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Extraction, Purification and Characterization of Transglutaminase from Silver Beet Leaves and its Effect on Sensory, Chemical and Rheological Properties of Kareish Cheese

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ABSTRACT

Strategy in transgluaminase (TGase) extracted from silver beet leaves (*Beta vulgaris* L.) performed by three stages. Ammonium sulfate precipitation (40-80%) is an important technique as an initial step in TGase purification, followed by an impure TGase sample is loaded into 2-(Diethylamino)ethyl ether sephadex A50. The final purification step was carried out by eluting dialyzed fractions into Sephadex G-100. The purified TGase was successfully purified by three purification steps with 224.2 U/mg of TGase specific activity, 3.4 fold increase in purification and 20.33% yields. The optimum temperature and pH of silver beet leaves TGase were 50°C and 5.0, respectively. The TGase completely lost its activity after 15 min at 80°C, however recovery of TGase after exposure to sodium chloride concentrations ranging from 2-6% does not affect its activity. The influence of different concentrations (5, 7.5 and 10U/g protein) of TGase on chemical, textural and sensorial of Kareish over storage time were detected. The yield and moisture content were reached the maximum values in cheese treated with TGase ($10Ug^{-1}$protein), in addition to the same treatment received maximum average scores in sensory evaluation. The concentration of TGase is negatively proportional with the gumminess, adhesiveness and hardnesss values of resulted cheese.

Keywords: transgluaminase - silver beet leaves – Kareish cheese - sensory evaluation

INTRODUCTION

Cardiovascular disease is one of important chronic disease associated with increasing dietary saturated fat intake (Fenelon and Guinee, 2000). This kind of association has enhanced reduced fat food to satisfy consumer demand (Dexheimer, 1992). However, the consumption of free or low fat food products is still very low resulted poor flavor, grainy and hard texture (Dexheimer, 1992; Muir *et al.*, 1992; Wilkinson *et al.*, 2001). Subsequently the challenge in improvement of reduced fat cheeses is to manufacture cheese has texture and flavors properties most closely to control treatment (Darwish and Taher, 2017).

Milk and dairy products consist of several components were resulted a high complicated structure and textures characteristics evaluated by consumers (Dickinson, 1997). Proteins have a significant influence on textural properties of cheese (Sakamoto *et al.*, 1994). The proteins structure modification could be performed by enzymatic, chemical and physical treatments (Darwish and Taher, 2017).

Enzymatic alteration of protein structure was exhibited as a preferable treatment because of high specificity of enzymatic reactions and high safety for human health. Transgltaminase (gamma-glutamyl transferase-EC2.3.2.13; protein glutamine; TGase) plays crucial role for deamidation, acyl transfer and cross-linking protein inter or intra chain glutamine (acyl donor) and acyl acceptor (lysine) (Romeih and walker, 2017). Further to it, this enzyme stimulates the free amines addition into polypeptides by binding the glutamine residue (Fig.1). Water is more essential part in the absence of free amine, where it becomes the acyl acceptor the ɣ-carboxamide groups are deamidated to glutamic acid residues (Heil *et al.*, 2016). The physic-chemical characteristics of protein quit significantly were affected by cross links (Khare and Gupta, 1987). TGase advantage in gelation, polymerization and formation of film (Nawong *et al.*, 2016). This enzymatic reaction are extremely interest to bioprocesses and food science research. TGase affects enormously on the proteins molecular structure in food matrices that enhance stability and texture, without affecting the color, pH and culinary of food, furthermore, making it more nutritious because of essential amino acids incorporation (Grossmann *et al.*, 2017; Darwish and Taher, 2017). TGase are widely distributed in many body tissues and fluids of vertebrates (Darwish and Taher, 2017). TGase was first detected in guinea pig liver (Wilhelm *et al.*, 1996). Further more TGase activity in genus *Steptoverticillium* such as *Streptoverticillium mobaraense* (Kanaji *et al.*, 1993; Darwish and Taher, 2017). TGase extraction from various plant source was widely distributed in pea, barley, wheat, soybean (*Glycine max*), rosemary leaves (*Rosmarinus officianlis* L.) and leaves of silver beet (Icekson and Apelbaum, 1987; Kang and Cho, 1996; Lilley *et al.*, 1998; Darwish and Taher, 2017).

Kareish cheese is popular type of Egyptian cheese that is similar to cottage cheese regarding the visual appearance and texture, is made from skim buffaloes' milk (Abou Donia, 1991). As with other types of free/ low fat cheeses , their consumption is still low due to inadequate texture and taste (Darwish and Taher, 2017). This study highlights the influence of plant TGase extracted from leaves of silver beet (*Beta vulgaris* L.) on sensory and texture charactistics of Kareish cheese.

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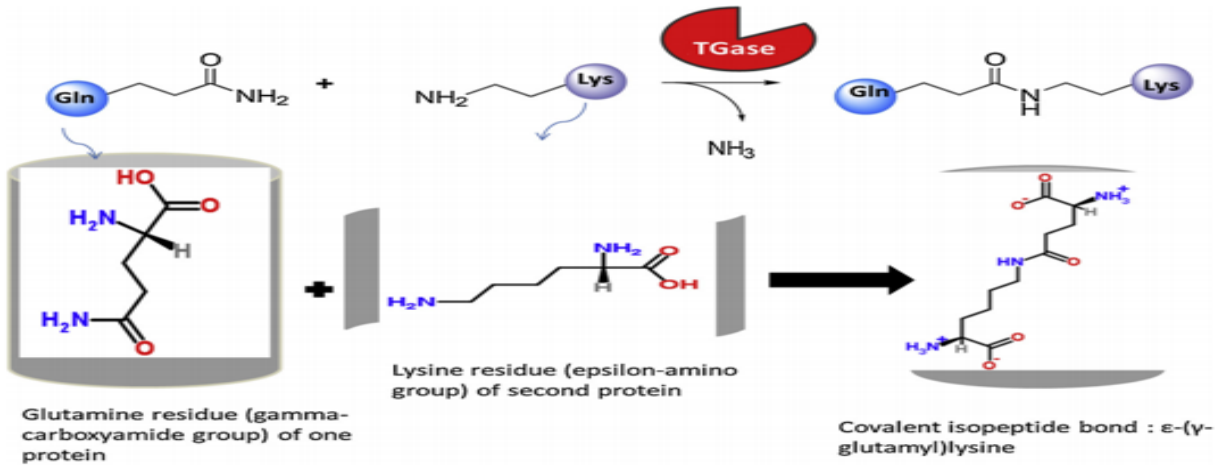

Fig 1. Transglutaminase mode of action: Reaction catalyzed by MTGase.

MTERIALS AND METHODS

Preparation of crude TGase

Crude extract of TGase was obtained by soaking 24 hours of 50 gm fine powder of *Beta vulgaris* L. leaves in 0.25 M Tris-Hydrochloride buffer pH 7.6 involving, (3% Polyvinylpyrrolidone, 10 mM EDTA, 0.05% β-mercaptoethanol and 25mM sucrose, and) then medley was centrifuged at 7000 g for 1/3 h at 4 °C. The greater part of the resultant supernatant was collected and kept at 4°C for further examination included total protein content and transglutaminase activity. (Darwish and Taher, 2017).

Precipitation of proteins using ammonium sulfate

The TGase precipitation by adding $(NH4)_2SO_4$ at 40% saturation. The admixture was held at 5°C for 60 min, and then centrifuged at 6500*g* for 15 min at 4°C, the supernatant solution was brought to 80% saturation by solid (NH4)2SO4 addition and then kept for 60 min at 4 °C. The greater part of supernatant was collected subsequently centrifuged at 6500*g* at 4°C g for 15min. The great part of the resultant supernatant was decanted and using Tris-Hydrochloride buffer (20 mM at pH 7.4) for dissolving the pellet. The crude extract was dialyzed overnight against the same buffer (Darwish and Taher, 2017).

Initial purification of TGase

The concentrated crude extract is applied to DEAE Sephadex A-50 (15 X 250 mm) equilibrated by passage of 20 mM Tris-HCl buffer pH 7.5. The same buffer elution removes non-targeted proteins. Then, targeted protein connected with DEAE Sephadex A-50 matrix is eluted at flow rate of 2 ml/min by salinity gradient consisted from 0 to 0.5M, dissolved in the same buffer. Fractions of protein (15 ml) were collected and activity of TGase and protein absorption at 280 nm were measured. Fractions were highly TGase efficient subjected to dialysis against Tris-Hydrochloride buffer (20mM, pH 7.5) for overnight (Darwish and Taher, 2017).

Ultimate purification of TGase

The dialyzed fraction was then subjected to further purification by Sephadex G- 100 (25 X 370 mm, Phamacia, Uppsala, Sweden). The chromatography column equilibration was carried out by Tris-Hdrocholoride buffer (pH 7.5, 20 mM). The same buffer was used for target protein elution at a flow rate 1.3 ml min^{-1}. The fraction associated with maximum efficiency of TGase considered as adequate purified TGase (Darwish and Taher, 2017).

TGase activity assessment:

The TGase activity was estimated according to previous studies (Folk and Cole, 1966). One unit of TGase activity was defined as the amount of enzyme which accelerates the reaction of hydroxylamine and Z-Gln-Gly for hydroxamic acid production (0.5 µmole min^{-1} at 37°C) considered as unit activity of TGase.

Determination of Total protein concentration

Coomassie G-250 (CBBG) using for determination of Total protein, the details about this method is given by Bradford (1976).

Optimum pH of TGase

The optimum pH of TGase was determined according to Darwish and Taher, (2017).

Optimum temperature of TGase

Screw cap tubes involved the substrate and purified TGase were held for 1/4 h at a board temperature range of 25°C to 85°C (Darwish and Taher, 2017).

Thermostability of TGase

The purified TGase was exposed for 10- 20 min at different temperature scales from 60-80°C, followed by rapid cooling and kept temperature around 37°C and then residual activity of TGase was determined (Darwish and Taher, 2017).

The impact of NaCl with various concentrations on TGase efficiency

The residual efficiency of TGase was determined after exposure to various concentrations of sodium chloride ranged from 3-15% (El-Hofi *etal.*, 2014).

Kareish cheese Production

Manufacture of Kareish cheese was carried out according to previous studies (Abou-Donia, 2008). The prepared reconstituted skim milk was distributed into four equal portions.

The TGase with different concentrations (5, 7.5 and 10U/g protein) were added to the first, second and third portions, respectively followed by incubation at 50°C for 1 hour after pasteurization. The forth portion was used for preparing of Kareish cheese without TGase (control). All samples were inoculated with 3% commercial yoghurt starter and then incubated at 45°C for 2.5-3.5 h. The finished curd may be duly broken for dipping into forms and molds,

followed by dry salting (2.5% W/W) and stored at 5°C for 16h in order to drain. The produced cheese was sharply cut into appropriate blocks and kept at 5°C for 2 weeks (Abou-Donia, 2008).

Determination of the physico-chemical quality of Kareish cheese

Kareish cheese was analyzed for pH, fat, moisture and protein (AOAC, 2005) at 0, 7 and 15 days of storage.

Texture profile analysis of tested cheese samples

Textural properties of tested cheese were analyzed by texture analyzer (TMS-Pro texture analyzer, USA). Adhesiveness springiness, hardness, cohesivenesss, chewiness and gumminess were assessed according to Szczesniak *et al*., (1963).

Sensory evaluation of tested cheese samples

Analysis of sensory properties of tested cheese samples were carried out according to Ahmed *et al.*, (2005).

Statistical analysis

The SPSS software version 15 was carried out for ANOVA. P values of less than 0.05 were regarded as statistically significant (SPSS, 2009).

RESULTS AND DISCUSSION

Transglutaminase purification

All parameter values related to purification steps of TGase included specific activity, total activity, purification fold and yield of TGase purified from *Beta vulgaris* L. are showed in Table (1). Yield and total activity of TGase are inversely proportional to purification progress steps, where the yield and total activity of crude TGase were 100% and 385.6 U respectively. In other words, the total activity that gets lower after each purification step, where the total activity of TGase after gel filtration step was reached to 78.47 U (Table 1). However the progress within development of the purification steps is directly proportional to purification fold and specific activity, where the purification fold and specific activity of TGase are intensively increased from 1 and 66.48 U/mg protein (crude TGase) to 3.4 and 224.2 U/mg protein (purified TGase) respectively (Table 1).

Table 1. Purification steps for TGase extracted from *Beta vulgaris* L. leaves

Parameters	Steps of purification			
	Crude TGase extract	TGase precipitation using Ammonium Sulfate	Initial purification (Diethylaminoethyl Sephadex)	ultimate purification (Sephadex G-100)
Volume (ml)	40	8	31	70
Concentration of protein (mg/ml)	0.145	0.082	0.016	0.005
Activity (U/ml)	9.64	13.2	2.88	1.121
Total TGase activity (U)	385.6	105.6	89.28	78.47
Total Protein (mg)	5.8	0.66	0.496	0.35
TGase specific activity (U/mg protein)	66.48	160	180	224.2
Yield	100	27.38	23.15	20.33
Purification fold	1	2.41	2.71	3.4

Total TGase activity is obtained by determine the activity of TGase in the fraction volume utilized in the assay and multiplying by fraction size
Specific TGase activity is the total TGase activity divided by total protein.
Yield is the a measurement of the efficiency retained after each purification stages as the total activity percentage in the crude extract.
Purification fold is obtained by dividing the specific TGase activity, calculated after each purification stage, by the specific TGase activity of the crude extract.

The elution through ion exchange DEAE- Sephadex A-50 column hall be continued until the A_{280} and TGase activity reach a maximum height in the fraction No. 30 and start to fall consistently (as shown in Fig. 2). This suggests that the high absorption at A_{280} (high protein content) was associated with TGase activity.

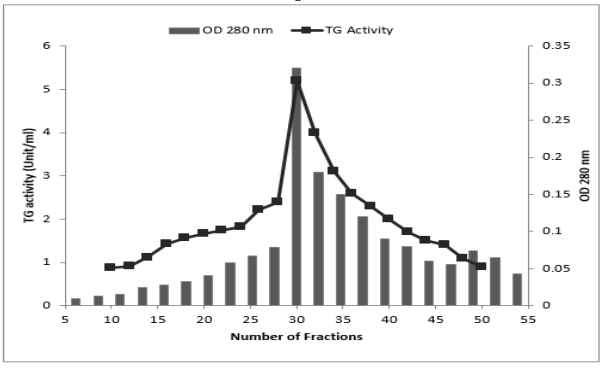

Figure 2. Initial purification step of TGase extracted from silver beet leaves by ion exchange DEAE-Sephadex A-50 column

In the same manner, the final purification stage of TGase that eluted enzyme activity and A_{280} gradually increased until reaching the maximum value in fraction No. 10 (as shown in Fig. 3).

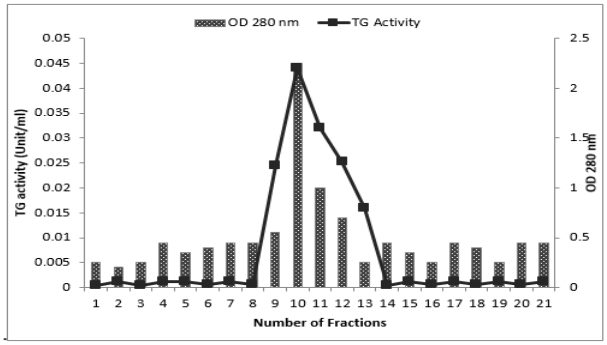

Figure 3. Final purification step of TGase extracted from silver beet leaves by gel filtration column.

Several prior studies were used various ion exchange resin types (DEAE - Sepharose, Diethylaminoethyl-Cellulose, Sulfopropyl-Sepharose and Quaternary amine-Sepharose) for critical step in TGase purification stages (El Hofi *et al.*, 2014; Darwish and Taher, 2017). The present research was consistent with other reports of Goldsmith and Martin (1995); Kwak *et al*., (1998); Darwish and Taher (2017). With regard to gel filtration as final step of TGase in this study was also in agreement with El Hofi *et al.*, (2014); Darwish and Taher (2017).

The impact of temperature variation and determination of optimum temperature of TGase activity

The effect of temperature variation ranging from 20 to 70°C on the TGase activity was determined after incubation at pH (5.0) for 15 min (Fig. 4). TGase showed that high efficiency at 50°C for accelerating reaction of Cbz-DL_Gln_Gly-OH and hydroxylamine (Fig.4). The TGase in this study has optimum temperature slightly decreased compare to previous studies. For instance, Darwish and Taher (2017) could determine the rosemary TGase has optimum temperature around 60°C. This might be related to the variation of enzyme origin. Lu *et al.*, (2003) who, also found that the TGse extracted from *Streptoverticillim mobaraense* has optimum temperature around 60 °C. However the optimum temperature of TGase in this study increased compare with the optimum temperature of TGase isolated from *Streptoverticillium hygroscopicus* and soybean, where their optimum temperature were 45°C and 37°C, respectively (Kang and Cho 1996; Cui *et al*., 2007).

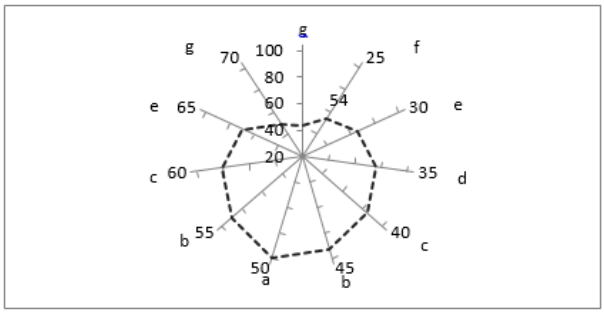

Figure 4. The temperature effect on the relative TGase activity (bars represent standard error of the mean).

The effect of pH variation and determination of optimum pH of TGase activity

The TGase activity was impacted by change in pH. The most appropriate pH value in which it exhibits maximum activity. Low or high pH values lead to loss of TGase efficiency. The stability of TGase was determined at different pH scale ranges from 2.0 to 10.0 (Fig. 5).

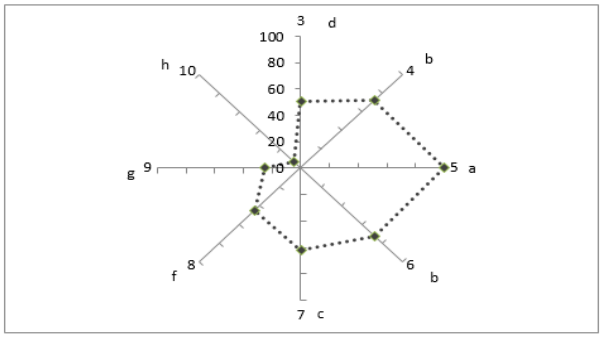

Figure 5. The influence of pH on the relative TGase activity (bars represent standard error of the mean).

The TGase activity for the stimulation Cbz-DL_Gln_Gly-OH reaction gradually raised in acidic condition until reaching maximum efficiency at pH 5.0. However the TGase activity reduced as the pH values exist near the alkaline range (Fig. 5). pH is below or above the optimum pH level was associated with decline in TGase activity due to change in molecular structure of enzyme. The optimum pH of TGase in the present study was diminished compare with optimal pH of rosemary TGase extracted by Darwish and Taher, (2017), who showed that the maximum efficiency of TGase at pH 6.0. El-Hofi *et al.*, (2014), who also detected the maximum efficiency of TGase at pH 7.0. This might be attributed to the amino acid nature at the active site, which undergoes deprotonation and protonation, in addition to conformational changes stimulated by amino acid ionization.

Thermostability of TGase

The thermostability of TGase was investigated between 60-85°C at various times (5, 10 and 15min). The TGase retains 100, 100, 66, 28% reactivity after heating 60, 65, 70 and 75°C, respectively (Fig. 6). The TGase completely lost its activity after 15 min at 80°C (Fig. 6). The activity of silver beet leaves TGase is adversely affected by high temperatures (Fig.6).These results are consistent with Darwish and Taher (2017), who reported that TGase extracted and purified from rosemary had high thermostability. However our findings were in disagreement with previous study, where the TGase extracted from liver of rats had low thermostability in which that the activity of TGase was completely lost while it was exposed to 52 and 60°C for 4 and 1 minutes, respectively (Wong *et al.*, 1990).

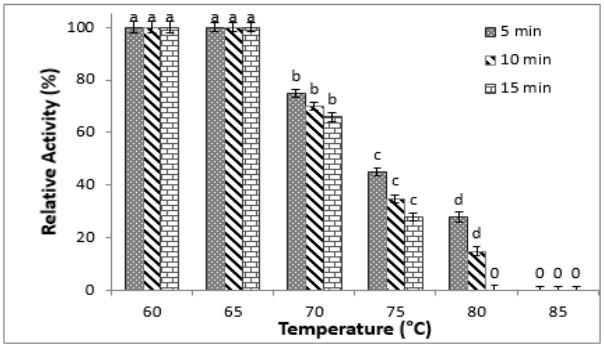

Figure 6. theromstability of TGase activity at different temperatures (bars represent standard error of the mean).

The impact of different sodium chloride concentrations on relative activity of TGase

For assessing the effect of different sodium chloride concentration on TGase efficiency, a wide array of sodium chloride concentration from 2-14% was investigated. The TGase activity was not affected by exposure to sodium chloride concentrations ranging from 2-6% (Fig. 7).

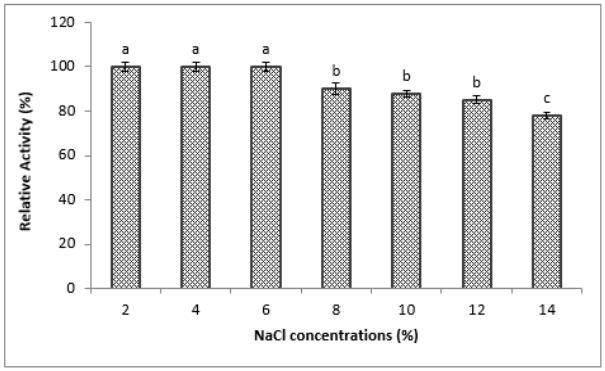

Figure 7. The influence of Sodium chloride on the relative TGase activity (bars represent standard error of the mean).

However high NaCl concentrations ranging from8-14% were partially inhibitory (Fig. 7), on the other hand, the relative activity of TGase is inversely proportional to high NaCl concentration (Fig. 7). This result was nearly in agreement with Darwish and Taher (2017), who detected that the relative efficiency of rosemary TGase was partially affected after exposure to high NaCl concentration from 5-14%. Tokunaga and Iwanaga, (1993); Worratao and Yongsawatdigul, (2005), who also detected that the NaCl concentration above 0.5 M was inhibited the TGase activity.

Chemical analysis of Kareish cheese

Cheese samples are subjected to chemical analysis to assess the effect of TGase on Kareish cheese production or as an index of quality. The total solid in all treatments progressively increased with increasing storage time. The fat and protein contents did not present any significant differences ($P<0.05$) in all treatments during storage time (Table 2). The influence of different TGase concentrations on Kareish cheese yield was listed in Table (2).

The Kareish cheese yield slightly reduced in all treatments over storage time (Table 2). The Kareish cheese was treated with different TGase concentrations coincided with statistically significant increased percentage yield of cheese ($P< 0.05$). The yield of Kareish cheese was used $10Ug^{-1}$ of TGase reached maximum (up to 24.36%).

The acidity of all samples progressively increased over storage time (Table 2). The concentration of TGase utilized for production of kareish cheese is coincided with decrease in pH values (Table 2). There were not significant effects (P< 0.05) of different TGase concentrations on protein and fat contents in Kareish cheese over storage time (Table 2).

Table 2. Chemical and physicochemical properties analysis performed on experimental cheese samples (means ± standard error) over storage time for 15 days at 5 °C

Treatments	Storage time (days)	pH	Total solid (%)	Protein (%)*	Fat (%)*	Yield (%)
Control	0	4.56 ± 0.06	29.94 ± 0.06	65.52 ± 0.14	0.33 ± 0.32	17.5 ± 0.32
	7	4.41 ± 0.08	30.34 ± 0.16	65.55 ± 0.09	0.34 ± 0.12	17.6± 0.23
	15	4.31 ± 0.09	30.64 ± 0.15	65.55 ± 0.11	0.32 ± 0.11	17.8 ± 0.41
	Mean	4.43^{a}	30.31^{a}	65.54^{a}	0.33^{a}	17.63^{d}
TGase (5U/g protein)	0	4.32 ± 0.03	27.94 ± 0.09	65.51 ± 0.12	0.35 ± 0.31	19.9 ± 0.15
	7	4.25 ± 0.04	28.14 ± 0.13	65.50 ± 0.23	0.35 ± 0.25	20.1± 0.24
	15	4.15 ± 0.10	28.44 ± 0.11	65.53 ± 0.26	0.31 ± 0.24	19.8± 0.04
	Mean	4.24^{b}	28.17^{b}	65.51^{a}	0.34^{a}	19.93^{c}
TGase (7.5U/g protein)	0	4.25 ± 0.12	25.34± 0.13	65.52 ± 0.31	0.36 ± 0.07	22.5 ± 0.09
	7	4.17 ± 0.04	25.64 ± 0.17	65.45 ± 0.29	0.31 ± 0.22	22.1 ± 0.27
	15	4.06 ± 0.12	25.94 ± 0.12	65.51 ± 0.32	0.34 ± 0.13	22.7 ± 0.24
	Mean	4.16^{bc}	25.64^{c}	65.52^{a}	0.34^{a}	22.43^{b}
TGase (10 U/ g protein)	0	4.18 ± 0.07	23.54 ± 0.23	65.57 ± 0.09	0.33 ± 0.19	24.2 ± 0.36
	7	4.00 ± 0.06	23.94 ± 0.31	65.56 ± 0.11	0.34 ± 0.12	24.7 ± 0.26
	15	3.86 ± 0.01	24.34 ± 0.18	65.53 ± 0.21	0.36 ± 0.34	24.2 ± 0.16
	Mean	4.01^{c}	23.94^{d}	65.55^{a}	0.34^{a}	24.36^{a}

*** Protein and fat contents are calculated in dry matter.**

Sensory character of Kareish cheese and its evaluation

For evaluation of sensory properties of Kareish cheese, the analysis of quantitative descriptive was utilized, which is generally carried out to study cheeses variety (Stone and Sidel 1993; Lawless and Heymann 2010).

Average sharpness ratings of descriptive features and the variation analysis are presented in Figure 8.

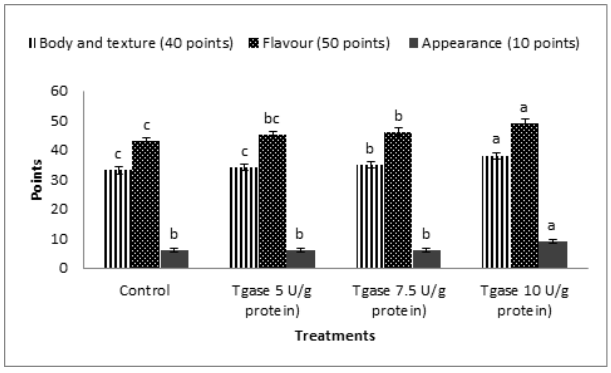

Figure 8.The effects of TGase at different concentration on Sensory properties of Kareish cheese (bars represent standard error of the mean).

ANOVA presented that there were statistically significant ($P< 0.05$) differences in the intensity sensory characteristics such as body, texture, flavor and appearance caused by TGase treatment (10U/g protein). The increase in sensory properties might be due to change in the mean moisture content, It can be noticed that, in the sensory profiles of Kareish cheese, the enhancement in body, texture, flavor and appearance from 5U/g to 10U/g of TGase agreed with the findings of Ibrahim,*et al.*,(2017), who reported that using TGase (5U/g protein) for making UF-white soft cheese leads to enhance sensory properties of cheese.

Texture profile analysis of experimentally cheese samples

For evaluation the effect of different TGase concentrations on texture characteristics of experimentally cheese samples, the texture profile analysis was used (Fig. 9, 10 and 11). The using different TGase concentrations significantly affected ($P<0.05$) on texture profile Kareish. A NOVA presented that there were significant ($P<0.05$) differences in the values of texture profile analysis such as gumminess, hardness, adhesiveness, cohesiveness and springiness (Fig. 9, 10 and 11). The values of cohesiveness and springiness significantly ($P<0.05$) increased with the increasing concentration of TGase, while values of gumminess, hardness and adhesiveness values were significantly ($P<0.05$) decreased (Fig. 9, 10 and 11).

The main reason for declining values of adhesiveness, hardness, and gumminess in experimentally samples were treated with TGase might be due to change in

the moisture level, where their high content of moisture. The high content of moisture leads to weak the protein network resulting soft resulted cheese that covers specific taste receptor cells in mouth over mastication (Bourne, 1978; Chen, *et al.*, 1978; Bryant, *et al.*, 1995; Maifrein *et al.*, 2002; Darwish and Taher, 2017). It can be seen that, the improvement in texture profile (adhesiveness, hardness, gumminess, cohesiveness and springiness) of Kareish cheese from 5U/g to 10U/g agreed with the findings of Ibrahim,*et al.*,(2017), who detected that improving of texture characteristics of UF-white soft cheese made by using 5U/g of TGase).

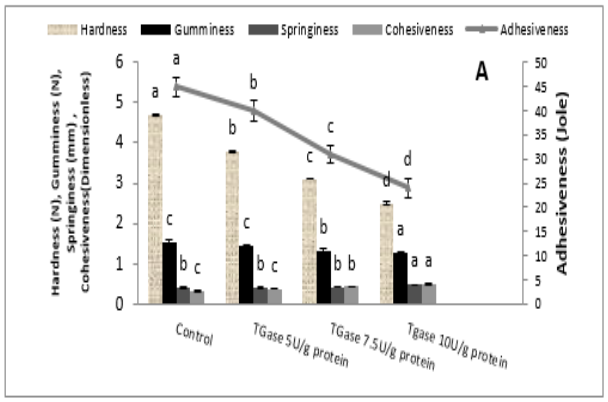

Figure 9. Texture profile analysis of fresh Kareish cheese made by different concentration of TGase, (bars represent standard error of the mean).

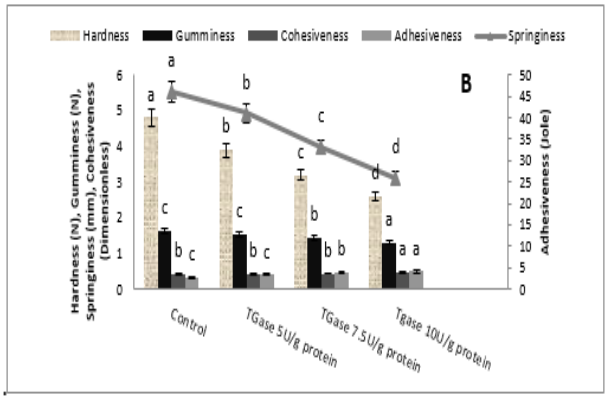

Figure 10. Texture profile analysis of Kareish cheese made by different concentration of TGase stored for 7 days at 7°C, (bars represent standard error of the mean).

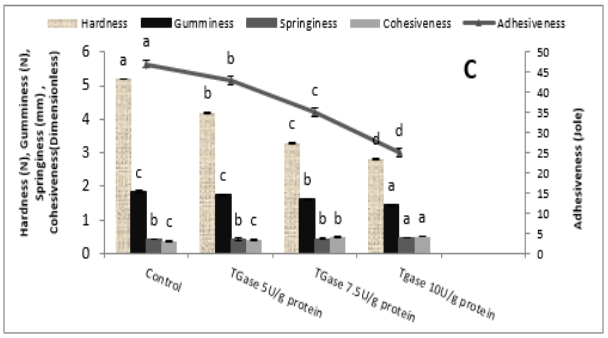

Figure 11. Texture profile analysis of Kareish cheese made by different concentration of TGase stored for 7 days at 15°C, (bars represent standard error of the mean).

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فصل وتنقية و توصيف إنزيم Transglutaminase من أوراق نبات البنجر ودراسة تأثيره علي الصفات الحسية والكيميائية والريولوجية للجبن القريش

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تستهدف الدراسة فصل وتنقية انزيم Transglutaminase من أوراق نبات البنجر 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 20.33 بمعدل تنقية يصل إلي 3.4، كما أشارت النتائج أن درجة الحرارة 50 °م بينما كانت درجة pH المثلي للإنزيم 5 ويفقد الانزيم نشاطه عند تعرضة علي درجة حرارة 80°م لمدة ربع ساعة. لا يتأثر الإنزيم عند معاملته بكلوريد الصوديوم بتركيز يتراوح من 2-6%. تم دراسة تأثير إضافة هذا الإنزيم علي الصفات الكيميائية والحسية و الريولوجية للجبن القريش باستخدام تركيزات مختلفة (5 و 7.5 و 10 وحدة / جم بروتين) خلال فترة تخزين تصل إلي 15 يوم . تميز الجبن الناتج بارتفاع محتواه من الرطوبة وانخفاض درجات pH مقارنة بالكنترول بينما لا تتأثر نسبة كلاً من البروتين والدهن بمعدلات إضافة TGase خلال فترات التخزين المختلفة. كما أشارت النتائج إلي وجود علاقة طردية بين تركيزات الإنزيم المستخدمة في التصنيع ومعدلات 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 إرتفاع معنوي في قيم Cohesiveness وSpringiness مقارنة بالكنترول علي الجانب الأخر حدث انخفاض معنوي في قيم Hardness و Adhesiveness و Gumminess مقارنة بالكنترول.