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QUALITY OF KAREISH CHEESE TREATED BY TRANSGLUTAMMINASE

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ABSTRACT

Kareish cheese was manufactured by using *Lc.Lactis* ssp *lactis* with Transglutamminase (TGase) at levels of 0.00, 0.02,0.03 and 0.04 %, stored in refrigerator at 5°C±1 for three weeks and examined periodically for chemical, microbiology and sensory properties. Protein fractionation profiles and microstructure were also studied. TGase treated fresh cheese gave higher yield and moisture contents and lower acidity value than the control. A minor decline of total bacterial viable count was found in cheese treated samples compared to the control one after one week of cold storage. Contaminating non-starter microorganisms (i.e., coliforms, yeasts & moulds) were not found in treated cheese samples during the 3-weeks of cold storage.

SDS-PAGE analysis showed clear differences in protein patterns and bands between the control and cheese treated with TGase. TGase caused aggregates of some proteins in β-casein region, increasing the TGase level led to more aggregates. Scanning electronic microscope showed that the use of TGase had a clear remarkable effect on the cheese protein matrix when compared to control cheese. Kareish cheese treated with TGase had a more compact structure and fine creamy gel texture.

The higher score of sensory evaluation was recorded for the higher levels of TGase treated cheese. Increasing the level of TGase increased the improvement of body and texture. It could be concluded that a good quality Kareish cheese could be made from milk treated with 0.04% TGase in order to maintaining the good body and texture. Moreover treated the cheese milk with TGase led to a product with pleasant consistency and mouth feeling.

Keywords: Transglutamminase, kareish cheese, yield, microstructure.

INTRODUCTION

Kareish cheese is an acid dairy product which is widely used in Egypt. It is a white, soft cheese containing about 70% moisture and not more than 10% fat , and is made from sour milk, either laban rayeb or buttermilk. Skim milk, fresh or slightly fermented, is also used at commercial scale for its manufacture in small dairy plants. Meanwhile, it becomes very popular because of its remarkable health quality as the only known fat free cheese consumed by the Egyptians (Hofi *et al.*, 2004). Kareish cheese made from laban rayeb is generally much better in quality and has a better flavor than that made from butter milk, which in turn is better than that made from skim milk . This can be attributed to favorable fermentations which may take place in milk during setting and to the higher fat content of laban rayeb compared to the other two products. The chemical composition of Kareish cheese varies from sample to sample, depending on the type of material used in cheese manufacture, and is as follows: moisture, 69.0%; total solids, 31.0%; protein, 17.0%; fat, 6.0%;

other organic materials, 0.2%; ash, 0.6%; salt, 4.5%; calcium, 0.2% and phosphorus 0.24% (El- Gendy, 2001).

Three-dimensional network of cheese is mainly linked together by physical cross-links, like hydrophobic interactions, van der Waal forces, hydrogen bonds and electrostatic interactions. These links are rather week and flexible compared to chemical cross-links, with permanent covalent bonds (Dickinson, 1997). Transglutaminase (TGase) is a protein-glutamine: amine γ-glutamyltransferase, which catalyses reactions, which could render corporation of an amide to the peptide chain or a protein cross-linking. Cross-linking reactions results in the formation of dimmers, trimmers and larger protein polymers. Without any interference, cross-linking will continue until glutamines or lysines become unavailable to the enzyme. The use of TGase in dairy products is so far only in the experimental stage, but there seems to be many applications results generally in products with higher strength and lower permeability and synersis (Faergemand and Qvist 1997; Lauber *et al.*, 2000; Lorenzen *et al.*, 1999; Nonaka, *et al.*, 1992; Abou El-Nour *et al.*, 2004). In the area of cheese production, TGase treatments can reduce cheese losses, improve water retention in reduced fat cheese and in turn increasing cheese yield, improve cheese quality and strongly improved the Body and texture of the cheese (Loranzen *et al.*, 2002 and Han *et al.*, 2003). TGase can be used in stabilizing products like yoghurt, fresh cheese, whipping cream and novel milk products (Lorenzen *et al.*, 2000; Abou El-Nour *et al.*, 2004 and Mleko *et al.*, 2004).

The aim of the present study was to investigate the possibility of improving the overall quality of Kareish cheese i.e. yield, microstructure and keeping quality by using TGase as a protein cross-linking enzyme.

MATERIALS AND METHODS

Materials

Milk: Buffalo's milk was obtained from Faculty of Agriculture, Suez Canal University and skimmed in Diary Science Department.

TGase: transglutaminase was obtained from Ajinomoto Europe Sales (Stubbenhuk 3, D-20459, Hamburg, Germany); the declared activity of the preparation was approximately 100 units/g.

Starter culture: *Lactococcus lactis* ssp. *lactis* (DSM 90501) was obtained from the culture bank of the institute of microbiology BFEL, Kiel, Germany.

Methods

Kariesh cheese Manufacture:

Kareish cheese was processed as shown in Figure (1). Resultant cheese was packed into plastic containers and stored in refrigerator at 5 ±1° C for three week. Samples were taken periodically after 24 hr (fresh), 1, 2 and three weeks. Whole experiments were repeated three times and the results are average of three replicates.

Chemical analysis:

Fat, total and soluble nitrogen, dry matter, was analyzed by standard methods of Marshall, (1992). Cheese samples were checked for pH using

(Jenway pH meter electrode No. 29010, Jenway limited, England). The cheese yield was recorded as kg of cheese/100kg of cheese milk. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE 15%) was performed by the method of Laemmli (1970). The proteins contained in cheese samples treated with or without TGase, was performed. The densitometric analysis of the stained bands was performed using the Gel Docu-Advanced Program (El-Manar Co. Cairo, Egypt).

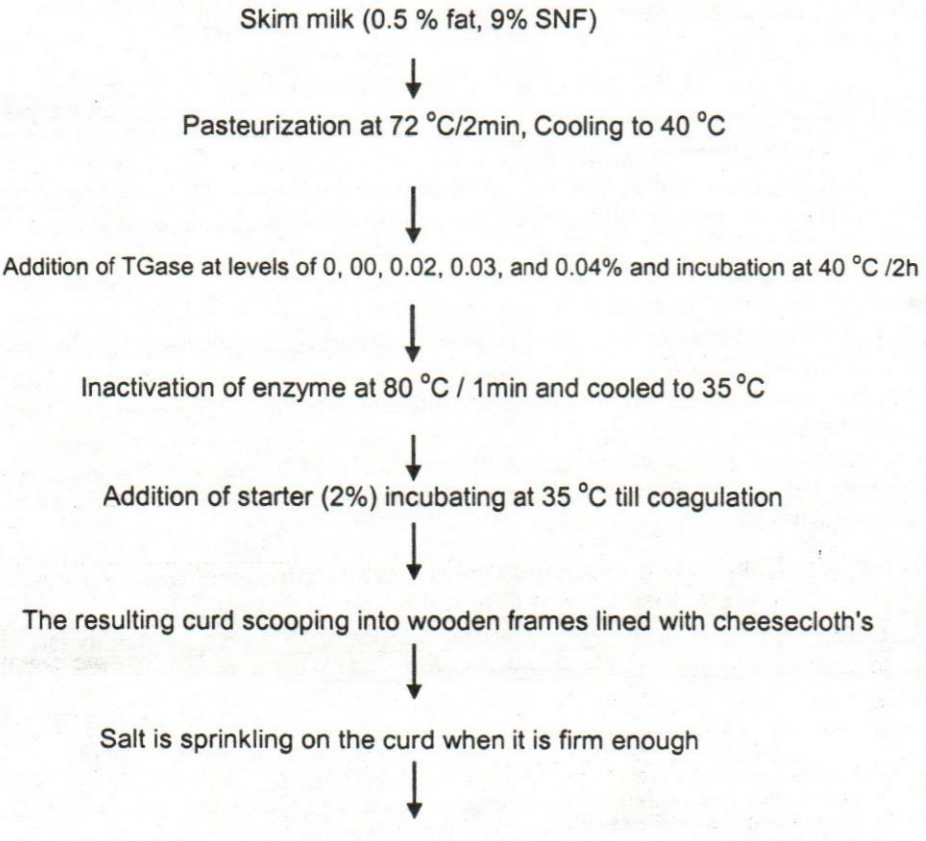

Fig (1): Flow diagram for the production of TGase treated Kareish cheese

Microbiological analysis:

Cheese samples were analyzed for total bacterial count using skim milk agar medium, for coliform count using McConkey agar medium as recommended by the American Public Health Association (1978), and for yeast & moulds count using YM agar medium (Merck).

Scanning Electron Microscopy (SEM) analysis:

Scanning electron microscopy studies were carried out by Etching method, which performed according to Reimer (1985). Cheese Samples

were cut to small right angle pieces, fixed with 3% glutaraldhyde for three hours, dehydrated with serial concentration of acetone, then critical point dried and spotted with gold for identification by "SEM". All specimens were observed with a SEM (Jeol T330A, Japan) and the images were taken at 15KV according to Reimer (1985).

Organoleptic assessment:

The sensory evaluations were assessed by the staff of the Dairy Department, Suez Canal University using the following scale for flavour, (40 points) and body and texture (60 points) according to Han (2003).

RESULTS AND DISCUSSION

Chemical composition of the whey obtained from Kareish cheese treated with different levels of TGase

Table (1) reveals total solids (TS), total nitrogen (TN), total protein (TP) contents, acidity and pH of whey drained from cheese of different treatments as affected by TGase levels. Results cleared that TS obtained form whey gradually decreased as the level of TGase increased. Also, TN and TP contents decreased in the recovered whey with increasing dosage of TGase compared to control. These results coincided with those of Han *et al.*, 2003 and El-Kholy, 2005; they reported that the decreased protein content in recovered whey is caused by the cross-linking of some of whey proteins to the cheese proteins by TGase. Slight differences were observed on acidity and pH of whey, acidity decreased with increasing the level of TGase, pH values had an opposite trend of acidity of the corresponding drained whey.

Table (1): Chemical composition of the whey obtained from Kareish cheese treated with different levels of TGase

Treatments	TS (%)	TN (%)	TP (%)	pH	Acidity (%)
T0	13.21	0.174	1.11	4.44	0.64
T1	12.87	0.152	0.996	4.50	0.62
T2	12.78	0.149	0.950	4.52	0.61
T3	12.74	0.146	0.931	4.53	0.60

T_0: Whey obtained from Kareish cheese containing 0.00 % TGase (control)
T_1: Whey obtained from Kareish cheese containing 0.02 % TGase
T_2: Whey obtained from Kareish cheese containing 0.03 % TGase
T_3: Whey obtained from Kareish cheese containing 0.04 % TGase
TS: total solids; TP: total protein; TN: total nitrogen

Yield and gross composition of TGase treated Kareish cheese.

Yield:

Data gave in Table (2) indicate that TGase treated fresh cheese gave higher yield than the control. This may be attributed to higher moisture content in the former than in the latter. Lorenzen and Schlimme, 1998 reported that TGase treatments increase the water binding capacity of cross linked protein. And could be attributed to whey protein retained in cheese curd. These results are in accordance with those given by Budtz, 2001and El-Kholy, 2005 they reported that TGase increased the amount of protein left in the coagulated cheese material as compared to cheese made without

TGase. Budtz, 2001 and Han, *et al.*, 2002 stated that TGase treated cured contained smaller protein particles with less empty space which reduced syneresis. The yield was increased with the increasing TGase level. Prolonged storage decreases the yield for all cheeses mainly due to the loss in moisture.

Acidity and pH:

It was noticed that TGase treated cheese had relatively low titratable acidity values than control which can be attributed to TGase capable to catalyzing the diamidition of glutamine residues where by water is used as nucleophil and ammonia liberated (Ikura *et al.*, 1980; Lorenzen and Schlimme, 1998) also O' Sullivan, *et al.*, (2001); Abou El-Nour, *et al.*, (2004) and El-Kholy, (2005) reported that the development of pH in products made from TGase treated milk was slower than control treatments. In addition, acidity of all samples increased gradually a long the cold storage to rich maximum values after 21 days. Similar observations were reported by (Abdel Kader, 2003; Salama, 2004 and El-Kholy, 2005).

The change in pH values were negatively related to the changes of acidity. Fresh cheese samples had pH values ranged from 4.56 to 4.63, in the 7^{th} day of storage, the pH did not change and after 21 days the pH dropped slightly to 4.47-4.54. Hence, the pH of the cheese remained almost stable (≈ 4.5) during the 21 days of storage which could be attributed to high buffering capacity of cheese proteins (Kailasapathy *et al.*, 1996 and Abbas *et al.*, 2006) reported similar results for yoghurt.

Total solids content:

The data presented in Table (2) show an increase in the TS content of the control than TGase cheese. This could be attributed to the considerable increase in water binding capacity of cross linked protein in cheese treated with TGase (Lorenzen and Schlimme, 1998). The TS content of cheese from all treatments increased as storage period progressed due mainly to the decrease of moisture content.

Nitrogen content:

Cheese milk treated with TGase led to an increase in the TN/TS content compared to control cheese and these were proportional to the amount of added TGase. These results are in accordance with those given by (Budtz, 2001and El-Kholy, 2005), they reported that TGase increased the amount of protein left in the coagulated cheese material as compared to cheese made without TGase. The TN contents of all samples increase throughout storage due mainly to the decrease in moisture content (Ezzat, 1990). On the other hand SN/TN decreased in cheese treated with TGase, this may be due to cross linking reactions between protein-protein, peptide-protein and amino acid-protein results in the formation of dimmers, trimmers and larger protein polymer Neve *et al.*, (2001). Further decrease in SN/TN of Kareish cheese was obtained by increasing the level of TGase added. In all samples the SN increased as storage progressed.

Microbiological properties

Results given in Fig (2) indicated that Kareish cheese made from milk treated with TGase (T3 and T4) had a slightly higher total count (TC) than other treatments when fresh and after 7 days of storage. A slightly decreased

in the total count was observed in T1, T2 and T3 than control during storage. However, the increase the addition of TGase at percentage ranged from 0.02 to 0.04 led to decrease in the total viable count during storage period. This may be due to cross linking reactions between protein-protein, peptide-protein and amino acid-protein results in the formation of dimmers, trimmers and larger protein polymer (Neve *et al.*, 2001), which cause shortage of some growth factors.

Table (2): Yield and gross composition of TGase treated Kareish cheese.

Treatments	Age (days)	Yield (%)	Acidity (%)	pH	TS (%)	SN (%)	TN (%)	SN/TN (%)
T0	Fresh	19.21	1.29	4.56	27.90	0.29	2.37	12.23
T1		23.92	1.25	4.60	25.92	0.25	2.36	10.77
T2		24.12	1.22	4.61	25.88	0.25	2.30	10.86
T3		24.19	1.21	4.63	25.80	0.24	2.32	10.61
T0	7	18.71	1.34	4.53	28.60	0.33	2.40	13.75
T1		23.56	1.28	4.60	26.20	0.26	2.40	10.83
T2		23.89	1.25	4.60	26.32	0.26	2.35	11.06
T3		24.10	1.23	4.61	26.40	0.25	2.33	10.72
T0	14	18.52	1.49	4.51	28.70	0.38	2.45	15.51
T1		23.11	1.32	4.58	26.30	0.29	2.50	11.60
T2		23.52	1.29	4.59	26.40	0.28	2.47	11.33
T3		23.77	1.24	4.60	26.50	0.26	2.40	10.83
T0	21	18.33	1.64	4.47	28.70	0.43	2.47	17.40
T1		22.85	1.42	4.49	26.35	0.33	2.52	13.09
T2		23.00	1.41	4.52	26.41	0.32	2.54	12.59
T3		23.22	1.37	4.54	26.52	0.29	2.60	12.23

T_0: Kareish cheese containing 0.00 % TGase (control)
T_1: Kareish cheese containing 0.02 % TGase
T_2: Kareish cheese containing 0.03 % TGase
T_3: Kareish cheese containing 0.04 % TGase

During storage total count of all treatments gradually decreased, this may be due to the effect of the acidity on the different microbial groups (Hammer and Babel 1957).

Coliform bacteria and yeasts & molds were not detected in 1.0 g of TGase Kareish cheese when fresh and during the first two weeks of storage period in all treatments as the results of high hygienic conditions during manufacturing and storage. However, a few amounts of yeast and moulds counts were observed in the control cheese at the end of storage.

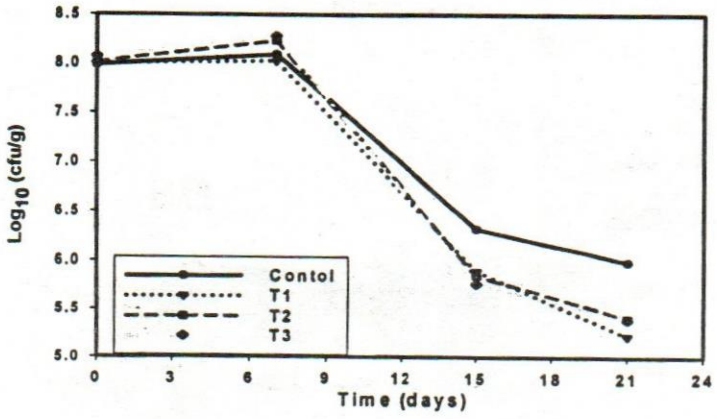

Fig (2): Changes in Total viable count of TGase treated Kareish cheese when fresh and during storage at 5°C ±1 for 21 days.
***See table (2) for samples designation**

Table (3): Coliform bacteria and yeast& moulds total viable count (x ×10 ^{3} cfu/g) of Kariesh cheese treated with TGase during storage period.

Treatments/ Time (days)	*E.coli*	Y&M	*E.coli*	Y&M	*E.coli*	Y&M	*E.coli*	Y&M
	Fresh		7		14		21	
T0	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.037
T1	N.D	N.D	N.D	N.D	N.D	N.D	N.D	ND
T2	N.D	N.D	N.D	N.D	ND	N.D	N.D	N.D
T3	N.D	N.D	N.D	N.D	ND	N.D	N.D	N.D

***See table (2) for samples designation**
Y&M: Yeast & Moulds
ND: Not Detected

SDS-PAGE profiles analysis of cheese samples treated with TGase.

Analysis for SDS electrophoratic patterns of fresh Kareish cheese samples made with different levels of TGase are presented in Fig (3) and Table (4). Analysis of all samples seemed to be divided into four regions, k-casein (in slow moving region) β-casein, αs casein and the fast moving region as monitored by El-Shibiny (1978) and Mahran *et al.*, (1988). SDS-PAGE analysis of all samples shows differences in density and intensity of all protein bands in all treatments and control. Protein aggregates in Kareish cheese treated with TGase was higher when compared to control. The highest aggregation in bands density and intensity were found in β-casein region. On the other hand the lowest aggregates in bands density and intensity were observed in control. Increased the level of TGase led to more aggregates of proteins in β-casein region.

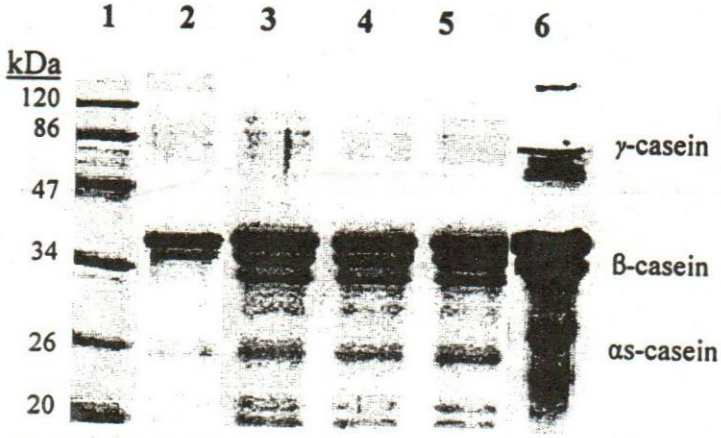

Fig (3):SDS-PAGE analysis of fresh Kareish cheese treated with TGase.

Lane 1: Low molecular weight protein marker; Lane 2: Kareish cheese treated with 0.00% TGase (control); Lane3: Kareish cheese treated with 0.02% TGase: Lane 4: Kareish cheese treated with 0.03% TGase; Lane 5: Kareish cheese treated with 0.04% TGase Lane 6: Sodium caseinte reference.

Table (4): Analysis of SDS-PAGE of fresh Kareish cheese treated with TGase.

Band	Mol.Wt* k.Da	Lane 2 %	Lane 3 %	Lane 4 %	Lane 5 %
1	100-105	23.74	14.18	19.98	14.03
2	55-70	32.14	48.85	50.29	57.07
3	10-14	31.26	25.95	21.95	22.45
4	6-7	12.86	11.04	7.78	6.46

See Fig (3) for samples designation

***Mol.Wt: molecular weight**

Scanning Electron Microscopy (SEM) analysis of Kareish cheese samples

The SEM of Kareish cheese samples treated with different levels of TGase are presented in Figures (4: a, b, c and d). The TGase treated cheese appeared to have a more compact structure compared to control one. Using TGase formed a delicate and fine creamy gel texture. On the other hand, control made using starter culture only without TGase associated with whey separation forming round voids and resulting contraction in smaller masses of casein aggregates with a more porous structure and nonreflecting zones around the compressed casein aggregate. As illustrated, casein appeared as large micelle aggregates separated by non reflective black space. A slight difference between the microstructure of TGase treated cheese can be detected. Increased

homogenous distributed clusters and continues microstructure were evident. Therefore, it could be noticed that the separated clusters or particles had positive effect and gave better closed tied microstructure.

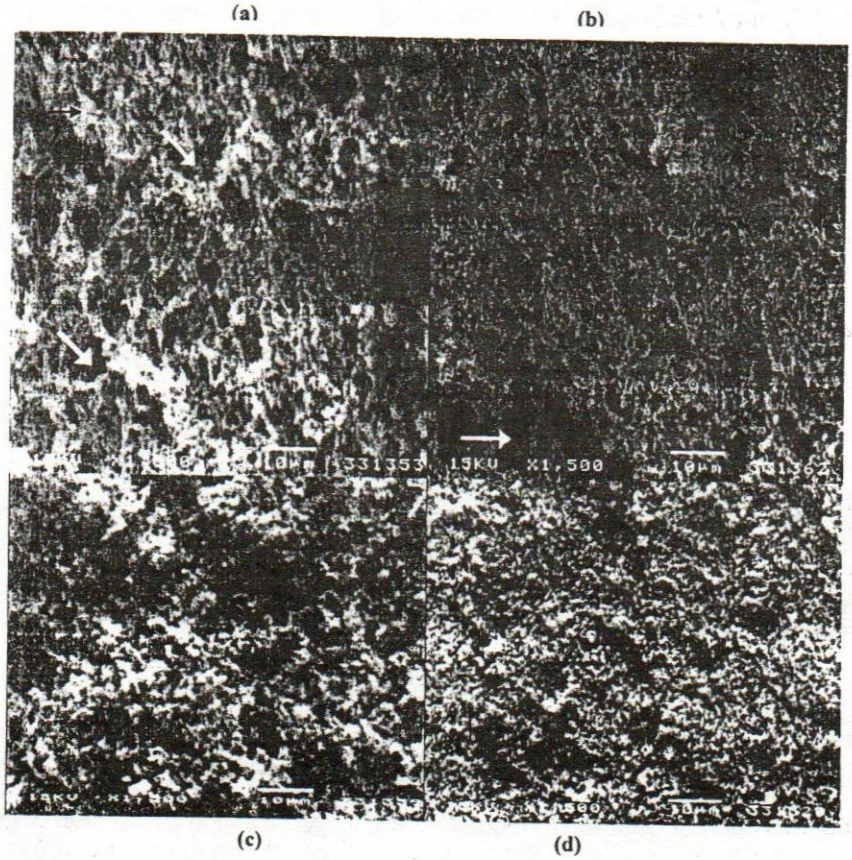

Fig (4: a, b, c and d): Scanning Electron Microscopy (SEM) analysis of TGase treated Kareish cheese samples

a: is control cheese (0.00 % TGase);b, c and d are Kareish cheese treatments treated with TGase (0.02, 0.03 and 0.04 %), respectively.

*** Black arrows: protein matrix, White arrows: serum pockets.**

Organoleptic properties

Table (3) represents the average results of the sensory evaluation of the Kareish cheeses. It was noticed a slight difference in flavour score between fresh control and TGase treated chesses. All cheeses were accepted for flavor by panelists. Prolonged storage improved flavour of all cheeses, but it was more pronounced for TGase cheeses. TGase treatments improved the body and texture of fresh cheese, the cheese had a soft curd and smooth body with no holes. TGase treated cheese had higher scores for body and texture compared to control. Increasing

the level of TGase added increased the improvement of body and texture.The best cheese quality was that made by adding 0.04% TGase. The results are in agreement with those of many investigators (Loranzen *et al.*,2002; Han *et al.*,2003; Abou El-Nour *et al.*, 2004 and El-Kholy, 2005), they reported that the texture of the products improved by TGase cross linking. Prolonged storage increase body and texture scores for all TGase treated samples. However, the body and texture score for control samples decreased during storage.

From the foregoing results it could be concluded that good quality Kareish cheese could be made from milk treated with 0.04% TGase in order to maintaining the good body and texture. Moreover treated milk cheese by TGase led to a product with pleasant consistency and mouth felling.

Table (5): Sensory properties of TGase treated Kareish cheese when fresh and during storage at 5°C±1 for 21 days.

Treatments	Storage period (days)											
	Fresh			7days			14 days			21days		
	B&T (60)	FI (40)	Total (100)	B&T (60)	FI (40)	Total (100)	B&T (60)	FI (40)	Total (100)	B&T (60)	FI (40)	Total (100)
T0	50	32	82	48	35	83	45	35	80	45	32	32
T1	51	33	84	53	35	88	54	35	89	54	33	33
T2	53	33	86	54	36	90	57	37	94	56	34	34
T3	56	33	89	58	37	95	58	37	95	57	35	35

B&T: Body and Texture; FI: Flavour
***See table (2) for samples designation**

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تأثير استخدام إنزيم الترانس جلوتاميناز على جودة الجبن القريش

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٢- جامعه قناة السويس- كليه الزراعة- قسم الالبان

يوجد انزيم الترانس جلوتاميناز Transglutaminase على نطاق واسع فى كثير من الكائنات الحية. ولهذا الانزيم القدرة على ربط البروتينات ببعضها أو ربط البروتينات بالأحماض الامينية مما يزيد من خواص البروتين الوظيفية وتحسين القيمة الغذائية والقوام والطعم والتصافى للناتج.

وفى هذا البحث تم استخدام هذا الانزيم فى صناعة الجبن القريش حيث تم عمل ٤ معاملات، المعاملة الأولى (معامله الكنترول) بدون أضافه أنزيم بينما المعاملات (٢،٣و٤) استخدم فيها الانزيم بتركيزات ٠,٠٢،٠,٠٣و٠,٠٤% على الترتيب. و تم تخرين الجبن الناتج على ٥ °م لمدة ٣ أسابيع و إجراء التحاليل الكيماويه و الميكروبولوجيه والحسيه وايضا تم دراسه التركيب البنائي للجبن الناتج بالميكروسكوب الاكترونى وشقوق البروتين المختلفه بالهجرة فى المجال الكهربي.

وقد زادت تصافى الجبن المعامل بالانزيم وارتفع المحتوى الرطوبي به عن الجبن الغير معامل. أظهرت الاختبارات الميكروبيولوجيه إن الجبن المعامل احتوى على عدد ميكروبى كلى اقل بعد اسبوع من التخزين، كذالك لم يظهر اى تلوث ناتج عن الفطريات اوالخمائر وبكتريا القولون طوال مدة التخزين.

تحليل SDS-PAGE لبروتين الجبن الطازج أظهرت اختلافات واضحة في شكل شقوق البروتين وكثافتها بين الجبن المعامل بالأنزيم وجبن الكنترول،كذالك وجد إن زيادة تركيز الإنزيم أدى إلى تأثير واضح خاصة فى منطقه الـ β-casein. أظهرت النتائج المتحصل عليها من فحص التركيب البنائى الدقيق بالميكروسكوب الأكترونى الماسح (SEM) وجود اختلافات في التركيب البنائى بين الجبن المصنع باستخدام إنزيم TGase و جبن عينة الكنترول، حيث تميزت عينات الجبن المعامل بتركيب بنائى مندمج متماسك وقوام ناعم كريمي مقارنه بعينه الجبن الغير معامل. هذا فضلا عن إن زيادة تركيز الأنزيم أدت إلى تحسن واضح في الشكل البنائى للجبن المتحصل عليه.

وأظهرت التقديرات الحسية حصول الجبن القريش المعامل بالانزيم على تقديرات أعلى بالنسبة للطعم والقوام ولم تكن لزيادة نسبة الأنزيم تأثير واضح على هذه الخواص .

وبذالك أمكن تصنيع جبن قريش له صفات جودة كيماوية، بنائية، تكنولوجية و حسية عالية وقوام مند مج متماسك ناعم وطعم مقبول باستخدام انزيم الترانس جلوتاميناز حتى تركيز ٠,٠٤%.