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INFLUENCE OF TRANSGLUTAMINASE (TGASE) ENZYME ON THE QUALITY OF LOW FAT TALLAGA CHEESE

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ABSTRACT

Low fat Tallaga cheese was made from cows milk standardized to 2% fat, heated to 75°C /15 sec., then treated with 0.2-0.5 g TGase/L cheese milk .Cheese was stored in the refrigerator ,for 21 days and analyzed at weekly intervals. Apparent viscosity , plastic viscosity and consistency index for cheese curd before molding were followed . Also , the organoleptic properties of low fat Tallaga cheese from different treatments were assessed.

Using transglutaminase significantly increase cheese yield and developed less syneresis . Total nitrogen and total solids of cheese from TGase treated milk were higher than cheese from untreated milk . Moisture content , total nitrogen of cheese decreased , while titratable acidity , fat , soluble nitrogen increased during storage . Total protein and total solids in the recovered whey decreased with increasing dosage of TGase compared to the control. Apparent viscosity , plastic viscosity and consistency index of cheese curd were increased with increasing TGase concentration and were higher than that of cheese curd (control) from untreated milk. Urea -PAGE showed some differences in protein degradation between control cheese and cheese treated with TGase when fresh and after 21 days of storage . Organoleptic scoring revealed higher scores for flavour , body and texture for cheese from milk treated with TGase . Cheeses treated with TGase exhibit firm and smooth texture , creamy taste with excellent appearance . Low fat Tallaga cheese of acceptable quality , firm texture, pleasant consistency and mouthfeeling can be made from heated milk treated with 0.4-0.5 g /L of cheese milk

Keywords : Tallaga cheese , transglutaminase , low fat cheese.

INTRODUCTION

The consumption of Tallaga cheese has been increased markedly during the last decade . Tallaga cheese is a variant of Domiati cheese characterizes by its low salt content and cold storage (Mehanna and Rashed , 1990 ; Shehata *et al.*, 1995). This variety has a spreadable mellow soft body with a pleasant creamy taste (Hofi *et al.*, 1979) .

The enzyme transglutaminase (TGase ; EC 2.3.2.13) catalyses an acyl group transfer reaction between γ- carboxyamide groups of peptide - bound glutamine residues (acyl donor) and the primary amino groups in a variety of amine compounds (acyl acceptor) , including peptide - bound ε-amino groups of lysine residues in protein molecules. Crosslinking of food proteins by TGase modifies the hydration ability , the gelation , rennetability , rheological and emulsifying properties of the present proteins (Lorenzen and Schlimme , 1998 ; Motoki and Segura , 1998). The rate of TGase cross-linking depends on the macromolecular structure of protein involved . The open conformation of the casein's makes them good substrates (Nio *et al.*, 1986 ; Han and Damodaran , 1996) for TGase cross -linking. As a result of

cross-linking high molecular polymers or aggregates are obtained (Singh , 1991 ; Ikura *et al.*, 1992). Enzymatic cross-linking is a method that has received increasing attention during the last 10 years. More than 80 patents and patent applications has been issued on TGase use in food which show clearly the importance of this tread (Nielsen , 1995). Besides its potentials for improving both the texture and the consistency of food systems , enzymatic cross-linking improved hydration ability and water binding of proteins , increased viscosity of protein solutions , decreased creaming of O/W emulsions and significantly improved heat stability of protein solutions (Mahmoud and Savello , 1990 ; Nonaka *et al.*, 1992 ; Faergemand *et al.*, 1997 ; Lorenzen *et al.*, 2000).

Transglutaminase can be used in stabilizing products like yoghurt , fresh cheese , whipping cream and novel milk products (e.g., spreads , low calorie foods) (Lorenzen *et al.*, 2000 ; Abou El-Nour *et al.*, 2004).In the area of cheese production, TGase treatment can reduce cheese lesses , improve water retention in reduced fat cheeses and in turn increasing cheese yield and improving cheese quality.

The aim of the present study was to investigate the manufacture of low fat Tallaga cheese from milk treated with TGase and to evaluate the effect of using this enzyme at different dosage on both cheese yield - cheese syneresis , chemical , rheological and organoleptic properties of obtained cheese.

MATERIALS AND METHODS

Materials :

Fresh raw cow's milk was obtained from a private farm in Ismailia governorate ; its chemical composition was as follow : 0.18% acidity , pH 6.58 , total solids 13.54% , fat 4.5% and total protein 3.38% . Table salt (NaCl dry coarse , El-Nasr Co., Alex.). Potassium chloride (pure) was obtained from El-Nasr Pharmecutical chemicals , microbial rennet powder , Hannilase L2235 (from *Rhizomucor miehei*) Chr. Hansen , Denmark (1-3g/100L milk).Transglutaminase (Activa MP contains 1% transglutaminase) was a gift from Ajinomoto Europe sales (Stubbenhuk, Hamburg, Germany). The declared activity of the preparation was approximately 100 units/g.

Experimental procedure :

Fresh cow's milk was standardized to 2% fat, milk then divided to five portions. Tallaga cheese was made by the process shown in Fig.(1). The first portion was regarded as control and was made as given in Fig. (1) except that no TGase was added . Four levels of TGase were used 0.2, 0.3, 0.4 and 0.5 g/L for the other four portions i.e. treatments 2,3,4 and 5 respectively. The levels, incubation and the inactivation temperature of TGase were used according to Ajinomoto applications data . Tallaga cheese was made from each portion by conventional method of making Domiati cheese (Fahmi and Sharara, 1950). The resultant low fat Tallaga cheese was packed in plastic cups filled with boiled whey from the respective treatment and stored in refrigerator for 3 weeks. The whole experiment was duplicated.

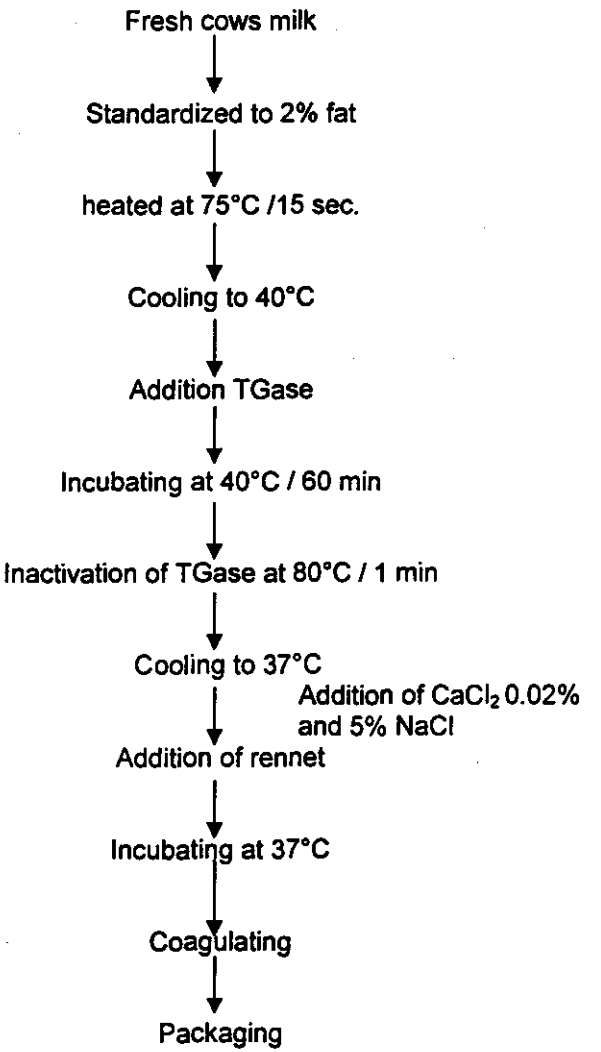

Fig (1) : Flow sheet of the production of Tallaga cheese treated with TGase

Methods of analysis :

Cheese sample were analyzed chemically when fresh and after 7,14 and 21 days of storage. Titratable acidity and moisture content were determined according to AOAC (1990); soluble nitrogen (SN), total nitrogen (TN) by micro Kjeldahl method and fat content by Gerber butyrometer according to Ling (1963), pH value was measured by using (Jenway digital pH meter , Jenway Limited, England). The yield of fresh cheese was recorded as kg of cheese /100kg of cheesemilk, whereas, syneresis was measured after a 20 hour incubation at 25°C by determine the amount of

separated aqueous phase. The rate of syneresis = net change in weight divided by original weight of sample after cutting . Viscosity and some rheological parameters of fresh curd (before molding) of Tallaga cheese were carried out using a Brookfield Digital Rheometer model DV-III+ (Brookfield Engineering Laboratories, Inc., MA , USA), equipped with a SC_4-21 spindle. Apparent viscosity was measured at 50 rpm. Measurements were carried out at temperature of 5°C in shear rate ranging from 23.3 to 232.5 S^{-1}. All rheological properties were performed in duplicates.

Gel electrophoresis (Urea -PAGE) :

U ea -PAGE was performed by the method of Andrews (1983). The gels were stained by the method of Blakesley and Boezi (1977). Cheese samples (0.1g) were dissolved in 1ml sample buffer and (11µl) were applied to the gel.

Organoleptic properties :

Organoleptic properties of cheese samples were evaluated according to the method of Pappas *et al.*, (1996) when fresh and after 14 and 21 days of cold storage (7°C ±1) . Cheese were assessed by means of six panelists from the staff of the Dairy Department, with maximum score points (50 points) for flavour, body and texture (40 points) and appearance (10 points).

RESULTS AND DISCUSSION

Gross component of cheese :

Table (1) shows the changes of moisture , fat content, acidity, total nitrogen , soluble nitrogen and total solids during cheese storage. The moisture content of cheese from all treatments decreased as storage period progressed also, the fat content of all Tallaga cheese treatments increased due mainly to the decrease in moisture content (Ezzat, 1990). Cheeses made from milk treated with TGase had slight lower moisture content compared with control cheese. Cheese milk treated with TGase increased significantly the TN content of Tallaga cheese, and this were proportional to the amount of added TGase compared to control cheese. These results are in accordance with those given by Budtz (2001) who reported that transglutaminase increased the amount of protein left in the coagulated cheese material as compared to cheese made without TGase treatment. The TN content of all cheeses decreased throughout storage, which might be due to the degradation of proteins into SN and subsequently the loss of some water soluble nitrogen (SN) from the degraded proteins. These results were in agreement with those reported for Domiati cheese by Kebary *et al.*, (1991) and Badawi and Kebary (1996). On the other hand the soluble nitrogen increased as storage progressed. Badawi and Kebary (1998) and Osman and Abou El-Nour (1998) reported similar results for traditional Domiati cheese. Further increase in SN content of Tallaga cheese was obtained by increasing the dosage of TGase added. Titratable acidity of cheeses made from milk treated with TGase were lower in comparison with control cheese.

Furthermore, during storage the development of acidity in cheese samples treated with TGase were slightly lower than control. The acidity of cheese was progressively increased during storage either the control or in treated cheese these results in agreement with Abdel Kader (2003) and Salama (2004).

Table (1) : Gross composition of low fat Tallaga cheese during storage at 7 ° C ± 2 °C .

Treatments	Age (days)	Moisture %	Fat %	TN %	SN %	TS %	Acidity %
Control	Fresh	64.98	8.60	2.64	0.19	34.98	0.28
	7	63.71	8.80	2.61	0.28	36.29	0.34
	14	62.40	9.10	2.53	0.40	37.6	0.41
	21	60.55	9.50	2.49	0.55	39.44	0.57
T2	Fresh	64.97	8.70	2.84	0.22	35.01	0.27
	7	63.70	8.90	2.81	0.33	36.30	0.31
	14	62.30	9.10	2.76	0.42	37.80	0.39
	21	60.54	9.30	2.72	0.57	39.46	0.54
T3	Fresh	64.95	8.80	2.87	0.24	35.04	0.26
	7	63.70	8.90	2.84	0.36	36.30	0.30
	14	62.10	9.10	2.72	0.44	37.90	0.38
	21	60.52	9.30	2.70	0.59	39.47	0.50
T4	Fresh	64.89	8.80	2.95	0.27	35.10	0.24
	7	63.68	9.00	2.92	0.38	36.32	0.28
	14	62.04	9.20	2.85	0.46	37.97	0.36
	21	60.50	9.30	2.75	0.64	39.48	0.47
T5	Fresh	64.80	8.90	3.03	0.28	35.21	0.24
	7	63.60	9.00	2.98	0.39	36.40	0.28
	14	61.95	9.30	2.90	0.47	38.06	0.34
	21	60.46	9.40	2.77	0.66	39.55	0.47

Each value in the table is the mean of two replicates .
T2, T3, T4, and T5 contain 0.2 ,0.3 ,0.4 and 0.5 g / l TGase respectively .

TGase treated cheese milk had a significant effect on the total solids (TS%) content of Tallaga cheese. The total solids recovered from TGase treated milk was higher than that from control. As storage period progressed the TS% of the cheese increased. Similar results were reported by Awad *et al.*, (2001) and Abdel - Kader (2003).

Table (2) reveals TS %, TN %, TP %, Fat %, pH and acidity of whey drained from cheese of different treatments as affected by TGase treated milk. Results cleared that total solids (TS%) obtained from Tallaga cheese whey gradually decreased as the level of TGase increased .Also TN content and TP decreased in the recovered whey with increasing dosage of TGase compared to the control. These results coincided with those of Han *et al.*, (2003) who reported that the decreased protein concentration in recovered whey is caused by the cross-linking of transglutaminase . No differences were observed on fat content of whey of all treatments and control one. Acidity of the whey drained from the resultant cheese decreased with increasing the amount of TGase , pH value had an opposite trend of acidity of the corresponding drained whey.

Table (2) : Effect of adding different levels of TGase on chemical composition of Tallaga cheese fresh whey .

Treatments	TS %	TN %	TP %	Fat %	pH Value	Acidity %
Control	12.43	0.1580	1.010	0.1	6.53	0.16
T2	12.00	0.1198	0.764	0.1	6.58	0.15
T3	11.98	0.1175	0.749	0.1	6.63	0.14
T4	11.96	0.1141	0.728	0.1	6.63	0.14
T5	11.93	0.1118	0.713	0.1	6.63	0.13

See table 1 for samples designation .

Cheese yield and syneresis :

Table (3) reveals that, yield of fresh Tallaga cheese increased with increasing the amount of TGase treated milk as compared with control .Cheese made from milk treated with 0.5g/l TGase (T5) had higher yield than control and other treatments .These results were in a good agreements with those reported by Kuraishi *et al.*, (1997). Han *et al.*, (2000) demonstrated that increasing yield of cheese could be due to whey protein retained in the cheese curd and established that the proportion of whey protein product incorporated into the cheese curd increases essentially as the amount of TGase added is increased .On the other hand, TGase treated milk decreased cheese syneresis and the syneresis decreased with increasing TGase level as compared with control . Similar results were reported by Han *et al.*, (2002). The syneresis of (T5) was about 40 % of that for the control indicating that , TGase Cross-linking of proteins minimized syneresis of the final products . Han *et al.*, (2003) stated that TGase - treated curd contained smaller protein particles with less empty space which reduced syneresis.

Table (3): Effect of TGase treated milk on cheese yield and cheese syneresis .

Treatments	Properties	
	Cheese yield (%)	Cheese syneresis (%)
Control	17.93	9.60
T2	22.28	8.59
T3	22.92	7.19
T4	23.24	6.53
T5	23.66	5.99

See table 1 for samples designation

Rheological analysis :

Transglutaminase treatment had markedly effects on the viscosity (Fig. 2) and the other rheological properties (Table 4), which were increased substantially by increasing TGase dosage.

Table (4) : Rheological parameters of low fat Tallaga cheese curd as affected by TGase .

Treatments	Plastic viscosity mPaS	Consistency index mPaS
Control	22.20	335.7
T2	35.50	551.9
T3	39.50	688.1
T4	66.20	776.7
T5	113.99	878.3

See table 1 for samples designation

Apparent viscosity of the TGase treated sample was much higher than that of the control sample. With (T5), viscosity was almost 55 % higher than that of the control. However, increased TGase dosage increasing the viscosity of cheese curd . These results coincided with those of Han *et al.*, (2003) who stated that increased TGase dosage , results in more extensive cross-linkage of proteins increased the viscosity of the products .

The result given in Table (4) showed that plastic viscosity and consistency index increased with increasing TGase levels .Moreover, results showed that plastic viscosity of the TGase treated sample (T5) was much higher than that of control sample , it was 5 times greater .Same trends were found with consistency index, which increased with increasing TGase dosage.

Polyacrylamide gel electrophoresis :

Elctrophretograms of low fat Tallaga cheese treated with TGase when fresh and after 21 days are shown in Fig. (3). There are a progressive breakdown of both α_{s_1}- and β- casein with time, and with increasing the concentration of TGase, β- casein was degraded more quickly in (T5) compared to control and other treatments .The intensity of β- casein band for (T5) after 21 days decreased more than that in control cheese (Lane 11) .A series of unidentified slowly migrating peptides appeared at the very top of the gels for all treatments. More degradation was observed in bands representing γ- casein in cheese samples treated with TGase, the intensities of these bands were decreased in (T5) and other treatments (T4, T3 and T2) respectively (Lanes 11, 9, 7 and 5) compared to control, these bands nearly disappeared after 21 day of storage.

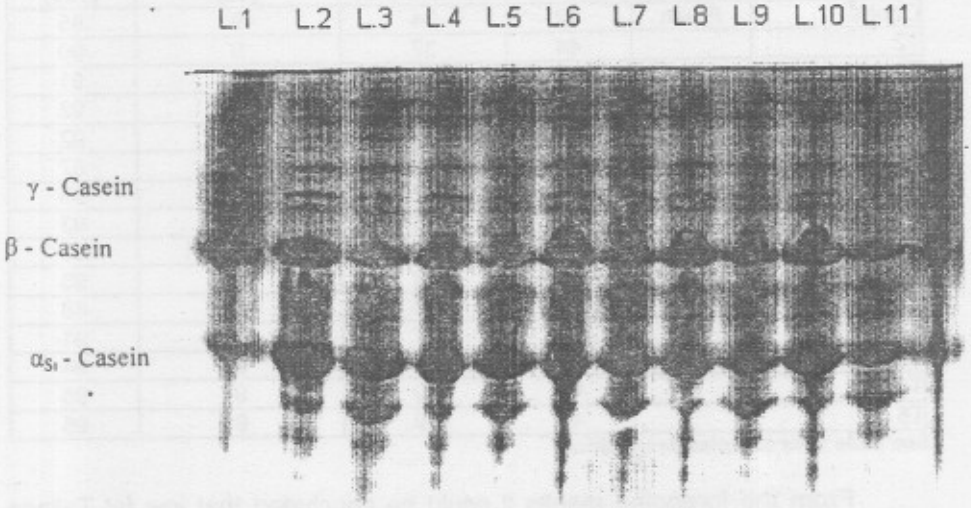

Fig (3) : Urea - PAGE of low fat Tallaga cheese treated with TGase
Lane (1) sodium caseinate (ST) ; Lanes (2&3) control ;Lanes (4 & 5) T2 ; Lanes (6 & 7) T3 ; Lanes (8 & 9) T4 ; Lanes (10 & 11) T5 .

Organoleptic evaluation :

Table (5) represents the average results of the sensory evaluation in terms of flavour, body & texture and appearance of the final product .

The results obtained revealed that cheeses made from cheese milk treated with TGase at levels 0.4-0.5 g /L had higher scores compared with control which received the lowest score for flavour, body and texture (slight harsh) and consequently for total scores .On the other hand, samples made from milk containing 0.2-0.3 g TGase /L milk received in between scores. Although all cheeses were accepted by panelists, the most acceptable cheese were cheese from T4 and T5 .The obtained cheese had an excellent flavour, texture and appearance , they are smooth , had a fat like emulsion and have a good taste. The effect of preheating of milk and addition of TGase and a suitable incubation period were more apparent on body and texture of low fat Tallaga cheese. The firmness of cheese curd significantly increased by the reaction of transglutaminase cross-linkage .Thus treatments 3, 4, and 5 received higher scores for firmness .Furthermore ,when the level of TGase added was increased smooth textured of Tallaga cheese were obtained. These results were in accordance with Han *et al.*, (2003) who reported that the texture of cheese significantly improved by transglutaminase cross - linking .Prolonged storage to 14 and 21 days increase flavour, body and texture scores for all cheese treatments .

Table (5): Organoleptic properties of low fat Tallaga cheese during storage

Treatments	Age (days)	Flavour (50)	Body & Texture (40)	Appearance (10)	Total (100)
Control	Fresh	42	34	9	85
T2		44	37	9	90
T3		44	38	9	91
T4		45	38	9	92
T5		45	38	9	92
Control	14	43	35	9	87
T2		44	37	9	90
T3		46	38	9	93
T4		46	38	9	93
T5		46	38	9	93
Control	21	44	35	9	88
T2		45	37	9	91
T3		46	39	9	94
T4		47	39	9	95
T5		47	39	9	95

See table 1 for samples designation

From the foregoing results it could be concluded that low fat Tallaga cheese can be made from milk treated with 0.4 - 0.5 g /L TGase in order to obtain cheese of acceptable texture , with high yield and less syneresis while maintaining the necessary firmness and good body .Moreover addition of TGase to cheese milk will lead to products having pleasant consistency and mouthfeeling .

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تأثير إنزيم ترانس جلوتاميناز (TGase) على جودة جبن الثلاجة المنخفضالدهن

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يوجد إنزيم Transglutaminase على نطاق واسع في كثير من الأنظمة الحيوية ، حيث يمكن الحصول علية من الكائنات الحية الدقيقة ، من مصادر نباتية ، من الأسماك ، من مصادر حيوانية و خاصة الثدييات و من الإنسان . لهذا الأنزيم القدرة على ربط السلاسل البروتينية ببعضها البعض عن طريق روابط معينة و تكوين شبكة بين جزيئات البروتين .

فى هذا البحث تم استخدام هذا الأنزيم في تصنيع جبن ثلاجة منخفض الدهن من لبن بقرى معدل إلى نسبة دهن ٢ % ، حيث تم تصنيع ٥ معاملات ، المعاملة الأولى (معاملة المقارنة) بدون اضافة الأنزيم و المعاملات الأخرى (٢ ، ٣ ، ٤ ، ٥) استخدم الأنزيم فيها بتركيزات ٠،٢ ، ٠،٣ ، ٠،٤ ، ٠،٥ جرام لكل لتر من اللبن على الترتيب . صنعت الجبن بالطريقة التقليدية لتصنيع الجبن الدمياطي . ثم تم تخزين الجبن على درجة ٧ ° م ± ٢ ° م لمدة ثلاثة أسابيع ، و أجري تحليل الجبن طازجا و أسبوعيا .

وجد أن إنزيم TGase لة تأثير كبير على زيادة تصافى الجبن و تقليل معدل انفصال الشرش . كما أظهرت النتائج زيادة نسبة الجوامد الصلبة الكلية و كذلك النيتروجين الكلى فى عينات الجبن المعامل بإضافة الأنزيم للبن المستخدم في صناعتها عن جبن المقارنة (بدون إضافة الأنزيم) . أدى تخزين الجبن إلى انخفاض كل من الرطوبة و النيتروجين الكلى بينما أدى إلى زيادة الحموضة و الدهن و النيتروجين الذائب .

و قد أظهرت النتائج انخفاض البروتين الكلى و الجوامد الكلية فى الشرش الناتج من الجبن المعامل بإضافة الأنزيم ، و تزداد درجة الانخفاض بزيادة تركيز الأنزيم مقارنة بالشرش الناتج من جبن المقارنة .

كما وجد إن إضافة الأنزيم تؤدى إلى زيادة اللزوجة الظاهرية و اللزوجة البلاستيكية و درجة اندماج الخثرة للجبن الناتج . كنتيجة لزيادة اللزوجة يقل انفصال الشرش (syneresis)

كما أظهرت التقديرات الحسية أن الجبن المصنع بإضافة الأنزيم حصل على تقديرات أعلى بالنسبة للقوام و التركيب و الطعم والنكهة – حيث تمتع الجبن المعامل بالأنزيم بقوام صلب مقفل و تركيب مندمج و ناعم و بطعم غنى و مقبول .

و بذلك يمكن تصنيع جبن ثلاجة منخفض الدهن باستخدام إنزيم Transglutaminase بتركيز ٤٫٠ – ٥٫٠ جرام / لتر لبن مسخن الى ٧٥ ° م / ١٥ ث .