

THE USE OF TG IN MANUFACTURING FULL FAT MOZZARELLA CHEESE

Metwally, M.M.E.¹; Hoda Elzeny¹ and Enas F. Gazar²

¹ Dairy Tech. Dept., Faculty of Agric., Cairo Uni., Giza, Egypt.

² Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

ABSTRACT

A method was devised to use transglutaminase enzyme (TG) in rennet coagulated cheeses. Two methods of mixing both enzymes were used. First Rennet was mixed with milk at 5°C for 30 min followed by the TG at 5° C and was left for this before using the temperature to 40° c for coagulation. The second method both enzymes were mixed with milk simultaneously at 5°C and the mixture was left for 2 hours before raising the temperature to 40°C. Full fat mozzarella cheese was manufactured using 2 level of TG according to the above first method. The two levels of enzymes (0.2 g/l , 0.5 g/l). Mozzarella cheese firmness meltability and stretchability as well as the other physical properties both TG levels. Resulted in softer body & richer flavor. particularly at the .5 u/l concentration. From the organoleptic & physical starchy of mozzarella. The 0.2 u/l level was recommended

INTRODUCTION

Currently, transglutaminase TG is the only commercial covalent cross linking enzyme available for dairy product improvements. Cross – linking reactions may lead to a modification of functional properties of proteins such as solubility emulsifying capacity, foaming and legation prosperities for example, in set yogurt, TG increased gel strength, reduced syneresis with a dry smooth gel surface. In Quarg cheese, TG resulted in fewer firms, less grainy and creamier cheese. (Mylarinen *et al.*, (2007), Jaros¹ *et al.*, (2006)

Mozzarella cheese has unique functionalities in both unmelted and melted states. In un melted state, credibility to uniform size and over all taste and in melted state multiplicity, free oil formation, stretchability and browning are the major functionalities these functionalities (Imm *et al.*, (2003) are governed by various factors such as composition, additives, processing so lido, first content.

Conditions as storage (Pastorino *et al.*, (2003), Sheehan *et al.*, (2004)) of the composition so lido first content sodium chloride & calcium contents are major factored thigh salt & low fat caused the hydration of protein, thus seducing the firmness and improving the meltability. For proper mizzen characteristics balance between salt, Ca& pH is needed.

Mozzarella microstructure is formed of protein fibers, as the backbone of the curd, which are Aome what paroled but with dentition due to but globules. Between these protein fifiers were channels which are filled with fat globules, bacterial cells and chaste serum. Fat globules forming a non-interacting filler preventing the condense of protein strands (Melko *et al.*, (2004) and Badawi, *et al.*, (2004)

Since TG affects product functionality such as forming smooth, firmer gel with lower why synerises and even resulting more product yield

Myllarinen *et al.*, (2007),), it definitely would help in improving mozzarella cheese performance.

However, the use of TG for processing ripened cheese faces a coagulation problem. Milk treated with TG does not coagulate with rennet, the enzyme, though the covalent cross-linking bridges blocks the primary coagulation step proximity by reducing the accessibility of k-casein in to rennet this is a steric hindrance effect, particularly TG cross-links the micelle intramolecularly as well as the dissociation of TG-casein in low this problem led scientists do think that it is unlikely that enzymatic modification with TG.

Will be useful in the manufacture of rennet coagulated cheese (Jaros1 *et al.*, 2007) However after finalizing this research, abatement was published on 2005 (Kumazawa *et al.*, 2005) which proposed two methods for milk coagulation with rennet and TG. Rennet was added to milk kept at low temperature for a period of time, then TG was added and the mixture was kept at cold temperature for a certain period of time then the mature temperature was period for coagulation. In the second method rennet and TG were added simultaneously to milk, the mixture was kept at low temperature for a period of time then the temperature was raised to 30°C for coagulation. Therefore this research was carried out to find a solution of the above problem and devise a methods that allows the use of TG in rennet coagulated cheeses- Secondly, the use of TG in processing full fat mozzarella cheese to study, the enzyme modification for cheese properties & functionality

MATERIALS AND METHODS

Fresh raw cow's milk was obtained from Dairy Science and Technology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. *Streptococcus salivarius subsp. Thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* were obtained from Hansen laboratory (Denmark). Calf rennet powder was obtained from Chr. Hansen's laboratories Denmark. Transglutaminase was a gift from Ajinomoto Europe Sales GmbH, Hamburg. Dry coarse commercial Sodium chloride was obtained from EL-Nasr Co., Alexandria, Egypt.

Fresh cow's milk was pasteurized (72°C/15s) and used for manufacture of mozzarella cheese according to the method of transglutaminase was added to milk with 2 levels (0.2 & 0.5 g/l of milk) and method of mixing was according to flow diagram in figure (1) cheese was stored at 4°C for 28 days. Cheese was sampled at 7, 15, 21 & 28 days for analysis

Moisture, Titratable acidity and salt were determined according to A.O.A.C (1990) ., Fat according to Ling (1963) and nitrogen contents and fractions using semi-microkjeldahl method according to SMEDP (1985).

Meltability of cheese was measured using the meltability test according to Olson and Price (1958) with the modification by Rayan *et al.*, (1980). Cylinders of cheese samples (15g) were put in melting tubes and placed at 110°C for 30 min. The distance of flow from the reference line to the

leading edge of the melting cheese was measured in cm and recorded as cheese meltability.

The stretchability of Mozzarella cheese was measured using an iron bar test as reported by Davis (1966).

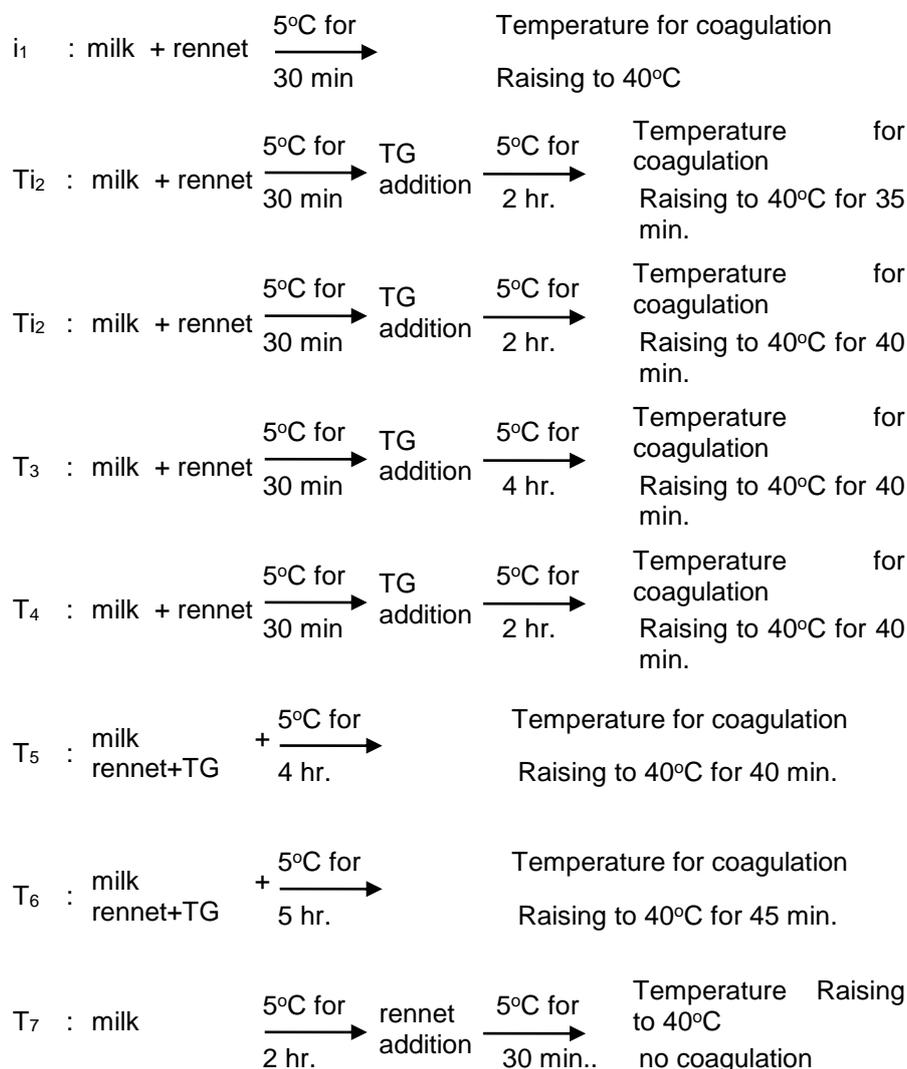


Fig (1): Methods of mixing milk with rennet & TG and coagulation conditions

The method described by Kindstedt and Fox (1991) was adopted for estimation of free oil (oiling off %) in melted mozzarella cheese using Gerber fat testing equipment.

Fat leakage of mozzarella cheese was evaluated as described by Bertola *et al.*, (1996)

Milk viscosity measurements on renneting were carried out in triplicates over temperature of 40°C using a concentric cylinder Brookfield Programmable viscometer (Model DV -11+; Brookfield Engineering Laboratories, USA) with UL adaptor and ULA spindle over a shear rate of 12.2 S⁻¹. The milk samples were allowed to temper at 40°C for 10 min. Prior to measurements, Win Gather version 1.1 (Brookfield Engineering Laboratories, Inc., copyright ©1995) Software was used to collect, store and plot the data on a personal computer to the viscometer.

Texture properties of curd (penetration test) and cheese (compression) samples were evaluated using texture analyzer (CNS-Farnell, Borehamwood, Hertfordshire, England). Cheese samples presented to the instrument were 1±0.1 cm cubes. ATA15-45° Perspex cone was used as the probe with a penetration of 10 mm at 0.5 mm/s. Samples were allowed to equilibrate at ambient temperature for approximately 30-45 min prior to testing.

For fixing cheese microstructure, cheese cubes (3x3x10cm) were prepared from samples of stretched cheese, which were fixed in 4% glutaraldehyde in 0.1M phosphate buffer at pH 7.2 for 2 hr to fix the protein. The cubes were washed several times in 0.1M phosphate buffer at pH 7.2 for 15 min intervals, then post fixed in 1% osmium tetroxide (OsO₄) in 0.1 M phosphate buffer for 1-2 hr for fat fixation. The cheese samples were re washed several times in 0.1 M phosphate buffer for 15 min intervals. Then specimens were dehydrated in series of aqueous ethanol solution (25%, 50%, 75%, 95% and 100%) for 15 min each. The samples were dried to critical point using CO₂ in a Critical point drier (Poland, Waterford, England), and mounted on aluminum SEM stubs, sputter-coated with gold (Spi module sputter coater, Spi Supplies division of Structure Probe, Inc.). Samples were examined at 5 KV through Scanning Electron Microscope JEOL-JSM5200 equipped with an IBM-compatible computer to recording the images.

The actual percentage of calcium, fat and protein recovered in cheese or lost in whey and stretching water were calculated. Theoretical yield was calculated with the modified Van Slyke formula as described by Metzger *et al.*, (2000). The original Van Slyke formula was based on cheddar cheese yield and was modified for mozzarella cheese. The modification included changing the assumed fat recovery from 0.93 to 0.85 and changing the constant factor from 1.09 to 1.13.

Yield (kilograms/100Kg of milk) = [(0.85 × milk fat %) + (milk casein% - 0.1) × 1.13] / [1 - (cheese moisture/100)].

Yield efficiency was determined by dividing the actual yield by the theoretical yield multiplied by 100.

The cheese samples were organoleptically evaluated, by dairy Dept. staff members, score points for flavor, body and texture as well as appearance were 50, 35, 15 respectively and this was according to the method of Scott (1981).

The two way statistical analysis of variance (ANOVA), mean separation, correlation and factorial was performed by running the MSTAT-C (ver.2.10, Michigan state university, USA.) package on a personal computer. The same program was used to analyze a factorial analysis of

variance completely randomized design. The statistical significance of the data was determined using *p* value less than 0.05.

RESULTS AND DISCUSSION

Results deal with the following 2 points

a- Methods for the use of TG in rennet coagulation of milk

b- The use TG in processing full fat mozzarella cheese and to study the enzyme modification for cheese properties and functionality.

a- The use of TG in rennet coagulation of milk

Figure (1) shows the schematic diagram of 7 experimental trials for methods of mixing.

Rennet and TG with milk and the procedures followed for coagulation.

These trials were run on two important facts. The first was that rennet and TG works at cold temperatures though at low velocity and the second was that cusion does not coagulate neither with rennet nor acid at cold temperature (5°C). However the order of mixing both enzymes were found to have an effect, so, if TD is mixed first with milk at 5°C for 5 hours and then rennet was added 5°C for 30 min, coagulation did not take place when the temperature was raised to 40°C so, rennet had to work on k-causin first replacing the glycomacropetides and then TG cross-linking let to work and this will not inhibit coagulation. Two mixing orders were tried, first rennet was added to milk for 30 min at 5°C followed by TG and the mixture was left for within 2 to or 4/T3 hours. Before raising the temperature to 40°C . in this trail milk coagulated in 35 & 40 min, respectively. The second order of mixing, rennet & TG were mixed with milk simultaneously and the mixture was kept at 5°C for 2(T4), 4 (T5) and 5 (T6) hrs. Before reusing the temperature for coagulation.

Milk of T4 & T5 was took 40 min, while TG took 45min these results indicated that there is a certain limit for the extent of T6 reaction other wise, coagulation started to be delayed. Actually when the released ammonia was measured as indication of the extant of TG reaction, these was slight increase . of the released ammonia by time (data not shown) Both mixing orders were found to be working and 2hrs for TG reaction was found to be proper.

Figure (2) presents the effect of the above 6 methods of mixing TG on milk coagulation profile as monitored by viscosity changes until coagulation. Compared to the control, coagulation time shifted to longer period with TG reaction and mixing method.

The control showed the regular coagulation profile of viscosity versus time on rennet action , viscosity increases then there is a slight dip followed by large increase until the coagulum is formed. Viscosity then declined due to coagulum deformation. The figure shows that as TG reaction period increased, coagulation period also increased. Therefore, T2 coagulated faster than T3 and T4 & T5 & T6. The coagulation was greatly delayed when TG reaction increased than 2 hrs so T5 & T6 are interfered in their time of coagulation and the shape of their curve were not normal. Since T2 curve was the closest to the control, it was adapted for mozzarella cheese manufacture.

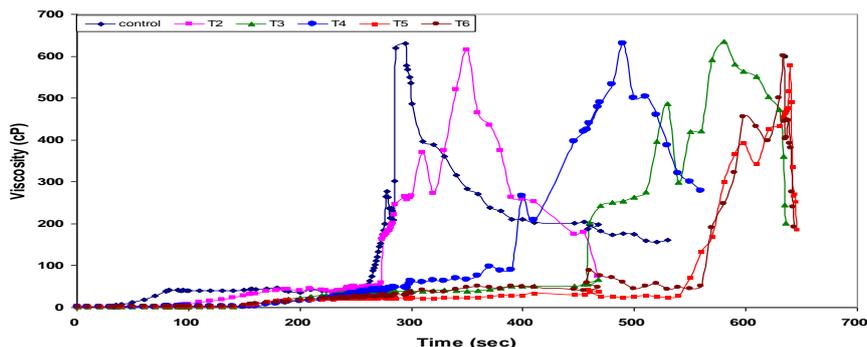


Fig (2).Effect of different coagulation trails on coagulation time and phases of milk coagulation.

b- the use of TG in full fat mozzarella cheese.

Two experimental mozzarella cheese using levels of TG were processed according to the manufacturing flow diagram (Fig3).

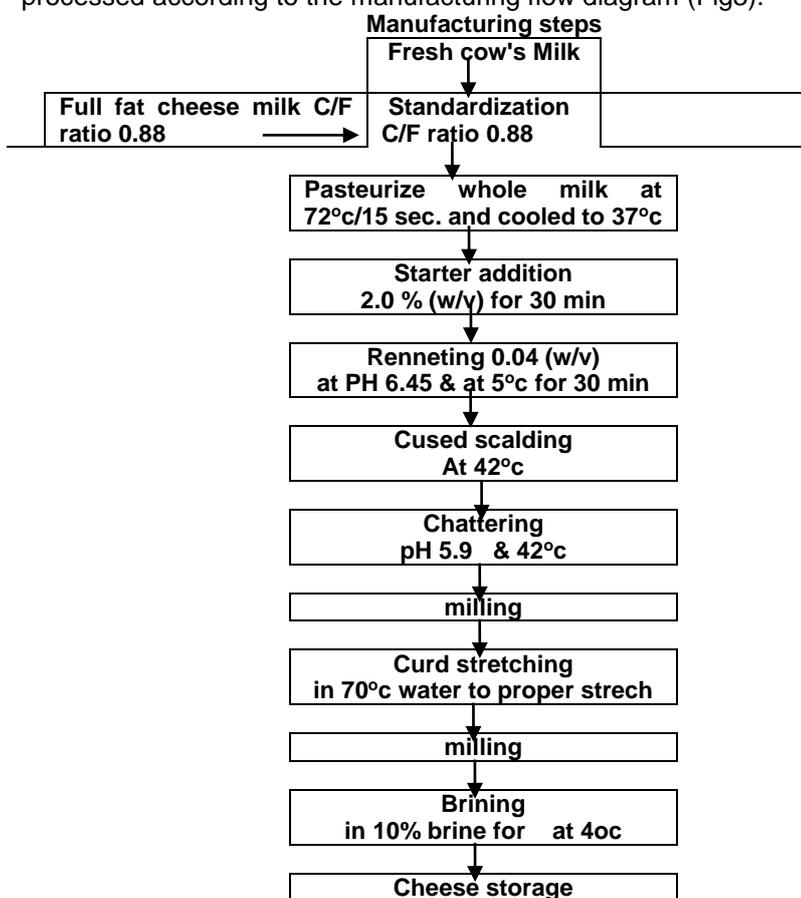


Fig (3) mozzarella cheese manufacture using TG flow diagram

Table (1) shows cows milk composition & Table (2) mozzarella cheese composition. As affected by TG addition and changes during cold storage. TG caused significant increase in moisture content fat and TN through storage. Moisture & fat contented pH decreased by storage. Cheese soluble & non-protein nitrogen contents were lower in TG chesses and effect increased by enzyme concentration.

Table (1) cross milk chemical composition

Component (%)	Full fat milk
DM	11.54
Fat	3.00
F/DM	26.00
Protein	3.38
P/DM	29.30
Casein	2.64
CN/ DM	22.88
C:F ratio	0.88
NPN	0.22
T.A	0.17
pH	6.61

Table (2) effect of TGase enzyme on chemical composition of full fat mozzarella cheese and during storage period.

Treatments	Storage period,(day)				
	Fresh	7	14	21	28
Moisture (%)					
Control	52.16c	51.74d	51.20e	50.77fg	50.03h
0.2 TGA	52.74b	52.32c	51.74d	51.29e	50.67g
0.5 TGA	53.13a	52.73b	52.18c	51.69d	50.95f
Fat/DM					
Control	40.76	40.95	40.99	40.89	40.61f
0.2 TGA	41.12abc	41.21ab	41.20ab	41.02	40.71
0.5 TGA	41.32a	41.08	41.17ab	40.99	40.66
PH					
Control	5.15 abc	5.10bcd	5.05	4.98gh	4.95h
0.2 TGA	5.16ab	5.11bcd	5.07def	5.00fgh	4.97h
0.5 TGA	5.19a	5.14abc	5.08cde	5.02	4.98gh
Acidity					
Control	0.67fgh	0.72	0.75de	0.78bcd	0.86ab
0.2 TGA	0.63h	0.67fgh	0.73def	0.76cde	0.84ab
0.5 TGA	0.62h	0.65gh	0.71efg	0.75de	0.83abc
TN					
Control full	3.37h	3.40gh	3.43fgh	3.47	3.52
0.2 TGA	3.42fgh	3.45efg	3.49	3.53bcd	3.58ab
0.5 TGA	3.48	3.52	3.54bc	3.58ab	3.62a
SN/TN					
Control	4.43m	5.40 j	6.97g	7.76 d	10.11 a
0.2 TGA	4.27n	5.13 k	6.60 h	7.74 e	9.71 b
0.5 TGA	4.05 o	4.99 l	6.31 i	7.23 f	9.09 c
NPN/TN					
Control	2.14 j	2.54 h	2.95 g	3.91 d	4.60 a
0.2 TGA	2.05 k	2.31 i	2.52 h	3.60e	4.18 b
0.5 TGA	1.95 l	2.17 j	2.38 i	3.37 f	4.01 c
Salt/M					
Control	2.33hi	2.41fg	2.55cd	2.68b	2.80a
0.2 TGA	2.24jk	2.34hi	2.47ef	2.68c	2.68b
0.5 TGA	2.18k	2.26ij	2.38gh	2.51de	2.61bc

The yield (table 3) increased by the enzyme addition and by its concentration, 0.5 TG concentration gave significant increase (from .2 into 13.6 %) . yield efficacies were significantly higher in both TG –chesses. The values were (99-8 , 106 and 110 % for control, .2 % TG & 5° TG respectively) Table (4) pints out milk constituents recovery. Significant increase in fat & protein recovery. With TG cheese over. The control these results are expected due to TG cross-linking action, the enzyme complex whey proteins into casein micelles surface.

Table (3) mozzarella cheese actual and theoretical yield as affected by TGase addition.

Treatment	Actual yield (kg/100kg milk)	Theoretical yield(kg/100kgmilk)	Efficiency (%)
Control full	12.00b	12.02b	99.83c
0.2 TGA	12.90ab	12.17a	106.00b
0.5 TGA	13.60a	12.27a	110.84a

Table (4) Recovery of milk constituents in mozzarella cheese as a function of TGase addition

Samples	Fat recovery (%)	Protein recovery (%)
Control	78.0c	76.33c
0.2 TGA	83.5 ^a b	83.39b
0.5TGA	87.81a	89.29a

Table (5) the effect is using TG on whey & starch water

a- whey

Samples	T.S	Fat	Protein
Control	7.04	0.50	0.89
0.2 TGA	6.60	0.43	0.63
0.5TGA	6.38	0.33	0.40

b- Stretch water

Samples	Fat	Protein
Control full fat	0.70	0.08
0.2 TGA	0.40	0.06
0.5TGA	0.27	0.03

More over cross-linking casein strengthen its fibers network these harvesting more moisture , fat & protein & other components, the effect was also manifested in SH/TN & NPN/TN ratios. The effect was clear in Table(5) which shows why & stretch water composition less fat & protein lost in both fluids, when & water.

Table (6) illustrates the functional stretchability & melt ability which are important properties of mozzarella are significantly increased by TG treatment and by enzyme concentration and the trend progressed by storage, stretchability increased from 160 into 200& 220 cm in the control, 2 TG & 0.5 TG, respectively, meltability also improved from 55 in the control cheese into 468 & 73 mm in .2 & .5 TG . chesses. There was no effect of the enzyme on oiling off property. However TG reduced fat leakage.

Table (7) illustrates the analysis of variance of cheese chemical composition & properties, and table(8) shows the analysis of variance of different coagulation trials.

Table (6). Effect of TGase enzyme on functional properties of full fat Mozzarella cheese

Treatments	Storage period (day)				
	Fresh	7	14	21	28
Stretchability (cm)					
Control full	160n	170m	183l	213i	233f
0.2 TGA	200k	210j	223g	241e	259c
0.5 TGA	220h	234f	247d	267b	278a
Meltability (mm)					
Control full	55f	63ef	72	79	87abc
0.2 TGA	68def	75	80	86	93ab
0.5 TGA	73	81	86	93ab	100a
Oil off %					
Control full	4.25e	4.42de	4.78c	5.20b	5.70a
0.2 TGA	4.22e	4.35de	4.63cd	4.97cd	5.63a
0.5 TGA	4.22e	4.33de	4.59cd	4.90bc	5.58a
Fat leakage (mm)					
Control full	57h	64f	73cd	80b	85a
0.2 TGA	55h	60g	68e	74cd	79b
0.5 TGA	51i	57h	64f	72d	75c

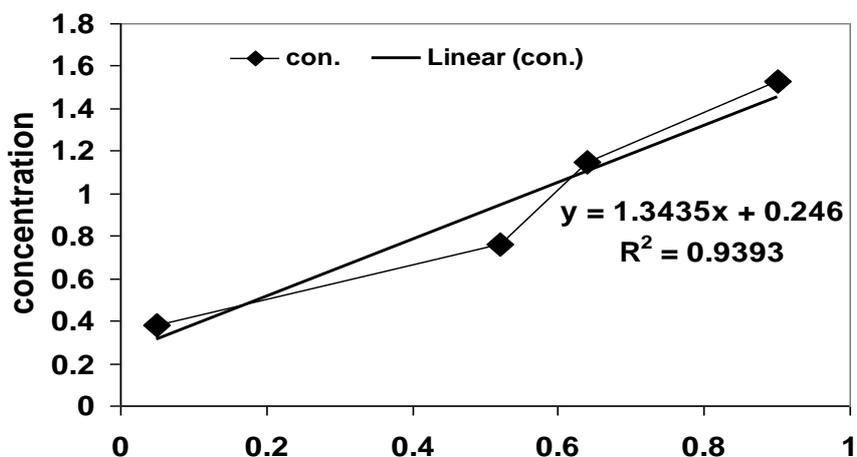
Table (7) Analysis of variances of mozzarella cheese – TG cheese chemical composition & probabilities

Source of variance	Component	P	Correlation	R ²	
					Whey chemical composition %
TGase concentration	Fat	**	-0.92	0.847	
	Protein	***	0.974	0.951	
	TS	**	-0.928	0.873	
	Stretching water chemical composition %				
	Fat	***	-0.854	0.732	
	Protein	NS	0.979	0.893	
	Whey Losses %				
	Fat	***	-0.995	0.989	
	Protein	***	-0.999	0.998	
	Stretching water losses				
	Fat	***	-0.973	0.947	
	Protein	***	-0.993	0.958	
Cheese chemical composition					
Moisture%	TGase con.	***	0.435	0.982	
	Period	***	-0.890		
Fat /DM	TGase con.	**	-0.97	0.941	
	Period	***	-		
pH	TGase con.	***	-	0.974	
	Period	***	-0.964		
Acidity	TGase con.	***	-	0.964	
	Period	***	0.952		
TN	TGase con.	***	0.642	0.975	
	Period	***	0.749		
SN/TN	TGase con.	***	-	0.972	
	Period	***	0.978		
NPN/TN	TGase con.	***	-	0.936	
	Period	***	0.943		
Salt/M	TGase con.	***	-	0.988	
	Period	***	0.908		
Cheese Yield					
Actual	TGase con.	*	0.934	0.876	
Theoretical		***	0.982	0.964	
Efficiency		***	0.997	0.995	
Cheese constituent recovery					
Protein	TGase con.	***	0.99	0.997	
Fat		***	0.997	0.993	

continue Table (7)

Cheese Functional properties				
Stretchability	TGase con.	***	0.706	0.974
	Period	***	0.69	
Meltability	TGase con.	***	-	0.734
	Period	***	0.709	
Oil Off	TGase con.	**	-	0.839
	Period	***	0.909	
Fat Leakage	TGase con.	***	0.536	0.98
	Period	***	0.832	
Cheese Texture				
Hardness	TGase con.	***	-0.997	0.995
Gumminess		***	-0.908	0.825
Cohesiveness		**	-0.873	0.759
Springiness		**	-0.952	0.918
Modulus		***	-0.99	0.998
Adhesiveness		***	-0.941	0.886
Chewiness		***	-0.941	0.886

***<0.001 **<0.01 *<0.05 NS=not significant



Amonia standard curve

Table (8): Analysis of variance of curd texture of different coagulation trails

Source of variance	Component	P	Correlation	R ²
			Cheese Texture	
Hardness	Coagulation trails	***	0.962	0.925
Gumminess		***	-0.966	0.939
Cohesiveness		***	-0.946	0.902
consistency		NS	-	-
Modulus		***	0.971	0.943

Fig(4) illustrates texture analysis of TG cheeses as compared with the control the TG enzyme particularly at 0.2 level improved the cheese physical properties followed by the 0.5 level with significant differences springiness.

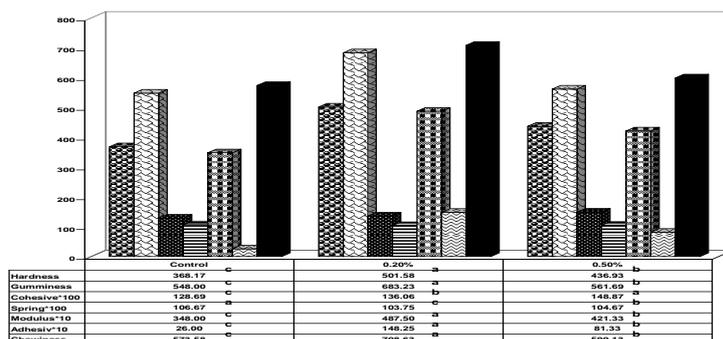


Fig (4): Texture profile of full fat Mozzarella cheese made with TGase enzyme

Table (9) presents the organoleptic properties of TG chesses. The chesses compared well with the control.

Table (9):Effect of TGase enzyme on organoleptic properties of full fat Mozzarella cheese

Organoleptic properties	Control	0.2 TGA	0.5 TGA
Flavor (50)	44a	42 a	43a
Body and texture (35)	33a	35a	34 a
Appearance (15)	14a	14. a	14a
Total	91	91	91

However TG imports strong softening effect on the cheese, particularly the 0.5 level. However, the 2 level was proffered by this is expected from the judges high moisture & contents. In general, TG work usually produces suffer gel with finer particles and with less tendency for 2 moisture .

Fig (6, a,b,c) presents cheese microstructure as viewed by electrons microscope. The control exhibited regular mozzarella structure, protein fibers were some what parallel with smooth surface and their existed between the fibers channels filled with cheese serum containing fat globules. In cheese with .2 % protein fibers intercrossing forming a close net causing the fat to surface on the fiber matrix giving richer flavor & softer body. TG at 0.5 level fat surfacing and covering all the cheese fiber matrix giving even softer body than the .2 TG level. In conclusion, the devised method for using TG in rennet coagulated cheeses proved to be working mixing milk with rennet at 5°C for 30 min then TG is added at 5°C and left for 2 hours to work before the temperature is raised to 40°C for coagulation. On rennet & TG were mixed with milk simultaneously at 5°C and the mixture was left for 2 hrs then temperature was raised to 40°C are the two devised methods. TG enzyme cannot be added before rennet nor the work of the enzyme should be longer than 2hur. Mozzarella cheese hardness & melting & stretch ability properties were improved by using the enzyme. TG level of 0.2 u/g gave the best results organoleptically as well as the other physical prosperities Actually, the enzyme accentuate the effect of fat in cheese and increased the retention of more overture Both components helped cheese mutability & stretchability.

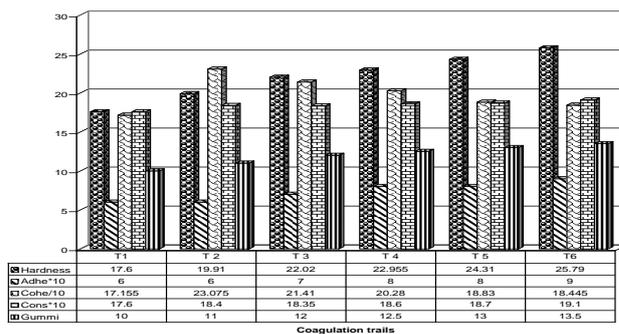


fig (5):Effect of different coagulation trails on the texture of Mozzarella cheese curds

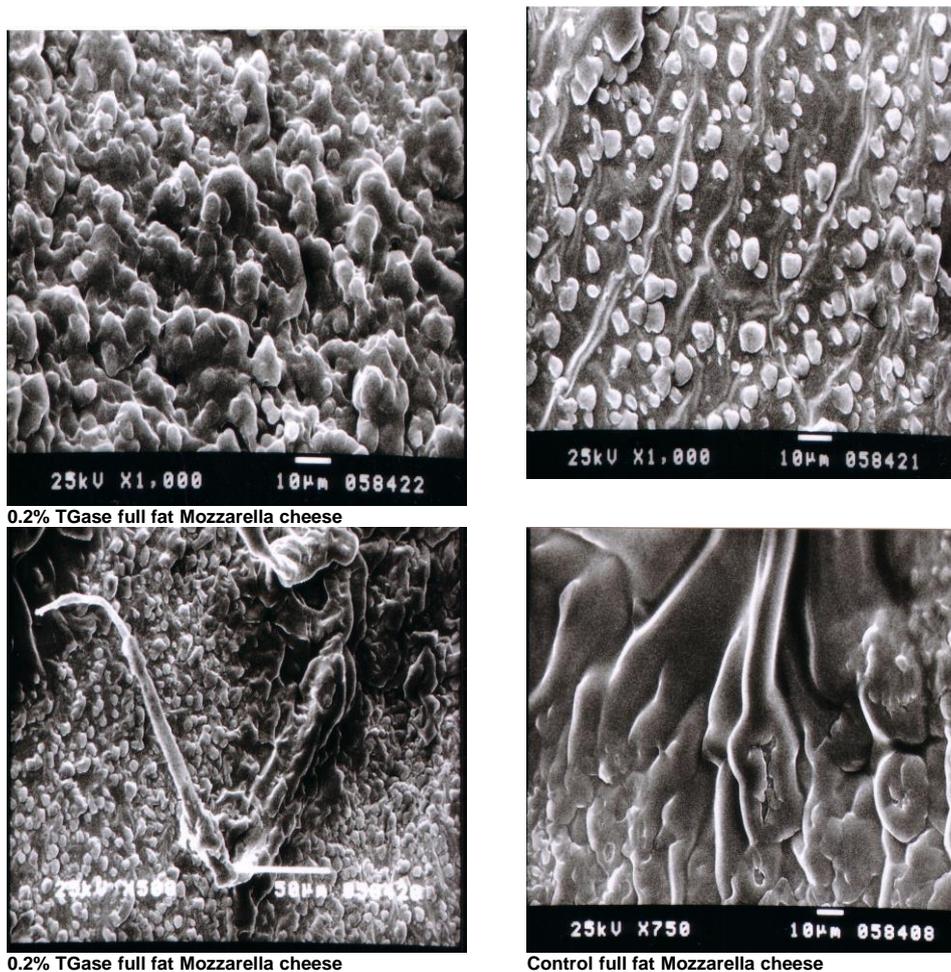


fig (6): cheese microstructure as viewed by electron microscope

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**إستخدام إنزيم الترانس جلودتاميناز فى تصنيع جبن الموزريلا كاملة الدسم
محمد محمد متولى*، هدى الزينى* و إيناس جزر**
*قسم تكنولوجيا الالبان – كلية الزراعة – جامعة القاهرة
** معهد بحوث تكنولوجيا الاغذية – مركز البحوث الزراعية بالجيزة**

تم استخدام الانزيم فى تصنيع جبن تجبن إنزيمى (بالمنفحه) . استخدمت طريقتين فى خلط الانزيم الطريق الاولى : خلط المنفحه مع اللبن على ٥ درجة مئوية لمدة ٣٠ دقيقه ثم إضافه الانزيم على ٥ درجة مئوية ويترك فترة قبل رفع الحرارة لدرجة حراره التجبن ٤٠ درجة مئوية بالطريقه الثانيه : يضاف المنفحه والانزيم معا ويتم خلطهم مع اللبن على ٥ درجة مئوية ويترك المخلوط لمدة ساعتين قبل رفع درجة الحرارة الى ٤٠ درجة مئوية . تم تصنيع جبن موزاريللا كامل الدسم باستعمال نسب الانزيم التاليه ٠,٢ جم / لتر و ٠,٥ جم / لتر حدث تغير فى صلابه ومطاطيه جبن الموزاريللا الناتجه وكذلك باقى الخواص . الجبن الناتج كان يتميز بقوام ناعم خاصه مع نسبه الانزيم المرتفعه . من التحكيم الحسى وخواص الجبن الموزاريللا الناتجه كانت نسبه ٠,٢ جم هى أفضل النسب