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Research Article
UF-white Soft Cheese Cross-linked by Rosemary Transglutaminase

[1]Osama A. Ibrahim, [2]Maher M. Nour, [1]Mohamed A. Khorshid, [1]Mahmoud A. El-Hofi, [1]El-Sayed E. El-Tanboly and [1]Nabil S. Abd-Rabou

[1]Department of Dairy Science, National Research Centre, Cairo, Egypt
[2]Department of Dairy, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

Abstract

Background: Enzymatic cross-linking of UF-white soft cheese by transglutaminase enzyme extracted from rosemary (*Rosmarinus officinalis* L.) leaves (RTGase) was evaluated. **Materials and Methods:** The RTGase was used in UF-white soft cheese at the level of 2.5, 5.0 and 7.5 U g^{-1} protein to examine the chemical, organoleptic properties and protein quality evaluation of cheese during storage period (1, 7, 15, 21 and 30 days). **Results:** The control cheese had the lowest acidity percentage and the highest total nitrogen/dry matter and dry matter values compared with other cheese treatments. However, water soluble nitrogen percentage of RTGase cheese was significantly ($p \leq 0.05$) lower than that detected in control cheese. Fat/dry matter of UF-white soft cheese treated with RTGase was significantly ($p \leq 0.05$) lower than that of control cheese during all storage period. After 30 days of storage period, the essential amino acids content, total essential amino acids/total amino acids ratio, protein efficiency ratio and biological value of RTGase cheese were higher than those of control cheese. The body and texture, flavor and total score of cheese treated with RTGase were higher than those of control cheese during all storage period. **Conclusion:** The RTGase treated cheese at the level of 5.0 U g^{-1} protein had significantly ($p \leq 0.05$) differences compared to control cheese in body and texture after 7 days until 30 days of storage period reflects the TGase cross-linking reaction and improves cheese textural, sensorial and nutritional properties.

Key words: Transglutaminase, rosemary, cross-linking, UF-white soft cheese

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Corresponding Author: Osama A. Ibrahim, Department of Dairy Science, National Research Centre, Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Transglutaminase (TGase, EC 2.3.2.13) is an enzyme catalyzes cross-linking of proteins at the molecular level as a promising tool of enzymatic modification, through an acyl transfer reaction using the γ-carboxamide group of peptide-bound glutamine residues as acyl donor and the ε-amino groups of lysine residues as acyl acceptor, intra-molecular and/or inter-molecular cross-links (isopeptide bonds) are formed, resulting in the polymerization of proteins[1,2].

The TGase cross-linking reaction functional effect is incorporation of amino acids or peptides into a protein as a useful tool for enhancing the nutritive value of foods[3]. Moreover, introduction of covalent cross-links by TGase leading to modify functional properties of proteins such as solubility, emulsifying capacity, foaming and gelation properties of proteins intended for human consumption, while chemical modification of proteins are not acceptable[4,5]. European Union (EU regulation 1332/2008) classified TGase as a processing aid and has Generally Recognized As Safe (GRAS) status in the USA[6]. Enzymatic modification of different food proteins has been used by means TGase enzyme such as gluten, globulin, soy proteins, casein and whey proteins[7].

The TGases are widespread in microorganisms, plants, vertebrate and invertebrate animals[8]. The TGase activity has been found in higher and lower plants[9]. The TGase activity has been detected in silver beet (*Beta vulgaris* L.) leaves[10]. Kang and Cho[11] purified TGase from soybean (*Glycine max*) leaves. The TGase was detected in root and shoot tissues of dicotyledonous [pea (*Pisum sativum*) and broad bean (*Vicia faba*)] and monocotyledonous [wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*)] plants[12]. Also, TGase was detected in the corolla of tobacco (*Nicotiana tabacum*) flowers and apple (*Malusdo mestica*) pollen[13-15].

El-Hofi *et al.*[16] purified and characterized TGase from rosemary (*Rosmarinus officinalis* L.) leaves. Rosemary is a condiment and medicinal herb widely used around the world as the natural antioxidant[17]. Rosemary TGase exhibited optimum activity at pH 7.0 and 55°C for the catalytic reaction of N-carbobenzoxy-L-glutaminylglycine and hydroxylamine[16].

Novel applications based on milk proteins enzymatic modification seems to be promising for increasing cheese yield and decrease syneresis. Cozzolino *et al.*[18] reported that the incorporation of whey proteins into cheese curd due to TGase treatment. The performance of TGase as a body stabilizer in tallaga cheese made from ultrafiltered cow's milk retentate, studied by Farahat *et al.*[19]. Di Pierro *et al.*[20] produced "cross-linked cheese" by TGase enzyme with increased water content and yield of the resulted cheese. De Sa and Bordignon-Luiz[21] studied the TGase impact on the rennet coagulation properties of milk gels and processed cheese while Ozer *et al.*[22] investigated the microbial TGase (mTGase) and rennet effect of renneting temperature on textural properties, proteolysis and yield of white-brined cheese. Ahmed *et al.*[23] studied the effect of fat replacer or mTGase on the quality of low-fat Gouda-like cheese while Salinas-Valdes *et al.*[24] studied yield and textural characteristics of panela cheeses produced with dairy-vegetable protein (soybean or peanut) blends supplemented with TGase. Moreover, Karzan *et al.*[25] effect of mTGase enzyme on some physicochemical and sensory properties of goat's whey cheese.

Most of enzymatic modification of milk proteins applications were used the available commercial microbial or animal TGase. Therefore, the objective of this study was to investigate the enzymatic modification of UF-white soft cheese by plant TGase covalent cross-linking of milk proteins extracted from rosemary (*Rosmarinus officinalis* L.) leaves. Also, chemical, organoleptic and biological evaluation of UF-white soft cheese during storage were studied.

MATERIALS AND METHODS

Raw materials: Rosemary (*Rosmarinus officinalis* L.) leaves were obtained from production and marketing of medicinal plants and their extracts unit, Medicinal and Aromatic Plants Research Department, National Research Centre, Dokki, Egypt. Milk retentate and permeate were obtained from Dairy Industry Unit, Animals Production Research Institute, Ministry of Agriculture, Giza, Egypt. Rennet, RENIPLUS, Microbial rennet powder from *Mucor miehei* was purchased from Gaglio Star, Spain. Cheese starter culture used in the cheese manufacture consisted of the mixture of *Lactococcus lactis* subsp., *lactis* and *Lactococcus lactis* subsp., *cremoris* were obtained from Egyptian Microbial Culture Collection (MIRCEN), Ain Shams University. The N-carbobenzoxy-L-glutaminylglycine (CBZ-Gln-Gly), hydroxylamine hydrochloride, L-glutamic acid γ-monohydroxamate, glutathione, reduced form, 98%, standard amino acids were purchased from Sigma-Aldrich, Steinheim, Germany. Other reagents and chemicals were used of analytical grade.

Preparation of RTGase enzyme: The RTGase enzyme extract was prepared from the rosemary (*Rosmarinus officinalis* L.) leaves according to El-Hofi *et al.*[16]. The prepared enzyme ectract had an activity of 10 U mL^{-1} measured by hydroxamate

method of Folk and Cole[26]. Each unit of TGase is defined in terms of its activity, which corresponds to the amount of the enzyme that catalyze the reaction of hydroxylamine and N-carbobenzoxy-L-glutaminylglycine to yield 1 μmol of hydroxamic acid min^{-1} at 37°C.

Cheese production and enzymatic treatment with RTGase: UF-white soft cheese manufacture was carried out as given by Hofi *et al.*[27].

Ultrafiltration of cheese milk: Fresh skim milk was heated to 72°C for ~15 sec and then ultrafiltrated (concentration factor ~ 4.5) using tubular concentration module DC2, supplied by amicon corporation, USA. The inlet and outlet pressure were ~5 and ~3 bar, respectively. Milk fat was added to the concentrated milk then it was homogenized at 50°C.

UF-white soft cheese manufacturing procedure: The homogenized retentate was heated at 72°C for ~15 sec then cooled to 37°C and inoculated with 2% active cheese starter culture (*Lactococcus lactis* subsp., *lactis* and *Lactococcus lactis* subsp., *cremoris*). Calcium chloride was added at 0.02 and 3% NaCl was added to pre-cheese milk and mixed well. The retentate was divided to four containers, the first served as a control; the others were served as T1, T2 and T3. The rennet was added to the control but the other containers the rennet was added plus RTGase at a level of 2.5, 5.0 and 7.5 U g^{-1} protein, respectively. The pre-UF white soft cheese was immediately distributed into suitable plastic containers. The containers were incubated at the same temperature (37°C), the curd is formed within ~ 40 min. The top of the curd in each container was covered with a volume of pasteurized salted permeate with then tightly closed with its lids and stored at 5°C for 4 weeks.

Analytical techniques of UF-white soft cheese

Chemical characterization: The following chemical analyses were carried out on cheese samples according to AOAC[28]: Moisture, ash, total solids, fat, total protein and the acidity was determined as lactic acid by titration with 1/9 N NaOH. The pH of the samples was measured by using pH-meter. Water Soluble Nitrogen (WSN) was estimated according to Ling[29]. All analyses were performed in triplicate and all experiment was repeated two trials.

Organoleptic properties evaluation: The organoleptic properties of UF-white soft cheese samples were evaluated by a regular score panels chosen from the staff members of the National Research Centre. Cheese samples were evaluated for body and texture (40 points), flavor (50 points) and color (10 points) according to Davis[30].

Determination of individual amino acids of cheese: To determine individual amino acids of UF-white soft cheese, sample corresponding to 40 mg protein. Aliquot (7.5 mL) of 6 N HCl was added purged with nitrogen for 60 sec. The tube was placed in oven at 110°C for 24 h. After cooling, the tubes content were quantitatively transferred to 25 mL volumetric flask and completed with HPLC grade water. About 1 mL of the solution was filtered through 0.45 μm sample filter. Ten microlitters of the filtered sample was dried in Waters Pico-tag workstation in 10-15 min. Aliquot (30 μL) of the freshly prepared derivatization reagent (350 μL methanol, 50 μL HPLC grade water, 50 μL triethylamine and 50 μL phenylisothiocyanate) was added to the dried sample and allow to react for 20 min and further dried in the Waters Pico-tag workstation for 15 min, 30 μL HPLC grade methanol was added and re-dried. The sample was diluted then filtered. Filtered sample (20 μL) was injected on to the HPLC column. The HPLC system was used to determine the individual amino acids. The standard amino acids of is prepared in the same sample condition. The analysis was carried out using a gradient of Pico-tag solvent at 40°C with a flow rate 1 mL min^{-1}.

Calculation of chemical score, protein efficiency ratio and biological value of cheese: Chemical score, Protein Efficiency Ratio (PER) and Biological Value (BV) of selected UF-white soft cheese treated with RTGase on their amino acid content was calculated according to Bhanu *et al.*[31], Alsmeyer *et al.*[32] and Oser[33], respectively.

Statistical analysis: Statistical analysis of the average values obtained from the chemical and organoleptic evaluation of cheese samples were analyzed by the Statistical Analysis System (SAS) using the ANOVA procedure for analysis of variance, using the General Linear Model (GLM) procedure of SAS software[34]. The results were expressed as Mean ± Standard Error and the difference between means were tested for significance using Duncan's multiple range at ($p \leq 0.05$).

RESULTS AND DISCUSSION

Addition of RTGase in UF-white soft cheese: RTGase extracted and characterized from rosemary (*Rosmarinus officinalis* L.) leaves in the laboratory scale[16] was used in UF-white soft cheese manufacture to study the chemical,

sensorial and nutritional effect of the plant TGase cross-linking on milk proteins during the cheese storage. The milk retentate was divided into four portions. The first portion was left without treatment and served as a control, while the others were treated with RTGase at the level of 2.5, 5.0 and 7.5 U g^{-1} protein and served as T1, T2 and T3, respectively. UF-white soft cheese samples were taken periodically when fresh and after 7, 15, 21 and 30 days of storage for analysis.

Cheese chemical composition: Titratable acidity and pH values of UF-white soft cheese treated with RTGase during storage at 5°C for 30 days are given in Table 1. The titratable acidity of UF-white soft cheese control had the lowest percent compared to all samples whilst its pH value was the highest value. Also, the obtained results indicated that cheese acidity was increased throughout the storage period together with the pH values of resultant cheese were decreased throughout the storage period and there are significantly ($p \leq 0.05$) differences between control and treated cheese after 15 days of storage. These results were accordance with Farahat *et al.*[19] who observed that the pH values of the experimental cheeses were decreased during the ripening period.

Total nitrogen/dry matter (TN/DM) of UF-white soft cheese treated with RTGase during storage at 5°C for 30 days are presented in Fig. 1. The control UF-white soft cheese had the highest TN/DM among all cheese samples. Also, the DM of all experimental cheeses was increased during storage period and there are significantly ($p \leq 0.05$) differences between control and treated cheese during all storage period. This is mainly due to increasing of water retention capacity of rennet gels formed by TGase with cross-linking bonds ε-(γ-glutamyl)-lysine[35]. The obtained results were agreement with Ozer *et al.*[22] who observed the mTGase in cheese making resulted in lower levels of total solids in experimental cheeses than control cheese. Also, similar results were found by Sayadi *et al.*[36] who reported that the TGase treated cheese possessed lower protein content compared to control cheese whilst its moisture content were highest. It is argued that milk proteins cross-linking by TGase limits the movement of protein chains and diminishes the rearrangement of bonds, which decreased wheying-off. Furthermore, TGase-treated cheeses both when TGase was added at the same time with chymosin and when it was added after the formation of the coagulum, show a significant increase in water content, it could be due to the different structure of casein micelles before and after rennet formation reaction. In fact, the cheese water content depends on the syneresis occurring during curd preparation, which in turn is influenced by both curd shrinkage and rearrangement of the network of the (para) casein micelle gel[20]. In addition, TGase application should be able to produce a stabilized protein network with a lower pore size that decreases syneresis[37]. Also, cross-links bonds resulted by TGase increases the free volume inside the cheese curd matrix leading to an increase in whey-holding. This effect should allow TGase to entrap into

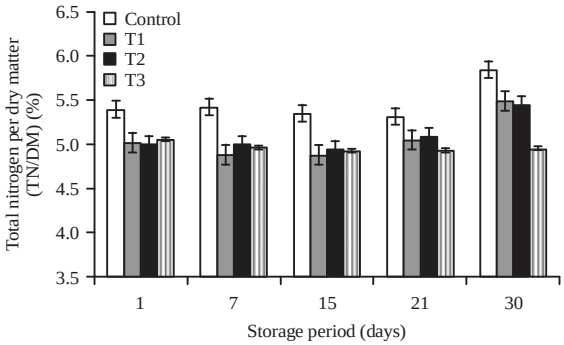

Fig. 1: Total nitrogen/dry matter (TN/DM) of UF-white soft cheese treated with RTGase during storage at 5°C for 30 days, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

Table 1: Titratable acidity and pH values of UF-white soft cheese treated with RTGase during storage at 5°C for 30 days

		Storage period (days)				
Parameters*	Cheese samples	1	7	15	21	30
Titratable acidity (%)	Control	0.46 ± 0.035^{h}	0.48 ± 0.009^{h}	0.54 ± 0.003^{g}	0.80 ± 0.003^{d}	0.90 ± 0.015^{c}
	T1	0.49 ± 0.003^{gh}	0.50 ± 0.020^{gh}	0.62 ± 0.012^{f}	1.05 ± 0.003^{b}	1.10 ± 0.029^{b}
	T2	0.50 ± 0.003^{gh}	0.51 ± 0.006^{gh}	0.69 ± 0.006^{e}	1.25 ± 0.003^{a}	1.30 ± 0.003^{a}
	T3	0.50 ± 0.049^{gh}	0.50 ± 0.003^{gh}	0.64 ± 0.003^{f}	1.10 ± 0.003^{b}	1.05 ± 0.026^{b}
pH	Control	6.31 ± 0.006^{a}	6.26 ± 0.023^{ab}	6.20 ± 0.015^{abc}	6.16 ± 0.023^{bc}	6.01 ± 0.064^{ed}
	T1	6.23 ± 0.020^{abc}	6.21 ± 0.009^{abc}	5.99 ± 0.049^{e}	5.89 ± 0.064^{ef}	5.81 ± 0.009^{fg}
	T2	6.21 ± 0.023^{abc}	6.12 ± 0.012^{cd}	5.71 ± 0.009^{gh}	5.80 ± 0.118^{fg}	5.79 ± 0.009^{fg}
	T3	6.20 ± 0.003^{abc}	6.16 ± 0.023^{bc}	5.91 ± 0.032^{ef}	5.90 ± 0.058^{ef}	5.61 ± 0.009^{h}

*All values correspond to the mean values Means±Standard Error obtained from two repetitions in triplicate. Means in the same column with different superscript letters are significantly ($p \leq 0.05$) different, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

cheese curd some free proteins present in the whey, such as casein fines or casein-macropeptide[38].

The results reported in Fig. 2 shows that the fat/dry matter (Fat/DM) of UF-white soft cheese treated with RTGase was significantly ($p \leq 0.05$) lower than the control cheese during all storage period but there are no significantly ($p \leq 0.05$) differences in control cheese during storage period. Contrary observation were reported by Farahat *et al.*[19] observed that the TGase treatment of retentate led to decrease in Fat/DM content of UF-cheese compared to control.

Figure 3 shows Water Soluble Nitrogen (WSN) percent occurring in UF-white soft cheese treated with RTGase during storage at 5°C for 30 days. The obtained results indicated that RTGase treated cheese had the significantly ($p \leq 0.05$) lower WSN percent than those detected in control cheese while WSN concentrations of all samples increased during storage period. Also, the WSN was decreased with RTGase concentration was increased. These results in agreement with Di Pierro *et al.*[20] and Ozer *et al.*[22] who observed that WSN of TGase treated cheese had the lower values than the control cheese. The WSN is primarily formed in cheese making by milk clotting enzymes (e.g., chymosin), plasmin or cell-wall envelope proteases at the early stage of proteolysis[39]. Therefore, it is fair to assume that both TGase cross-linking of milk proteins and partly restricted action of milk clotting enzymes in the new cheese matrix leading to slower degradation of milk proteins by proteolytic agents[22]. In fact, protein breakdown is an important factor in cheese technology for both flavors and texture development during cheese ripening[39].

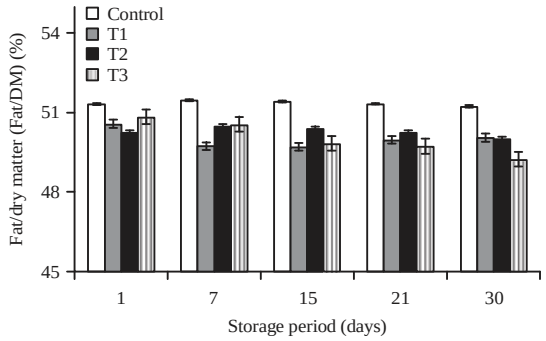

Fig. 2: Fat/dry matter (Fat/DM) of UF-white soft cheese treated with RTGase during storage at 5°C for 30 days, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

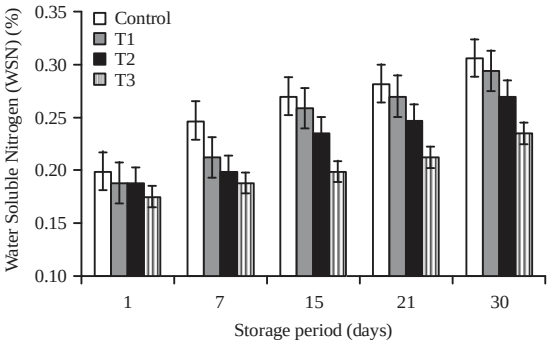

Fig. 3: Water Soluble Nitrogen (WSN) occurring in UF-white soft cheese treated with RTGase during storage at 5°C for 30 days, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

Individual amino acid content of cheese: Individual amino acid contents of 30 days experimental UF-white soft cheese treated with RTGase are given in Table 2. After 30 days of cheese storage period the results indicated that Essential Amino Acids (EAA) and the total essential amino acids to total amino acids ratio of the RTGase treated cheese higher than control cheese. It could be due to RTGase created

Table 2: Individual amino acid contents of 30 days experimental UF-white soft cheese treated with RTGase

	Cheese samples (mg/100 g cheese)			
Amino acids	Control	T1	T2	T3
Threonine	9.43	8.22	2.86	4.90
Tyrosine	19.65	17.91	4.02	8.05
Valine	6.37	25.80	21.15	6.57
Methionine	2.84	70.32	26.82	58.99
Cystine	3.66	3.56	0.39	4.97
Isoleucine	5.72	11.70	5.50	91.91
Leucine	9.07	27.21	37.11	60.17
Phenylalanine	14.08	65.78	62.02	11.38
Lysine	125.72	19.11	66.93	74.60
Aspartic	7.08	7.32	2.90	3.50
Glutamic	37.20	31.14	16.45	20.17
Serine	12.41	12.26	6.16	8.12
Glycine	10.11	9.91	4.15	6.04
Histidine	7.02	5.97	3.03	3.55
Arginie	25.88	22.56	34.07	13.43
Alanine	16.95	16.25	6.86	9.86
Proline	14.46	12.90	3.92	6.12
Total EAA	183.91	237.67	225.81	317.04
Total amino acids	327.65	367.92	304.34	392.33
EAA/TAA	0.561	0.646	0.742	0.808

EAA: Essential amino acids, EAA/TAA: Total essential amino acids to total amino acids ratio, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

ε-(γ-glutamyl)lysine bonds with cross-linking of inter and intra-molecular of the milk proteins[40]. In fact, liberation of individual amino acids in cheese is primarily controlled by starter peptidases and degree of amino acids liberation depending on the starter enzyme systems and degree of autolysis in cheese[41]. Similar findings were also reported by Ozer *et al.*[22].

Chemical score, protein efficiency ratio and biological value of cheese: Chemical score is a comparison of the amount of the limiting amino acid in a food with the amount of that same amino acid in a reference food. Chemical score of 30 days experimental UF-white soft cheese treated with RTGase was given in Fig. 4, the results indicated that the leucine was the first limiting amino acid in control cheese while the amino acids threonine, threonine and valine were the first limiting amino acids in treated cheese with RTGase of T1, T2 and T3, respectively.

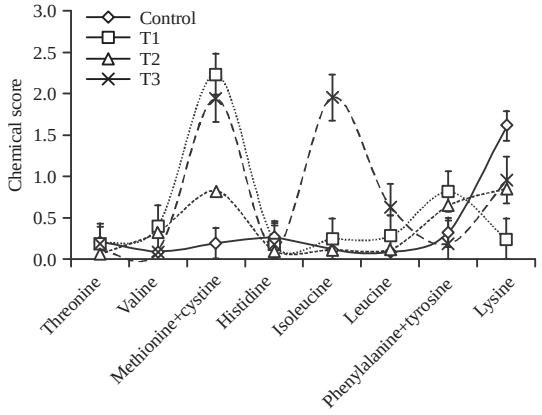

Fig. 4: Chemical score of 30 days experimental UF-white soft cheese treated with RTGase, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

Protein Efficiency Ratio (PER) determines the effectiveness of protein in 30 days experimental UF-white soft cheese treated with RTGase was given in Fig. 5, the PER results indicated that after 30 days of cheese storage period of treated cheese with RTGase higher than control cheese.

Biological Value (BV) measures the proportion of absorbed nitrogen which is retained and presumably utilized for protein synthesis and therefore reflects true protein quality. The concept of BV has the merit that it can be used to assess protein requirements derived from foods with known quality differences, because BV is directly related to the efficiency of protein utilization. Food with a high value correlates to a high supply of the essential amino acids which animal proteins typically possess higher biological value than vegetable sources due to the vegetable source's lack of one or more of the essential amino acids. The BV of 30 days experimental UF-white soft cheese treated with RTGase was shown in Fig. 6, BV results indicated that throughout the cheese ripening and after 30 days of storage period of RTGase cheese higher than control cheese.

Organoleptic properties of cheese: Organoleptic properties of UF-white soft cheese treated with RTGase during storage at 5°C for 30 days are presented in Fig. 7. The results indicated that body and texture and flavor of treated cheese had the

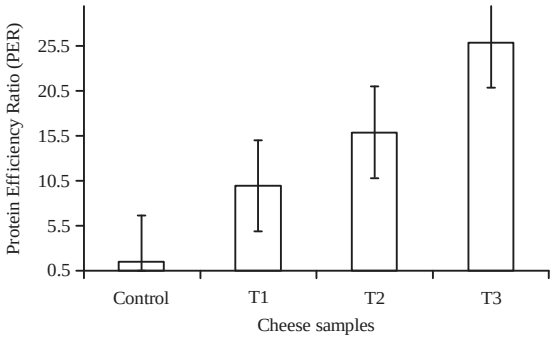

Fig. 5: Protein Efficiency Ratio (PER) of 30 day experimental UF-white soft cheese treated with RTGase, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 unit RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

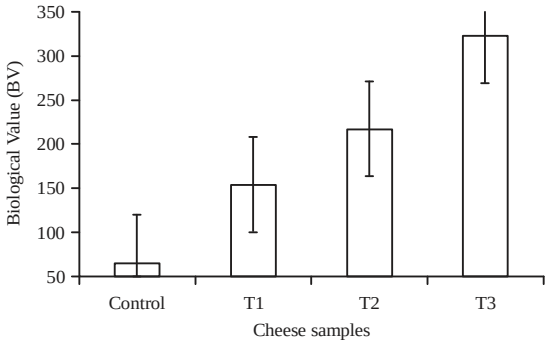

Fig. 6: Biological Value (BV) of 30 day experimental UF-white soft cheese treated with RTGase, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein

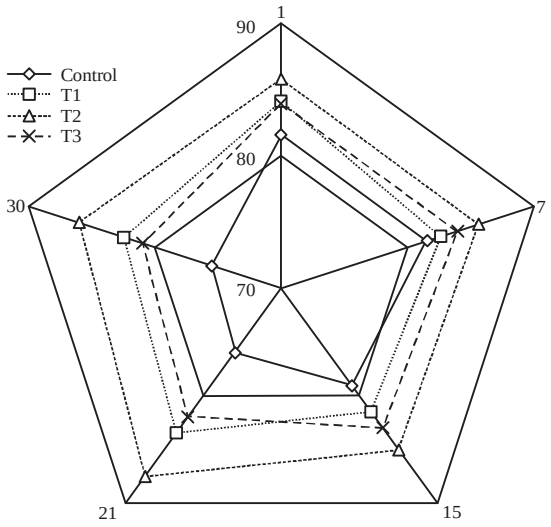

Fig. 7: Organoleptic properties of UF-white soft cheese treated with RTGase during storage at 5°C for 30 days, Control: Cheese sample without RTGase, T1: Cheese sample treated with 2.5 U RTGase g^{-1} protein, T2: Cheese sample treated with 5 U RTGase g^{-1} protein, T3: Cheese sample treated with 7.5 U RTGase g^{-1} protein, Each point describe collective score for each sample per day including flavor, body and texture and color

higher scores than control cheese during all storage period. Also, the total score of organoleptic properties of treated cheese with RTGase significantly ($p \leq 0.05$) were higher than control cheese during all storage period. The RTGase cheese (T2) had significantly ($p \leq 0.05$) differences compared to control cheese in body and texture from 7 days until 30 days of storage period, it could be due to both inter and intra-molecular bonds formation between the protein molecules with improves the textural properties of cheese[42]. These results were accordance with Ahmed *et al.*[23] who reported that the TGase addition improved the organoleptic properties of the resultants cheese "especially the body and texture" as a result of increasing its water-holding capacity and the cross-linking bonds between protein molecules. Ozrenk[43] stated that the introduction of additional covalent cross-linking by TGase represents a promoting tool to improve the functional properties for casein-based dairy products.

CONCLUSION

It could be concluded that RTGase cheese at the level of 5.0 U g^{-1} protein had significantly ($p \leq 0.05$) differences compared to control cheese in body and texture after 7 days until 30 days of storage period reflects the TGase cross-linking reaction and improves cheese textural properties. Moreover from the nutritional point of view, the essential amino acids content, total essential amino acids to total amino acids ratio, PER and BV of RTGase cheese higher than control cheese due to RTGase cross-linking of milk proteins with improving the biological value of the treated cheese. Hence, novel plant RTGase extracted in the laboratory scale with low cost compared to commercial TGase could be used to improve the chemical, sensorial and nutritional properties of UF-white soft cheese.

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