J. Food and Dairy Sci., Mansoura Univ., Vol. 8 (2): 79 - 86 , 2017

Characterization of Transglutaminase Isolated from Rosemary Leaves, and Development of Chemical, Rheological and Sensory Properties of Kareish Cheese Made with Transglutaminase.

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ABSTRACT

Purification of transglutaminase (TGase) isolated from leaves of rosemary (*Rosmarinus officinalis* L) carried out by three steps including protein precipitation with using ammonium sulfate (40-80%), followed by elution into ion exchange column (DEAE-Sephadex A-50). The further purification of dialyzed fractions were applied on Sephadex G-100. The purified enzyme eluted from gel filtration column (Sephadex G-100) had 26.51 yield associated with increased about 6.3 in fold of purification. The optimum pH for rosemary TGase was 6.0 and the highest activity of this enzyme associated with incubated at 60°C for catalytic reaction of Z-Gln-Gly and hydroxylamine. The TGase was stable following heating up to 70 for 15 min, while a complete loss of TGase activity was associated with exposing to 90°C for 5 min. The exposure of TGase to salt concentrations ranging from 2-4% did not effect on its activity. However increased NaCl concentrations of 5-14% caused a decline in the activity. The effects of cross-linked by different concentrations of TGase (2.5, 5 and 10U/g protein) on chemical, sensorial and textural of Kareish during 15 days of storage period were studied. The Kareish cheese chemical composition presented to be affected by using different concentration of TGase. The moisture and yield were the highest values in cheese made by 10U/g protein of TGase, while pH was the lowest value in the same treatment, compare with other concentrations of TGase and control. Kareish cheese made by using 10 10U/g protein of TGase receive high scores in sensorial properties. Hardness, adhesiveness, gumminess and chewiness values were decreased significantly ($P<0.05$) in Kareish cheese made with TGase than control.

Keywords: Kareish cheese- Transglutaminase – Rosemary – Cross-linked – Purification – Texture properties

INTRODUCTION

Dietary fat consumption is associated with many chronic diseases such as cardiovascular diseases (Fenelon and Guinee, 2000). This association has resulted increased consciousness among consumers and corresponding an increase in demand for low- fat dairy products (Dexheimer, 1992). However, the low fat cheese consumption is still low (Broadbent *et al.,* 1997). Reduced fat cheese has low typical flavor intensity and rubbery, hard and grainy texture (Emmons et al 1980; Jameson, 1990; Olson and Johnson, 1990; Muir *et al.,* 1992). Thence, the challenge in enhancement of low fat cheese is to make cheese has a flovour and texture profile more closely to full fat cheese (Wilkinson *et al.,* 2001).

Dairy products are multicomponent materials with high complex structure and properties of textures that are evaluated by consumers (Dickinson, 1997). Dairy industries worldwide are designing to enhance dairy products or new ingredients with novel functional and physical properties (Sharma *et al.*2001). Proteins are the important part of the major groups of building blocks for textural characteristics of dairy products and also for nutritional value of human (Sakamoto *et al.,* 1994). Several food proteins are used as functional components for enhancing the stability, texture and handling characteristics of dairy products. It has reported that proteins structure could be modified by physical, chemical or enzymatically methods (Yildirim 1998; Faergemand *et al.,* 1998). Modification of protein structure by enzymatic reaction has been presented as a best method due to high enzymatic reactions specificity and low risk for human health. Cross-linked enzyme is a way that has received increasing attentiveness over the last 15 years (Motoki and Seguro, 1998; Faergemand *et al.,* 1998; Sorensen *et al.,* 1999). Transglutaminase (TG) is the only cross-linking enzyme that catalyzes formation of covalent bond between protein particles on a commercial scale (Dickinson 1997). The transglutaminase catalyzed reactions are presented in Fig. 1 (Zhu *et al.,* 1995; Motoki and Seguro 1998; Sharma *et al.,* 2001). TGases are vastly found in many body fluids and tissues of vertebrates. TG was first investigated in liver of guinea pig and considered as an enzyme catalyzing polyamines incorporation into glutamine residues of peptides or proteins (Wilhelm *et al.,* 1996). Moreover TG activity has been detected in actinomycetes belong to genus Streptoverticillium such as *Strep. Mobaraense* (Ando *et al.,* 1989; Kanaji *et al.,* 1993). Plant TGase was found in pea seedlings (Icekson and Apelbaum 1987), and also TGase activity has been detected in the silver beet leaves (*Beta vulgaris* L.).In a related study, Kang and Cho (1996) purified the same enzyme from leaves of soybean (*Glycine max*.) The TGase activity was also found in barley, pea, wheat (*Lilley et al.,* 1998). TGase was recently purified and identified from leaves of rosemary (*Rosmarinus officianlis* L.) (El-Hofi *et al.,* 2014).

Kareish cheese is a traditional cheese in Egypt, is made from skimmed milk (Abou Donia, 1991) and therefore presented unfavorable textural properties of reduced fat cheeses. The objective of this study was to evaluate the sensory and texture properties and Kareish cheese yield made using cross-linking of milk proteins by transglutaminase extracted and purified from rosemary (*Rosamarinus officinalis* L.).

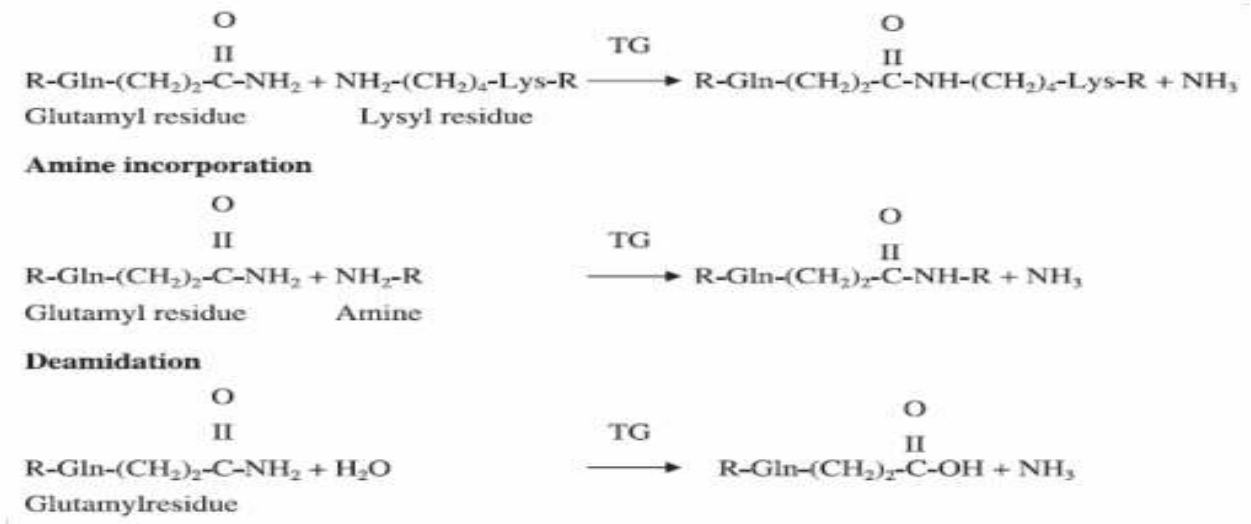

Figure 1. Mechanism of Transglutaminase (TG) action that catalyzed formation of covalent bond between protein particles.

MTERIALS AND METHODS

Crude enzyme preparation

Transglutaminase in a crude extract was prepared by overnight soaking of 40 gm *Rosmarinus officinalis* L. powdered leaves in 0.2 M Tris-HCl buffer pH 7.5 including, (25mM sucrose, 0.05% β-mercaptoethanol, 3% Polyvinylpyrrolidone and 10 mM EDTA), then mixture was filtrated and centrifuged at 6000 g for 15 min at 5 °C . Resultant supernatant was gathered and placed in screw cap tube for further determinations included transglutaminase activity and protein content (El-Hofi *et al.,* 2014).

Protein precipitation of crude extract

To precipitate transglutaminase, ammonium sulfate at 40% saturation was added to the previous crude extract; mixture was kept for 1h at 5°C, and then centrifuged at 6000 g for 10 min at 5°C. The supernatant is collected and added gradually up to 80% saturated $(NH_4)_2SO4$ for additional precipitation. The mixture was kept for 1h at 5°C, subsequently centrifuged at 6000 g for 10 min at 5°C. The supernatant is removed and resultant pellet is resolved in 20 mM Tris-HCl buffer pH 7.5 (8ml). The mixture was dialysed in the same buffer for overnight.

Partial purification of enzyme crude

The dialyzed portion is placed onto Column (15 X250 mm) of DEAE Sephadex A-50. Column equilibration by passage of 20 mM Tris-HCl buffer pH (7.5) through DEAE Sepahdex particles in the column. Elution of the same buffer eliminates unbound proteins. Then , targeted protein bound to the matrix is eluted with gradient concentration of sodium chloride (0 to 0.5 M), made by dissolving in the same buffer at flow rate of 1.5 ml/min. Protein fractions (10 ml) were combined and their absorbance of protein at 280 nm was determined and activity of TG was estimated. Fractions with high activity of TG were dialyzed against Tris-HCl buffer (20mM, pH 7.4) for 18 h.

Final purification of enzyme crude

The further purification of dialyzed fractions were applied on column (25 X 370 mm) of Sephadex G-100 (Phamacia, Uppsala, Sweden), equilibrated with Tris-HCl buffer (20 mM, pH 7.5). The target protein was eluted with the same buffer at a flow rate of 1.5 ml/min and different fractions (as 10 ml) were collected. The fraction with high activity of TG considered as purified enzyme.

Assessment of TG activity:

The activity of TG was determined according to Folk and Cole., (1966). Each unit activity of TG is expressed as the enzyme amount which catalyzes the reaction of Z-Gln-Gly and hydroxylamine for production of hydroxamic acid (0.5 µmole/min at 37°C).

Content of protein determination

Total protein was determined by using Coomassie brilliant blue G-250 dye described by Bradford (1976).

Characterisitics of TG biochemical

Optimum pH

The determination of optimum pH of purified enzyme were performed according to El-Hofi *et al.,* 2014.

Optimum temperature

Screw cap tubes including the enzyme extract and substrate were kept for 10 min at various temperatures ranging from 30-80 °C (El-Hofi *et al.,* 2014).

Thermal stability of TG

The extract of enzyme was heated at various temperature ranging from 60-80°C for 10 and 20 min, subsequently rapid cooling to 37°C and determined for residual activity of enzyme (El-Hofi *et al.,* 2014).

The influence of different concentration of NaCl on TG activity

The enzyme extract was treated with different NaCl concentration ranged from 3-15% the activity of enzyme was calculated as relative activity (El-Hofi *et al.,* 2014).

Manufacture of experimental Kareish cheese

Kareish cheese was made as described by Abou-Donia, (2008). Skimmed milk was divided four parts. First part was used for manufacture of control Kareish

cheese (free TGase). The second, third and fourth parts was incubated with 2.5, 5 and 10 U/ g protein of TGase, respectively at 50°C for 60 min after pasteurization. Milk samples were inoculated with 2% of yogurt starter culture, followed by incubated at 42°C for 3-4 hours. The cheese curd was dipped into moulds, after complete coagulation, and then salting of cheese with dry salt (3% w/w) and kept at 4°C to drain for overnight. The resultant cheese was then cut into suitable blocks and stored at 4°C for 15 days

Chemical analysis of experimental Kareish cheese samples

The moisture, fat, protein, pH of Kareish cheese were assessed according to (AOAC, 2005).

Texture profile assessment of experimental Kareish cheese samples

Textural characteristics of experimental samples were assessed using texture analyzer (TA 1000, Lab pro (FTC TMS-Pro),USA). Hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness were determined according to Szczesniak *et al.,* (1963).

Evaluation of sensory

Assessment of sensory properties of experimental Kareish cheese were performed according to Ahmed *et al.,* (2005).

Statistical analysis

Data presented are the mean of thress separate of measurements. The SAS software (SAS Institute, 2004) was performed for ANOVA. Differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Transglutaminase purification

Total activity, specific activity, yield and purification fold of TG purified form *Rosmarinus officinalis* L. is presented in Table (1). Total activity of enzyme and yield were decreased during the progress of purification steps, where the total activity and yield of crude enzyme were 270 U and 100% respectively, whereas the purified enzyme activity war reached to 71.57 U. However the specific activity and purification fold were increase during the purification steps progress, where the specific activity and purification fold of crude enzyme were 44.62 U/mg protein and 1 respectively, whereas the specific activity and purification fold of purified enzyme were 280.66 U/mg protein and 6.3 respectively. It could be noticed from Fig. 2 that A_{280} and TGase activity increased progressively till reaching the highest rate in the fraction 38, but it reduced subsequently. This suggests that this maximum TGase activity was associated with high protein content. However in the final step of TGase purification that A_{280} and partially purified enzyme activity eluted from gel filtration sephadex G100 increased till reaching the highest value in fraction 15 (Fig. 3).

Many previous studies have been used different types of ion-exchange chromatography (SP-Sepahrose, DEAE-cellulose, Q Sepharose and DEAE-Sepharose) for purification of TGase (Ha and Iuchi, 1997; Kumazawa *et al.,* 1997). The current study was in agreement with Goldsmith and Martin, (1995) and Kwak *et al.,* (1998). And also the present study for purification of TGase by gel filtration Sephadex G 100 was in agreement with Folk, (1970); Tokunaga and Iwanaga (1993) and El Hofy *et al.,* (2014).

Table 1. Transglutaminase purification separated from leaves of *Rosmarinus officinalis*.

Parameters	Purification steps			
	Crude enzyme	Precipitation of Ammonium sulfate	Partial purification (Sephadex A-50)	Final purification (Sephadex G-100)
Volume (ml)	50	10	40	85
Protein concentration (mg/ml)	0.121	0.098	0.011	0.003
Activity unit/ml	5.4	9.3	1.871	0.842
Total activity unit	270	93	74.8	71.57
Total Protein mg	6.05	0.98	0.44	0.42
Specific activity units/mg protein	44.62	94.89	170.1	280.66
Yield	100	34.44	27.70	26.51
Purification fold	1	2.12	3.8	6.3

Total activity = activity of enzyme x fraction size, Specific activity = Activity of enzyme/ protein concentration, yield =total activity of purification steps/ starting total activity x 100, purification fold= specific activity of purification steps/ starting specific activity.

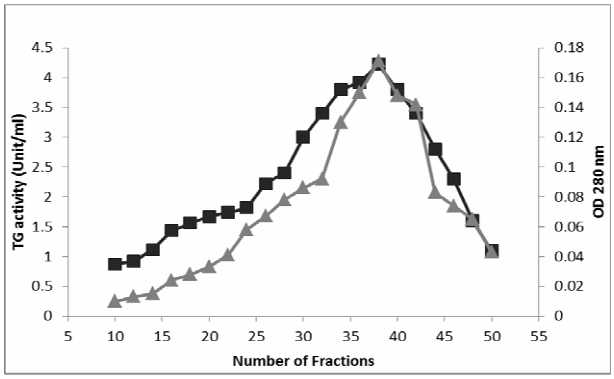

Figure 2. Activity (closed squares) and absorbance at 280 nm (closed triangles) of TGase fractions eluted from a ion exchange DEAE-Sephadex A-50 column.

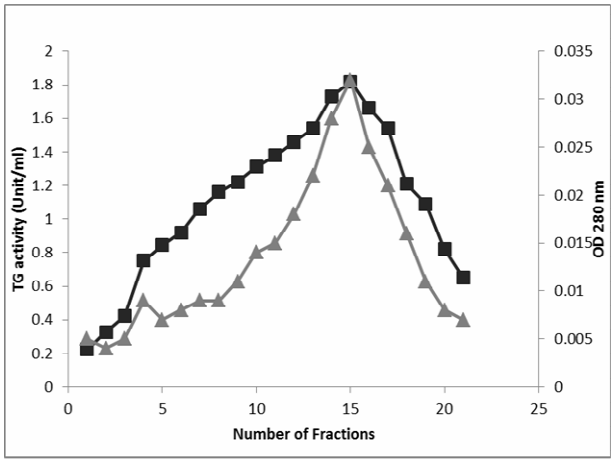

Figure 3. Activity (closed squares) and absorbance at 280 nm (closed triangles) of TGase fractions eluted from a gel filtration Sephadex G-100 column.

The Influence of temperature on transglutaminase activity

The activity of TGase was determined at different temperature ranging from 30 to 80 °C after incubation form 15 min at optimum pH (6.0) with the same reaction mixtures as pointed out previously. Transglutaminase presented high activity at 60 °C for the catalytic reaction of hydroxylamine and Z-Gln-Gly (Fig. 4). The optimum temperature of TGase in the present study was slight higher than that recorded by El-Hofey *et al* (2014), who reported that optimum temperature of rosemary TGase was 55 °C. This might be attributed to the variety of rosemary from which TGase was obtained and may also due to regional variations. The rosemary TGase optimal temperature almost was the same as TGase isolated from *Streptoverticillim mobaraense* (Lu *et al.,* 2003) and it was nearly harmony to that of TGase isolated from *Bacillus subtilis* and *Streptoverticillium ladakanu*, which had optimum activity at 60°C (Ho *et al.,* 2000; Suzuki *et al.,* 2000). However It was totally different, comparing with the optimum activity of enzyme isolated from soybean and *Streptoverticillium hygroscopicus* at 37°C and 45°C, respectively (Kang and cho 1996; Cui *et al.,* 2007).

The influence of pH on transglutaminase activity

The activity of transglutaninase was measured at different values of pH ranging from 3.0 to 10.0 (Fig. 5). The transglutaminase activity for the catalytic reaction of hydroxylamine and Z-Gln-Gly increased progressively in acidic pH till reaching to the optimum activity at pH 6.0. The activity of enzyme was decreased significantly at alkaline pH (Fig.5). Optimum PH in the present study , was little lower than that indicated by El-Hofy et al (2014), who found the optimum activity of transglutaminase isolated from rosemary at pH 7.0.

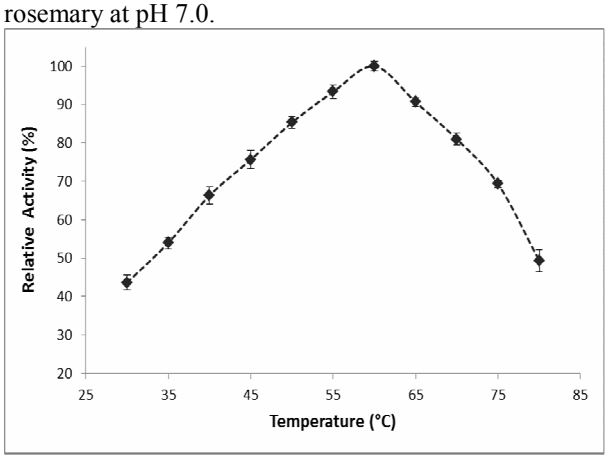

Figure 4. Effect of temperature on the activity of transglutaminase isolated from rosemary leaves.

However the optimum pH for the activity of rosemary isolated transglutaminase is different, compare with other mammals transglutaminase, that had an optimum activity at pH 9.0 (Wong *et al.*1999). Microbial transglutaminase isolated from *Bacillus subtilis* that has an optimal activity at pH 8.2 (Suzuki *et al.,* 2000).

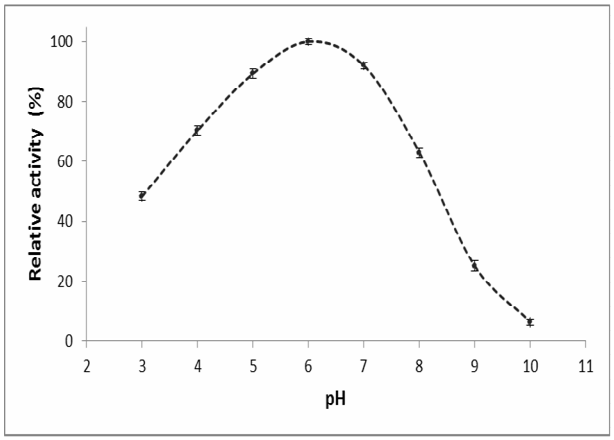

Figure 5. Effect of pH on the activity of transglutaminase isolated from rosemary leaves.

Thermal stability of transglutaminase

The thermal stability of enzyme was assessed between 65-90°C for different times (5, 10 and 15min). It could be noticed from Fig. 5 that TGase was stable following heating up to 70 for 15 min. A decline in activity of TGase was detected with exposing to 75-85°C for 5, 10 and 15 min (Fig 6). The decline in activity rate of TGase is directly proportional to the temperature change. However a complete loss of TGase activity was associated with exposing to 90°C for 5 or 15 min. These results conflict with the previously obtained on other TGase isolated from rat liver, where it had low thermal stability and exposing to 52°C for 4min or 1min at 60°C was associated with a complete loss of TGase activity (Wong *et al.,* 1990).

The influence of NaCl on the activity of transglutaminase

For examining the influence of various NaCl concentration of the relative activity of TGase, a wide range of NaCl concentrations from 2 to 14% was studied. It could be seen from Fig. 7 that exposing the TGase to salt concentrations ranging from 2-4% did not impact its activity. However increased NaCl concentrations of 5-14% caused a decline in the activity. The rate of decline in the activity of TGase is directly proportional to concentrations of salt. This result was nearly consistent with Tokunaga and Iwanaga (1993) and Worratao & Yongsawatdigul, (2005), who found that the activity of TGase inhibited by salt concentrations above 0.5 M. High NaCl concentration might induce changes in conformation of an enzyme, resulting in a decline of transglutaminase activity (Kishi *et al.,*1991; Kumazawa *et al.,* 1997).

Chemical composition of Kareish cheese

The moisture in all cheese samples decreased slightly during the storage period (Table 2). Treating cheese with different concentration of TGase in the present study had significant effect on the moisture content of Kareish cheese (Table 2). The moisture content is directly proportional to concentration of TGase. The moisture content of Kareish cheese treated with 10U/g Protein of TGase was the highest, compare with control and other concentration of TGase (Table 3). This result is consistent with Faergemand and Qvist

(1997), who noted an increase in water-holding capacity of cheese supplemented with TGase. Furthermore the dairy products treated with TGase had fine microstructure and much lower rate of permeabilities than nontreated dairy products.

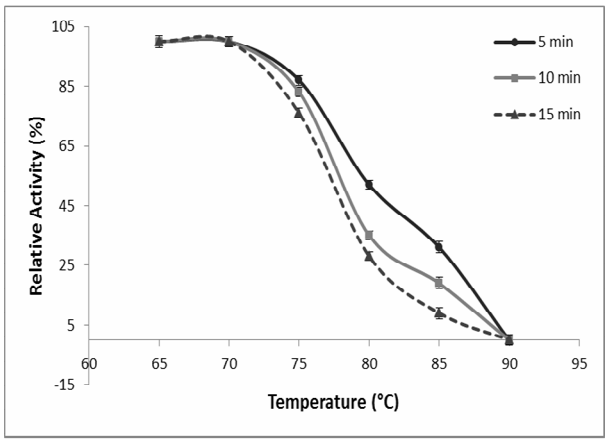

Figure 6.Thermal stability profile of transglutaminase isolated from rosemary leaves.

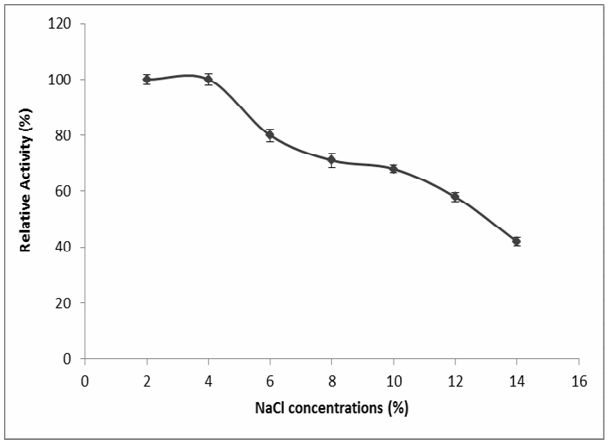

Figure 7. Effect of NaCl concentrations on activity of transglutaminase isolated from rosemary leaves.

The protein and fat content in Kareish cheese samples were detected to be related to the content of moisture in cheese samples over storage period. There were no significant differences ($P < 0.05$) in the content of protein and fat on a dry matter basis between the treatments during storage period (Table 2).

Table 2. Chemical composition analysis of Kareish cheese samples (means ± standard error) made with different concentrations of TGase isolated from rosemary during storage period at 5 °C for 15 days

Treatments	Storage period (days)	Yield (%)	pH	Moisture (%)	Protein/Dry matter (%)	Fat/Dry matter (%)
Control	0	18.3 ± 0.12	4.44 ± 0.03	70.2 ± 0.16	65.50 ± 0.11	0.34 ± 0.12
	7	17.9± 0.15	4.31 ± 0.05	69.8 ± 0.23	65.56 ± 0.12	0.36 ± 0.15
	15	17.6 ± 0.32	4.21 ± 0.02	69.5 ± 0.12	65.57 ± 0.15	0.36 ± 0.18
	Mean	17.93^{d}	4.32^{a}	69.83^{d}	65.55^{a}	0.35
TGase (2.5U/g protein)	0	20.6 ± 0.45	4.29 ± 0.07	72.2 ± 0.12	65.47 ± 0.14	0.36 ± 0.22
	7	20.3± 0.14	4.23 ± 0.08	72.0 ± 0.15	65.50 ± 0.17	0.36 ± 0.33
	15	19.8± 0.12	4.17 ± 0.02	71.7 ± 0.14	65.51 ± 0.15	0.35 ± 0.14
	Mean	20.23^{c}	4.23^{b}	71.96^{c}	65.49^{a}	0.36
TGase(5U/g protein)	0	22.8 ± 0.17	4.22 ± 0.04	74.8	65.48 ± 0.19	0.36 ± 0.17
	7	22.6 ± 0.20	4.19 ± 0.01	74.5 ± 0.11	65.49 ± 0.22	0.35 ± 0.12
	15	22.2 ± 0.18	4.16 ± 0.02	74.2 ± 0.16	65.50 ± 0.26	0.35 ± 0.33
	Mean	22.53^{b}	4.19^{bc}	74.5^{b}	65.49^{a}	0.35
TGase (10 U/ g protein	0	24.9 ± 0.16	4.12 ± 0.03	76.6 ± 0.33	65.51 ± 0.34	0.34 ± 0.35
	7	24.6 ± 0.22	4.08 ± 0.02	76.2 ± 0.24	65.55 ± 0.25	0.35 ± 0.17
	15	24.1 ± 0.25	3.97 ± 0.01	75.8 ± 0.12	65.45 ± 0.41	0.34 ± 0.12
	Mean	24.53^{a}	4.06^{c}	76.2^{a}	65.50	0.34

The effect of different concentrations of TGase on yield percentage of Kareish were presented in Table (2). The yield (%) in all cheese samples decreased slightly during storage period (Table 2). The used TGase for making Kareish cheese was associated with significant increase ($P < 0.05$) yield (%) of cheese. The yield (%) of Kareish cheese treated with 10U/g Protein of TGase was highest, compare with other concentration of TGase and control (Table 2).

The pH values of Kareish cheese samples were determined over storage period are presented in Table (2). The pH values of all samples decreased gradually during progress of storage period (Table 2). The pH values are inversely proportional to TGase concentration used for making Kareish cheese. The lowest pH value of cheese sample made by 10U/g protein of TGase, compare with other concentration and control.

Sensory characterization of Kareish cheese

The mean grades for sensory properties (appearance, flavour and body and texture) of Kareish cheese samples during storage period are presented in Table 3. Panelists found significant differences ($P < 0.05$) in sensory properties of Kareish cheese during storage period(Table 3), generally, the cheese treated with TGase (10 U/ g protein) during different times in storage period received the highest appearance, flovour, body & texture and total sensory properties scores, compare with other treatments and control, but other treatments and control of cheeses were considered accept cheeses. This might because of their relatively higher content of moisture in cheese samples treated with different concentrations of TGase as previous mentioned in Table (2). High moisture content leads to give soft, moist and creamy cheese which reflected the ability of TGase to enhance the texture properties of Kareish cheese. These results are in agreement with those of Ibrahim,*et al.*,(2017), who found that improving of textural, sensorial and nutritional properties of UF-white soft cheese by treated with TGase (5U/g protein).

Table 3. Sensory assessment of Kareish cheese samples (means ± standard error) made with different concentration of TGase during storage period at at 5 °C for 15 days

Treatments	Storage period (days)	Body and texture (40)	Flavour (50)	Appearance (10)	Total (100)
Control	0	36 ± 0.52	47 ± 0.31	7 ± 0.1	90
	7	34 ± 0.83	44 ± 0.28	6 ± 0.92	84
	15	33 ± 0.2	43 ± 0.49	5 ± 0.57	81
	Mean	33.5^{d}	44.7^{c}	6^{b}	84.17^{d}
TGase (2.5U/g protein)	0	37 ± 0.66	48 ± 0.52	7 ± 0.74	92
	7	35 ± 1.32	44 ± 0.73	5 ± 0.82	87
	15	33 ± 0.4	42 ± 0.52	5 ± 0.91	80
	Mean	35^{c}	45.3^{c}	6^{b}	86.33^{c}
TGase (5 U/g protein)	0	38 ± 0.33	48 ± 0.83	8 ± 0.76	94
	7	36 ± 0.86	46 ± 0.55	5 ± 0.35	87
	15	35 ± 0.47	45 ± 0.43	5 ± 0.33	85
	Mean	36.3^{b}	46.3^{b}	6^{b}	88.67^{b}
TGase (10U/g protein)	0	39 ± 0.1	48 ± 0.29	8 ± 0.43	95
	7	38 ± 0.3	47 ± 0.63	7 ± 0.58	92
	15	36 ± 0.63	46 ± 0.32	6 ± 0.72	88
	Mean	37.7^{a}	47^{a}	7^{a}	91.67^{a}

Texture properties of Kareish cheese evaluation

Different textural parameters were assessed in Kareish cheese made with different concentration of TGase isolated from rosemary (Table 4). The textural characteristics of Kareish cheese were influenced significantly ($P < 0.05$) by using different concentration of TGase. Hardness, adhesiveness, gumminess and chewiness values were decreased significantly ($P < 0.05$) in Kareish cheese made with TGase than control (Table 4). The decline rate of hardness, adhesiveness, gumminess and chewiness values are inversely proportional to concentration of TGase while values of springiness and cohesiveness were increased significantly ($P < 0.05$) in Kareish cheese made with TGase than control (Table 4). The low values of hardness, adhesiveness, gumminess and chewiness in Kareish cheese made with TGase might be related to its high moisture content weakens the network of protein resulting smooth cheese that covers specific taste buds in mouth during chewiness (Bourne, 1978; Chen, *et al.,* 1978; Bryant, *et al.,* 1995 and Maifrein *et al.,* 2002). These results are consistent with Ibrahim,*et al.,*(2017), who investigated that enhancing of textural properties of UF-white soft cheese by treated with TGase (5U/g protein).

Table 4. Textural parameters of Kareish cheese samples (means ± standard error) made with different concentration of TGase during storage period at at 5 °C for 15 days

Treatment	Pickling period (days)	Hardness (Newton)	Adhesiveness (Jole)	Springiness (Millimeter)	Cohesiveness (Dimensionless)	Gumminess (Newton)	Chewiness (Jole)
Control	0	4.8± 0.12	45 ± 0.34	0.40 ± 0.05	0.32 ± 0.03	1.54 ± 0.41	0.61± 0.03
	7	4.9± 0.33	46 ± 0.51	0.41 ± 0.07	0.33 ± 0.06	1.62 ± 0.31	0.66 ± 0.04
	15	5.1± 0.22	48 ± 0.62	0.42 ± 0.05	0.36 ± 0.07	1.84 ± 0.45	0.77 ± 0.02
	Mean	4.93 ^{a}	46.33^{a}	0.41^{d}	0.34^{d}	1.66^{a}	0.68^{a}
TGase (2.5U/g protein)	0	3.8 ± 0.23	40 ± 0.32	0.41 ± 0.01	0.38 ± 0.03	1.44 ± 0.32	0.59 ± 0.03
	7	3.9 ± 0.12	41 ± 0.17	0.42 ± 0.05	0.40 ± 0.04	1.56 ± 0.13	0.66 ± 0.05
	15	4.3 ± 0.15	43 ± 0.18	0.43 ± 0.04	0.41 ± 0.03	1.76 ± 0.13	0.76 ± 0.04
	Mean	4^{b}	41.33^{b}	0.42^{c}	0.4^{c}	1.59^{b}	0.67^{b}
TGase (5 U/g protein)	0	3.1 ± 0.23	31 ± 0.46	0.42 ± 0.02	0.43 ± 0.08	1.33 ± 0.17	0.56 ± 0.01
	7	3.2 ± 0.25	33 ± 0.37	0.44 ± 0.02	0.46 ± 0.04	1.47 ± 0.22	0.65 ± 0.02
	15	3.4 ± 0.17	35 ± 0.15	0.45 ± 0.03	0.48 ± 0.01	1.63 ± 0.27	0.73 ± 0.03
	Mean	3.23^{c}	33^{c}	0.44^{b}	0.46^{b}	1.48^{c}	0.65^{c}
TGase (10U/g protein)	0	2.6 ± 0.31	24 ± 0.12	0.43 ± 0.03	0.48 ± 0.04	1.25 ± 0.19	0.54 ± 0.07
	7	2.7 ± 0.27	26 ± 0.10	0.45 ± 0.02	0.49 ± 0.08	1.32 ± 0.31	0.59 ± 0.08
	15	2.9 ± 0.14	27 ± 0.11	0.47 ± 0.04	0.51 ± 0.06	1.48 ± 0.42	0.69 ± 0.06
	Mean	2.73^{d}	25.67^{d}	0.45^{a}	0.49^{a}	1.35^{d}	0.61^{d}

CONCLUSION

It is concluded from this study that treatment of milk with different concentration of TGase yielded Kareish cheese sample with high moisture content and low pH. The rheological parameters of cheese treated with TGase included hardness, adhesiveness, gumminess and chewiness were lower than control. The important role of TGase for manufacture of dairy products for improving textural and sensorial characteristics for Kareish cheese.

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توصيف إنزيم Transglutaminase المفصول من أوراق نبات الروزماري واستخدامه في تحسين الصفات الكيميائية والريولوجية والحسية للجبن القريش

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تستهدف الدراسة تنقية انزيم Transglutaminase من أوراق نبات الروزماري والتي تمت علي ثلاث مراحل تتمثل المرحلة الأولي في الترسيب باستخدم كبريتات الأمونيوم (40-80%) ثم يلي ذلك التنقية باستخدام المبادل الأيوني (DEAE –SephadexA-50) وفي نهاية مراحل التنقية يتم استخدام الترشيح بالجل (Sephadex G-100) وقد أشارت النتائج أن معدل إنتاج الإنزيم بتقنية الترشيح بالجل وصل إلي 26.51 بمعدل تنقية يصل إلي 6.3، كما أشارت النتائج أن درجة pH المثلي للإنزيم كانت 6 و درجة الحرارة المثلي 60 ° م . يتميز هذا الإنزيم بقدرته العالية علي تحمل درجات الحرارة المرتفعة، حيث يظل محتفظاً بنشاطه كاملاً عند تعرضه إلي 70 ° م لمدة 15 دقيقة بينما يفقد الإنزيم نشاطه كلياً عند معاملته بدرجة حرارة 90 ° م لمدة خمس دقائق . لا يتأثر الإنزيم عند معاملته بكلوريد الصوديوم بتركيز يتراوح من 2-4% بينما نجد أن نشاط الأنزيم يتأثر في مدي يتراوح من 5-14%. تم دراسة تأثير إضافة هذا الإنزيم علي الصفات الكيميائية والحسية الريولوجية للجبن القريش باستخدام تركيزات مختلفة (2.5 و 5 و 10 وحدة / جم بروتين) خلال فترة تخزين تصل إلي 15 يوم . تميز الجبن الناتج بارتفاع محتواه من الرطوبة وانخفاض درجات pH مقارنة بالكنترول. كما أشارت النتائج إلي ارتفاع معدلات تصافي الجبن الناتج. حصل الجبن القريش الناتج من المعاملة بتركيز 10 وحدة/ جم بروتين من الإنزيم علي أعلي درجات التقييم الحسي. كما لوحظ أن هناك انخفاض ملحوظ في Hardness و Adhesiveness و Gumminess و Chewiness بينما ارتفعت معدلات كلاً من Cohesiveness و Springiness مقارنة بالكنترول.