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UTILIZATION OF TRANSGLUTAMINASE IN MOZZARELLA CHEESE MANUFACTURE

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ABSTRACT

A method was devised to use transglutaminase enzyme (TG) in rennet coagulated cheeses. Two methods of mixing both enzymes (rennet and TG) 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 2 hrs before raising the temperature to 40°C for coagulation. In the second method, both enzymes were mixed with milk simultaneously at 5°C and the mixture was left for 2 hrs before raising the temperature to 40°C. Full fat mozzarella cheese was manufactured using 2 levels (0.02 and 0.05%) of TG according to the above first method. The two levels of enzymes improved Mozzarella cheese firmness, meltability and stretchability as well as the other physical properties. Both TG levels, resulted in softer body and richer flavor particularly at the 0.05% concentration. From the organoleptic and physical structure of Mozzarella cheese, the 0.02% level was recommended.

INTRODUCTION

Currently, transglutaminase (TG) is the only commercial covalent cross linking enzyme available for improving dairy products. Cross–linking reactions may lead to a modification of functional properties of proteins such as solubility, emulsifying capacity, foaming and gelation properties. For example, in set yogurt, TG increased gel strength, reduced syneresis with a dry smooth gel surface. In Quarg cheese, TG resulted in lower firmness, less grainy and creamier cheese (Myllarinen *et al.* 2007; Jaros *et al.* 2006).

Mozzarella chasse has unique functionalities in both unmelted and melted states. In un- melted state, shredability to uniform size and overall texture and in melted state meltability, free oil formation, stretchability and browning are the major functionalities (Imm *et al.* 2003). These functionalities are governed by various factors such as composition, additives, processing conditions and storage (Sheehan *et al.* 2004; Pastorino *et al.* 2003; McMahon and Oberg 1999). Of the composition, solids, fat content, sodium chloride and calcium contents are the major factors. High salt and low Ca^{++} caused the hydration of protein, thus reducing the firmness and improving the meltability. For proper Mozzarella characteristics a balance between salt, Ca^{++} and pH is needed (Guinee *et al*. 2002; McMahon and Oberg 1999).

Mozzarella microstructure is formed of protein fibers, as the backbone of the curd, which are somewhat parallels but with dentations due to fat globules. Between these protein fibers, channels filled with fat globules, bacterial cells and cheese serum. Fat globules forming non-interacting filler preventing the coalescence of protein strands (Mleko *et al.* 2004; Badawi *et al.* 2004)

Since TG affects product functionality such as forming smooth, firmer gel with lower whey syneresis and even resulting in 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, through the covalent cross-linking bridges blocks the primary coagulation step probably by reducing the accessibility of k-casein to rennet. This is a steric hindrance effect; particularly TG cross-links the micelle intra-molecularly as well as the dissociation of TG-casein is low. This problem led scientists to think that, it is unlikely that enzymatic modification with TG will be useful in the manufacture of rennet coagulated cheese (Jaros *et al,* 2006). However, after finalizing this research, a patent was published on 2005 (Kumazawa and Miwa 2005) which proposed two methods for milk coagulation with rennet and TG. Rennet was added to milk and 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 mixture temperature was raised 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 device a method 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 and functionality

MATERIALS AND METHODS

Materials

Fresh raw cow's milk was obtained from Dairy Science and Technology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. *Streptococcus thermophilus, Lactobacillus dlbruckii subsp. bulgaricus* and Calf rennet powder were obtained from Hansen's laboratory (Denmark). Transglutaminase was a gift from Ajinomoto Europe Sales Gmbh, Hamburg. The powder preparation contains a 100 units/g. Dry coarse commercial sodium chloride was obtained from EL-Nasr Co., Alexandria, Egypt.

Methods of mixing milk with rennet & TG and gel formation conditions

Seven trials were carried out as shown in Table (1)

Table (1). Mixing trials of milk with rennet & TG and coagulation conditions

Trials	Method of mixing milk with rennet* and TG*	CT**
T1(control)	Milk+ rennet at 5 °C / 30 min	40 °C
T2	Milk + rennet at 5 °C/ 30 min. + TG at 5 °C / 2 hrs.	40 °C
T3	Milk + rennet at 5 °C /30 min. + TG at 5 °C / 4 hrs.	40 °C
T4	Milk + rennet + TG simultaneously at 5 °C / 2 hrs.	40 °C
T5	Milk + rennet + TG simultaneously at 5 °C / 4 hrs.	40 °C
T6	Milk + rennet + TG simultaneously at 5 °C / 5 hrs.	40 °C
T7	milk + TG at 5 °C / 2-5 hrs + rennet at 5 °C / 30 min.	40 °C

***0.04% (w/v) rennet and 0.05% TG **CT: Coagulation temperature**

Manufacture of Mozzarella cheese:

Fresh cow's milk was pasteurized (72°C/15s) and used for manufacture of Mozzarella cheese according to the method of Kindstedt (1993) as shown in Fig. (1).

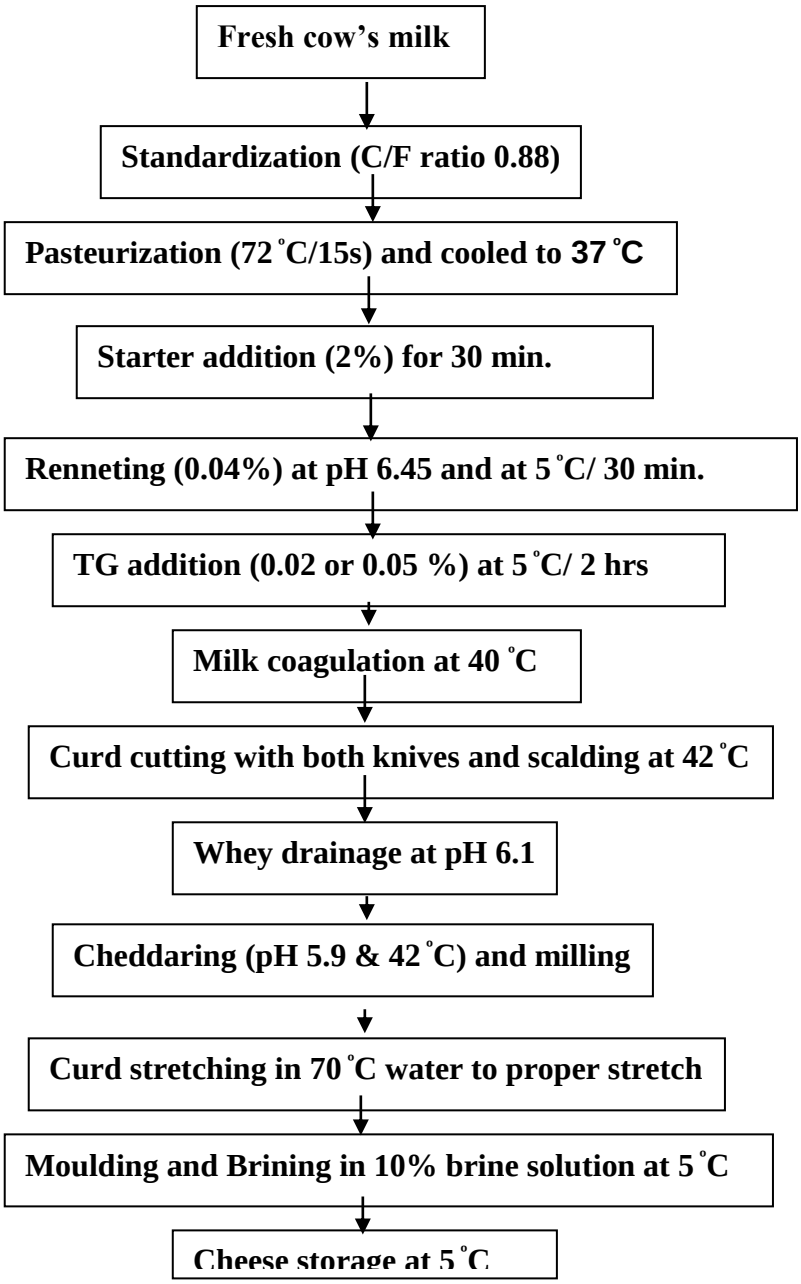

Fig. (1). Flow diagram of Mozzarella cheese manufacture using TG

Transglutaminase was added to milk with 2 levels (0.02 and 0.05%) and method of mixing was according to the flow diagram in Fig. (1). Cheese was stored at 5°C for 28 days. Cheese was sampled at 7, 15, 21 & 28 days for analysis.

Chemicals analysis:

Moisture, Titratable acidity and salt were determined according to A.O.A.C (1990). Fat content was determined according to ling (1963). Total nitrogen (TN), soluble nitrogen (SN), casein (CN) and non protein nitrogen (NPN) contents were determined using semi-microkjeldahl method according to SMEDP (1985). pH was measured using pH-meter (Jenway 3305, England).

Functional properties:

Meltability of cheese was measured using the meltability test according to Olson and Price (1958) with the modification by Rayan *et al.* (1980). Stretchability was measured using an iron bar test as reported by Davis (1966). Free oil formation was estimated by modified Gerber test as described by Kindstedt and Fox (1991). Fat leakage was evaluated as described by Bertola *et al.* (1996).

Viscosity measurements

Milk viscosity measurements on renneting were carried out in triplicates using a Brookfield viscometer (Model Dn-11+; Brookfield Engineering Lab., USA) according to Metwally and El-zeini (2004).

Texture properties

Texture parameters of coagulum were evaluated using texture analyzer (CNS Farnell, Borehamwoad, Hertfordshimre, England), as described by Ahmed *et al*. (2005). Cheese cubes 10.0± 0.1 mm were submitted to two successive compressions to 50% of their initial height using flat-headed plunger (20 mm diameter) at a constant rate 0.5 mm/s. Samples were allowed to equilibrate at ambient temperature approximately 30-45 min prior to testing. Texture characteristics such as hardness, cohesiveness, springiness, Adhesiveness, Modulus of elasticity, gumminess and chewiness were calculated. Average of four measurements was reported.

Cheese microstructure

Cheese cubes were prepared according to Lobato-Calleros (2006). Samples were examined at 5 KV through Scanning Electron Microscope (JEOL-jsm 5200) equipped with an IBM- compatible computer to record the images.

Milk components recovery and yield calculations:

The actual percentage of fat and protein recovered in cheese or lost in whey and stretching water were calculated as percentage of that in milk. Theoretical yield was calculated with the modified Van Slyke formula as described by Metzger *et al.* (2000).

Yield (kg of cheese /100Kg of milk) = [(0.85x milk fat %) + (milk casein%- 0.1) x1.13] /1-(cheese moisture/100).

Yield efficiency = (Actual yield / theoretical yeild) x 100.

Organoleptic properties

The cheese samples were organoleptically evaluated, by dairy Dept.-staff members, according to the method of Scott (1981). Score points for flavor, body & textures and appearance were 50, 35, and 15, respectively.

Statistical analysis:

Experiments were conducted in triplicate as a completely randomized design. The treatments were methods and conditions of mixing rennet & TG and TG concentration, along with a control coagulum and cheese (made without TG). Treatment levels were one to six, according to the number of TG mixing method, and one to three according to TG concentration in cheese. Statistical analysis, ANOVA, was performed using MSTAT-C (ver.2.10, Michigan state university, USA.) package on a personal computer, Individual comparisons, contrast or LSD between treatments and correlations were also performed. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

The use of TG in rennet coagulation of milk

Seven experimental trials were carried out (Table 1). These trials were run on two important facts. The first is that rennet and TG work at cold temperatures with low velocity and the second is that casein does not coagulate neither with rennet nor acid at cold temperature (5 ˚C). However, the order of mixing both enzymes was found to have an effect on the coagulation process as well as the properties of the produced gel. So, if TG is mixed first with milk at 5 ˚C prior to rennet addition at the same low temperature for 30 min, no coagulation took place till 5 hours when the temperature was raised to 40 ˚C. Therefore, rennet had to work on K-casein first releasing the glycomacropeptide 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 either 2 (T_2) or 4 (T_3) hrs before raising the temperature to 40 ˚C. In the second order of mixing, rennet & TG were mixed with milk simultaneously and the mixture was kept at 5 ˚C for 2 (T_4), 4 (T_5) and 5 (T_6) hrs, before raising the temperature for coagulation.

Viscosity

Fig. (2) presents the effect of methods of mixing milk with rennet and TG on coagulation profile as monitored by viscosity changes during coagulation. The coagulation time was the lowest for T_2 (35 min) followed by T_4 (49 min), while, T_3, T_5 and T_6 took longer coagulation time (58, 64 and 63.4 min, respectively) and no coagulation was observed for T_7 (not showed).These results indicated that there is a certain limit for the extent of TG reaction otherwise, coagulation started to be delayed. Actually, when the released ammonia was measured as indication of the extent of TG reaction, there was slight increase in the released ammonia by time (data not shown). Both mixing orders were found to be working and 2 hrs for TG reaction was found to be proper.

Compared to the control, coagulation time shifted to longer period with TG reaction and mixing methods ($P<0.001$). The control (T_1) showed the regular coagulation profile of viscosity versus time on rennet action. Viscosity increases then a slight dip took place followed by a high increase reaching the maximum as the coagulum is formed, viscosity then declined due to coagulum deformation by shearing. The figure shows a positive correlation

(0.973) between TG reaction period and coagulation time. Therefore, T_2 was coagulated faster than T_3 and T_4 than T_5 & T_6. The coagulation was greatly delayed when TG reaction increased more than 2 hrs. So, T_5 & T_6 interfered in their coagulation time and the shape of their curves was not normal. Since T_2 curve was the closest to the control, it was adapted for Mozzarella cheese manufacture. O'Sullivan *et al*. (2002) reported that TG through the covalent cross linking bridges blocks the primary coagulation step probably by reducing the accessibility of Κ-casein to rennet. Cozzolino *et al.* (2003) stated that adding TG prior to and concomitantly with rennet to pasteurized cheese milk resulted in no coagulation at all and a pronounced increase of coagulation time, respectively. The results also agree with those of Kumazawa & Miwa (2005).

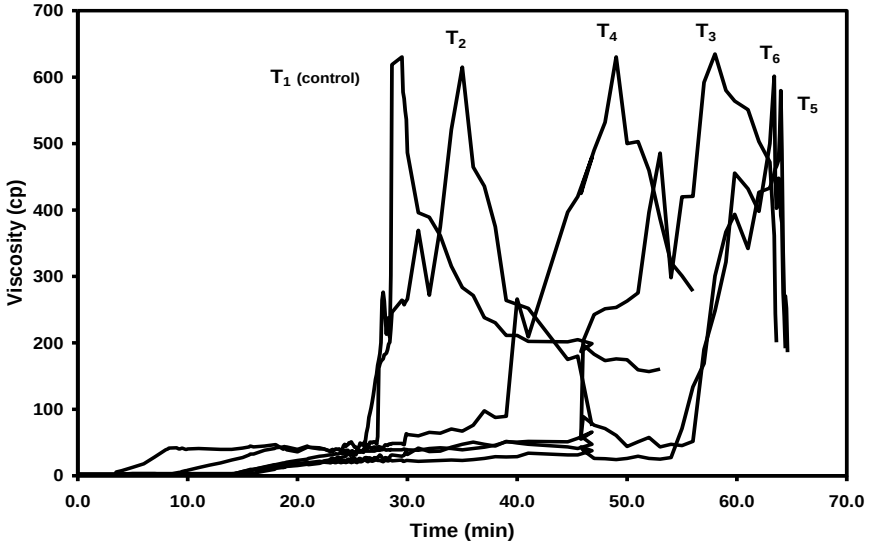

Fig. 2. The effect of mixing methods of rennet & TG with milk on viscosity during coagulation process

Texture

The changes in the six textural coagulum parameters obtained from the methods for mixing TG and rennet with milk are shown in Fig. (3). Statistical significant ($P<0.001$) differences were found in the texture parameters measured due to the TG mixing methods. From the results of the penetration test, the gel of T_2 was much more resistant to penetration by the cone plunger than the gels of the other trails. Therefore, the maximum hardness was obtained with T_2 trial (26.16 g) which insignificantly differed from T_3 and T_4 ($\alpha=0.05$). Also, insignificant differences were found in hardness of T_5 and T_6. At the same α level, the adhesiveness force (AF) differed insignificantly for the control & T_2, and also for T_4 & T_5. The highest AF was obtained with T_6 which indicates a higher sticking character than the other trials. A substantial difference was recognized between the cohesiveness of T_2, T_3 & T_4 gels compared to the control. The lowest

cohesiveness was maintained with T_5 (1.817) which insignificantly differed from the control. The internal strength of the bonds of T_2 was the highest with significant difference compared to the rest of the trials (α=0.05). T_5 and T_6 showed insignificant differences in consistency. T_2 and T_4 gumminess insignificantly differed as well as T_5 and T_6. So, the results suggested the capability of the TG to produce gel, in the presence of rennet, with many different physical characteristics through which T_2 introduced the best texture properties.

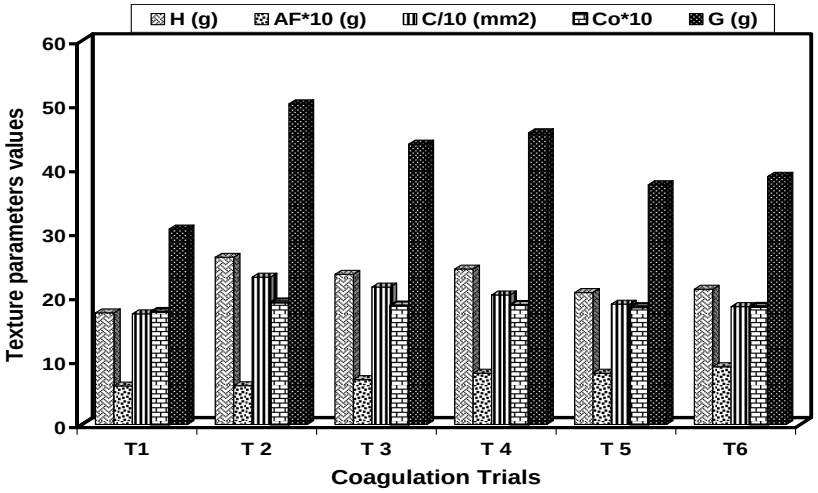

Fig. 3. The effect of mixing methods of rennet & TG with milk on texture parameters (Hardness (H), Adhesiveness force (AF), Consistency (C), Cohesiveness (Co) and Gumminess (G) of the coagula.

These results were in accordance with those of Kumazawa & Miwa (2005); Han *et al.* (2003); Lorenzen *et al.* (2002); Neve *et al.* (2001) and Faergemand & Qvist (1997) for acid and rennet gels.

The use of TG in full fat mozzarella cheese manufacture

Two experimental Mozzarella cheeses using two levels (0.02 & 0.05%) of TG were processed. Chemical composition of cow's milk used in cheese making is shown in Table (2).

Table (2). Cow's milk chemical composition

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

Mozzarella cheese composition

The mean Mozzarella cheese composition changes as a function of TG and cold storage is given in Table (3). Not only the presence of TG but also the storage period caused significant ($p<0.001$) differences in all chemical components measured.

Table (3). Effect of TG on chemical composition of fresh cheese and during storage period

Treatments TG(%)	Storage period,(day)				
	Fresh	7	14	21	28
(%)	Moisture (%)				
Control	52.16^{c}	51.81^{d}	51.29^{e}	50.76^{fg}	50.18^{h}
0.02 TG	52.74^{b}	52.35^{c}	51.79^{d}	51.32^{e}	50.71^{g}
0.05 TG	53.13^{a}	52.70^{b}	52.15^{c}	51.62^{d}	50.98^{f}
Fat/DM (%)					
Control	40.76	40.95	40.99	40.89	40.61^{f}
0.02 TG	41.12^{abc}	41.21^{ab}	41.20^{ab}	41.02	40.71
0.05 TG	41.32^{a}	41.08	41.17^{ab}	40.99	40.66
PH					
Control	5.15 ^{abc}	5.10^{bcd}	5.05	4.98^{gh}	4.95^{h}
0.02 TG	5.16^{ab}	5.11^{bcd}	5.07^{def}	5.00^{fgh}	4.97^{h}
0.05 TG	5.19^{a}	5.14^{abc}	5.08^{cde}	5.02	4.98^{gh}
Acidity (%)					
Control	0.67^{fgh}	0.72	0.75^{de}	0.78^{bcd}	0.86^{ab}
0.02 TG	0.63^{h}	0.67^{fgh}	0.73^{def}	0.76^{cde}	0.84^{ab}
0.05 TG	0.62^{h}	0.65^{gh}	0.71^{efg}	0.75^{de}	0.83^{abc}
TN (%)					
Control	3.37^{h}	3.40^{gh}	3.43^{fgh}	3.47	3.52
0.02 TG	3.42^{fgh}	3.45^{efg}	3.49	3.53^{bcd}	3.58^{ab}
0.05 TG	3.48	3.52	3.54^{bc}	3.58^{ab}	3.62^{a}
SN/TN (%)					
Control	4.43^{m}	5.40 ^{j}	6.97^{g}	7.76 ^{d}	10.11 ^{a}
0.02 TG	4.27^{n}	5.13 ^{k}	6.60 ^{h}	7.74 ^{e}	9.71 ^{b}
0.05 TG	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}
0.02 TG	2.05 ^{k}	2.31 ^{i}	2.52 ^{h}	3.60^{e}	4.18 ^{b}
0.05 TG	1.95 ^{l}	2.17 ^{j}	2.38 ^{i}	3.37 ^{f}	4.01 ^{c}
Salt/M (%)					
Control	2.33^{hi}	2.41^{fg}	2.55^{cd}	2.68^{b}	2.80^{a}
0.02 TG	2.24^{jk}	2.34^{hi}	2.47^{ef}	2.68^{c}	2.68^{b}
0.05 TG	2.18^{k}	2.26^{ij}	2.38^{gh}	2.51^{de}	2.61^{bc}

Values without superscript letters have more than 3 insignificant interaction letters.

Moisture content positively correlated (Table 7) with TG concentration, the highest increase was maintained with 0.05% TG, while moisture decreased with storage (-0.89). Compared to the other cheeses, 0.05% TG cheese had a significantly higher ($P<0.001$) pH, and TN as fresh and during storage, while fat/DM increased ($P<0.001$) in 0.02% TG cheeses over 0.05% during storage. SN/TN, NPN/TN, acidity and salt/M percentage significantly ($P<0.001$) increased through storage and decreased in TG cheeses and the reductions were enhanced with increasing TG concentration. Changing in moisture content and proteolytic break down during storage has been suggested to be the major factors caused these changes. The increase in acidity and the decrease in pH values could be attributed to acid producing activity of starter culture.

Moreover, the increase in TN and the decrease in SN and NPN of TG cheeses may be due to the additional cross-links induced by TG, which inhibits proteolysis (Cozzolino *et al.* 2003; O'Sullivan *et al*. 2002; Dickinson *et*

al. 1999), and has a negative effect on metabolism of starter organisms (Lorenzen 2000; Faergemand *et al.* 1999). The effect of storage on cheese composition concurs with the results of others (El-Batawy *et al.* 2004; Hamad 2004; Zammar 2002).

Cheese yield and recovery

Table (4) presents the effect of TG on cheese yield and recovery. Actual yield increased by TG ($P<0.05$) as well as theoretical yield and efficiency ($P<0.001$). Insignificant increase in yield of 0.05% TG cheese over that of 0.02% at 0.05 α level (13.6 & 12.9%, respectively) was observed. Yield efficiencies were significantly higher in both TG cheeses than control (99.8, 106 and 110% for control, 0.02 & 0.05% TG, respectively).

Table (4). Effect of TG on Mozzarella cheese yield and recovery

Yield & recovery	Control	0.02 %TG	0.05% TG
Yield (%)			
Actual	**12.00^{b}**	**12.90^{ab}**	**13.60^{a}**
Theoretical	**12.02^{b}**	**12.17^{a}**	**12.27^{a}**
Efficiency	**99.83^{c}**	**106.00^{b}**	**110.84^{a}**
Recovery (%)			
Fat	**78.0^{c}**	**83.5^{b}**	**87.81^{a}**
Protein	**76.33^{c}**	**83.39^{b}**	**89.29^{a}**

Significant increase ($P<0.001$) in fat & protein recovery with TG cheeses over the control was observed (Table 7). These results are expected due to TG cross-linking action, the enzyme complex whey proteins into casein micelles surface. Moreover, cross-linking casein strengthen its fibers network, thus harvesting more moisture, fat & protein and other components. These results agree with those of Kumazawa & Miwa (2005).

Losses of fat and protein in whey and stretch water

The effect of using TG on fat and protein losses in whey & stretch water of Mozzarella cheese was clear in Table (5).

Table (5). Effect of TG on fat and protein losses in why and starch water

Component (%)	Control	0.02 %TG	0.05%TG
Whey			
Protein	**22.91^{a}**	**16.03^{b}**	**9.94^{c}**
Fat	**14.50^{a}**	**12.33^{b}**	**9.24^{c}**
Stretch water			
Protein	**0.71^{a}**	**0.53^{b}**	**0.27^{c}**
Fat	**7.00^{a}**	**4.00^{b}**	**2.70^{c}**

Significantly ($P<0.001$) less fat & protein were lost in both fluids, whey and stretch water, as a result of increasing TG concentration as indicated by negative correlations (Table 7). Least significant test showed significant differences within control and 0.02 & 0.05% TG of protein and fat losses in whey and stretch water (Table 5) at 0.05 α level. Han *et al.* (2003) confirmed that the decreased protein concentration in recovered whey is caused by the cross-linking of TG.

Functional properties

Table (6) illustrates the functional properties of Mozzarella cheese manufactured with TG. Stretchability & meltability which are important

properties of mozzarella cheese, significantly (P<0.001) increased by TG treatment and concentration and the trend was progressed by storage.

Table (6) Effect of TG on functional properties of Mozzarella cheese

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

Stretchability of cheeses increased from 160 to 200& 220 cm in the control, 0.02 & 0.05% TG, respectively. Storage of the cheeses increased the stretchability (P<0.001). Meltability also improved from 55 in the control cheese into 68 & 73 mm in o.02 & 0.05% TG cheeses, respectively. Within treatments, insignificant effect of the enzyme on oiling off property at 0.05 α level was noticed. In addition, no correlation was found between TG concentrations and oiling off (Table 7).

While, TG reduced fat leakage, storage period significantly increased it (P<0.001) with high positive correlation (0.832). Not only fat leakage, but also stretchability, meltability and oil off significantly increased (P<0.001) by storage period. During storage, expressed water content (water located in the interstitial between fat globules in the fat serum channels) which is proportional to fat content, is absorbed by protein matrix. That causes an expansion of the protein matrix into the fat globules interstitial spaces and brings about a disappearance of the fat-serum channels. The results are in concomitant with those of Guinee *et at.* (2002), O'Sullivan *et al.* (2002), McMahon & Oberg (1999), Faergemand & Qvist (1997), Faergemand *et al.* (1999)

Texture

Variations in the chemical composition caused by incorporating TG in making cheese, resulted in different structural arrangements that produced different ($p \leq 0.001$) textural characteristics of hardness, springiness, chewiness, modulus, adhesiveness, gumminess and cohesiveness (Table 7).

Table (7) Analysis of variances of Mozzarella cheese properties

Source of variance	Component	P	Correlation	R^2
	Cheese Yield			
TG (%)	Actual	*	0.934	0.876
	Theoretical	***	0.982	0.964
	Efficiency	***	0.997	0.995
	Milk constituents recovery			
	Fat	***	0.997	0.993
	Protein	***	0.99	0.997
	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				
TG (%)	Moisture%	***	0.435	0.982
Storage		***	-0.890	
TG (%)	Fat /DM	**	-0.97	0.941
Storage		***	-	
TG (%)	pH	***	-	0.974
Storage		***	-0.964	
TG (%)	Acidity	***	-	0.964
Storage		***	0.952	
TG (%)	TN	***	0.642	0.975
Storage		***	0.749	
TG (%)	SN/TN	***	-	0.972
Storage		***	0.978	
TG (%)	NPN/TN	***	-	0.936
Storage		***	0.943	
TG (%)	Salt/M	***		0.988
Storage		***	0.908	
Cheese Functional properties				
TG (%)	Stretchability	***	0.706	0.974
Storage		***	0.69	
TG (%)	Meltability	***	-	0.734
Storage		***	0.709	
TG (%)	Oil Off	**	-	0.839
Storage		***	0.909	
TG (%)	Fat Leakage	***	0.536	0.98
Storage		***	0.832	
Cheese Texture				
TG (%)	Hardness	***	-0.997	0.995
	Gumminess	***	-0.908	0.825
	Cohesiveness	**	0.873	0.759
	Springiness	*	0.87	0.774
	Modulus	***	-0.99	0.998
	Adhesiveness	***	-0.997	0.994
	Chewiness	***	-0.941	0.886

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

Fig. (4) illustrates the changes in texture parameters as a result of using two concentrations (0.02 & 0.05%) of TG as compared with the control.

Hardness, gumminess, modulus, adhesiveness and chewiness inversely proportioned (-0.997, -0.908, -0.99, -0.997 and -0.941, respectively)

with increasing TG concentration over 0.02%. However, cohesiveness and springiness positively correlated (0.873 & 0.87, respectively) with increasing TG concentration, due to the increase in the internal bonds number which caused by TG action on milk proteins (Li-Chan 2004). The hardness recorded 36.2 and 14.8% increases with 0.02% TG cheeses over that of 0.05% TG and control, respectively. Insignificant increase in springiness was obtained with increasing TG concentration over control at 0.05 α level. Modulus of elasticity followed the same trend as hardness.

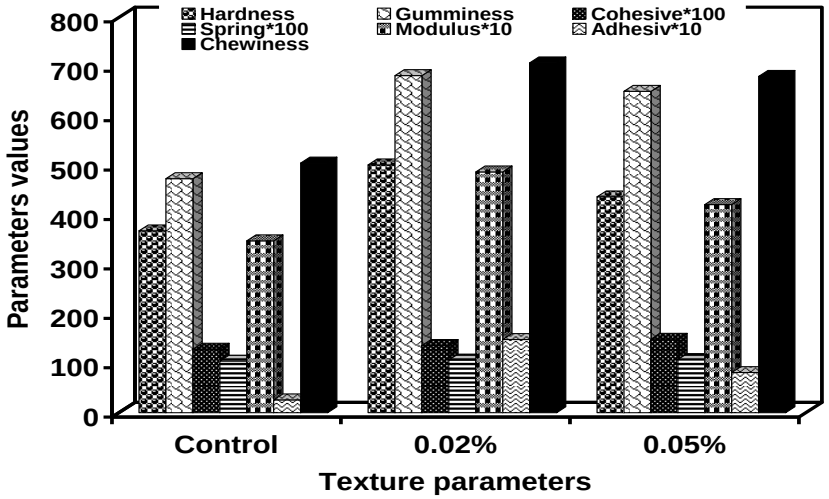

Fig (4) Texture profile of control and TG Mozzarella cheeses

Based on these observations, 0.02% TG is recommended for making Mozzarella cheese, since cheeses made with that concentration showed improvement in physical properties followed by that of 0.05% level. The results agree with those of Li-Chan (2004); Kuraishi *et al* (2001).

Microstructure

Fig (5) shows the changes in microstructure of Mozzarella cheeses caused by using TG in the manufacture. The control natural Mozzarella cheese exhibited highly oriented fibrous structure. Longitudinal sections showed that protein formed continuous phase and was in the form of linear fibers (Fig. 5, a) parallel with smooth surface and existed between fibers channels filled with cheese serum containing fat globules.

In cheese with 0.02 % TG (Fig. 5, b), the increase of protein intercrossing forming a close net causing the fat to surface the fiber matrix giving richer flavor. The intercrossing protein fibers intercepted with each other in a circular form giving a compact shape in 0.05% level TG (Fig. 5, c). More fat and milk serum entrapped within the protein matrix fiber channels giving softer body than that of 0.02% TG cheese.

Fig. (5) Mozzarella cheese SE micrographs: Control (a), 0.02% TG (b) and 0.05% TG (c)

Sensory evaluation

Table (8) presents the organoleptic properties of TG cheeses. In spite of that 0.05% imparts softer texture than 0.02% TG, the two levels compared well with the control. This is expected, as increasing the TG concentration, increases moisture contents. In general, TG usually produces softer gel with finer particles and less tendency for moisture exudation. However, 0.02% level was preferred by judges. Similar results were found by Han *et al.* (2003) in soft cheeses.

Table (8) Effect of TG on organoleptic properties of Mozzarella cheese

property	Control	0.02% TG	0.05% TG
Flavor (50)	44	43	43
Body & Texture (35)	33	34	32
Appearance (15)	14	14	14
Total (100)	91	91	89

CONCLUSION

The devised methods for using TG in rennet coagulated cheeses proved to be working. The first method, of mixing milk with rennet at 5 ˚C for 30 min then TG is added at 5 ˚C and left for 2 hrs to function before the temperature is raised to 40 ˚C for coagulation, and the second method, of mixing rennet & TG 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 should the working time of the enzyme be longer than 2hrs. Mozzarella cheese hardness, melting and stretch ability properties were improved by using the enzyme. TG level of 0.02% gave the best results organoleptically as well as the other physical properties. Actually, the enzyme accentuates the effect of fat in cheese and increases the retention of more moisture. Both components helped cheese meltability and stretchability.

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إستخدام إنزيم الترانس جلوتاميناز في تصنيع جبن الموزاريللا كامل الدسم

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اجري هذا البحث بهدف الإستفادة من خصائص إنزيم الجلوتاميناز في تصنيع الجبن ذات التجبن الأ نزيمي والتغلب علي صعوبة تجبن اللبن بإنزيمات التجبن في وجود إنزيم الجلوتاميناز. وقد استخدمت طريقتان لخلط إنزيمي الجلوتاميناز والرنين (المنفحه) مع اللبن. في الطريقة الأولي: تم خلط المنفحة مع اللبن علي ٥ْم حيث ترك علي هذة الحرارة لمدة ٣٠ق ثم أضيف إنزيم الجلوتاميناز علي ٥ْم أيضا وترك علي هذة الحرارة لمدة ٢ـ ٤ ساعات ثم رفعت درجة حرارة اللبن إلي ٤٠ْم حتي تمام التجبن. وفي الطريقة الثانية: تم خلط المنفحة و إنزيم الجلوتاميناز معا باللبن علي ٥ْم ثم ترك علي هذه الحرارة لمدة ٢ـ ٥ ساعات بعدها رفعت حرارة اللبن إلي ٤٠ْم حتي تمام التجبن. وتم تتبع اللزوجة أثناء التجبن وتقدير زمن التجبن وكذلك خواص القوام للخثرة المتكونة.

وأشارت النتائج إلي أفضلية الطريقة الأولي حيث تم إستخدامها في تصنيع جبن موزاريللا كامل الدسم مع إضافة إنزيم الجلوتاميناز بمعدل ٠,٠٢ ٪ , ٠,٠٥٪ وقد تميزت الجبن الناتج بخواص القوام الجيد والخواص الوظيفية والطبيعية الأخري التي تفوقت فيها علي جبن المقارنة. وتشير نتائج التحكيم الحسي والتركيب الطبيعي للجبن إلي أفضلية الجبن المصنع بإستخدام إنزيم الجلوتاميناز بمعدل ٠,٠٢ ٪ , لذلك يوصى بإستخدام الإنزيم بهذا المعدل في صناعة جبن الموزاريللا كامل الدسم.