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USE OF SOME LACTIC ACID BACTERIAL STRAINS IN ENHANCING RAS CHEESE RIPENING

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ABSTRACT

This work was done to use some mixtures of lactic acid bacterial strains to enhance the flavour development and accelerate Ras cheese ripening. *Streptococcus thermophiles, Lactococcus lactis* ssp. *lactis, Lactococcus lactis* ssp. *cremoris, Lactobacillus delbrueckii* ssp. *bulgaricus Lactobacillus helveticus and Lactobacillus paracasei* were added in different mixtures to the cheese milk during production. Data showed that the mixture of the 6 strains gave the best sensory evaluation and very good ripening indices. Therefore, the third treatment showed the highest organoleptic properties scores after two months.

Key words: Enhancement, Ras cheese, lactic acid bacteria, nonstarter lactic acid bacteria.

INTRODUCTION

One method of flavour manipulation is careful selection of the microorganisms used in cheese. Typically lactic acid bacteria are selected as starters and adjuncts that contribute much to flavour profiles and the rate of flavour development (El-Soda *et al.*, 2000).

Starter cultures such as *Lactococcus lactis* are the primary drivers of acid during cheese manufacture and cause the drop in milk pH from about 6.7 to an acidic final cheese pH of about 5.2. Increased acid functions not only to expel whey and promote or inhibit bacterial growth, but also contributes to characteristic flavours by influencing the environment for bacterial and enzymatic processes and their subsequent activity (Broome, 2007; Johnson *et al.*, 2009). of primary importance for flavour development is the autolytic nature of the starter strain. Hickey *et al.* (2007) found highly autolytic starter cultures positively influenced flavour by increasing free amino acids whereas poorly autolytic strains promoted off-flavours. This highlights the need for careful strain selection. The starter is essential for overall Ras cheese flavour. Cheese made without a starter culture in a controlled microbial setting fails to develop flavours in Ras cheese. Another factor in the cheese microorganism environment is the non starter lactic acid bacteria (NSLAB) which may be incorporated through post-pasteurization contamination.

Adjunct culture use is one method for controlling NSLAB and improving flavour (Broadbent *et al.,* 2003; Johnson and Lucey, 2006). Adjuncts are microorganisms intentionally added to cheese milk that positively impact cheese sensory quality. Adjuncts often were NSLAB isolated from mature cheese and identified as contributing to cheese quality. Now propagated for flavour enhancement, adjunct use is common practice in cheese making today (Johnson and Lucey, 2006). Cultures used as adjuncts should multiply sufficiently to produce the desired benefit to cheese quality, causeing no defects such as off-flavours or gas formation (Di Cagno *et al.,* 2003). Contribution of adjunct cultures to flavour is generally thought to be due

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to increased proteolysis and the subsequent increase in small peptides and free amino acids.

According to O'Sullivan (2007), lactic acid bacteria play the biggest part in cheese manufacture. It is believed that only five genera of LAB actually contribute to cheese flavour: *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Enterococcus*. Thus, this work was done to use some mixture of lactic acid bacterial strain as adjunct culture for Ras cheese and study their impact on cheese ripening.

MATERIALS AND METHODS

Materials

Lactococcus lactis subsp. *lactis*; *Lactococcus lactis* subsp. *Cremoris*; *Streptococcus thermophilus*; *Lactobacillus delbrueckii* subsp. *bulgaricus*; *Lactobacillus helveticus*, *Lb. paracasei* subsp. *paracasei* and *Lactobacillus delbrueckii* subsp. *lactis.* were obtained from Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt.

Milk: Raw cow's milk (3.7% fat and 9% SNF) was obtained from El- Gemaiza Research station.

Rennet: Cow's rennet powder obtained from Chr. Hansen's laboratory, Denmark.

Methods

Traditional Ras Cheese Making

Ras cheese was manufactured according to the method described by Abd El-Tawab (1963) and was ripened at 13 ± 2° C for 3 months. Four samples of Ras cheese (3 treatments and control) were made in El-Gemaiza Research station, which were taken from fresh cheese (after salting) and then monthly up to three months. Remainder cheese was waxed and coated and restored at 13 ±2°C. The control and the three treatments were as follows:

Control = Ras cheese contained: *Lactococcus lactis subsp. lactis*, *Lactococcus lactis* subsp. *cremoris*, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus.*

T1 = Ras cheese containing (the control mixture) and *Lb. paracasei* subsp. *paracasei*.

T2 = Ras cheese containing (the control mixture), *Lb. paracasei* subsp. *Paracasei* and *Lb. helveticus*.

T3= Ras cheese containing (the control mixture), *Lb. paracasei* subsp. *paracasei*, *Lb. helveticus* and *Lactobacillus delbrueckii* subsp. *lactis.*

Chemical Analyses

Moisture content was determined as described by AOAC (1998). The fat content, titratable acidity (TA), total nitrogen (TN), soluble nitrogen (SN), non protein nitrogen (NPN), were determined by using the method described by Ling (1963). The salt content of cheese was determined according to Atherton and Newlander (1978). Total volatile fatty acids (TVFA) content were determined according to the method described by Kosikowski (1978).

Microbiological Examination

Total bacterial count, coliform, moulds, and yeasts were determined as described by (American Public Health Association 1992). Total proteolytic bacterial count was carried out as described by Frank *et al*. (1992). Lipolytic bacterial count was determined according to the method described by Luk (1981).

Organoleptic Evaluation

The organoleptic evaluation properties of Ras cheese samples were assessed as recommended by El-Koussy (1966).

RESULTS AND DISCUSSION

Ras cheese was examined for the total bacteria, coliform, moulds and yeasts counts in addition to the proteolytic and lipolytic bacterial counts.

Total Bacterial Count

Data presented in Table 1 show the changes in the total bacterial count between the control and the treated samples. The total bacterial count in treated sample were higher than the control. T.C. of all cheese treatments were decreased with the progress of the ripening period. T.C. in T_3 sample was the highest. The gradual decrease in T.C. may be due to the effect of high acidity and the presence of lactic and acetic acids, which declined the T.C. during ripening period. (Lankaputhra *et al*., 1996).

Table 1. Changes in total bacterial count × 10^6 cfu/g of Ras cheese made with different lactic acid bacterial mixture during ripening period

Treatment	Ripening period (days)				
	Fresh	15	30	60	90
Control	182	79.4	42.5	27.6	20.4
T1	197	95.3	49.5	32.6	25.1
T2	202	90.1	50.2	33.9	26.3
T3	206	101	50.9	35.8	26.4

Coliforms Counts

Data presented in Table 2 show that the coliform count were detected in the samples up to 30 days, then after that, it was not detected. The control sample had higher as compared with the other samples. These counts results are in agreement with .Bartels *et al* (1987).

Mould and Yeast Counts

The results of moulds and yeasts count in the resultant Ras cheese are given in Table 3. Results show that no moulds or yeasts were detected up to the second month of ripening. The highest counts were recorded for cheeses of T1 and T2 .These are in agreement with El-Soda *et al.,* 1990.

Lipolytic and Proteolytic Bacterial Counts

Data presented in Tables 4 and 5 show the changes in lipolytic and proteolytic bacterial counts in Ras cheese made with starter culture and non starter bacteria. It is clear that, the lipolytic and proteolytic bacterial counts were higher in the treated than that of the control samples, and increased with increasing the ripening period until the first month followed by decreased until the end of ripening period of all Ras cheese samples. The highest lipolytic and proteolytic bacterial counts along the ripening period were recorded for Ras cheese samples of T_3, T_2 and T_1. These results are in agreement with those reported by Choi and Kim (1988).

Chemical composition of cheese

Compositional properties of Ras cheese made with different starter and non starter bacteria that of the control are compared as shown in Table 6. Data presented in Table 6 show that the moisture content decreased with the progress of ripening in control and treated samples. Most of the moisture loss occurred during first month of ripening, the reason for this loss may be attributed to the second stage of salting and before cheese waxing. There was a gradual increase in salt content in the first month of ripening, and then slight increased until the end of ripening period. Decreasing the moisture content and increasing the salt level during ripening of Ras cheese were reported by Awad, (2006) and Hofi *et al* (1970)

From the same table, it was observed that the titratable acidity of all cheese showed a gradual increase along the ripening period, the acidity was higher in the treated samples than the control. This may be due to the increase in total lactobacilli count. Therefore, lactic acid might have contributed directly to the increase in (T.A.) (Lau *et al*., 1991). However, as cheese ages, more caseins and high molecular weight peptides are hydrolyzed into low molecular weight peptides that increase their carboxyl groups, which could interfere with the titration process. (Fox *et al*., 2000). The fat and protein content in Ras cheese were found to be related to the moisture content in cheeses during ripening. The fat and protein content of all resultant cheeses showed a gradual increase during the ripening period. This may be due to the loss of moisture content and the increase of dry mater content in all cheeses along the ripening period. The obtained results were confirmed by the data given by Awed *et al* (2003).

The Ripening Indices

The ripening indices examined in Table 7 were the TN and TVFA as percentages of dry matter basis, SN and NPN as percentages of the total nitrogen. Data presented in Table 7 show that the TN, SN, NPN, and TVFA were higher in treated samples than the control. The TN, and SN in T_3 were higher as compared with the other

Table 2. Changes in coliform count × 10^3 cfu/g of Ras cheese made with different starter and non starter lactic acid bacteria during ripening period

Treatment	Ripening period (days)				
	Fresh	15	30	60	90
Control	1.5	0.7	0.3	ND	ND
T1	1.3	0.5	0.2	ND	ND
T2	1.1	0.4	0.2	ND	ND
T3	1.0	0.4	0.2	ND	ND

ND: not detected

Table 3. Changes in moulds and yeasts × 10^3 cfu/g of Ras cheese made with different starter and non starter lactic acid bacteria during ripening period

Treatment	Ripening period (days)				
	Fresh	15	30	60	90
Control	ND	ND	ND	ND	2.8
T1	ND	ND	ND	ND	3.3
T2	ND	ND	ND	ND	3.1
T3	ND	ND	ND	ND	2.9

ND: not detected

Table 4. Changes in lipolytic bacterial count in Ras cheese made with different starter and non starter lactic acid bacteria during ripening period (×10^3 cfu/g)

Treatment	Ripening period – days				
	Fresh	15	30	60	90
Control	40.1	47.4	49.9	16.6	4.1
T 1	42.6	51.3	55.8	19.0	4.2
T2	43.5	58.6	62.7	21.3	6.6
T3	44.2	60.1	65.1	22.9	5.5

Table 5. Changes in proteolytic bacterial count in Ras cheese made with different starter and non starter lactic acid bacteria during ripening period (× 10^3 cfu/g)

Treatment	Ripening period (days)				
	Fresh	15	30	60	90
Control	53	70.9	81.2	20.5	3.5
T 1	54.2	72.0	83.9	22.8	3.7
T2	55.3	77.1	89.5	25.1	4.8
T3	55.9	79.1	90.2	26.8	5.2

Table 6. Changes in chemical composition of Ras cheese made with different lactic acid bacterial mixtures

*Treatment	Ripening period (days)	Moisture %	Acidity %	Fat %	Protein %	Salt / DM
Control	Fresh	43.60	1.56	28.40	23.15	3.38
	15	38.70	1.74	31.10	24.50	3.55
	30	36.90	1.85	32.00	26.30	3.68
	60	35.20	2.15	33.20	26.59	3.83
	90	34.50	2.20	33.25	26.75	3.89
T1	Fresh	41.15	1.58	28.45	23.41	3.38
	15	37.90	1.79	31.70	25.12	3.58
	30	35.70	2.00	32.30	25.70	3.71
	60	34.10	2.22	33.10	26.36	3.85
	90	33.85	2.25	33.30	26.45	3.92
T2	Fresh	41.50	1.58	28.50	23.10	3.45
	15	37.60	1.80	31.80	24.70	3.60
	30	36.00	2.10	32.00	26.00	3.71
	60	35.00	2.24	33.00	26.44	3.85
	90	3415	2.27	33.20	26.49	3.92
T3	Fresh	41.10	1.60	29.00	23.30	3.42
	15	38.20	1.85	31.50	24.95	3.65
	30	37.15	2.15	23.00	26.00	3.70
	60	35.30	2.26	33.10	26.22	3.84
	90	34.10	2.29	33..20	26.50	3.92

Table 7. Changes in the ripening indices of Ras cheese made with different starter and non starter lactic acid bacteria during ripening period

*Treatment	Ripening period (days)	TN / DM%	SN / TN%	NPN / TN%	TVFA / DM%
Control	Fresh	6.61	6.48	2.402	3.09
	15	6.42	8.27	2.901	3.55
	30	6.82	10.16	4.401	4.84
	60	6.89	11.74	6.569	5.87
	90	7.10	12.91	8.723	6.44
T1	Fresh	6.65	6.63	2.250	3.50
	15	6.51	8.92	3.599	3.99
	30	6.82	11.94	4.122	5.21
	60	6.93	13.93	5.413	6.10
	90	7.21	14.15	6.803	6.69
T2	Fresh	6.67	6.90	2.246	3.52
	15	6.53	8.98	2.593	4.13
	30	6.83	12.07	3.910	5.32
	60	6.94	13.98	5.201	6.30
	90	7.22	14.17	6.724	6.85
T3	Fresh	6.72	6.98	2.201	3.53
	15	6.60	9.19	2.407	4.14
	30	6.87	12.29	3.872	5.95
	60	6.98	14.10	5.101	7.12
	90	7.31	14.22	6.435	7.26

TN: total nitrogen DM: dray matter SN: soluble nitrogen

NPN: non protein nitrogen TVFA: total volatile fatty acid

treatments, the results may be due to the differences in the total viable and non viable cells and the differences in the activities of the starter and non starters enzymes (Hannon *et al*., 2007; Courtin *et al*., 2002 and El-Soda *et al*., 2000). The increased in non protein nitrogen content in all cheeses throughout the ripening period may be due to the protein breakdown occurred through the growth of microflora and proteolysis with proteolytic enzyme (Lynch *et al*., 1996 and Hannon *et al*., 2007). TVFA content for cheese containing strains of lactic acid especially T_3 had the highest effect during the ripening period. The results indicate that non starter lactic acid bacteria have different lipolytic activity. (El-Soda *et al.,* 1990).

Organoleptic Properties

Data presented in Table 8 show that the organoleptic score increased in the treated samples after two months compared with the control during ripening period. The maximum total scores was recorded in T_3, these results indicated that the proteinase and peptidases of starter and non starter bacteria was very important for the production of small peptides in Ras cheese during ripening. Fox *et al.* (2000) reported similar results for Cheddar cheese.

Table 8. Average score of organoleptic properties of Ras cheese made with different starter and non starter lactic acid bacteria

Treatment	Ripening period (days)	Flavour 50	Body & texture 40	Appearance 10	Total score 100
Control	**Fresh**	33	30	7	70
	15	34	32	8	74
	30	36	33	8	77
	60	39	35	8	82
	90	44	37	9	90
T1	**Fresh**	34	32	7	73
	15	36	32	8	78
	30	38	35	8	84
	60	42	37	8	91
	90	44	38	9	93
T2	**Fresh**	35	33	7	75
	15	37	33	8	80
	30	41	35	8	85
	60	44	38	9	93
	90	45	39	9	93
T3	**Fresh**	35	33	8	76
	15	38	34	8	80
	30	42	35	9	86
	60	47	39	9	95
	90	47	39	9	95

REFERENCES

Abd El-Tawab, G. (1963). Studies on Ras cheese. M. Sc. Thesis. Fac. Agric., Ain Shams Univ., Egypt.

American Public Health Association (1992). Standard methods for the examination of dairy products, Inc. 16th (Ed.) New York.

AOAC (1998). Official Methods of Official Analysis Chemists. 15th Ed. William Horitz, (edj pub) Association of official Agric. Chemsits, Washington, D.C., U. S. A.

Atherton, H.V. and J.A. Newlander (1978). Chemistry and testing of Dairy product. 4th Ed. Avi publishing company L.N.C. west part connective.

Awad, S., A. El Attar, A.H. Ayad, and M. El-Soda, (2003). Characteristic of Egyptian market Ras cheese; 1-sensory evaluation, rheological, physico-chemical properties and microbiological analysis. Egyptian J. Dairy Sci, 31, 289–303.

Awad, S. (2006). Texture and flavour development in Ras cheese made from raw and pasteurised milk. Food Chemistry, 97, 394–400.

Bartels, H.J., M.E. Johnson and N.F. Olson, (1987). Accelerated ripening of Gouda cheese. I. Effect of heat-shocked thermophilic lactobacilli and streptococci on proteolysis and flavour development Milchwiassenschaft 42 (2): 83-88.

Broadbent, J.R., K. Houck, M.E. Johnson and C.J. Oberg (2003). Influence of adjunct use and cheese microenvironment on nonstarter bacteria in reduced-fat Cheddar-type cheese. J. Dairy Sci., 86: 2773–2782.

Broome, M.C. (2007). Starter culture development for improved cheese flavour. Pages 157–176 in Improving the flavour of cheese. B.C. Weimer, ed. Woodhead Publishing Limited, Cambridge, England.

Choi, C.K. and H.U. Kim (1988). Accelerated ripening of Cheddar cheese using *Streptococcus lactis* Lac-mutants. Korean-J.-of-Dairy-Sci.; 10 (4): 189-200.

Di Cagno, R., M. De Angelis, V.K. Upadhyay, P.L.H. McSweeney, F. Minervini, G. Gallo, and M. Gobbetti (2003). Effect of proteinases of starter bacteria on the growth and proteolytic activity of *Lactobacillus plantarum* DPC2741. Int. Dairy J., 13:145–157.

El-Koussy, L.A. (1966). Studies on soft cheese manufactured from pasteurized milk. Ph. D. Thesis, Ain Shams Univ. Cairo, Egypt.

El-Soda, M., N. Ezzat, A. Salam and A. Khamis (1990). Acceleration of Ras cheese ripening. III. Evaluation of commercial enzyme mixtures. Egyptian J. of Dairy Sci., 18 (2): 273-279.

El-Soda, M., S.A. Madkor and P.S. Tong (2000). Adjunct cultures: Recent developments and potential significance to the cheese industry. J. Dairy Sci., 83 (4): 609–619.

Fox, P.F., T.P. Guinee, T.M. Cogan, P.L.H. Mscweeney (2000). Fundamentals of cheese Science. Aspen Publishers, Inc.,Permissions Department, Gaithersburg, Maryland, 1260-8342.

Frank, J.F., G.L. christen and L.B. Bullerman (1992). Tests for groups of microorganisms. In: R.T. Marshal (Ed.). Standard methods for the Examination of Dairy products. (16th Ed.), 271 – 286. American public Health Association, Washington, DC, USA.

Hannon, J.A., K.N. Kilcawley, M.G. Wilkinson, C.M. Delahunty and T.P. Beresfoed (2007). Flavour precursor development in Cheddar cheese due to lactococcal starters and the presence and lysis of lactobacillus helveticus. Int. Dairy J., 17 (4): 316-327.

Hickey, D.K., K.N. Kilcawley, T.P. Beresford, E.M. Sheehan and M.G. Wilkinson (2007). Starter strain related effects on the biochemical and sensory properties of Cheddar cheese. J. Dairy Res., 74: 9–17.

Hofi, A.A., E.H. Youssef, M.A.Ghoneim and G.A. Tawab (1970). Ripening changes in Cephalotyre ‘‘Ras” cheese manufactured from raw and pasteurised milk with special reference to flavour. J. of Dairy Sci., 53: 1207–1212.

Johnson, M.E. and J.A. Lucey (2006). Major technological advances and trends in cheese. J. Dairy Sci., 89: 1174–1178.

Johnson, M.E., R. Kapoor, D.J. McMahon, D.R. McCoy and R.G. Narasimmon (2009). Reduction of sodium and fat levels in natural and processed cheeses: Scientific and technological aspects. Compr. Rev. Food Sci., Food Saf., 8:252–268.

Kosikowski, F.V. (1978). Cheese and fermented milk foods 2nd Ed. Published by the Author. Cornell Univ., Itacha, N.Y.

Lankaputhra, W.E.V., N.P. Shah and M.L. Britz, (1996). Survival of bifidobacteria during refrigerated storage in the presence of acid and hydrogen peroxide. Milchwissenschaft. 51: 65-70.

Lau, K.Y., D.M. Barbano and R.R. Rasmussen (1991). Influence of pasteurization on protein breakdown in Cheddar cheese during ripening. J. Dairy Sci. 74, 727-740.

Ling, E.R. (1963). A text book of dairy chemistry. Vol. 11, Champan and Hall Ltd. London.

Luk, H. (1981). Quality control in the dairy industry. In: R.K. Robin son (Ed). Dairy Microbiology (Vol. II) Applied Sci. Publishers, Ltd., England. PP. 313-315

Lynch,C.M., P.L.H. Mcsweeney, P.F. Fox, T.M. Cogan and F.D. Drinan (1996). Manufacture of Cheddar cheese with and without adjunct lactobacilli under controlled microbiological. Int. Dairy J. 6:851-867.

O'Sullivan, D.J. (2007). Techniques for microbial species identification and characterization to identify commercially important traits. In Improving the Flavour of Cheese. B.C. Weimer, ed. Woodhead Publishing Limited, Cambridge, England. PP 199-218.

اســـتخدام بعض ســـلالات بكتـــريا حمض اللاكتيك فى إسراع تســـوية الجبن الراس

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يهدف هذا البحث إلى دراسة بعض سلالات بكتيريا حمض اللاكتيك والتى شملت

Lactococcus lactis subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Streptococcus thermophilus; Lb. delbrueckii* subsp. *Bulgaricus; Lactobacillus helveticus, Lactobacillus paracasei* subsp. *paracasei* and *Lactobacillus delbrueckii* subsp. *lactis.*

وقد تم إجراء الاختبارات الكيميائية والميكروبيولوجية والحسية على عينات الجبن الراس وذلك خلال فترة التسوية (طازجة – ١٥ -٣٠ -٦٠ -٩٠يوم) ولقد أوضحت النتائج المتحصل عليها أنه لا يوجد أى تأثير على التركيب الكيميائى بصفة عامة بين معاملات الجبن المختلفة. وقد وجد أن هناك زيادة في كل من الحموضة والمحتوى الملحي والنيتروجين الكلي والنيتروجين الذائب والنيتروجين غير البروتيني والأحماض الدهنية الطيارة بينما لوحظ أن هناك نقص في كل من المحتوى الرطوبي والعدد الكلي للبكتريا وكذلك البكتريا المحللة للبروتين والبكتريا المحللة للدهون. وقد تناقص عدد بكتيريا الكوليفورم في جميع المعاملات بالمقارنة بالكنترول بعد ٣٠ يوم من التسوية. بينما ظهرت الفطريات والخمائر بعد ٦٠ يوم من التسوية في جميع المعاملات والكنترول كما أظهرت المعاملة الثالثة أفضل تحكيم حسي بعد شهرين من التسوية.

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