Improvement of Low Fat Kashkaval Cheese Quality by Using Transglutaminase: b- The Impact on some Rheological Characteristics

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Abstract: Improvement of low fat Kashkaval cheese quality by using different percentages of Transglutaminase has been studied. Low fat Kashkaval cheese was made from mixture of buffalo's and cow's milk (1:1) standardized to 1% fat, treated by adding Transglutaminase (TGase) at the rate of 0.3, 0.5 and 0.7 g/Liter respectively. The resultant cheese was stored for 45 days at 10°C and 80% relative humidity. Data showed that using Transglutaminase in low fat cheese increased moisture %, TN % and fat %, while decreased acidity % as compared to low fat control cheese. Using Transglutaminase had significant positive effect on the rheological characteristic of low fat kashkaval cheese. A linear relationship was found between the concentration of TGase used and the improvement of cheese rheological properties. It could be concluded that low fat Kashkaval cheese can be made from milk treated with 0.7 g TGase/L in order to obtain cheese of acceptable texture and good body.

Keywords: Cheese rheology, Texture profile, Transglutaminase, Kashkaval cheese

INTRODUCTION

Kashkaval cheese name is derives from the Italian cheese "*Caciocavallo*". It is particularly popular in Eastern Europe and Mediterranean region. The Romanians call this cheese *cascaval*, the Greeks *kasseri* while the Turkish prefer to interpret it as *Ksara*. Kashkaval belongs to the pasta filata cheeses. This means that the curd is given a hot bath during the production process (Alichanidis and Polychroniadou, 2008).

Low fat products especially low fat cheese had a great attention from consumers all over the world as a result of the desire to decrease the calories intake to avoid health problems and diseases. Because of that the market of low fat cheese has been grown and the manufacturers compete each other to produce high quality low fat cheese (Johansen *et al*., 2011). For cheese, fat is not only a nutrition factor but it is also a very important component that contributes to the cheese characteristics such as taste, functionality, appearance and rheological properties. Removing or reducing fat from cheese lead to have low quality cheese as a result of appear many defects, lacking in the distinctive cheese flavor and texture is not comparable to full fat cheeses (Koca and Metin, 2004; Ahmed *et al*., 2015).

One of the major problems with fat reduction in cheese is inadequate breakdown of casein and, the development of a firm texture that does not break down during mastication, unlike that observed in full-fat cheeses (Rogers *et al*., 2010). Creamy mouthfeel and texture provided by fat can be achieved by increasing the moisture content beyond that of full- fat cheese (Mistry, 2001). Researchers have been trying to find different ways to develop low fat cheese versions of the known cheese varieties. Approaches that have been used to improve low-fat cheese texture involve decreasing protein concentration (*i.e.*, increasing moisture to protein ratio of the gel phase), causing greater hydrolysis of the proteins, altering protein - protein interactions or creating a bigger filler phase (Mistry, 2001; Rogers *et al*., 2010). One of these ways is using transglutaminase to make cross-linking bonds between milk proteins to improve the water holding capacity, texture stability, and the rheological properties of cheese (Gauche *et al*., 2010).

Transglutaminase (TGase) is a useful tool to change the rheological properties of caseins without damaging their special functional properties (Ozrenk, 2006). The rate of TGase cross-linking depends on the macromolecular structure of protein involved.

TGases (transglutaminases; EC 2.3.2.13) belong to a family of enzymes that catalyse an acyl-transfer reaction between the γ -carboxamide group of peptide-bound glutamine residues and various primary amines, including the ε-amino group of endoprotein lysine residues (Özrenk, 2006).

Therefore, the aim of this study was to investigate the impact of using Transglutaminase at different dosage on some rheological properties of obtained low fat kashkaval cheese and to monitor the changes of cheese quality during the ripening period.

MATERIALS AND METHODS

Materials:

Fresh Cow's and buffalo's milk were obtained from the herd of Faculty of Agriculture, Suez Canal University, Ismailia governorate. Direct Vat yoghurt Starter (DVS) culture containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* and Rennet powder, CHY-MAX were obtained from Chr-Hansen's laboratories, Denmark. Commercial salt was obtained from the local market. Calcium chloride was obtained from El-Naser pharmaceutical and chemical company, Cairo governorate, Egypt. Transglutaminase was obtained from Ajinomoto Europe Sales GmbH, Hamburg, Germany.

Experimental procedure:

Five treatments were carried out. Treatment 1 (T1) was made from mixture of buffalo's and cow's milk (1:1) standardized to 3.25% fat to serve as full fat cheese control. The other four treatments were made from mixture of buffalo's and cow's milk (1:1)

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standardized to 1% fat, milk then divided to four portions: the first portion treatment 2 (T2) was regarded as low fat Kashkaval cheese control. Treatment 3 (T3), treatment 4 (T4) and Treatment 5 (T5) were made by adding Transglutaminase (TGase) at the rate of 0.3, 0.5 and 0.7 g/Liter respectively. Kashkaval cheese was made according to Simov and Ivanov (2005) as shown in Fig. (1). The levels, incubation and the inactivation temperature of TGase were used according to Ajinomoto applications data. The resultant cheese was stored for 45 days at 10°C and 80% relative humidity. The whole experiment was thrice. The chemical composition of standardized milk used to make full and low fat Kashkaval cheese is shown in Table (1).

Table (1): Chemical composition (%) of standardized milk used to make low fat Kashkaval cheese (Mean value of three replicates)

Type of milk	TS%	Fat%	Casein	C/F
Buffalo's and cow's milk (1:1)	12	3.25	2.6	0.8
Low fat buffalo's and cow's milk (1:1)	9.5	1	2.6	2.6

Cheese samples were analyzed when fresh, 15, 30 and 45 days for the chemical composition. The rheological characteristics were determined when fresh, 15 and 45 days.

Milk
↓
Standardized to C/F=0.8/or 2.6
↓
Heating at 75°C/15 sec
↓
Cooling to 40°C
↓
Addition TGase
↓
Incubating at 40°C/60 min.
↓
Inactivation of TGase at 80°C/ 1 min.
↓
Cooling to 33°C
↓
Addition of Starter culture (1U/100L)
↓ 30 min.
Addition of Starter culture (1U/100L)
↓
Addition of $CaCl_2$ (0.02%w/v)
↓
Addition of rennet
↓ 30-40 min.
Addition of rennet
↓
Cutting curd (2 cm)- stirring curd
↓
Scalding to 38-40°C for 40 min.
↓
Drainage-off the whey
↓
Cheddaring (36°C- 2h.)
↓
Kneading of curd (hot 12% NaCl solution 72°C) for 2 min.
↓
Moulding of cheese curd
↓ 15 h.
Waxing
↓
Storage at 10°C and 80% relative humidity for 45 days

Fig (1): Flow sheet of the production of Kashkaval cheese treated with TGase

Methods of analysis:

Analysis of cheese milk (Total solids; Fat; and Casein content) was determined according to the methods described in AOAC (2007). Cheese sample were analyzed chemically when fresh and after 15, 30 and 45 days of storage. Moisture content and total nitrogen content (TN) was measured by Kjeldahl method using semi-micro Kjeldahl were carried out according to the methods described by AOAC (2007). Acidity was determined by the titration method according to Vujicic *et al.* (1996) and fat content by Gerber butyrometer described by Ling (1963).

Rheological properties:

Texture profile analysis test of cheese samples (which shape was 2×2×2 cylindrical) was done using a Universal Testing Machine (TMS-Pro) Food Technology Corporation, Sterling, Verginia, USA) equipped with 1000 N (250 lbf) load cell and connected to a computer programmed with Texture Pro^{TM} texture analysis software (program, DEV TPA With holding time between cycle two second). A flat rod probe (49.95 mm in diameter) to uniaxially compresse the "cheese samples with the following parameters conduction to 30% of their original height. Each sample was subjected to two subsequent cycles (bites) of compression-decompression.

Data were collected on computer and the texture profile parameters were calculated from (program, DEV TPA) texture analyzer and computer interface. Calculation described by Szczesniak *et al.* (1963) and Bourne (1978) was used to obtain the following texture profile parameters (Hardness, Cohesiveness, Springiness, Gumminess and Chewiness). The rheological characteristics were determined when fresh, 15 and 45 days of ripening.

Statistical analysis:

All measurements were done in triplicate and analysis of variance with two factorial (treatments and storage period) were conducted by the procedure of General Linear Model (GLM) using CoStat (1998) under windows software version 6.311 and least significant difference (LSD) test were employed to determine significant difference at $p<0.05$.

RESULTS AND DISCUSSION

Gross component of cheese:

Moisture content is one of the macro components of any type or variety of cheese. Table (2) shows the average of moisture content of low fat Kashkaval cheese treatments. Moisture content of low fat Kashkaval cheese treatments pronouncedly ($p\leq 0.05$) higher than full-fat cheese (T1). According to Özrenk (2006) and Gauche *et al*. (2008), TGase-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, and therefore the moisture content increases. Furthermore, the differences in moisture content between the full fat and the low fat cheese may be attributed to their protein content, *i.e.* a higher protein content of low fat cheese may contribute to increase water binding capacity of the cheese matrix (Romeih *et al.,* 2002; Ahmed *et al.,* 2015). On the other hand, the moisture content of all treatments showed a marked ($p\leq 0.05$) decrease throughout the storage period. Similar trends were observed by Abd El-Gawad *et al.* (2007), and Santa and Srbinovska (2014).

Data illustrated in Table (2) showed that total nitrogen content of the resultant low fat Kashkaval cheese treatments significantly ($p\leq 0.05$) higher than that of control full-fat (T1) when fresh and throughout the storage period. Adding Transglutaminase at all levels studied were increased significantly TN content ($p\leq 0.05$) as compared with control full-fat (T1) and control low-fat (T2) cheese. Same results were recorded by El-Kholy (2005) for low fat Tallaga cheese and Metwally *et al.* (2018) for low fat Mozzarella cheese. During the 45 days of storage, the TN content increased significantly ($p\leq 0.05$) among all treatments. This increament might be due to the loss of moisture content during the storage period. These results in agreement with (Abd El-Gawad *et al.,* 2007; Santa and Srbinovska, 2014; Talevski *et al.,* 2017).

Fat has a great effect on the palatability of any type of cheese; it can also affect the smoothness and richness of the body and texture. With increasing the level of Transglutaminase, there was a significant ($P\leq 0.05$) increase in fat content as compared with control low-fat Kashkaval cheese (T2).These results are in agreement with those reported by (El-Kholy, 2005; Ya-nan Hu *et al.,* 2013). Lorenzen *et al.* (2000), and Ya-nan Hu *et al.* (2013) indicated that transglutaminase can increase the gel strength of dairy products by catalyzing the covalent bond of Ɛ-(γ-glutamyl) lysine. So, a stronger rennet gel catalysed by TGase can prevent rennet curd from being smashed and fracturing and hence fat loss reduced. Fat content of all treatments increased significantly ($P\leq 0.05$) during the storage time. These variations in fat content may be due to the changes in moisture content of different cheese treatments during storage period.

It is obviously clear from Table (2), that the acidity of low fat cheese treatments was lower than full fat control cheese (T1). There was a significant ($p<0.05$) decrease in titratable acidity (TA) with the increase of Transglutaminase levels added. Similar results also observed by El-Kholy (2005). TA of all cheese samples had the same trend. There was a continuous increase in titratable acidity with the progressive ripening period either in the control or in the TGase treated cheese. These results were in accordance with the results for low fat Tallaga cheese given by El-Kholy (2005); Santa and Srbinovska (2014) for Galichki Kashkaval and Ahmed *et al*. (2015) for low fat Gouda cheese.

Table (2): Effect of using different percentages of Transglutaminase on gross component of low fat Kashkaval cheese during ripening period (Mean value of three replicates)

Treatments	Storage period (days)				Mean
	Fresh	15	30	45	
	Moisture content %				
T1	43.5	42.11	41.60	41.13	42.09^{E}
T2	45.70	43.67	43.03	42.41	43.70^{D}
T3	46.60	45.10	44.53	44.00	45.06^{C}
T4	47.23	45.80	45.26	44.77	45.77^{B}
T5	47.85	46.37	45.85	45.37	46.36^{A}
Mean*	46.18^{a}	44.61^{b}	44.05^{c}	43.54^{d}	
Treatments	TN%				
	Fresh	**15**	**30**	**45**	**Mean**
T1	4.11	4.25	4.3	4.36	4.255^{D}
T2	6.22	6.36	6.49	6.53	6.400^{C}
T3	6.27	6.38	6.52	6.57	6.435^{C}
T4	6.29	6.44	6.56	6.61	6.475^{B}
T5	6.33	6.48	6.59	6.65	6.513^{A}
Mean*	5.844^{d}	5.982^{c}	6.092^{b}	6.144^{a}	
Treatments	Fat%				
	Fresh	**15**	**30**	**45**	**Mean**
T1	27.0	27.9	28.5	28.8	28.05^{A}
T2	7.9	8.3	8.4	8.7	8.33^{D}
T3	8.1	8.4	8.5	8.7	8.43^{D}
T4	8.5	8.7	8.9	9.0	8.78^{C}
T5	9.0	9.2	9.4	9.5	9.28^{B}
Mean*	12.10^{d}	12.50^{c}	12.74^{b}	12.94^{a}	
Treatments	Acidity%				
	Fresh	**15**	**30**	**45**	**Mean**
T1	0.99	1.30	1.33	1.40	1.26^{A}
T2	0.85	0.94	1.00	1.20	0.99^{B}
T3	0.80	0.87	0.92	0.96	0.89^{C}
T4	0.78	0.84	0.88	0.93	0.86^{D}
T5	0.75	0.81	0.86	0.89	0.83^{E}
Mean*	0.83^{d}	0.95^{c}	0.99^{b}	1.08^{a}	

T1: control full fat, T2: control low fat, T3: 0.3 gTGase/L, T4: 0.5 g TGase/L, T5: 0.7 g TGase/L.
*a, b, c & d and A, B, C & D: means with the same letters among treatments and storage period respectively are not significantly different (p> 0.05)

Rheological Characteristics:

Rheology of cheese, defined as the study of their deformation and flow when subjected to a stress or strain. The behavior of the cheese when subject to these stresses or strain is referred by descriptive terms such as hardness, firmness, springiness, crumbliness or adhesiveness. Cheese rheology is a function of its composition, microstructure, the physicochemical state of its components and the macrostructure (Fox *et al.* 2017).

Hardness of cheese can be defined as high resistance to deformation by applied stress (Fox *et al.* 2000). From Table (3), It is clear that the decreasing of fat affect the hardness of the kashkaval cheese. low-fat kashkaval cheese treatments had higher (P<0.05) hardness values than the full-fat control (T1). These results are coincided with Ya-nan Hu *et al.* (2013), Ahmed *et al.* (2015). Rudan *et al.* (1999), explained that the increase of hardness was due to the decrease of the fat content and total filler volume and the increase of the amount of matrix (protein). Although the moisture content increased, it did not completely offset the decrease in fat as indicated by the steady increase in protein content with the reduction of fat content.

Adding TGase decreased the hardness of the low-fat cheese significantly (P≤ 0.05). Transglutaminase improved the hardness of low fat treatments due to the cross-linking of protein occurred by TGase in the resultant cheese (Bonisch *et al*., 2007). Moreover, the use of TGase in the production of low-fat cheese increased the water-holding capacity, emulsification, foaming, viscosity and solubility in the final product (Li- Chan, 2004). These results in accordance with Ya-nan Hu *et al.* (2013) and Ahmed *et al.* (2015). During the storage period the hardness increased up to the day 15 and then decreased significantly (P<0.05) at the end of the storage. The decrease in hardness at the end of the storage period is according to cheese proteolysis. Pierro *et al*. (2010) reported that the reduction of hardness of TG- treated cheeses during early ripening was probably because of proteolysis.

Fox *et al.* (2000) defined the springiness as the tendency of a deformed material to recover to its undeformed condition after removal of deformation stress. It can be noticed from Table (3) that full fat control cheese (T1) had the highest (P≤ 0.05) springiness value among all treatments while, low fat control cheese (T2) had the lowest (P≤ 0.05) value. This result may be due to that decreasing fat content in cheese matrix have more compact protein matrix with less open spaces that would be occupied by milk fat globule (Bryant *et al.,* 1995). Fox *et al.* (2000) stated that as concentration of casein in cheese matrix increases, the intra- and inter-strand linkages become more numerous and the matrix displays greater elasticity and more difficult to deform and more Springer. In contrast of the current study Koca and Metin (2004) reported that full- fat Kashar cheese had significantly lower springiness values than low fat cheese during ripening. Adding Transglutaminase increased springiness values in the low fat Kashkaval cheese treatments T3, T4 and T5 respectively. These results in agreement with Ya-nan Hu *et al.* (2013). In all treatments springiness values decreased with storage progressed.

The cohesiveness is explained as the ratio of the shape of the resistance to the second compression to the behavior of the compression (Fox *et al.,* 2000; Tunick*,* 2000). It was found that the mean of cohesiveness value of low fat control cheese was higher than that of full-fat cheese (Table 3). Adding Transglutaminase to the low fat Kaskaval cheese treatments decrease the cohesiveness. This may be due to the increase in the moisture content by adding Transglutaminase. During ripening periods the cohesiveness increased up to 15 days then decreased by the end of ripening.

Table (3): Effect of using different percentages of Transglutaminase on the hardness, Springiness and Cohesiveness of low fat Kashkaval cheese during ripening period (Mean value of three replicates)

Treatments	Storage period (days)			Mean
	Fresh	15	45	
		Hardness (N)		
T1	19.2	33.0	24.8	25.67^{E}
T2	25.3	49.5	37.2	37.33^{A}
T3	22.9	45.5	33.0	33.80^{B}
T4	21.1	44.7	29.5	31.77^{C}
T5	20.5	43.3	25.3	29.70^{D}
Mean*	**21.8^{c}**	**43.2^{a}**	**29.96^{b}**	
		Springiness (mm)		
T1	4.52	2.36	1.82	2.90^{A}
T2	4.30	1.97	1.62	2.63^{D}
T3	4.37	2.2	1.67	2.75^{C}
T4	4.40	2.25	1.71	2.79^{C}
T5	4.44	2.29	1.77	2.83^{B}
Mean*	**4.41^{a}**	**2.21^{b}**	**1.72^{c}**	
		Cohesiveness (Ratio)		
T1	0.70	0.90	0.73	0.78^{D}
T2	0.79	0.99	0.85	0.88^{A}
T3	0.77	0.96	0.83	0.85^{AB}
T4	0.76	0.95	0.80	0.84^{BC}
T5	0.74	0.93	0.78	0.82^{C}
Mean*	**0.75^{c}**	**0.95^{a}**	**0.80^{b}**	

See Table 2 for treatments designation

*a, b & c and A, B, C, D & E: means with the same letters among treatments and storage period respectively are not significantly different (p> 0.05)

Gumminess is described as breaking force required to ingest a semi-solid food easily (Raphielides *et al.,* 1995). Table (4) shows that gumminess values of low fat control cheese (T2) and low fat treatment (T3) exhibit higher (P≤ 0.05) values of Gumminess than full fat control cheese (T1). This may be due to the high hardness values corresponding to those low fat cheese treatments. Full-fat cheese had significantly (P≤ 0.05) lower Gumminess values than low fat cheese during storage. Adding Transglutaminase decreased the gumminess of the low fat cheese treatments T3, T4 and T5 respectively as compared to low fat control cheese (T2). This may be due to the reduction of hardness by adding Transglutaminase. Gumminess of all treatments increased significantly (P≤0.05) up to 15 days and then decreased significantly (P≤0.05) at the end of ripening period.

Chewiness is defined as required chewing force to make a solid food ready for ingesting (Raphielides *et al.,* 1995). Chewiness values (Table 4) of low fat Kashkaval cheese treatments are higher (P≤ 0.05) than that of full fat control cheese (T1). This may be due to the higher hardness values of low fat cheese treatments. It could be noticed that adding Transglutaminase decrease significantly (P≤0.05) the chewiness values of treatments T3, T4 and T5 respectively. Chewiness of all treatments increased significantly (P≤0.05) With prolonged ripening period up to 15 days then decline significantly (P≤0.05) at the end of ripening period.

It was noticed that treatment 5 (0.7 g TGase/L) gained the highest score for all texture profile properties and overall acceptability among all low fat Kashkaval cheese treatments when fresh and throughout storage period.

Table (4): Effect of using different percentages of Transglutaminase on the Gumminess and Chewiness of low fat Kashkaval cheese during ripening period (Mean value of three replicates)

Treatments	Storage period(days)			Mean
	Fresh	15	45	
	Gumminess (N)			
T1	13.44	29.70	18.1	20.41^{E}
T2	19.99	49.05	31.62	33.55^{A}
T3	17.63	43.68	27.39	29.57^{B}
T4	16.04	42.47	23.60	27.37^{C}
T5	15.17	40.27	19.73	25.06^{D}
Mean*	**16.46^{c}**	**41.03^{a}**	**24.09^{b}**	
	Chewiness (N)			
T1	60.75	70.09	32.94	54.59^{E}
T2	85.96	96.54	51.22	77.91^{A}
T3	77.04	96.10	45.74	72.96^{B}
T4	70.58	95.56	40.36	68.83^{C}
T5	67.35	92.22	34.92	64.83^{D}
Mean*	**72.34^{b}**	**90.10^{a}**	**41.04^{c}**	

See Table 2 for treatments designation
*a, b & c and A, B, C, D & E: means with the same letters among treatments and storage period respectively are not significantly different (p> 0.05)

The results obtained in this study indicate that using Transglutaminase improved low fat Kashkaval cheese rheological characteristics when fresh and along storage period. A linear relationship was found between the concentration of TGase used and the improvement of cheese rheological properties. The cross-linking of protein occurred by TGase led to decrease the hardness of the resultant cheese. A significant positive correlation among these three TPA parameters (hardness, gumminess and chewiness) was found. It was observed that both the difference of means and the changes in the hardness values of all cheeses showed a similar trend to the changes in the gumminess and chewiness values during storage.

The addition of Transglutaminase to low-fat kashkaval cheese highly improved overall acceptability. It could be concluded that low fat cheese can be made from milk treated with 0.7 g TGase/L in order to obtain cheese of acceptable texture and good body.

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تحسين جودة جبن الكشكفال المنخفض الدهن باستخدام الترانس جلوتامينيز: ب- التأثير على بعض الخصائص الريولوجية

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في هذا البحث تم دراسة جودة جبن الكشكفال المنخفض الدهن باستخدام نسب مختلفة من إنزيم ترانس جلوتامينيز. صُنع جبن كشكفال منخفض الدهن من خليط من اللبن الجاموسى والبقرى (1: 1) معدل إلى نسبة 1٪ دهن، حيث تم تصنيع 5 معاملات، المعاملة الأولى جبن كامل الدسم (معاملة المقارنة) بدون إضافة الإنزيم والمعاملة الثانية جبن منخفض الدهن (معاملة المقارنة 2) بدون إضافة الإنزيم والمعاملات الأخرى استخدم الإنزيم فيها بمعدل 0.3 و 0.5 و 0.7 جم/لتر من اللبن على الترتيب. تم تخزين الجبن الناتج لمدة 45 يومًا عند 10 درجات مئوية ورطوبة نسبية 80٪. أظهرت النتائج أن استخدام إنزيم الترانس جلوتامينيز في الجبن المنخفض الدهن أدى إلى زيادة نسبة الرطوبة ٪ والنيتروجين الكلى ونسبة الدهن، بينما انخفضت الحموضة ٪ مقارنة بالجبن منخفض الدهن (معاملة المقارنة 2). كان لاستخدام إنزيم ترانس جلوتامينيز تأثير إيجابي ومعنوي على الخصائص الريولوجية لجبن الكشكفال منخفض الدهن. وجدت علاقة خطية بين تركيز الإنزيم المستخدم وتحسين الخواص الريولوجية للجبن. يمكن أن نستنتج أن جبن الكشكفال المنخفض الدهن يمكن أن يصنع من اللبن المعامل بـ 0.7 جرام ترانس جلوتامينيز/لتر لبن للحصول على جبن ذو قوام وتركيب جيد.