THE USE OF TRANSGLUTAMINASE IN THE PRODUCTION OF LOW FAT MOZZARELLA CHEESE

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ABSTRACT

Low fat mozzarella cheese was made from standardized (C:F ratio, 2.05), pasteurized (72 $^{\circ}$ C/15 s) cow's milk treated with 2 levels (0.02 and 0.05%) of transglutaminase (TG). Both TG and control cheeses were stored at 5 C /28 days. Cheese was sampled at 7, 14, 21 and 28 days for chemical analysis, sensory attributes and functional properties determination.

Results indicated that incorporation of TG into cheese milk significantly increased cheese yield, moisture and fat and protein recovery compared to those of the control. Cheese functionality also improved by both levels of TG. Stretchability and meltability increased from 100 cm & 30 mm in control to 140 cm& 45 mm and 160 cm & 50 mm in 0.02 & 0.05% TG cheeses, respectively. While, oiling off and fat leakage values were decreased in TG cheeses. Cheese proteolysis was enhanced by cold storage, which in turn, improved cheese functionality. Hardness, gumminess, modulus of elasticity and adhesiveness inversely proportioned with increasing TG concentration. Organoleptically, TG cheeses competed well with control and were more acceptable particularly that of 0.05%.

INTRODUCTION

The popularity of pizza widened Mozzarella cheese market. However, large consumption of pizza with regular fat Mozzarella caused the consumer concern about the consumption of high fat product. This led scientists to investigate the possibility of production of low fat Mozzarella.

Low fat Mozzarella has inferior organoleptic and physical properties. Cheese with less than 10% fat usually has hard, rubbery and translucent with poor flavor development and poor meltability and performance when cooked on pizzas (Jaros et al., 2006; Badawi et al., 2004; Guinee et al., 2002). Since fat not only acting as smoothing factor and a good substrate for flavor but also the globules forming a non-interacting filler between protein fibers particularly when are closely packed as cheese go through the stretching. Thus, fat globules prevent the coalescence of protein strands allowing more serum in between. So, if fat is reduced, it is difficult to maintain the same water-to-protein ratio in the cheese and fat no longer acts as blocking agent. The presence of proper percent of water is important, since it acts as lubricant or plasticizer between protein molecules. Number of measures were tried to maintain the same water-to-protein ratio in low fat Mozzarella, either by modifying processing procedure or by adding certain additives such as whey proteins or fat replacer. Fat replacer blocks protein coalescence during cooking and create openness in the matrix allowing more moisture to be retained.

Transglutaminase enzyme cross-links protein molecules through covalent bonds and modifies the functional properties of protein and product composition. It has been suggested that various dairy products with reduced

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fat and protein could be produced having texture similar to the full fat and high total solids products with TG treatment (Faergemand *et al.*, 1999). TG was used in processing low fat (5%) ice-cream. The enzyme treatment compensated for the low fat and the product was superior in all physical and organoleptic properties than their corresponding controls. Actually, the enzyme changed time-viscosity curve at constant shear rate (61.2 s⁻¹) of low fat ice-cream from no change in viscosity (time-independent) into rheopectic flow (time-thickening). The required physical properties could be obtained with the enzyme treatment (Metwally, 2007). The TG treatment of regular fat Mozzarella cheese increased moisture content, yield and recovery of fat and whey proteins. TG cheese physical properties and sensory attributes were superior to their corresponding controls; the cheese was creamier and had softer body (Metwally *et al.*, 2007).

For the above improvements imparted by TG treatment particularly the retention of more whey protein, moisture and fat and the increase in cheese softness, it was thought that the use of TG would help in ameliorating Mozzarella cheese low fat physical problems.

Therefore, the objective of this research was to use TG in the production of low fat Mozzarella cheese and to study the physical and organoleptic properties of the resulting cheese.

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 (Stubbenhuk 3, D-20459, Hamburg, Germany), the declared activity of the powder preparation was100 units/g. Dry coarse commercial sodium chloride was obtained from EL-Nasr Co., Alexandria, Egypt.

Low fat Mozzarella cheese Manufacture:

Fresh cow's milk was standardized to C:F ratio of 2.05 then pasteurized (72° C/15 s) and used for cheese manufacture according to the method of Kindstedt (1993). Transglutaminase (TG) was added to milk with 2 levels (0.02 and 0.05%), rennet was added to the milk for 30 min. at 5°C followed by TG and the mixture was left for 2 hrs at 5°C. before raising the temperature to 40°C for complete coagulation (Metwally *et al.*, 2007). Control cheese was made following the same procedure without TG. Cheeses were stored for 28 days at 5°C. Samples were taken from cheeses at 7, 14, 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 Standard Method of Examination of Dairy Products (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) as modified 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.

Texture properties

Cheese cubes (10.0±0.1) mm were submitted to two successive compressions to 50% of their initial height, using texture analyzer (CNS Farnell, Borehamwoad, Hertfordshimre, England), as described by Ahmed *et al.* (2005) with flat-headed plunger (20 mm diameter) at a constant rate of 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 samples 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.

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) = $[(0.85x \text{ milk fat }\%) + (\text{milk casein}\%-0.1) \times 1.13] /1-(cheese moisture/100).$

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

Organoleptic properties

Cheese samples were organoleptically evaluated according to the method of Scott (1981). Score points for flavor, body & textures and appearance were 50, 35, and 15, respectively. The evaluation was carried out by 10 staff members of the Dairy Science and Technology Department, Faculty of Agriculture, Cairo University.

Statistical analysis:

Experiments were conducted in triplicate as a completely randomized design, which incorporated the 3 treatments (control, 0.02 and 0.05% TG levels) and used to analyze the response variables relating to composition, yield and recovery, fat and protein losses in whey and stretching water, functional properties, texture and sensory evaluation of the cheeses. An 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. The level of significance was determined at P < 0.05.

RESULTS AND DISCUSSION

Composition of milk and cheese

Low fat Mozzarella cheese was processed from standardized cows' milk and the composition of milk is reported in Table (1).

Table (1). Cheese milk composition

C	Component (%)	DM	Fat	F/DM	Protein	P/DM	Casein	CN/ DM	C:F ratio	NPN	T.A	PH
	Value	9.96	1.3	13.05	3.42	34.34	2.67	26.81	2.05	0.22	0.16	6.63

Milk was treated with TG at 0.02 & 0.05 % concentrations and processed into Mozzarella cheese. Table (2) shows treated cheese composition. The enzyme treated cheeses contained higher fat, moisture and TN and less SN/TN and NPN/TN than the control. The effect was enzyme concentration dependent and the increase in fat and moisture was only significant (P<0.001) at the 0.05 % TG level. However, the effect on TN and other N fractions were significant at both TG concentrations. The enzyme treatment exerted no effect on cheese pH or acidity.

Table (2). Effect of TG on che	emical composition of fresh cheese and
during storage period	l

Tractmente		Sto	prage period,(d	lay)	
Treatments	Fresh	7	14	21	28
(%)			Moisture (%)		
Control	54.48 ^b	53.78 ^{cd}	53.40 ^{de}	52.95 ^{fg}	52.26 ^h
).02% TG	54.71 ^{ab}	54.02°	53.67 ^{cd}	53.16 ^{ef}	52.54 ^{gh}
).05% TG	55.10ª	54.42 ^b	54.05 ^{cd}	53.54 ^{cd}	52.87 ^{fg}
		Fat/DM	(%)		
Control	21.16 ⁱ	21.23 ^{hi}	21.5 ^{ghi}	21.55 ^{fgh}	21.68 ^{efg}
).02 TG	21.46 ^{ghi}	21.61 ^{fg}	21.81 ^{efg}	21.86 ^{ef}	22.01 ^{de}
).05 TG	21.90 ^{ef}	22.26 ^{cd}	22.46 ^{bc}	22.72 ^b	23.08 ^a
	•	PH	•	•	
Control	5.16 ^{abc}	5.12	5.07 ^{ef}	5.03 ^{fgh}	4.97 ^h
).02 TG	5.19 ^{ab}	5.14	5.08 ^{def}	5.05 ^{efg}	4.99 ^{gh}
).05 TG	5.19 ^a	5.15	5.10	5.07 ^{ef}	4.99 ^{gh}
	•	Acidity	(%)	•	
Control	0.64 ^{gh}	0.68 ^{efg}	0.72 ^{def}	0.77 ^{bcd}	0.85ª
).02 TG	0.6 ^{1h}	0.66 ^{fgh}	0.7 ¹	0.75 ^{cde}	0.83 ^{ab}
).05 TG	0.60 ^h	0.64 ^{gh}	0.69 ^{efg}	0.74 ^{cde}	0.81 ^{abc}
	•	TN (%	5)	•	•
Control	3.88 ^h	3.91 ^{gh}	3.94 ^{fgh}	3.97 ^{efg}	4.01
).02 TG	3.96 ^{efg}	4.02 ^{cde}	4.05 ^{bcd}	4.07 ^{abc}	4.11 ^{ab}
).05 TG	3.99 ^{def}	4.05 ^{bcd}	4.08 ^{abc}	4.11 ^{ab}	4.14 ^a
	•	SN/TN (%)		•
Control	3.65 ¹	4.09 ^j	5.33 ^g	6.30 ^d	8.48 ^a
).02 TG	3.37 ^m	3.73 ^k	5.17 ^h	6.13 ^e	7.49 ^b
).05 TG	3.25 ⁿ	3.19 ⁿ	4.97 ⁱ	5.54 ^f	7.14 [℃]
	•	NPN/TN	(%)		•
Control	1.90 ⁱ	2.16 ^h	2.52 ^f	3.38 ^b	3.84ª
).02 TG	1.75 ^j	1.96 ⁱ	2.34 ^g	2.96 ^d	3.40 ^b
).05 TG	1.55 ^k	1.78 ^j	2.18 ^h	2.77 ^e	3.25°
	•	Salt/M (•
Control	2.43 ^f	2.54 ^d	2.65°	2.75 ^b	2.84 ^a
.02 TG	2.32 ^{gh}	2.4 ^f	2.51 ^{de}	2.64 ^c	2.76 ^b
).05 TG	2.25 ^h	2.33 ^g	2.46 ^{ef}	2.55 ^d	2.68 ^c

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

The above trends continued through the 28 days of cold storage. The above effects on cheese composition were expected due to the crosslinking effect of the enzyme on milk casein which was done in different levels.

Intra-molecular cross-linking of the core of the micelles rendering the micelles more stable under different treatments (e,g. acidification, heating, cooling). Also the cross-linking between individual micelles prevents k-dissociation and forming complex with whey proteins. Formation of crosslinking at the surface of the micelles strengthen the gel formed and resulting in the formation of small pores giving better moisture retention, reduces whey syneresis and entrapping more fat. Moreover, the retention of more whey proteins increases moisture contents by absorption. Though moisture contents of the curd increases, curd firmness also increases due to the compactness effect of TG cross-linking (Myllarinen *et al.*, 2007; Jaros *et al.*, 2006). The above mechanism explains the changes occurred in cheese composition.

During cold storage the increase in moisture, TN and fat were in equal percentage in all cheeses. However, the percentage increase in SN during storage was less in TG treated cheese than the control (119 and 132%, respectively). Cross-linked proteins may resist proteolysis to some extent (O'Sulivan, 2002) and exerts negative effect on starter activity (Ozer *et al.*, 2007; Lorenzen *et al.*, 2002).

Cheese yield and recovery

Table (3) represents cheese yield which increased significantly (P<0.001) by TG treatment and the increase was TG concentration dependent. This was caused by more retention of protein, fat and moisture in cheeses (Table 2). Cheese yields of the control, 0.02 & 0.05% TG were 9.9, 10.7 and 11.4%, respectively. Of course, the efficiency as well as fat and protein recovery was increased with the enzyme treatment. Highest recovery of fat and protein (84.9 & 84.45%, respectively), was with 0.05% TG treatment.

		Cheese treatment							
Yield & recovery	Control	0.02 %TG	0.05% TG						
	Yield (%)								
Actual	9.90°	10.70 ^b	11.40ª						
Theoretical	9.14 ^b	9.18 ^{ab}	9.26 ^a						
Efficiency	108.32°	116.56 ^b	123.11ª						
	Recovery (%)							
Fat	74^.6°	80.41 ^b	84.9 ^a						
Fat Recovery	747.0°	00.41~	04.9						
Protein	71.62°	78.97 ^b	84.45 ^a						

Table (3).	Effect	of [•]	ΤG	on	Mozz	arella	cheese	yield	and	recovery	/

Table (4) shows the fat and protein loss in cheese whey and stretch water. The loss was significantly (P<0.001) reduced by the enzyme treatment.

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Component		Cheese treatment					
(%)	Control	0.02 %TG	0.05%TG				
	Whey						
Protein	27.58ª	20.35 ^b	15.09 ^b				
Fat	18.48 ^a	15.39 ^b	13.23 ^c				
	Stretch wate	•	•				
Protein	0.53ª	0.26 ^b	0.18 ^b				
Fat	7.62 ^a	5.31 ^b	3.0 ^c				

 Table (4). Effect of TG on fat and protein losses in whey and stretch water of Mozzarella cheese

Functional properties

The functional characteristics of Mozzarella cheese treated with TG and their changes during cold storage are reported in Table (5). TG treatment improved cheese stretchability, meltability, oiling off and fat leakage and the degree of improvement was enzyme concentration dependent. The increase in stretchability and meltability was significant (P<0.001) at both TG concentrations. Stretchability increased from 100 cm in the control cheese to 140 & 160 cm and meltability increased from 30mm in the control cheese to 45 & 50 mm in the 0.02 & 0.05% TG treatments, respectively. Oiling off decreased from 1.72% in the control cheese to 1.65 & 1.63 % and fat leakage from 38 mm in the control cheese to 36 & 35 mm in the 0.02 & 0.05% TG treatments, respectively. The differences were significant only with the 0.05% TG cheese.

 Table (5). Effect of TG on functional properties of Mozzarella cheese

Treatments		Storage period (day)							
(%)		sh 7	14	21	28				
	:	Stretchabilit	ty (cm)						
Control	100 ^m	119 ⁱ	134 ^k	160 ^h	179 ^f				
0.02 TG	140 ^j	153 ⁱ	174 ^g	198 ^d	214 ^b				
0.05 TG	160 ^h	174 ^g	189 ^e	210°	223ª				
		Meltability	(mm)						
Control	30 ^k	42 ^j	57 ^g	65 ^e	77°				
0.02 TG	45 ⁱ	57 ^g	69 ^d	78°	85 ^b				
0.05 TG	50 ^h	61 ^f	77°	83 ^b	92 ^a				
		Oil off	%						
Control	1.72 ^{kl}	1.89 ^{hi}	2.15 ^f	2.63°	3.05 ^a				
0.02 TG	1.65 ^{lm}	1.82 ^{ij}	2.02 ^g	2.44 ^d	2.98 ^{ab}				
0.05 TG	1.63 ^m	1.77 ^{jk}	1.94 ^h	2.36 ^e	2.95 ^b				
		Fat leakage	(mm)						
Control	38 ^{gh}	64 ^f	53 ^d	60 ^b	67 ^a				
0.02 TG	36 ^{hi}	60 ^g	49 ^e	54 ^d	59 ^{bc}				
0.05 TG	30 ⁱ	38 ^{gh}	45 ^f	50 ^e	57°				

These properties increased by cold storage in all cheeses and the improvement contributed through storage. 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 the disappearance of the fat-serum channels giving higher values of meltability (O'Sullivan *et al.*, 2002;

McMahon & Oberg, 1999; Faergemand & Qvist, 1997; Faergemand *et al.*, 1999).

Texture

Reduction in fat content in fresh cheese causes hardness, cohesiveness, adhesiveness, gumminess, springiness and modulus to increase than those of full fat cheese. Modulus of elasticity increased since TG increased the active bridges between polypeptides, results of low fat Mozzarella cheese showed that effect (Fig. 1).

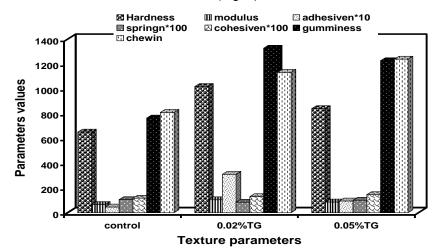


Fig (1). Texture profile of control and TG low fat Mozzarella cheeses

Cheese with TG had higher texture parameters above those of the control cheese; however the increase was inversely proportional to the enzyme concentration. Therefore, the 0.05% TG resulted in more balanced texture properties than those of either the control or 0.02% TG. Though, the 0.05% TG would form more bonds causing a strong protein network, as shown by the slight increase in the cohesiveness. The signified increase in fat, whey protein recovered in the cheese as well as the higher moisture content modified and balanced the texture to more desirable one. Therefore, from point of texture 0.05% TG is preferable.

Organoleptic properties

Table (6) presents the organoleptic properties of TG cheeses. No significant differences were found in flavor and appearance between TG and control cheeses. TG cheeses body and texture showed higher scores compared with the control one (α =0.05). The 0.05% TG cheese was more acceptable and preferable than the other two samples.

	Cheese treatment					
property	Control	0.02% TG	0.05% TG			
Flavor (50)	40 ^a	40 ^a	40 ^a			
Body & Texture (35)	30°	31 ^b	32ª			
Appearance (15)	13ª	13ª	13ª			
Total (100)	83°	84 ^b	85ª			

 Table (6). Effect of TG on organoleptic properties of Mozzarella cheese

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Microstructure

Scanning electron micrographs of control and TG low fat Mozzarella cheeses are shown in Fig. (2, a, b& c).

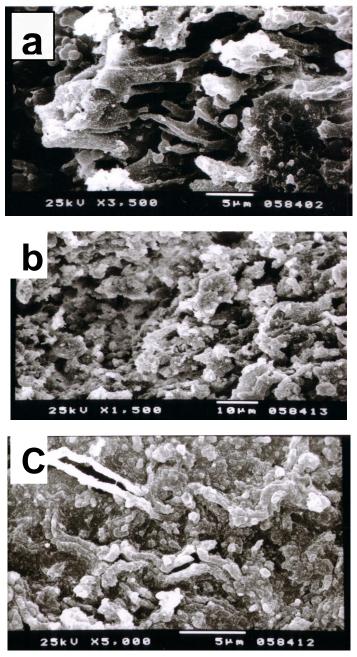


Fig. (2) Low fat Mozzarella cheese SE micrographs: (a) Control (b) 0.02% TG (c) 0.05% TG

Control cheese had a typical microstructure of a continuous protein matrix and the strands had no particular orientation interspersed with serum channels (Fig. 2, a). The cheese had several well-distributed pores, most of which were presumably serum pockets with few fat globules. The addition of TG to cheese milk during manufacturing greatly affected its microstructure. Protein matrix intercrossed and fibers showed no particular direction and were fewer as expected for the cross-linking action of TG. Cheese had some serum pockets, but they were smaller and less than control cheese (Fig. 2 b). When 0.05% TG was used in making the cheese, serum pockets became very small and fewer (Fig. 2 c). Cheese matrix showed fibrous structure with many strands oriented in a straight direction. The matrix became more compact with few elongated holes and the appearance was dense.

CONCLUSION

The use of TG in making low fat Mozzarella modified cheese physical properties in a direction that ameliorate the bad effect of low fat. The enzyme at 0.05% concentration increased significantly cheese yield, moisture and the recovery of fat and whey proteins. The enzyme improved low fat Mozzarella meltability, stretchability, oiling off and fat leakage. Organoleptically, treated cheese was more acceptable and the structure parameters were more satisfactory than the control. Previous results of Metwally *et al.*, (2007) and the current results point out that when TG is used in dairy products, the proper concentration should be selected according to properties required.

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> إستخدام إنزيم الترانس جلوتامينيز في تصنيع جبن الموزاريللا قليل الدسم هدى الزينى* ، محمد متولى* و ايناس جزر** * قسم علوم و تكنولوجيا الالبان – كلية الزراعه – جامعة القاهره – الجيزه. ** معهد بحوث تكنولوجيا الاغذيه – مركز البحوث الزراعيه – الجيزه – القاهره.

كان الهدف من هذا البحث هو انتاج جبن موز اريللا قليل الدسم باستخدام إنزيم الترانس جلوتامينيز لتحسين خواصة الطبيعية والوظيفية والحسية. وتم تصنيع الجبن من اللبن البقرى المعدل (C:F= 2.05) والمبستر (٢٢ م/١٥) حيث أضيفت المنفحة اليه على ٥ م ثم ترك علي هذة الحرارة لمدة ٣٥ ق ثم أضيف إنزيم الترانس جلوتامينيز بمعدل ٢,٠٠ ٪, ٥,٠٠٪ علي ٥ م وترك علي هذة الحرارة لمدة ساعتين ثم رفعت درجة حرارة اللبن إلي ٤٠ م حتي تمام التجبن. وتم تصنيع جبن المقارنة بنفس الطريقة مع عدم اضافة الأنزيم وتم تخزين الجبن لمدة ٢٨ يوم على ٥ م. وتم تحليل الجبن لتقدير التركيب الكيماوى والخواص الريولوجية والوظيفية والحسية وكذلك التركيب البنائي الدقيق.

أدى إستخدام الإنزيم الى زيادة الدهن والرطوبة والنتروجين الكلى ، وأنخفاض النتروجين الذائب الى الكلى فى جبن الأنزيم الطازج والمخزن عن جبن المقارنة ، وارتبط ذلك بتركيز الأنزيم المستخدم. كما زادت تصافى الجبن بدرجة معنوية فكانت ٩,٩٪ ، الماريزيز الأنزيم المستخدم. كما زادت تصافى الجبن بدرجة معنوية فكانت ٩,٩٪ ، أعلى نسبة أستخلاص للدهن والبروتين فى جبن الأنزيم ٥٠,٠٪ ، أشارت النتائج أيضاً إلى تحسين الخـواص الوظيفية وخـواص القـوام لجـبن الأنـزيم حيـث زادت المطاطية معنين الخـواص الوظيفية وخـواص القـوام لجـبن الأنـزيم ٢٠،٠٥ سم وكذلك الأنصهار من ٢٠,٠ مار ٢٠ مم فى جبن المقارنة الى ١٦٠ مم لكل من جبن الأنزيم ٢٠,٠ ، م.٠.٢ من ٢٠ مم فى جبن المقارنة الى ٢٠ ، ٢٠ سم وكذلك الأنصهار ب ٢٠,٠٠ من ٢٠ مم فى جبن المقارنة الى ٢٠ ، ٢٠ سم وكذلك الأنصهار ب ٢٠,٠٠ من ٢٠ مم فى جبن المقارنة الى ٢٠ ، ١٦٠ سم وكذلك الأنريم ٢٠,٠ البارد. وزادت قيم جميع خواص القوام التى كانت فى تناسب عكسى مع تركيز الأنزيم ب وكان جبن الأنزيم ٥٠,٠٪ الأفضل فقد حصل على أعلا الدرجات من حيث القبول العام. وكان جبن الأنزيم م.٠٠٪ الأفضل فقد حصل على أعلا الدرجات من حيث القبول العام. وخلام هذا الجن بتركيب ليفى ذو شبكة بروتين كثيفة المظهر أكثر أندماجاً ذات جيوب قليلة ولمهر هذا الجن بتركيب ليفى ذو شبكة بروتين كثيفيا الماهم أكثر أدماجاً ذات العول العام.