for all the tested probiotic strains within the next 2 hours (**Figure 2a**). After the first four hours, acidity developed with wide different rates for the tested strains, and ***Bifidobacterium breve*** ISO8 recorded the highest acidity development among the last incubation time (8-10 hr), ***Lactococcus lactis*** also recorded the highest acidity within the middle incubation time (4-10 hr). The lowest acidity development was recorded for ***Bifidobacterium breve*** ISO8 within the first incubation time (4-8 hr) (**Figure 2a**). On the other hand, the pH values recorded by all tested strains (**Figure 2b**) took an opposite trend to that of acidity as mentioned by **EL-Alfy *et al.* (2018).** Increasing of acidity followed by decreasing of pH attributed to formation of acids during incubation especially lactic acid as a result of microorganisms' metabolism **(Abd EL-Gaber 2019).**

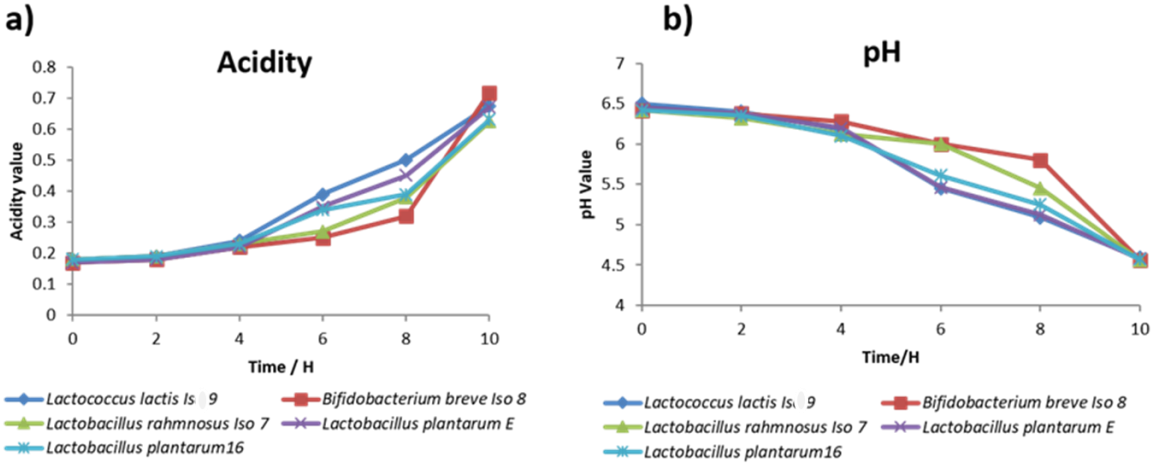


Figure 2. (a and b): Development of acidity and the change in pH produced by some probiotic bacteria

Chemical composition of cheese slurry made with some probiotic strains:

The gross chemical composition of cheese slurry during incubation for 7 days at 40ºC displayed in **Table (1)**.

The total solid contents of fresh prepared cheese slurry were slightly higher than that of the control and recorded 21.26, 21.48, 21.33, 21.13, 21.16 and 21.20% for treatments 1-6, respectively. The total solid contents of incubated slurry for 7 days at 40ºC were slightly increased compared with same treatments at the beginning of incubation period and recorded 22.15, 21.93, 22.49, 22.43, 22.62 and 22.64% for the same previous order, such increase due to the loss of some moisture by evaporation during incubation and this are confirmed by **EL-Alfy *et al.* (2011).**

The fat content for fresh prepared cheese slurry of different treatments with different probiotic strains were 10% for the control and all other samples, whereas the fat content of cheese slurry at the end of incubation (7 days at 40ºC) was slightly increased. This could be attributed to the increase of total solids ***via*** loss of some moisture **(Abd EL- Gaber 2019).**

The protein content of fresh and incubated cheese slurry for 7 days at 40˚C of different tested strains which were increased after incubation for 7 days with a slight difference among the treatments, such increase suggested to be due to the increase in total solids **(EL-Alfy *et al*., 2018).** The variance of protein content among treatments may be attributed to the proteolysis take place during cheese slurry incubation.

Table 1. Gross chemical composition and pH values & Acidity of cheese slurry during incubation at 40ºC up to 7 days

Storage period (days)	Sample NO	T.S%	Fat%	Protein%	SN%	Ash%	CHO%	Acidity%	pH
Start	Control	21.26	10.00	8.50	0.26	1.51	1.47	0.79	6.20
	T1	21.48	10.00	8.40	0.28	1.52	1.43	0.81	5.95
	T2	21.33	10.00	8.40	0.25	1.53	1.42	0.78	5.84
	T3	21.13	10.00	8.65	0.25	1.53	1.37	0.82	5.66
	T4	21.16	10.00	8.50	0.26	1.55	1.35	0.84	5.44
	T5	21.20	10.00	8.35	026	1.56	1.47	0.85	5.47
End	Control	22.15	10.50	8.15	0.27	1.45	1.63	1.16	4.22
	T1	21.93	10.50	8.35	027	1.45	1.80	1.43	4.21
	T2	22.49	10.50	8.25	0.27	1.49	2.04	1.14	4.14
	T3	22.43	11.00	8.20	0.27	1.53	1.46	1.67	3.47
	T4	22.62	11.00	8..05	0.28	1.52	1.57	1.61	3.45
	T5	22.64	11.00	8.00	0.28	1.56	1.43	1.44	3.68

Control; Yoghurt starter (T1) Control+ ***Lc. lactis*** IS9; (T2) Control + ***B. breve*** ISO8; (T3) Control + ***L. rhamnosus*** ISO7; (T4) Control + ***L. plantarum*** E; (T5) Control + *L.* ***plantarum*** ATCC14917.

The carbohydrate content of cheese slurry slightly decreased during the incubation for 7 days at 40ºC, and this may be due to the CHO hydrolysis and formation of acid mainly lactic acid. The obtained results are in accordance with that obtained by **(Abdel-Baky *et al*.., 1982 and EL-Shafei (2015).**

Microbiological Properties of the prepared cheese slurry made with some probiotic strains:

Total bacterial, Proteolytic and lipolytic bacterial counts in fresh and incubated cheese slurry at 40ºC for 7 days are given in **Table (2)**. The total bacterial count of the tested strains recorded 7.2, 6.67, 6.85, 7.0, 6.93 and 7.0 in the first day of incubation of the cheese slurry. The obtained results revealed that there are an increase of total bacterial counts during the incubation time up to 7 days at 40ºC to be 7.65, 7.58, 7.88, 8.3, 8.11 and 8.18 for C, T1 , T2, T3, T4 and T5 log cfu g^{-1}, in the same order. The proteolytic and lipolytic bacterial counts of cheese slurry also increased and reached its highest counts at the end of incubation after 7 days at 40ºC, and the experimental cheese slurry treatments recorded the highest counts than the control.

In general, the counts of all bacterial groups (Total bacterial, proteolytic and lipolytic bacterial counts) were increased throughout the incubation period at different rates and there were difference in counts among the control and other treatments of cheese slurry. Moreover, the highest counts were recorded for all the tested microorganisms, the total bacterial counts and lipolytic and proteolytic bacterial counts, by the end of incubation time. The obtained results agreed with that obtained by **EL-Sayed and Abbas (1992), and EL- Shafei (2015).**

Table 2. Total bacterial counts*, proteolytic and lipolytic counts (log cfu g^{-1}) of cheese slurry made with different probiotic strains during incubation for 7 days at 40°C

Treatments	Total bacterial counts*		Proteolytic bacterial count (End of incubation)	Lipolytic bacterial count (End of incubation)
	1st day of incubation	End of incubation (7days)		
Control	7.20	7.65	7.30	7.00
T1	6.67	7.58	7.20	7.30
T2	6.85	7.88	7.56	7.48
T3	7.00	8.30	7.20	7.72
T4	6.93	8.11	8.60	8.52
T5	7.00	8.18	8.56	8.41

*Counts are Three replicate, Control: yoghurt starter (***L. delbruckii*** subsp ***bulgaricus*** and ***Str. thermophiles***), T1: yoghurt starter + ***Lactococcus lactis*** IS9, T2: yoghurt starter + ***B. breve*** ISO8, T3: yoghurt starter +***L. rhamnosus*** ISO7, T4: yoghurt starter + ***L. Plantarum*** E, and T5: yoghurt starter + ***L. plantarum***. ATCC14917.

Sensory evaluation:

The cheese slurry samples were evaluated for different organoleptic properties and the panelist score are presented in **Table (3)**. According to the obtained results, the control recorded the lowest score than the other slurry treatments. The fresh samples of slurry recoded lower score than that incubated for 7 days at 40ºC, and it was found that T5 ***L. plantarum*** ATCC14917 recorded the highest score either when fresh or after incubation time. Much lower score values were observed for treatment 2 (***Lactococcus lactis*** IS9. The incubation for 7 days at 40ºC recorded the maximum score values for the control and all other treatments. On the other hand, increasing of incubation time will produce undesirable flavour with excessive acidity and it seemed to be unacceptable. Also, it may be related with the appearance of bitterness or rancidity (over ripening) as recorded by **Mehanna *et al* .(2009)** and **EL-shafei (2015).**

Fatty acids profile of cheese slurries made with different probiotic strains:

Table (4) and Fig (3) shows the fatty acids profile of cheese slurries made with some probiotic lactic acid bacteria. The results of fatty acids revealed that, the saturated fatty acids (SFAs) in control slurry recorded the lowest proportion (62.55%), while slurry made with ***Lactococcus lactis*** recorded the highest proportion (64.78%). Moreover, the proportion of short and medium chain fatty acids were increased, particularly, butyric acid (C4:0) which increased from 0.45% in control slurry to be 3.08, 1.27, 1.16 and 1.43% in slurries made with ***Lac. Lactis*** IS9, ***B. breve*** ISO8**, *L. rhamnosus*** ISO7, ***L. plantarum*** E and ***L. plantarum*** ATCC14917, respectively.

The benefit of the produced butyrate that it plays essential roles in cell growth control differentiation and prevention of tumor genesis in colon cells **(Dhankhar *et al*., 2016)**, butyric acid also contributes to the maintenance of the gut barrier functions and has anti-inflammatory and immunomodulatory properties **(Riviere *et al*., 2016).** On the other hand, short chain fatty acids have high rate of digestibility and play an important role in reduction of triglycerol levels as well as cholesterol level in blood and liver (**Hara *et al*., 1999)** and **(Fushimi *et al* .,2006).**

From technological and organoleptic aspects, short-chain fatty acids are considered to be the most components responsible for flavor development in slurries and cheese **(Kwak *et al*., 2002)** and that was noticeable from organoleptic testing. The mono-unsaturated and poly-unsaturated fatty acids nearly had no change or kept around their level in the control slurry which have health benefits for human body and play an important roles in preventing and/ or treatment of different diseases ***i.e.*** hypertension, coronary heart diseases, inflammatory, cancer, thrombotic diseases and autoimmune disorders and as well as type II diabetes, ulcerative colitis and crohn's disease. **Tokusoglu and Zcan (2005)**; **Simopoulos, (2009) and Ortega *et al*. (2018).**

Table 3. Sensory evaluation score of cheese slurry made with different probiotic strains during incubation period up to 7 days at 40ºC

Treatment	Storage period (day)	Flavour (60)	Body Texture (40)	Total	Average score
Control	1	54	24	78	77
		53	23	76	
		52	25	77	
	7	53	24	77	77
		52	26	78	
		54	23	77	
T1	1	52	25	77	78
		53	25	78	
		55	23	78	
	7	55	24	79	78
		54	23	78	
		51	25	76	
T2	1	55	35	90	90
		56	34	90	
		54	36	90	
	7	56	35	91	92
		55	36	91	
		56	37	93	
T3	1	57	35	92	93
		56	37	93	
		58	36	94	
	7	56	36	92	93
		57	36	93	
		56	37	93	
T4	1	55	38	93	94
		56	38	94	
		58	37	95	
	7	57	36	93	94
		57	37	94	
		56	37	94	
T5	1	60	37	97	97
		59	38	97	
		58	38	96	
	7	58	38	96	96
		59	37	96	
		57	38	95	

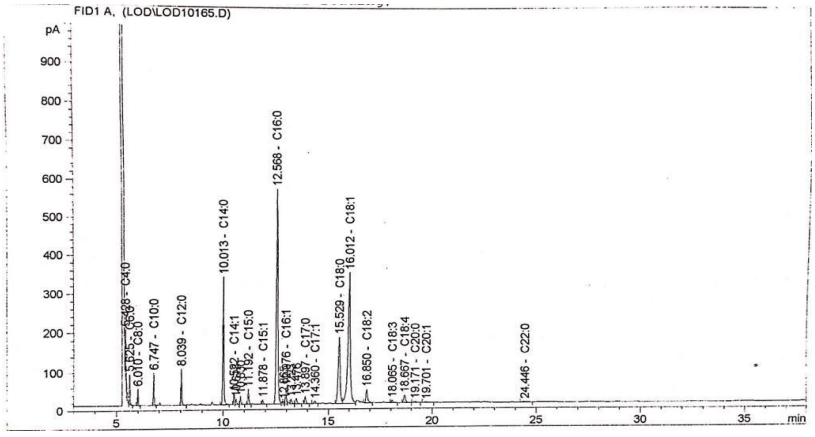
Control; Yoghurt starter (T1) Control +***Lc. lactis*** Is 9; (T2) Control + ***B. breve*** Iso8; (T3) Control + ***L. rhamnosus*** Iso 7; (T4) Control+ ***L. plantarum*** E; (T5) Control + ***L. plantarum*** ATCC14917.

Table 4. Fatty acids composition of cheese slurries made with different probiotic strains

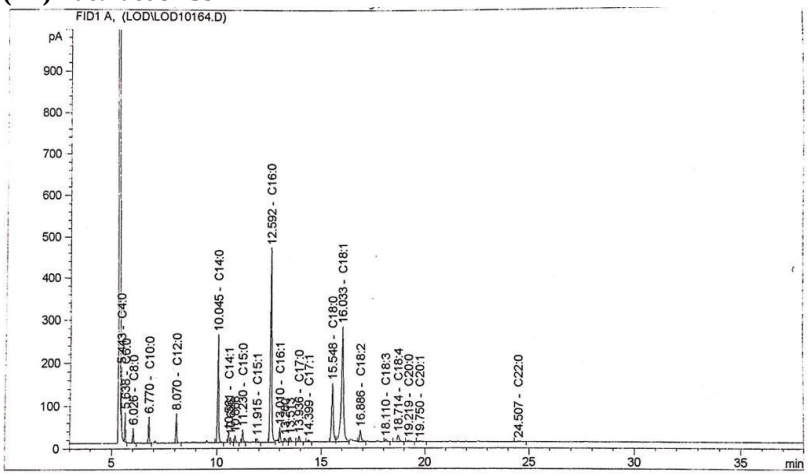
Fatty acids		Fatty acids of slurries (%)					
Common name	**Formula**	**Control**	**T1**	**T2**	**T3**	**T4**	**T5**
SFAs							
Butyric	**C4:0**	0.45	3.08	1.27	1.81	1.16	1.43
Caproic	**C6:0**	1.81	2.15	1.59	1.75	1.54	1.68
Caprylic	**C8:0**	1.02	1.17	0.92	0.99	0.91	0.95
Capric	**C10:0**	2.07	2.29	1.93	2.05	1.92	1.97
Lauric	**C12:0**	2.61	2.77	2.45	2.57	2.49	2.53
Myristic	**C14:0**	11.33	11.65	10.84	11.23	11.12	11.22
Pentadecanoic	**C15:0**	1.43	1.39	1.37	1.41	1.39	1.41
Palmitic	**C16:0**	28.86	28.17	28.76	28.61	28.72	28.72
Margaric	**C17:0**	0.84	0.78	0.9	0.85	0.9	0.84
Stearic	**C18:0**	11.68	10.9	12.36	11.84	12.05	11.93
Arachidic	**C20:0**	0.28	0.26	0.34	0.31	0.34	0.31
Behenic	**C22:0**	0.17	0.17	0.19	0.18	0.19	0.18
TSFAs		**62.55**	**64.78**	**62.92**	**63.6**	**62.73**	**63.17**
MUFAs							
Myristoleic	**C14:1**	0.91	0.89	0.51	0.92	0.86	0.95
Pentadecenoic	**C15:1**	0.45	0.43	0.44	0.44	0.45	0.45
Palmitoleic	**C16:1**	1.55	1.58	1.59	1.52	1.66	1.51
Heptadecenoic	**C17:1**	0.29	0.27	0.36	0.30	0.35	0.30
Eicosenoic	**C20:1**	0.28	0.26	2.34	0.33	0.33	0.32
Oleic	**C18:1**	27.24	25.63	27.02	26.56	27.11	26.97
TMUFAs		**30.72**	**29.06**	**32.26**	**30.07**	**30.76**	**30.50**
PUFAs							
Linoleic	**C18:2**	2.22	2.06	2.08	2.08	2.19	2.08
α-Linolenic	**C18:3n3**	0.45	0.41	0.45	0.44	0.47	0.44
Stearidonic acid	**C18:4**	1.33	1.24	1.35	1.33	1.37	1.35
TPUFAs		**31.24**	**29.34**	**30.9**	**30.41**	**31.14**	**30.84**
TUFAs		**34.72**	**32.77**	**36.14**	**33.92**	**34.79**	**34.37**
Total unknown		**2.730**	**2.430**	**2.940**	**2.470**	**2.480**	**2.460**

Control: Yoghurt starter (T1): Control +***Lc. lactis*** Is 9; (T2): Control + ***B. breve*** Iso8; (T3)*:* Control + ***L. rhamnosus*** Iso 7; (T4): Contol+ ***L. plantarum*** E; (T5): Control + ***L. plantarum*** ATCC14917(16). (SFAs) Saturated fatty acids; (MUFAs) Mono unsaturated fatty acids; (PUFAs) Poly unsaturated fatty acids; (UFAs) Unsaturated fatty acids.

Control



(T1) *Lac. lactis* IS9



(T2) *B. breve* ISO8

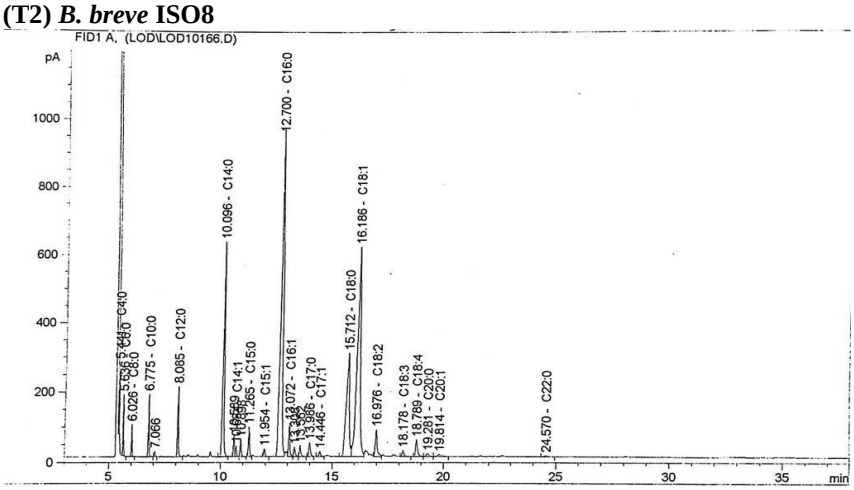
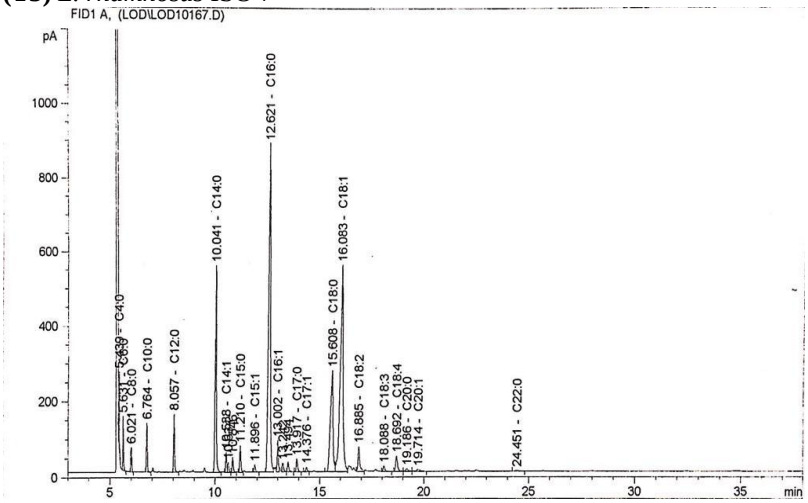
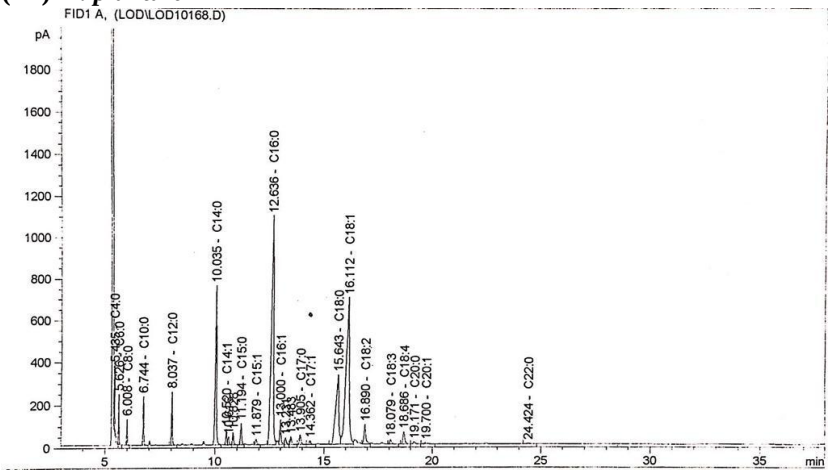


Fig (3) Chromatogram of fatty acids composition from cheese slurries made with different probiotic strains Control: Yoghurt starter (T1) Control + *Lc. lactis* IS9; (T2) Control + *B. breve* ISO8.

(T3) *L. rhamnosus* ISO 7



(T4) ***L. plantarum*** **E**



(T5) ***L. plantarum*** ATCC14917 (**16**)

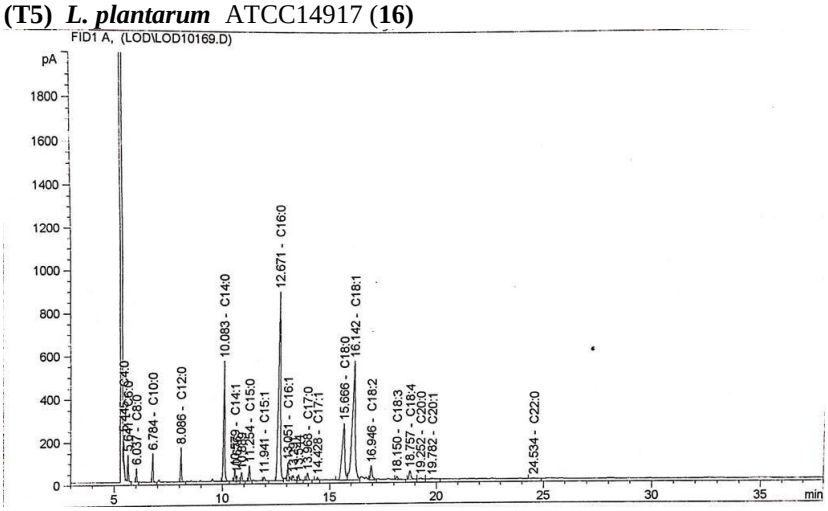


Fig. (3) Continued Chromatogram of fatty acids composition from cheese slurries made with different probiotic isolates. (T3)*:* Control + ***L. rhamnosus*** ISO7; (T4): Control+ ***L. plantarum*** E; (T5): Control + ***L. plantarum*** ATCC14917.

Individual Free Amino Acid Composition

The content of free amino acids in test samples of cheese slurry on 7th day of ripening is illustrated by **Table (5)**. The results showed that essential free amino acids in the samples of cheese slurry were, Histadine, Lysine, Leucine, Isoleucine, Methionine, Valine, Phenylalanine and Therionine, respectively. With the most pronounced differences noted for Lysine, Leucine Valine acids.

Total non- essential amino acid contents of the cheese slurry are presented in **Table (5)** had significant effects on total free amino acid contents of the cheeses. Total free amino acid contents of the cheese slurry differ due to the differences of probiotic strains used in making the cheese Faster increases were also reported in total free amino acid levels throughout the ripening process overall characteristic flavor of different strains varieties and describe how far the ripening has proceeded. As compared to the control essential amino acids significantly higher in all the treatments contains probiotics amino acid concentrations on the 7th day of ripening. On the other hand, non- essential amino acids ***i.e*** (Aspartic, Serine, Glutamic, Glycine, Alanine, Tyrosine, Argnine, Proline and Cysteine). Also, there was an increase in Glutamic and Proline. **Shenana and Patel (2020).**

Table 5. Amino acids profile of cheese slurry made with different probiotic strains

Amino acid	**Amino acids profile of cheese slurry (%)**					
Essential	**Control**	**T1**	**T2**	**T3**	**T4**	**T5**
Histadine (HIS)	0.22	0.26	0.29	0.22	0.22	0.18
Lysine (LYS)	**0.58**	**0.70**	**0.76**	**0.58**	**0.57**	**0.49**
Leucine (LEU)	**0.67**	**0.83**	**0.91**	**0.65**	**0.64**	**0.55**
Isoleucine (ILE)	0.38	0.48	0.49	0.33	0.33	0.29
Methionine (met)	0.26	0.24	0.31	0.23	0.22	0.18
Valine (VAL)	**0.50**	**0.58**	**0.53**	**0.40**	**0.40**	**0.34**
Phenylalanine (PHE)	0.39	0.46	0.49	0.37	0.37	0.30
Therionine (THR)	0.32	0.36	0.41	0.30	0.28	0.26
Non-essential						
Aspartic (ASP)	0.52	0.61	0.74	0.49	0.45	0.41
Serine (SER)	0.39	0.46	0.47	0.37	0.33	0.32
Glutamic (GLU)	**1.46**	**1.73**	**2.23**	**1.43**	**1.40**	**1.25**
Glycine (GLY)	0.16	0.17	0.17	0.13	0.12	0.11
Alanine (ALA)	0.31	0.38	0.31	0.22	0.21	0.19
Tyrosine (TYR)	0.16	0.25	0.36	0.11	0.15	0.04
Argnine (ARG)	0.25	0.29	0.28	0.25	0.24	0.21
Proline (PRO)	**0.89**	**1.07**	**1.20**	**0.85**	**0.82**	**0.74**
Cysteine (cys)	0.05	0.09	0.30	0.09	0.17	0.06

Control; Yoghurt starter (T1) Control + ***Lc. lactis*** Iso 9; (T2) Control + *B. breve* Iso8; (T3) Control + *L. **rhamnosus*** Iso 7; (T4) Control + ***L. plantarum*** E; (T5) Control + ***L. plantarum*** ATCC14917.

Conclusion

The use of probiotic bacteria *e.g. B. breve, L. rhamnosus, L. plantarum* strongly develops cheese slurry system with good proteolytic and lipolytic properties**.** Cheese slurry can be an effective vehicle to deliver the probiotic microorganisms to cheese. From technological and organoleptic aspects, cheese slurry containing *L. rhamnosus* Iso7 and *L. plantarum* ATCC 14917 are the most suitable candidates to be applicable to wide variety of cheeses.

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تأثير بعض بكتريا الداعمة للحيوية علي الطعم والنكهه و جودة خثرة الجبن الطازج

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ويهدف هذا البحث الى دراسة تحمل خمسة من السلالات الداعمة الحيوية لظروف النمو والتصنيع (تحمل ملح كلوريد الصوديوم – الحموضة – الـpH) و دراسة تأثير استخدامها كبادئات مساعدة في صناعة خثرة الجبن والسلالات المستخدمة هي:
Lactococcus lactis IS9, *Bifidobacterium breve* ISO8, *Lactobacillus rhamnosus* ISO7, *Lactobacillus plantarum* E and *Lactobacillus plantarum* ATCC 14917.

هذا وقد تم دراسة تأثر السلالات بالتركيزات المختلفه لكلوريد الصوديوم وخلصت النتائج الي أن كل السلالات المستخدمة لها القدرة علي تحمل تركيز كلوريد الصوديوم حتي 4% دون التأثير علي معدل النمو وايضا تم دراسة قدرة السلالات على معدل تطور الحموضة و الـ pH و بعد ذلك تم استخدام السلالات في صناعة الـــ cheese slurry من اللبن الخليط (بقري + جاموسي) 1 : 1 والتحضين علي 40°م لمدة 7 أيام ثم اجراء التحليل الكيماوي – الميكروبيولوجي – الحسي.

وسجلت النتائج زيادة طفيفة وبمعدلات مختلفة أثناء فترة التحضين لكل من الجوامد الكلية، الدهن ، النتروجين الكلى، لكل المعاملات أيضا اظهر التحليل الكيميائى للـــــ cheese slurry الذى تم تحضيرة زيادة مطردة و خصوصا فى الحموضة ، النتروجين الذائب و الأحماض الدهنية الكلية الطيارة والاحماض الأمينيه الحرة وهذا يعكس مدى تحلل البروتين والدهن خلال التحضين على 40°م لمدة 7 أيام. العد الميكروبيولوجى لمجاميع البكتريا المختلفة (العد الكلى للبكتريا ، البكتريا المحللة للبروتين، البكتريا المحللة للدهن) زاد بمعدلات مختلفة أثناء التحضين حتى مرور 7 ايام على 40°م و سجل العد الكلى للبكتريا أعلى قيمة. و نستنتج من هذه الدراسة انه يمكن استخدام الـ cheese slurry المحتوي على تلك السلالات في صناعة الجبن حيث يعتبر وسيلة فعالة جدا لمرور البكتريا الداعمة للحيوية و ايضا نواتج تحلل الدهن و البروتين الناتجة عنها الى الجبن.