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The use of transglutaminase enzyme in processing ras cheese

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ABSTRACT

To overcome the problem of the delayed ripening of ripened cheeses made with Transglutaminase enzyme, a number of starters were selected for their proteolysis activity. Ras cheese slurry was made using the selected cultures, incubated at 30C for 30 days and was chemically and organoleptically tested.Two cultures combinations resulted of good quality slurry.*L. Helveticus* and *L. Lactis, and L. helveticus, L. lactis* and *L. casei* resulted in good Ras cheese slurry, particularly the former one.

Keyword: Transglutaminase, Lactic Acid Bacteria, Ras Cheese, Ripened Cheese

INTRODUCTION

Transglutaminase enzyme is used in production of different dairy products since it improves the yield as well as the body and structure of the product particularly low fat products. In yogurt, the enzyme increased gel strength and decreased syneresis particularly in low fat yogurt,(Iuliana *et al*., 2012).In soft cheese, the enzyme gave higher gel firmness, increased the yield, decreased syneresis and improved organoleptic properties,(Ieva, 2013). In low fat mozzarella cheese, the enzyme increased cheese yield by increasing moisture, fat and protein recovery. The enzyme improved stretchability and meltability as well asorganoleptic properties (Metwally*et al*., 2007). In low fat ice cream, the enzyme compensated for the low fat to produce a product superior in all physical and organoleptic properties than control (Metwally, 2007, Priscilla*et al.,* 2012).

However, the use of the enzyme in ripened cheese was limited due to the delayed ripening process resulting from slow microbial proteolytic activity leading to a harder texture and low meltability (Özer, 2007). This is a result of the enzyme cross-linking reaction which reduces the availability of low molecular weight peptides used as nitrogen source by the starter (Fæaergemand, 1999).

Aaltonen *et al* (2014) used the enzyme for making Edam cheese from ultrafiltrated milk. Ultrafiltration of milk was used to remove the enzyme inhibitor present in milk. The enzyme was incubated with the retentate at 17□C for 2h, then the mix was standardized with raw milk which containing the inhibitor. The resulted cheese was harder in texture than the control. Therefore, they suggested the use of peptidase to overcome the low free amino acids present in UF cheese (Aaltonen and Huumonen 2013).

To overcome the slow activity of ripening culture in Transglutaminase (TG) cheese, this research tried to select culture strains that can be active and help in normal ripening of the cheese.

MATERIALS AND METHODS

Materials:

Starter cultures of *Lactobacillushelveticus*(1654$^{T)}$, *Lactobacillus casei*subspcasei (1093^{T}),*Lactobacillus delbreuckii bulgaricus* (761N),*Lactococcuslactis*subsp*lactis*(1106)and*Streptococcus salivarius subsp. thermophilus*(1043)were obtained from Microbiological Resources Center, Cairo MIRCEN, College of Agriculture, Ain Shams University.

Cow milk was obtained from Dairy Department pilot plant, College of Agriculture, Cairo University.

A microbial Transglutaminase (EC: 2.3.2.13) obtained from streptoverticillum mobaraense, commercially available as (ACTIVA® YG) was used. The enzyme (declared activity is 100U/g) was a gift from Ajinomoto Europe Sales Gmbh, Hamburg Germany.

Chemicals used were of high purity.

Methods

Selecting the proper culture:

Activated culture strains, single or in combinations with ratio 1:1, were tested for their proteolytic activity using the method of Church *et al*., 1983.One percent,either ofa single or in 1:1 combination of microorganisms,of the active culture was inoculated into 100 ml of sterile skim milk, incubated for 24 h at 37□C.

Combinations of the strains were selected for their high proteolytic activity and were used in making cheese slurry.

Milk rennet coagulation using the TG enzyme:

Milk was coagulated with rennet in presence of TG following the method of Metwally et al (2007).

Ras Cheese Slurry Making:

Cheese slurry was made according to the normal Ras cheese manufacturing protocol followed the procedure of Abdelfattah 1998. Fresh cow's milk was pasteurized at 72˚C/15s, and inoculated with 2%starter of the selected combination. Milk was kept for 30 min for development of the proper acidity (0.18%) then cooled down to 5˚C. Liquid rennet was added tomilkat a rate of2.5 ml/kg and left for 30 min at 5 ˚C. Then 0.05% of Transglutaminase was addedand the mixture was left for 2 h at 5 ˚C before raising the temperature to 40˚C to complete coagulation.The curd was cut vertically and horizontally with sterilized knives and the temperature was raised to 45˚ C within 15 min. When the curd particles reached the grain size, they were allowed to settle down in the bottom of the cheese vat. Half of the whey was drained off using a sterilized cheesecloth, and then salt was added at rat of 20 g per kg of milk. Mixingcontinued for 10 min, and then the remaining whey was drained off completely usingcheesecloth. The curd was then filled into a sterilized glass jars and covered with sterilized screw plastic cap. The jars

were completely filled with curd leaving no air space. Empty glass jars and their caps were sterilized by boilingin water for 20 min before use. Control cheese was made following the same procedure without TG enzyme.

The slurry was incubated at 30°C for 30 days and their chemical and organoleptic properties were tested.

Slurry Chemical Tests:

Slurry Total Protein andSoluble Nitrogen was determined by semi-micro Kjeldahl method according to SMEDP (1985),acidity by the method of Ling (1963), fat by the Gerber method, sodium chloride by the method of IDF (1982) and total volatile acids by the methods of Kosikowski (1978).

Organoleptic Properties:

Cheese slurry was judged by number of the Department staff members, and the average of their score was reported.

Statistical Analysis:

All measurements were done in triplicate then reported as the arithmetic mean. Analysis of variance (one way Anova) was used for multiple comparisons over the different treatments. The statistical significance of the data was determined using Fisher's LSD post hoc test. P-value 0.05 was considered sufficient to reject the null hypothesis. Statistical analysis was performed by running the SPSS 20 (IBM Corp., Copyright© 2011) package on a personal computer.

RESULTS AND DISCUSSIONS

The problem of using TG enzyme in ripened cheese is the slow and incomplete ripening due to low starter activity. Thiswas attributed to the lack of small peptides that can be used as nitrogen source for their activity. Thisresearch was carried out to select cultures that can grow in TG cheese overcoming the above problem through their proteolytic activity.

Cheese slurry was used to screen microorganisms for their growth and effect on cheese ripening. Because of the lengthy ripening time required for flavor development, evaluation of each strain on quality is time consuming and costly. Therefore, cheese slurry is used to rapidly evaluate flavor and proteolytic potential of the starter in an efficient way.

Five strains of microorganisms, grouped in seven different combinations, were grown in sterile skim milk, and their proteolytic activities were determined and results are presented in Table (1).

The mixture of *L. casei* and *L. lactis* produced more proteolysis than other combinations followed by *L. helveticus* and *L. lactis*. Therefore, both combinations were used as starter for processing Ras cheese slurry.

Table (1): Proteolytic activity of combination of strains of lactic acid bacteria

Strains[123]	**Absorbance** at 340 *nm*
L. helveticus and *L. casei*	0.591
L. helveticus and *L. bulgaricus*	0.639
L. helveticus and *S. thermophilus*	0.548
L. helveticus and *L. lactis*	0.663
L. casei and *S. thermophilus*	0.611
L. casei and *L. bulgaricus*	0.546
L. casei and *L. lactis*	0.784

1. Ratio of mixing was 1:1
2. Incubation temperature was 30□C for 24h.
3. Inoculation rate was 1% in sterile skim milk.

The above two starters were formed of *L. helveticus*, *L. lactis* and *L. casei*microorganisms. Therefore, the three microorganisms were mixed together to form a third group to find out the effect of mixing on cheese.*Streptococcusthermophilus* and *L. bulgaricus* starter was used as a control with and without TG enzyme. Table (2) presents Ras cheese slurry chemical composition made with the above three cultures and compared with the control. *Lactobacillushelveticus* and *L. lactis* starter resulted in the highest protein, fat, soluble nitrogen (SN) and total volatile fatty acids than other starters. This is followed by the control without the enzyme, since TG enzyme reduced the control protein and SN. The other two groups were almost similar in their chemical composition.

Table(2):Chemical composition of Ras cheese slurry with Transglutaminase enzyme and with different starters

Strains	**Chemical Composition**						
	Total Protein	**Fat**	**Moisture**	**Salt**	**Acidity**	**S.N.**[2]	**T.V.F.A**[3]
	%						**mg/l**
Control[1]	21.43	32	44.52	1.2	2	0.64ace	4.86ace
L. bulgaricus and *S. thermophilus*	20.8	34	48.38	1	1.9	0.54b	4.64b
L. casei and *L. lactis*	20.72	35	42.18	1.3	3.06	0.64ace	4.86ace
L. helveticus and *L. lactis*	23.58	35	41.94	1.5	3.25	0.72d	5.46d
L. helveticus and *L. lactis* and *L. casei*	20.54	35	41.43	1.2	2.9	0.66ace	4.88ace

- **Incubation temperature was 30□C for 30 days.**
- **Inoculation rate was 1% in sterile skim milk.**
- **Microorganisms were mixed in equal rates.**
- **Means followed by same letter(s) within each column are not significantly different at 5% levels of probability**

1. The control used was *L. bulgaricus* and *S. thermophilus* without the enzyme
2. Soluble Nitrogen
3. **Total volatile fatty acids**

The culture with the three microorganisms did not compare well with other two groups. However, to emphasize the above results and to find the effect the Enzyme on the final product, the two cultures, the *L. helveticus*and

L. Lactis culture, and the *L*. *helveticus* and *L*. *lactis* and *L*. *casei* culture with and without the enzyme were compared and reported in Table (3).*Lactobacillus helveticus* and *L. lactis* improved slurry composition made with the enzyme. The enzyme increased the yield, SN and total volatile fatty acids in the slurry compared to its control.

The other culture did not compare well with the above culture,but still the culture improved the yield, SN over its control.

Table(3):The effect of selected cultures on chemical composition of Ras cheese slurry made with Transglutaminase enzyme.

Culture		Chemical Composition						
	Yield	Total Protein	Fat	Moisture	Salt	Acidity	S.N.[1]	T.V.F. A[2]
	g	%						mg/l
L. helveticus and *L. lactis*(Control)	401	26.61	35	64.66	1	2.5	0.66	5.14
L. helveticus and *L. lactis*	406	26.97	36	66.66	1	3	0.92	6.24
L. helveticus and *L. lactis* and *L. casei*(Control)	444	21.25	28	66.66	1	3.5	0.72	4.56
L. helveticus and *L. lactis* and *L. casei*	448	22.73	29	66.66	1	3.56	0.78	4.74

- **Microorganisms were mixed in equal rates.**
- **Controls were processed without the enzyme.**

1. Soluble Nitrogen
2. Total volatile fatty acids

Table (4) compares slurry sensory evaluation of the five used cultures. Once again, *L. helveticus* and *L. lactis* and *L. helveticus*, *L. casei* and *L. lactis*, produced best result, in flavor, body and texture, acceptability and in turn total score. The difference from the control was great, whether the control contains the enzyme or not.

Table(4):Sensory evaluation of Ras cheese slurry made with Transglutaminase enzyme and selected cultures.

Strains	Average Score of			
	Flavor	Body & Texture	Acceptability	Total Score
	(50)	(35)	(15)	(100)
Control	32.1	26.27	9.85	68.22abe
L. bulgaricus and *S. thermophilus*	32.3	22.75	9.85	64.90 ae
L. casei and *L. lactis*	40.8	28	11.87	80.67bcd
L. helveticus and *L. lactis*	43.4	28.37	12.62	84.39cd
L. helveticus and *L. lactis* and *L. casei*	44.3	28	12.75	85.05bcd

- **The control used was *L. bulgaricus* and *S. thermophilus* without the enzyme**
- **Slurries were incubated at 30□C for 30 days.**
- **Number of banalities were ten**
- **The starters were compared with 1:1 ratio of each microorganism.**
- **Means followed by same letter(s) within each column are not significantly different at 5% levels of probability**

Cultures used improved the organoleptic properties of the slurry as compared to the control which is usually used for making Ras cheese. The use of the enzyme with the control did not improve the cheese but was less acceptable than the control without the enzyme.

To be sure of this good performance of both cultures, another batch of slurry was made using both cultures, with and without the enzyme and the sensory evaluation are shown in Table (5).

Table (5): Effect of selected cultures on the sensory evaluation of cheese slurry made with Transglutaminase enzyme.

Strains	Average Score of			
	Flavor	Body & Texture	Acceptability	Total Score
	(50)	(35)	(15)	(100)
L. helveticus and *L. lactis*(Control)	42.75	29.00	11.5	83.25
L. helveticus and *L. lactis*	45.75	31.00	13.0	89.75
L. helveticus and *L. lactis* and *L. casei*(Control)	37.75	24.25	9.75	71.75
L. helveticus and *L. lactis* and *L. casei*	39.25	25.75	9.00	74.00

- **Slurries were incubated at 30□C for 30 days.**
- **Number of banalities were ten**
- **The starters were compared with equal ratio of each microorganism.**

Both cultures produced slurry with good acceptability, particularly *L. helveticus* and *L. lactis* culture, and the product matches Ras cheese flavor. The other culture also produced slurry with good acceptability as compared to the control reported in Table (4).

Therefore, the selected cultures used in this research, which had some proteolytic activity, solved the problem of using the TG enzyme in processing ripened cheese.

Other types of ripened chesses mayuse this procedure to select their proper starter that solve the problem of using the TG enzyme.

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استخدام انزيم الـ Transglutaminase في تصنيع الجبن الراس

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من المعرف ان هناك بعض المشاكل التي نعوق استخدام انزيم الـ Transglutaminase في صناعة الجبن الجاف المسوى على نطاق واسع حيث يؤدي لتأخير فترة التسوية، وللتغلب على تلك المشكلة، تم استخدام عدد من البادئات التي تتميز بقدرتها على تحليل البروتين. ولاختبار ذلك تم تصنيع حبيبات slurry الجبن الراس بواسطة تلك البادئات وتم التخزين بالتحضين على درجة حرارة ٣٠ °م لمدة ٣٠ يوم. أثبت النتائج أن خليط البادئ من *L. Helveticus* and *L. Lactis* أعطى أفضل النتائج تلها خليط البادئ من *L. helveticus, L. lactis* and *L. casei*.