Comparative Study on the Effect of Adding Transglutaminase to Cow's Milk on the Properties of Low-Fat Gouda-Like Cheese

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ABSTRACT

Three batches of Gouda - like cheese were prepared from whole fat (3.5% fat), reduced fat (2.5% fat) and low fat milk (1.5% fat). Another three batches were similarly made with the addition of Transglutaminase (TGase) at a rate of 0.5 g/ liter (50 unit) of milk to study the effect of enzyme on the quality of Gouda - like cheese with three levels of fat content. The obtained results showed that addition of enzyme , markedly increased the moisture content of treated milk cheese as compared with the control. The highest moisture contents for fresh and ripened cheese were found in low- fat cheese treated with the enzyme. The addition of enzyme to cheese milk led to slight decrease in fat content of the fresh and ripened cheese, while an increase in total protein content was detected for all treatments. Reducing fat content (2.5 or 1.5%) of cheese milk reduced the ripening indices (WSN, NPN, PTA-SN and TVFA) of the resultant cheeses comparing with the control. On the other hand, addition of the TGase, markedly increased the values of all ripening indices including the whole milk cheese. The addition of TGase, showed slight effect on either decreasing the acidity or raising the pH values. Reducing fat content of cheese milk lowered the enumeration of viable total bacterial counts, lipolytic and proteolytic bacteria. The incorporation of TGase increased the three bacterial groups during the first month of ripening and continued for lipolytic and proteolytic bacteria up to the end of ripening period, while TC decreased after the first month. The addition of the TGase, highly improved the organoleptic properties of the three groups of cheese.

Keywords: Transglutaminase (TGase), low-fat Gouda-like cheese, cow milk

INTRODUCTION

Gouda cheese is a semi hard Dutch cheese which first made in the town of its name in Holland. Gouda is a wheel-shaped cheese typically in size from 8 to 45 pounds. Gouda cheese is a sweet curd cheese with a limited number of eyes (Wong, 1974, Zall, 1992, Kosikowski & Mistry, 1997). The texture of Gouda cheese is firm, flexible and round or oval gas scattered throughout the curd. The colour varies from ivory white to yellow. The flavour mild becoming piquant but not sour. Gouda cheese is one of the most popular semi-hard types consumed in Egypt.

Increasing awareness of customers on fitness and healthy lifestyle has led to an increased demand for low-calorie food in particular for low and reduced fat cheeses (Konkular *et al.,* 2004). Since cheese is one of the most widely consumed food products, nutritional sciences and food industry play a prominent role in the development of healthier food ingredients. Fat content is responsible for many desirable functional, textural, and sensory properties in cheese, and its decrease alters physical properties and flavour lowering cheese quality . Changes in functional qualities are, presumably, due to loss of plasticizing property of the fat and increased cross-linking of proteins within the curd and, hence, in the cheese. Though, presence of additional moisture present (Gunasekaran & Ak, 2003) can improve functionality of low-fat cheese, low and reduced fat cheeses have certain disadvantages stipulated by reduction in proportion of moisture in nonfat substances (NFS), level of proteolysis activity, amount of free oil and increased proportion of intact casein proportion (Sheehan & Guinee, 2004). Various techniques used to improve the texture of low and reduced fat cheeses include process modifications, use of special starter cultures and fat replacer

In recent years, an attempt to improve the qualities of low or reduced fat dairy products by using transglutaminase (TG: E.C.2.3.2.13) has been made (Yokoyama *et al.,* 2004, Ozer *et al.,* 2007). Transglutaminase (TGase) is a transferase naturally pre-

sent in most animal tissues and body fluids, and can form both inter- and intra- molecular iso-peptide bonds (γ-glutamyl) lysine between many proteins by cross-linking of the amino acid residues of protein-bound glutamine and lysine (Yokoyama *et al.,* 2004).Many food proteins are good substrates of TGase, especially casein , which is the principle protein in milk .The introduction of additional covalent cross-linking by TGase represents a promising tool to improve the functional properties for casein-based dairy products (Őzrenk, 2006).

The aim of the present investigation is to study the effect of TGase addition on the chemical composition, proteolysis, lipolysis and organoleptic properties of full, reduced and low-fat Gouda-like cheese.

MATERIALS AND METHODS

Materials:

All chemicals used in the present study were of analytical grade supplied by BDH, Sigma and Prolabo Chemical Companies. Fresh cow's milk was obtained from the herd of Sides Experimental Station, Animal Production Research Institute (Cairo). The TGase**:** from *Streptoverticillium mobaraense* (ACTIVA MP, with activity of 100 units /g powder) was obtained from Ajinomoto Europe Sales GmbH, Hamburg, and Germany. Cheese starter consisted of *Lactococcus lactis subsp. Lactis and Lactobacillus helveticus* was obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Table salt used in cheese salting was obtained from El-Nasr Company, Alexandria, Egypt. Hansen's powder rennet was obtained from CHr. Hansen's Laboratories, Copenhagen, Denmark. Trypton Glucose Extract Agar Mediam Code CM127 (TGEA), Nutrient Agar Medium were bought from Oxoid Division of Oxoid LTD., London.

Cheese making: Six batches of Gouda cheese were made as follows:

1- Milk fat was standardized to 3.5%, 2.5% and 1.5% fat content without addition of enzyme and manufactured to (G1, G3 and G5) Gouda-like cheese, respectively.

2- Transglutaminase was added with ratios 0.5/ 1kg of milk batches which contained 3.5, 2.5 and 1.5% fat to prepare Gouda-like cheeses (G2, G4 and G6), respectively. Milk was pasteurized at 63ºC for 30 min., then cooled to 30ºC and $CaCl_2$ (0.02 %) was added. Transglutaminase was added to milk. Gouda -like cheeses were made from the three batches according to Scott (1981). Cheeses were ripened at 15ºC for 3 months at a relative humidity of 85%. Samples for analysis were periodically taken at 0, 1, 2, and 3 month. of ripening. Cheeses were made in duplicate in 2 batches.

Analytical methods:

The pH values of milk and cheese samples were measured using a combined calomel glass electrode pH-meter model HANNA instruments H 18424. Titratabel acidity, moisture and Fat % of milk and cheese were determined according to Ling (1963)**.** Salt was determined according to Simov (1980).

Total nitrogen (TN) was determined using the macro-Kjeldahle method (block digester) as described by Ling (1963). Proteolysis in cheese was followed by the determination of water soluble nitrogen (WSN) content of cheese according to Kucroo & Fox (1982). Non protein nitrogen (NPN) was estimated as described by (Ling,1963) after precipitation proteins with phosphotungstic acid(PTA,5%). Soluble Nitrogen (PTA-SN) was determined according to Jarrett *et al.* (1982). To an aliquot (5 ml.) of WSN, 3.5 ml. of 3.95 M. H_2SO_4 and 1.5 ml. of 33.3% phouphotungstic acid were added and left overnight under refrigeration for complete precipitation of the protein. The mixture was then filtered through Whatman No. 42 filter paper, and 10 ml. of the filtrate were taken for nitrogen determination. Polyacrylamide gel electrophoresis (PAGE) was performed in a slab gel electrophoresis (Gel electrophoresis Apparatus G.E 2/4 LS Pharmacia Sweeden) using the stacking gel method described by Andrews (1983).

Total volatile fatty acids (TVFA) were determined using the method described by Kosikowski (1982) by measuring the total volatile fatty acids (TVFA) in 10 g of cheese sample and were expressed as: mls of 0.1N NaOH required for the titration of water solution.

Microbiological tests:

1- Total bacterial count was determined by the plate count method, according to American Public Health Association Method (APHA, 2004).

2- Proteolytic bacterial count was determined using Nutrient agar medium to which 1 ml of sterile skim milk was added just before pouring the medium .Plates were incubated at 30ºC for 5 days, according to Sharaf(1970). The plates were flooded with 10% hydrochloric acid before recording**.**

3- Lipolytic bacterial count was determined as given by Davis (1955).One ml of emulsified pure butter fat was added toNutrient agar medium, and the plates were incubated at 30ºC for 7 days. Copper sulphate solution 10% was flooded on the plates to detect the fat hydrolytic organisms.

Organoleptic properties: The cheese samples were scored according to Pappas *et al.* (1996) for appearance, body & texture and flavour by a regular score panel including the staff members of Sides Experimental Station and the Dairy Technology Department, Animal Production Research Institute. The scoring was based on the following scale. Flavour: 50 points, Body & Texture: 40 points, Appearance:10 points from Total 100 points.

RESULTS AND DISCUSSION

Chemical composition:

The chemical composition of the control full and low fat Gouda-like cheeses as well as those with transglutaminase during ripening are as follows :

Moisture content:- It is clear from the data in Table(1), that the moisture content of fresh cheese is higher in reduced and low-fat cheeses as compared with full-fat milk cheese . As the ripening period preceded, the moisture content decreased for the six treatments. The addition of TGase markedly increased the moisture content of the three treatments as compaired with the control samples. At the end of ripening period for the enzyme treatments still had the higher moisture contents than the control samples.It were (41.72, 42.13 and 44.87 % for full fat milk (3.5 %) , reduced fat(2.5%) and low fat(1.5 %)) , respectively. The main reason for that was the variation of fat content. The same trend was shown by Sahan *et al.* (2008). As the ripening advanced, the moisture contents of all treatments were found to decrease to reach minimum values after 3 months. Generally, it was noted that the moisture contents of low fat cheese treated with TGase were higher than that in the control (untreated with TGase) either fresh or during ripening. According to Őzrenk (2006) and Gaucge *et al*.(2009). TG-catalyzed cross-linking in casein micelles show a better water –holding capacity, meaning that more free water can be entrapped in the rennet gel network, therefore the moisture content increases. Additionally, this effect was more obvious for low-fat cheese than full-fat cheese. Similar results were observed for yoghurt with low-fat content (Lorenzen *et al.,* 2002).

Fat content: The fat contents of G1 and G2 Gouda cheese were obviously higher than the other treatments (G3, G4,G5and G6) along the ripening period (Table 1). As the ripening period progressed, the fat contents of all treatments were gradually increased reaching to the maximum values after 90 days of ripening. This could be attributed to the reduction of its moisture content throughout the ripening period. Addition of transglutminase had slight lower fat content of the resultant fresh cheese or during ripening comparing with the control untreated TGase samples (G1,G3andG5) .This may be due to the addition of TGase to cheese milk which led to relatively higher moisture retention in the resultant cheese .El-Aidie (2005) mentioned that addition of fat replacers to Edam cheese had no pronounced effect on the fat/dry matter(F/DM) contents of the resultant cheeses as compared to the control during ripening.

As the ripening period progressed, the F/DM contents of all treatment samples were gradually increased reaching to the maximum values after 90 days of ripening. The rate of increase was according to the following order: G2 < G4 < G6.

Total Proteins: As shown from the data in Table (1), the protein contents of the treated cheeses were higher than that of the control for fresh and along the ripening period. Treatment samples contained TGase possessed the highest values according to the following order G2<G4 < G6.

Moreover the data revealed that there is a contradictory relationship between the fat content of the milk and the protein content of cheese during ripening, as the fat content increased , the protein content decreased. This could be attributed to the reduction of cheese moisture content. This phenomenon was confirmed previously by Guinee *et al.* (1998) and Sahan (2008).

Salt content: The salt content of G1and G2

was relatively closer to the low-fat cheese G3, G4, G5 and G6, along the ripening period (Table 1). The salt contents of the treated cheeses G2, G4 and G6 was slightly higher than the untreated cheeses G1, G3 and G5 during the ripening period.

The data in Table (2) indicated that higher acidity was observed for higher fat content cheeses. This was observed in the control cheese (G1) which had acidity of 0.82% in fresh sample, compared to 0.76% and 0.74% in G3and G5,respectively.These results were confirmed by Fenelon & Guinee (2000). Fat content in the resultant cheeses of the both treatments, plays the essential role for this relationship. This trend was continued along the repining period.

Similar findings were noted when transglutaminase was added. The treatments G2, G4 andT6 showed lower percentage of TA during ripening as compared to G1,G3 and G5. The trends of these results agreed with those found in several TGase treated dairy products by Neve *et al.* (2001), Lorenzen *et al.* (2002), Abou-El-Nour *et al.* (2004), Ozer *et al.* (2007) and Masoud *et al.* (2008). In addition, the observed long acidification time for TGase treated milk, is in agreement with the study of Faergemand *et al.* (1999). The availability of low molecular weight peptides needed for the growth and activity of bacteria (Tamime & Robenson 1985) was found to decrease due to the arises

Table 1: Effect of adding transqlutaminase on the chemical composition of Gouda-like cheese during 3 months of ripening

Ripening period (months)	Treatments*					
	Full-fat		Reduced-fat		Low-fat	
	G1 (Control)	G2	G3 (Control)	G4	G5 (Control)	G6
			Moisture %			
Fresh	42.75	44.62	44.65	46.60	47.32	49.37
1	41.40	43.15	42.96	44.88	45.93	47.82
2	40.50	42.44	41.18	43.15	44.77	46.60
3	39.80	41.72	40.26	42.13	42.68	44.87
			Fat %			
	G1	G2	G3	G4	G5	G6
Fresh	28.70	28.30	22.20	21.70	15.80	15.20
1	29.44	29.10	23.10	22.50	16.60	16.30
2	29.90	29.55	24.00	23.40	17.20	16.90
3	30.30	30.00	24.80	24.10	18.80	18.40
			Total Protein %			
	G1	G2	G3	G4	G5	G6
Fresh	22.25	22.57	26.70	27.14	30.22	30.73
1	22.80	23.22	27.52	28.20	31.60	32.18
2	23.22	23.54	28.44	29.18	32.37	33.06
3	23.58	24.02	28.92	29.74	33.49	33.99
			Salt %			
	G1	G2	G3	G4	G5	G6
Fresh	2.15	2.25	2.10	2.20	2.08	2.19
1	2.54	2.73	2.58	2.71	2.54	2.68
2	2.89	3.12	2.82	3.07	2.80	3.01
3	3.04	3.27	2.99	3.22	2.97	3.18

* G1 (control): Full-fat untreated cheese
G2: Full-fat TGase treated cheese
G3 (control): Readuced-fat untreated cheese
G4: Readuced-fat TGase treated cheese
G5 (control): Low-fat untreated cheese
G6: Low-fat TGase treated cheese

cross-linking bonds which may explain the slow growth and activity of yoghurt starter.

Also, it can be seen from Table (2) that the TA of all cheese treatments had the same trend of increase along the ripening period, as it increased with variable rates during ripening to reach the highest values after 3 months of ripening.

The data for the pH values given in Table (2) show that the pH values had an opposite trend towards the TA. It decreased gradually in all treatments up to the end of the ripening period.The data, also, revealed that reduction of cheese fat or adding TGase elevated the pH value comparing to the control, along the ripening period.

In general, it can be concluded that the reduction of cheese fat content, led to the decrease in the TA, comparing to the control during ripening, whereas an opposite trend was observed when TGase was added.

Ripening indices:

Table (3) includes WSN/TN, NPN/TN and PTA-SN / TN for the six different treatments. Control whole milk, reduced milk and low-fat cheese treatments had lower ripening indices as compared with those produced from added TGase . On the other hand, as the fat content decreased the three ripening indices decreased. For the six treatments as the ripening period progressed, the three ripening indices gradually increased to reach the maximum values after 3 months of ripening These variations may be due to the higher moisture content and the lower salt content and proteolytic bacterial counts noted in reduced and low-fat cheeses. These results are in agreement with those found by Haque *et al.* (2007) in Cheddar cheese and Sahan (2008) in Kashar cheese.

Reducing fat content in low-fat cheese resulted in decreasing the (WSN/TN), NPN/TN and PTA-SN/TN contents in the control cheeses (G3, G5) during ripening, compared to G1 cheese, in spite of the relatively higher percent of protein in the former samples (G3, G5) , than the control cheese (G1). The degree of proteolysis of low-fat cheeses was lower than that of full-fat cheeses, which may be partly due to the decrease of both water/protein(W/P) and moisture in non fat substances(MNFS). The decrease in W/P and MNFS, which means decrease in the available moisture, decreased the activity of enzymes and microorganisms and thereby the degree of proteolysis (Lane *et al.,* 1997 , Rudan *et al.* 1999**).** The results also indicate that in the TGase-treated samples, the degree of proteolysis was higher during the first month and lower at later ripening periods than the corresponding control cheeses. Compared with G5, G3 and G1 (TG-untreated), cheeses obtained treated by TGase showed higher W/P and MNFS, which led to more proteolysis initially

Table 2: Titratable acidity (%) and pH values of Gouda-like cheese as affected by transglutaminase addition during 3 months of ripening .

Ripening period (months)	Titratable acidity (TA) (%)					
	Treatments*					
	G1 (Control)	G2	G3 (Control)	G4	G5 (Control)	G6
Fresh	0.82	0.77	0.76	0.75	0.74	0.72
1	0.99	095	0.94	0.91	0.92	0.90
2	1.30	1.26	1.23	1.16	1.15	1.09
3	1.87	1.80	1.68	1.63	1.53	1.50
			pH values			
	G1(Control)	G2	G3(Control)	G4	G5(Control)	G6
Fresh	5.61	5.62	5.72	5.73	5.80	5.83
1	5.54	5.57	5.65	5.66	5.67	5.68
2	5.49	5.54	5.51	5.57	5.61	5.63
3	5.04	5.10	5.12	5.16	5.20	5.22

*G1 (control): Full-fat untreated cheese
G2: Full-fat TGase treated cheese
G3 (control): Readuced-fat untreated cheese
G4: Readuced-fat TGase treated cheese
G5 (control): Low-fat untreated cheese
G6: Low-fat TGase treated cheese

Table 3: Proteolysis in Gouda-like cheese as affected by transglutaminase addition during 3 months of ripening.

Ripening period (months)	WSN /TN					
	Treatments*					
	G1(Control)	G2	G3(Control)	G4	G5(Control)	G6
Fresh	8.64	9.50	7.28	7.81	6.42	7.35
1	9.92	10.41	8.82	8.67	7.85	8.22
2	11.61	11.75	9.63	9.24	8.77	8.42
3	12.64	12.18	10.78	10.32	9.36	8.85
	NPN /TN					
	G1(Control)	G2	G3(Control)	G4	G5(Control)	G6
Fresh	3.87	4.98	3.16	4.36	2.88	3.45
1	4.72	5.55	4.33	4.42	3.94	3.96
2	5.12	5.62	4.82	4.65	4.17	4.06
3	5.99	5.67	4.90	4.77	4.52	4.33
	PTA-SN /TN					
	G1(Control)	G2	G3(Control)	G4	G5(Control)	G6
Fresh	1.12	1.53	1.01	1.42	0.86	1.26
1	1.75	2.06	1.54	1.58	1.43	1.48
2	2.84	2.98	2.63	2.16	2.32	2.09
3	3.36	3.27	2.99	2.51	2.47	2.28

*G1 (control): Full-fat untreated cheese
G2: Full-fat TGase treated cheese
G3 (control): Readuced-fat untreated cheese
G4: Readuced-fat TGase treated cheese
G5 (control): Low-fat untreated cheese
G6: Low-fat TGase treated cheese

as discussed previously. At later ripening months, TGase catalyzed additional cross-linking of proteins and TG interference with the action of the coagulant enzyme may lead to the slower degradation of the TG-treated cheeses (Pierro *et al.,* 2010). Moreover, the inhibitory effect on the proteolysis of low-fat cheese was more obvious than that of full-fat cheese.

As the ripening period progressed, the values of WSN/TN, NPN/TN and PTA-SN /TN increased gradually in all samples reaching the maximum levels at the end of the ripening period. The treatments contained TGase recorded the highest values than the control cheeses. Variations in microbiological and chemical compositions among the treatments, as well as the protein breakdown occurred through the growth of cheese microflora and/ or the proteolytic enzyme activity may be the main reasons responsible for this increase.

The intensity of low-fat Gouda-like cheeses treated with TGase decreased with the progress of ripening period. This result confirmed the level of PTA-SN which was higher at low levels of fat cheeses treated withTGase than the others.

Polyacrylamide Gel Electrophoresis (PAGE): The electrophoretic patterns (Fig.1) of Gouda-like cheese made from cow milk of different fat content with or without TGase were performed to monitor the ripening of cheeses.

The electrophoretic patterns of Gouda-like cheese treated with or without TGase at different fat levels appeared to have some protein bands in different regions. The intensity of αs1- casein molecular weight (~27 KDa) and β-casein (~25 KDa), γ -casein(~20.7KDa), proteose peptone fraction 5 (~19 KDa) were identified in polyacrylamide gels. The molecular weights of these bands correspond to those published by Eigel *et al.* (1984) & Fox (1989). Number of bands decreased during ripening. Many bands could be detected in the fast region that could be derived from the degradation of αs1- casein and also, the patterns revealed great degradation of β-casein.

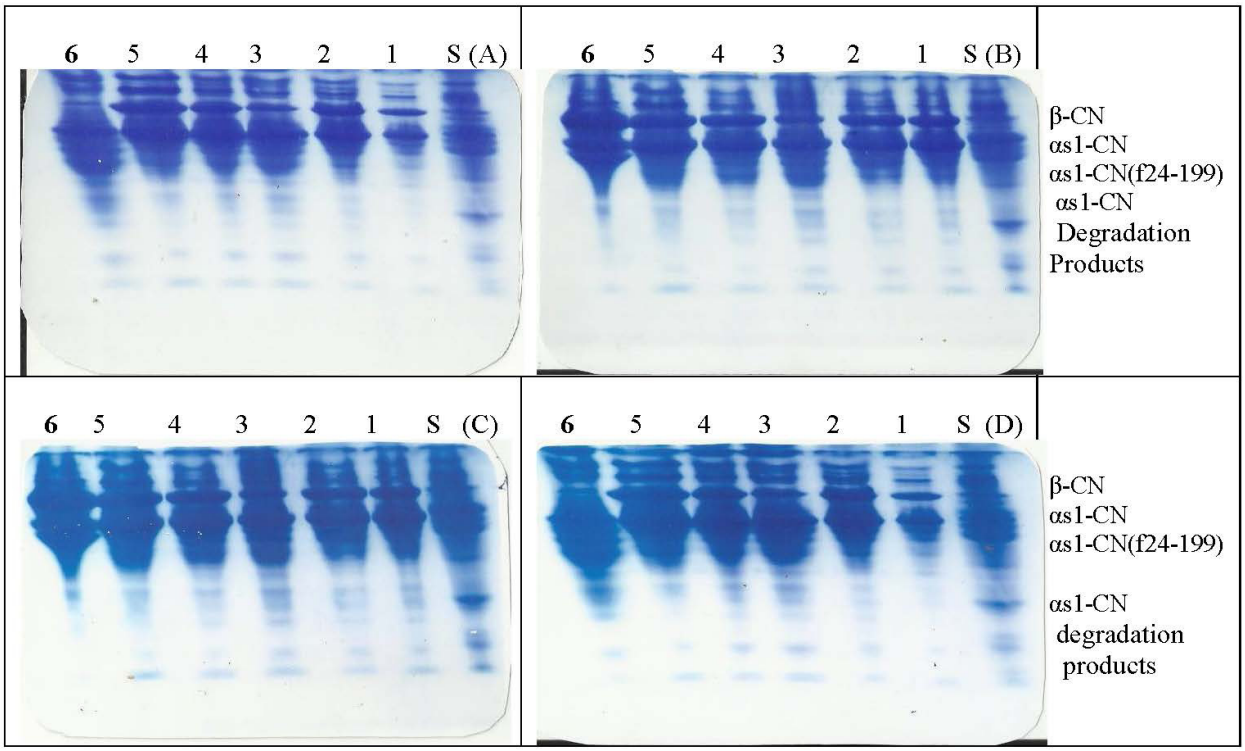

Fig. 1: Polyacrylamide gel electrophoretic patterns of fresh and ripened Gouda cheese.

(A: fresh, B: 1 month., C: 2 month. and D: 3 month Ripening period);
Slots: S: Sodium caseinate
1, 3 and 5 (Control): without added TGase (G1, G3 and G5)
2, 4 and 6 : with added TGase (G2, G4 and G6)

Total Volatile Fatty Acids (TVFA): Table (4) shows that there is a direct relationship between the fat content of cheese and its content of TVFA, when fresh and along the ripening period. It means that as the fat content of cheese increased the level of TVFA increased. These results were demonstrated by Abd El-Kader *et al.* (2001), Khalil (2003), Shehata *et al.* (2004) and Ali (2006).

Addition of TGase to cheese milk of low –fat cheese raised the TVFA contents in the resultant cheese (G2, G4 and G6) to the highest values during ripening, compared with the control (G1, G3 and G5). Moreover, Table (4) shows that cheese containing TGase had higher TVFA contents, during ripening, than that found in the control cheese (G1, G3 and G5). Decreasing of acidity and in-

Table 4: Total Volatile Fatty Acid (TVFA) contents of Gouda-like cheese as affected by transglutaminase during ripening .

Ripening period (months)	Total Volatile Fatty Acids (TVFA)*					
	Treatments**					
	G1	G2	G3	G4	G5	G6
Fresh	7.7	8.4	6.9	7.9	6.6	7.8
1	12.1	13.5	11.8	12.89	10.9	12.6
2	16.4	18.3	14.6	16.9	12.2	16.5
3	19.1	21.7	16.7	19.8	14.3	19.6

* Milliliters of 0.1 N NaOH/ 100g cheese
**G1(control): Full-fat untreated cheese
G2: Full-fat TGase treated cheese
G3 (control): Readuced-fat untreated cheese
G4: Readuced-fat TGase treated cheese
G5 (control): Low-fat untreated cheese
G6: Low-fat TGase treated cheese

creasing of moisture contents of these treatments (G2, G4 and G6) than the controls may be responsible for differences in TVFA.

Microbiological tests: Counts of bacteria, yeasts and moulds, were present in cheese throughout ripening and contribute, in a positive manner, to the maturation process either directly through their metabolic activity or indirectly through the release of enzymes into the cheese matrix through autolysis.

Table (5) shows that the total viable bacterial count (TC) of cheese was found to decrease, during ripening, as the fat content decreased. Variations in the chemical composition between full-fat cheese (G1) and reduced-fat or low-fat cheese (G3&G5) may be the reason behind this decrease.The TC of full-fat cheese (G1&G2) were 7.6 cfu x$10^7$and 8.8 cfu x10^7 at the beginning of ripening, decreased to 6.2 cfu x10^7 and 7.3 cfu x10^7 at the end of ripening period. Counts in G3, G4 were 6.7cfu x10^7 /g, 7.5 cfu x10^7 /g declined to 5.1, 6.6 cfu x10^7 /g for TC. This trend of decrease in the bacterial count as the fat content of cheese decreased was approved previously by Laloy *et al.* (1996), Fenelon *et al.* (2001) and Ali (2006). Total bacterial count began to increase after ripening and continued until the cheese was 4 weeks old. Then, the number of bacteria rose slowly with storage. The overall total bacteria count was higher in full-fat Edam cheese (Erdogan & James, 1999).In accordance, Haque *et al*. (2007) found similar trend in low-fat Cheddar cheese.

The use of TGase in the production of low-fat Gouda cheese reduced the TC in the resultant cheese during ripening compared to full-fat cheese .Variations in moisture, acidity among these treatments play crucial role in bacterial activity. Banwart (1981) referred to the reduction of water activity because of the higher water holding capacity and subsequently inhibiting the bacterial growth. Moreover, Ozer *et al.,* (2007) demonstrated that addition of TGase to yoghurt decreased the rate of starter culture activity, owing to the cross-linking of protein molecules occurred. (Cross-linking of milk proteins by MTGase had a growth-slowing

Table 5: Effect of adding transglutaminase to Gouda-like cheese milk on the total bacterial count, total proteolytic and lipolytic bacterial counts in the resultant cheese, during 3 months of ripening .

Ripening period (months)	Total bacterial count (cfu10^7/g)*					
	Treatments**					
	G1	G2	G3	G4	G5	G6
Fresh	7.6	8.8	6.7	7.5	5.6	6.7
1	9.4	10.3	8.5	9.1	7.3	8.4
2	8.1	9.2	7.2	8.4	6.2	7.5
3	6.2	7.3	5.1	6.6	4.4	5.0
	Proteolytic bacterial count (cfu10^3 /g)					
	G1	G2	G3	G4	G5	G6
Fresh	41	43	35	36	31	32
1	79	80	64	66	52	55
2	99	102	78	84	63	69
3	118	124	101	106	84	88
	Lipolytic bacterial count (cfu10^3 /g)					
	G1	G2	G3	G4	G5	G6
Fresh	28	30	16.5	19	11.5	13.5
1	49	56	28.5	37	22.5	29
2	57	61	39	42	30.5	35
3	64	69	43	47	33	39

*Cfu: colony forming units

**G1(control): Full-fat untreated cheese
G2: Full-fat TGase treated cheese
G3(control): Readuced-fat untreated cheese
G4: Readuced-fat TGase treated cheese
G5(control): Low-fat untreated cheese
G6: Low-fat TGase treated cheese

effect on yogurt starter bacteria).

During ripening, TC of all treatments were found to increase up to 30 days of ripening then declined gradually reaching the lowest ones at the end of the ripening period. Decreasing the moisture content and increasing acidity participate in this reduction. Furthermore Fox *et al.* (2004) added that starters provide the most significant contribution to the microbial mass in young curd, typically attaining densities of >=10^8cfu/g within one day of manufacture. This biomass represents considerable biocatalytic potential for cheese ripening reactions. However, the majority of the starter systems are intracellular and do not have immediate access to the cheese matrix. During cheese ripening, many starters loose viability and release their intracellular enzymes due to autolysis. The counts of proteolytic and lipolytic bacteria were decreased by decreasing the fat content in the cheese, along the ripening period, (Table 5).

Addition of TGase to cheese milk of full –fat and low fat caused an increase in the lypolytic and proteolytic bacteria comparing to the control samples (Table 5).

As the ripening period advanced, the counts of proteolytic and lipolytic bacteria of all treatments gradually increased up to the end of the ripening period. This trend of increase was found to be opposite to that happened in TC. These results are in agreement with that found by Effat *et al.* (1992) and Kebary *et al.* (2002). Moreover, variations in the count of the microorganisms among the treatments were due to the manufacturing process influences the gross composition of the cheese which is best defined by the four parameters-salt-in-moisture (S/M), moisture in non-fat substances (MNFS), fat in dry matter (F/DM) and pH (Gilles & Lawrence, 1973). These parameters in turn influence the environment in which the microorganisms proliferate. The primary environmental factors controlling growth of microorganisms in cheese include water and salt content, pH, presence of organic acids and nitrate, redox potential and ripening temperature (Beresford *et al.,* 2001).

Cheese Structure: The relationship between cheese composition and quality of cheese has long been recognized (O'Conner, 1974 & Fox, 1975). However it appeared that the only scheme for assessing cheese quality on the basis of compositional analysis which has been put commercially was that proposed by Gilles & Lawrence (1973), who mentioned a number of factors , fat in dry matter(F/DM), moisture in non fat substances (MNFS) , pH and salt in moisture (S/M) .The MNFS is the most single important factor affecting cheese quality. The results in Table (6) showes the changes in MNFS and moisture to protein M/P which are the major factor responsible for ripening process and cheese texture. The present study indicated that changes in major components of cheeses due to the reduction of fat content in the cheese milk treated with TGase showed relatively near value of MNFS compared with(G1) full-fat cheese. Because the moisture did not replace the fat on an equal basis, there was a decrease in the moisture in non fat substances (MNFS) and in the moisture to protein in the cheese, which is in agreement with results reported by Fenelon & Guinee (2000). The results in Table (6) showed that MNFS and M/P were higher in treated TGase cheese than untreated TGase cheeses .The literature indicated that bitterness develops early in the aging process and is a common defect in aged low-fat cheeses, partly because of low-salt content and high moisture. Hydrophobic compounds produced by proteolysis are perceived with greater intensity of bitterness in low-fat cheeses than in full-fat cheeses because these compounds are obscured by fat (Olson & Johnson, 1990). Bitterness in low-fat cheese may be lowered by increasing the salt in moisture phase of cheese to > 4.5 % to control microbial activity, but this also make the cheese harder (Banks *et al.,* 1993, Mistry & Kesperson, 1998).

Organoleptic properties: Significant differences were noted between full-and low-fat cheeses during ripening ,Table (7). Full-fat Gouda-like cheese is characterized by smooth body ,open texture and clean acid flavour. Awad *et al.* (2003) stated that no fracture was observed when full-fat Gouda cheese samples compressed in the hand and it was of a pliable texture.The control cheese (G1&G2) gained the highest scores along the ripening period as compared to the other treatments contained or free from enzyme. These results are consistent with that found by Drake *et al.* (1996) and Fox *et al.* (2000).

Notwithstanding, low-fat cheese was of the lowest sensory properties among the other treatments, along the ripening period. It is characterized by flat flavour , more firmer and elastic texture than the control cheese (El-Neshawy *et al*., 1986, Madeson & Ardo, 2001).

Table 6: Changes in the structural properties of Gouda-like cheese during 3 months of ripening.

Ripening period (months)	Treatments*					
	Full-fat cheese		Reduced-fat cheese		Low-fat cheese	
	M /P %**					
	G1	G2	G3	G4	G5	G6
Fresh	1.92	1.98	1.67	1.72	1.57	1.60
1	1.82	1.87	1.57	1.61	1.45	1.49
2	1.76	1.81	1.48	1.50	1.39	1.41
3	1.71	1.76	1.42	1.46	1.32	1.33
	MNFS %**					
	G1	G2	G3	G4	G5	G6
Fresh	59.46	62.23	57.39	59.51	56.20	58.22
1	58.67	60.86	55.86	57.91	55.07	56.99
2	57.77	60.24	54.18	56.33	54.07	55.94
3	57.10	59.61	53.54	55.51	52.56	54.85
	S/M%**					
	G1	G2	G3	G4	G5	G6
Fresh	5.03	5.04	4.70	4.72	4.40	4.44
1	6.14	6.33	6.01	6.04	5.53	5.60
2	7.14	7.35	6.85	6.98	6.25	6.31
3	7.64	7.84	7.43	7.64	6.96	7.08
	F/DM %**					
	G1	G2	G3	G4	G5	G6
Fresh	50.13	51.10	40.11	40.64	29.99	30.02
1	50.17	51.19	40.49	40.82	30.70	30.85
2	50.25	51.25	40.80	41.16	31.14	31.27
3	50.33	51.48	41.51	41.65	32.79	33.01
	P/DM %**					
	G1	G2	G3	G4	G5	G6
Fresh	38.86	40.75	48.24	50.82	57.37	60.69
1	38.91	40.84	48.25	51.16	58.44	61.67
2	39.03	40.90	48.35	51.33	58.61	61.91
3	39.17	41.21	48.41	51.39	58.43	61.64

* G1 (control): Full-fat untreated cheese
G2: Full-fat TGase treated cheese
G3 (control): Readuced-fat untreated cheese
G4: Readuced-fat TGase treated cheese
G5 (control): Low-fat untreated cheese
G6: Low-fat TGase treated cheese
** M /P %: moisture to protein
MNFS %: moisture in non fat substances
S/M%: salt in moisture
F/DM %: fat in dry matter
P/DM %: protein in dry matter

For low-fat cheese, Awad *et al.(*2003) added that it had very hard and brittle body , crumbling under compression forces, when cut with the knife distinct layers are observed as the sample crumbles. .Moreover, Rogers *et al.* (2010) found that one of the major problems with fat reduction in Cheddar cheese is the development of a firm texture that does not breakdown during mastication , unlike that observed in full-fat cheeses. It appeared that the diminished texture quality in low-fat cheese is attributed to changes in the breakdown patterns during chewing, as altered by fat disrupting the

Table 7: Effect of adding transglutaminase to cheese milk on the organoleptic properties of the Gouda-like cheese, during 3 months of ripening .

Treatments*	Ripening period (months)	Appearance (10)	Body & texture (40)	Flavour (50)	Total Scores (100)
G1 (control)	Fresh	9.0	30	27	66.0
	1	8.0	31	32	71.0
	2	8.0	32	41	81.0
	3	9.0	35	45	89.0
G 2	Fresh	8.0	31	27	66.0
	1	8.0	32	36	76.0
	2	8.0	33	42	83.0
	3	8.0	32	48	90.0
G3 (control)	Fresh	7.5	27	25	59.5
	1	7.5	29	30	66.5
	2	7.7	30	37	74.5
	3	7.5	32	41	80.5
G 4	Fresh	7.0	26	26	59.0
	1	7.0	30	33	68.0
	2	7.0	30	40	77.0
	3	7.0	31	44	82.0
G5 (control)	Fresh	7.0	24	25	56.0
	1	7.0	25	27	59.0
	2	7.0	26	35	68.0
	3	7.0	29	38	74.0
G 6	Fresh	7.0	25	26	58.0
	1	6.5	26	30	62.5
	2	6.5	27	37	70.5
	3	7.0	28	41	76.0

*G1(control): Full-fat untreated cheese
G2: Full-fat TGase treated cheese
G3(control): Readuced-fat untreated cheese
G4: Readuced-fat TGase treated cheese
G5(control): Low-fat untreated cheese
G6: Low-fat TGase treated cheese

cheese network. The microstructure of reduced fat and low-fat cheeses may explain their excessively firm texture. Drake *et al.* (2010) carried out a study to document and compare flavor development in Cheddar cheese with different fat contents so as the quantitatively characterized how flavour and flavour development in cheese are altered with fat reduction. The results showed that differences documented between full-fat and low-fat Gouda cheese and are not due solely to differences in matrix and flavour release but also to distinct differences in ripening biochemistry, which leads to an imbalance of many flavour contributing compounds . Correlation between sensory properties of low fat cheese and its microstructure was noted, also, by Mistry & Anderson (1993). They found that the microstructure of reduced and low –fat cheeses may explain their excessively firm texture. Microstructure of reduced and low –fat have large stretches of uninterrupted protein matrix with few fat globules scattered between them. Full fat cheese exhibit a protein matrix interspersed liberally with fat globules. The lack of fat globules in low-fat cheese resulted in the firm, undesirable texture (Bullens *et al.,* 1994). Data in the same Table (6) indicated, moreover ,that low-fat gained the lowest scores during ripening.

The action of TGase on the organoleptic properties of low fat cheese was found to improve the texture of low fat cheese during the first month and lower it at later ripening times comparing with the corresponding TG-untreated cheeses. The panelist scores displaying in the former Table declared that TGase cheese containing the enzyme had total scores near to the control cheese and higher than the other treatments, during ripening. It is characterized by good body and texture and clean acid flavour. The structure was improved greatly than low-fat, being more open, soft, pliable and have the highest body and texture scores in comparison with the other treatments, with the exception of the control cheese

which had the highest scores for all properties of cheese quality.Bohdziewicz & Bohdziewicz (2010) reported that the use of TGase in the manufacture of cheese increased slightly the hardness and sweet taste and decreased the whey leakage of Twarog cheese in comparison with the control products **.**

From the previous results it is worth to note generally that:

- Reduction of cheese milk fat% had an adverse effect on the organoleptic properties of the resultant cheese.
- Addition of TGase enzyme greatly improved the sensory properties of cheese specially the body and texture .
- Full-fat cheese was found to be the best treatment followed by TGase treatments .
- Organoleptic properties of all cheeses gradually improved as the ripening period proceeded.

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دراسة مقارنة على تأثير إضافة أنزيم ترانس جلوتاميناز إلى اللبن البقرى على صفات الجبن شبيه الجودا

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يهدف هذا البحث الى دراسة تأثير اضافة انزيم ترانس جلوتاميناز إلى اللبن البقري على الخواص الكيماوية والميكروبيولوجية والحسيه للجبن شبيه الجودا

تم تصنيع الجبن شبيه الجودا من لبن بقرى كامل ٣,٥٪ دهن ، ومنخفض الدهن الى ٢,٥ ٪ ، ومنخفض الدهن الى ١,٥ ٪ بدون اضافة الانزيم واعتبرت المعاملات الثلاث بمثابة كونترول وتم تصنيع ثلاث معاملات مماثلة مع إضافة الانزيم بمعدل ٥ و ٠ جم/ ١كجم لبن.

تشير النتائج المتحصل عليها الى أن خفض نسبة الدهن باللبن أدى الى ارتفاع نسب الرطوبة ، البروتين ،والـ pH وانخفاض نسبة الدهن و الحموضة فى الجبن وكذلك انخفاض بسيط فى نسبة الملح/ الرطوبة، من ناحية أخرى أدى خفض الدهن الى انخفاض قيم دلائل التسوية متمثلة فى النيتروجين الذائب فى الماء ، النيتروجين غير البروتينى، النيتروجين الذائب فى حامض الفوسفوتنجستك وكذلك الهجرة الكهربائية للبروتينات ، بينما أدى اضافة انزيم ترانس جلوتاميناز الى اللبن للمعاملات الثلاث إلى ارتفاع ملحوظ فى قيم دلائل التسوية وكذلك زيادة ظاهره فى ألأحماض الدهنيية الطيارة وكانت هذه الزيادات أكثر وضوحا فى الجبن منخفض الدهن عن الجبن كامل الدسم .كما أدى الانخفاض الدهن الى تقليل الاعداد البكتيرية الكلية وأيضا المحللة للدهن والبروتين أما إضافة الأنزيم أدى الى زيادة أعداد البكتريا المحللة للدهن و المحللة البروتين حتى نهاية فترة التخزين بينما زاد العدد الكلى للبكتريا فى الشهر الاول ثم أنخفض بعد ذلك فى كل المعاملات .

حصل الجبن كامل الدسم على أعلى درجات التحكيم الحسى مقارنة بالجبن منخفض الدهن، تبين أن أضافة أنزيم ترانس جلوتاميناز قد حسنت بشكل كبير الخواص الحسية للجبن .