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The effect of microbial transglutaminase enzyme on some physicochemical and sensory properties of goat's whey cheese

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Abstract

The effect of the microbial transglutaminase (MTGase) enzyme treatment on some properties of whey cheese made from goat's milk whey was studied; the results shows that the yield of whey cheese increased about 0.7 and 1.01% by using the concentration of 4 and 8U gm^{-1} whey protein respectively, the addition of MTGase for producing whey cheese caused a clear increase in hardness and the higher hardness was reached by using 8U gm^{-1} protein which represented 79gm cm^{-2} compared to untreated samples (34gm cm^{-2}) after 7 days of storage at 4◦C. On the other hand, using this enzyme for producing whey cheese affect some sensory properties, bitterness and texture affected significantly ($p \leq 0.05$) while other properties (color, flavor, holes and consistency) not significantly affected.

Introduction

The dairy products from goat milk, are becoming more interesting in the worldwide because of many reasons such as economic for the rural regions (Voors and D'Haese, 2010; Gaspar *et al*., 2011) a medical reasons, and a gastronomic aspects (Slaˇcanac *et al*., 2010). The consuming of these products from goat milk has some beneficial properties, goat milk proteins are more digestible (Haenlein, 2004) and at the same time its less allergenic (Sanz Ceballos, 2009). Goat's milk fat is also more digestible (Haenlein, 2004). So there is a growing interest in producing different products from this milk.

Whey proteins (WP) are important food ingredients that are used for producing some food products like confectionary, desserts and also including dairy products. WP compose about 20% of bovine milk and are generally produced as a by-product of the cheese industry. Whey cheese is one of the dairy products that are produced by denaturation and aggregation of these proteins using heat treatment.

This type of cheese has a weak and crumbly texture and it gives a little yield, this is mainly due to the absence of the strong covalent chemical bonds. An alternative method for creating chemical crosslinks is enzyme-catalyzed crosslinking. Transglutaminase enzyme (EC 2.3.2.13) (TG) has been used to crosslink a wide range of food protein types (Dickinson, 1997). TGase used for catalyzing covalent bond formation during an acyl transfer reaction between γ-carboxyamide groups of peptide bond glutamine residues and the ε-amino groups of lysine residues, leading to the formation of intra and intermolecular isopeptide bonds between protein molecules (Dickinson, 1997; Motoki and Seguro, 1998). Such chemical crosslinks bring favorable textural and rheological properties and increasing the hardness behavior of the final food products (Anon, 1995; Dickinson, 1997).

Many studies focused on the impact of microbial TGase-induced protein crosslinking on dairy products from cow milk (Lauber *et al*., 2000; Bonisch *et al*., 2007; Jaros *et al*., 2007) only few data are available for goat milk (Farnsworth *et al*., 2006; Rodriguez-Nogales, 2006). The objectives of the present study were to using microbial TGase for whey cheese production and the study of the effect of this enzyme on some physicochemical and sensory properties of it.

Materials and Methods

Fresh raw goat milk was obtained from dairy filed unit (Animal Resource department, College of Agriculture, Salahhadin University Erbil-Iraq), Microbial transglutaminase (MTGase) was supplied by Ajinomoto Co., Inc., Tokyo, Japan. Other chemicals used were of analytical grade.

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Whey cheese manufacturing

Cheese whey of goat milk which was supplemented by 5% of whole goat milk was pasteurized at 72°C for 15 second and cooled to 35°C, and then the enzyme treatments (0, 4 and 8 U gm^{-1} protein) were applied followed by incubation for 1 hour at the same temperature. Heating treatment was applied for goat cheese whey until the curd was produced, then the whey cheese samples were stored at 4ºC for physicochemical analysis and sensory evaluation during 1 and 7 days of storage.

Milk protein estimation

Micro Kjeldahl method was used for milk and cheese protein determination according to A.0.A.C (2000).

Total solid of goat milk estimation

The total solids of milk and cheese samples were determined using approved A.O.A.C (2000) Methods.

Determination of cheese hardness

The hardness of whey cheese was measured by using a texture analyzer (Steven-LFRA Mechtric Steven, England) with a probe TA 6 penetration speed 1.0, 0.5 mm s^{-1} and penetration distance 10, 0.5mm respectively. The measurements were performed as soon as the samples were removed from the refrigerator. Cheese hardness was expressed in gm indicative of the force required to breaking the curd (Bourne, 1978).

Whey cheese yield

Whey cheese yield percentage was calculated, using the following equation.

$$\%\ \text{Cheese yield} = \frac{\text{Weight of cheese}}{\text{Weight of cheese whey}} \text{X100}$$

Identification of protein cross-linking in whey cheese

PolyAcrylamide gel electrophoresis (SDS-PAGE) technique was used for identification of protein cross-linking in whey cheese samples. The analysis was conducted for whey cheese samples using the procedure described by Laemmli (1970)

Sensory evaluation

Sensory evaluation analysis of whey cheese was carried out for flavor, texture, holes, consistency, bitterness and color, overall acceptability was based on a 60 points. Samples were coded with random numbers.

Statistical analysis

The data were statistically analyzed according to the method of analysis of variance as a general test. Factorial experiment with three and six replications was used by XLSTAT program ver. 7.5.2 and conducted using Complete Randomized Design (CRD). All possible comparisons among the means were carried out by using Least Significant Difference (LSD) test at significant level of 0.05 or 0.01 after they show their significance in the general test.

Results and Discussion

Whey cheese yield

The results showed that the treating whey with MTGase caused the increasing in the total protein content and the total solids of the produced cheese at first day of storage which contained 15.86 and 17.85% protein; 23.5 and 25% total solids for 4U and 8U treatment respectively compared to 14.43% protein and 22.5% total solids for control. Concerning the yield of the product, it was found that the treating cheese whey with MTGase increased the yield of whey cheese about 0.7 and 1.01% by using MTGase concentration of 4 and 8U gm^{-1} whey protein respectively, while this addition were convoyed by decrease in whey protein content (Table 1).

These results may be due to the cross linking of hydrophilic part of the κ-casein known as caseomacropeptide (CMP) which splits during chymosine action through coagulation process of milk and it remains dissolved in the whey. Tolkach and Kulozik (2005) reported that this part of κ-casein is highly reactive towards MTGase, in addition the pre-heated treatment of whey has an important role in increasing accessibility of whey proteins toward TGase (Zhang and Zhong, 2009) because heat treatment induced denaturation of these proteins leading to unfolding of their structure and exposure of additional sites that were unavailable in the native form (Jooyandeh, 2009) and can be explained by the enhancement in serum binding to the gel network reinforced by additional covalent bonds and incorporation of casein fines into the gel network (Bönisch *et al*., 2008). In the same way, Bönisch *et al*. (2008) and Sayadi *et al.* (2013) found a marked increase in yield of casein gels and Iranian low fat white cheese when TGase and rennet were used at once, respectively .

Whey cheese hardness

Figure 1 shows there is a high significant

Table 1. Composition and yield of MTGase treated whey cheese during storage time.

MTGase conc. U. mg^{-1} protein	Storage time (day)	Total solids %	Protein %	Yield %	Protein in whey %
Control	1	22.5	14.43	4.71	0.160
	7	23	15.10		
4U	1	23.5	15.86	5.41	0.138
	7	24.5	16.76		
8U	1	25	17.85	5.72	0.129
	7	26	18.86		

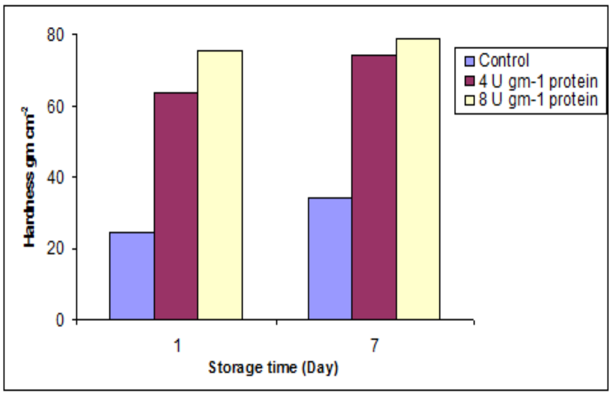

Figure 1. Whey cheese hardness at different storage time for untreated and treated samples with MTGase

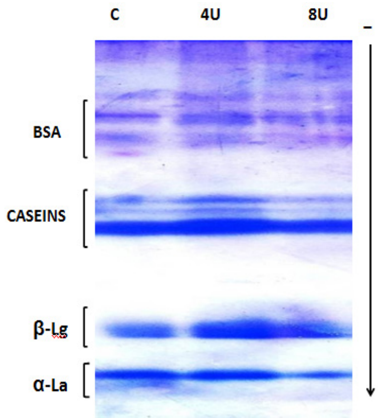

Figure 2. SDS-PAGE separation of proteins in whey cheese samples

increasing (p≤0.01) in hardness of whey cheese made by using MTGase. The higher value of hardness was reached 79gm cm^{-2} for 8U gm^{-1} protein compared to untreated samples (34gm cm^{-2}) after 7 days of storage at 4^{o}C. The results were in agreement with those of Han and Spradin (2000), Mahmood and Sebo (2009) and Di Pierro *et al.* (2010) who found that the curd treatment with MTGase after cutting had enhanced the efficiency of protein retaining and improving the hardness of cottage cheese, Iraqi white cheese and cross linked cheese, respectively.

Identification of protein cross-linking in whey cheese

SDS electrophoresis was carried out for whey cheese samples produced from three treatments after one day of storage (Figure.2). Observable differences between MTGase-treated with 8U gm^{-1} protein sample and untreated sample were observed. Result indicates decreasing in protein band size or density for both β-lactoglobulin and α-lactoalbumin of treated samples. Polymerization reaction catalyzed by MTGase resulted in the formation of high molecular weight protein polymers. Similar result was founded by Gauche *et al*. (2008). These changes in electrophoretic profiles of cheese proteins upon MTGase-treatment which were accompanied by decrease in whey protein content of whey cheese and conversely, the parallel increase in whey cheese protein content emphasize the cross-linking of these proteins with each others. Also, the intense of bands became less by increasing enzyme concentration for caseins, caseins band occurring related to the addition of 5% milk to whey protein before whey cheese manufacturing. The decreasing of proteins bands density with enzyme concentration increasing ensures the occurrence of cross linking reaction. The obtained results were in accordance with the improvement of protein, total solids, yield and hardness of treated cheese.

Effect of MTGse addition on color

The sensory evaluation of the control and MTGase treated whey cheese samples were studied and the results are illustrated in Table 2. The results shows that no significant differences were observed among treatments, the highest score was recorded for treated sample with MTGase (4U gm^{-1} protein). Similar results for soft cheese were attended by Mahmood and Sebo (2009). While Aloğlu and Öner (2013) illustrated that the results of sensory evaluation for labneh samples showed that significant differences (P ≤ 0.05) were observed for brightness, and the highest score was recorded by using 2U gm^{-1}.

Effect of MTGse addition on bitterness

As shown in Table 2, the bitterness score was

Table 2. Sensory evaluation of stored whey cheese treated with MTGase

Treat.	Storage time (day)	Color 10	Bitterness 10	Holes 10	Consistency 10	Texture 10	flavor 10	Total 60	Notes
Control	1	9.33	9.50	9.66	8.16	7.33	8.66	54.39	Whey separation
	7	9.16	10.0	10.0	9.16	8.50	9.33		
4U	1	9.66	9.83	9.66	9.00	8.50	8.50	56.32	Creamy taste no whey separation
	7	9.66	10.00	9.83	9.50	9.00	9.50		
8U	1	9.16	10.00	10.0	9.33	9.66	8.83	56.82	More cohesive and creamy taste without whey separation
	7	9.00	10.00	9.83	9.83	9.16	9.83		
$LSD_{(0.05)}$		0.95	0.448	0.62	1.35	0.974	1.31	6.37	

significantly less ($P \leq 0.05$) in control sample as compare to sample with high MTGase concentration (8 U gm^{-1} whey protein) after one day of storage. No significant differences for the addition of MTGase were obtained in soft cheese bitterness and the lowest scores were recorded for untreated samples Mahmood and Sebo (2009).

Effect of MTGse addition on holes and consistency

Table 2 shows that there was no significant increase for the effect of MTGase treating on the holes of whey cheese as compared to control sample, Mahmood and Sebo (2008) reported that the holes in soft cheese was significantly affected by subjecting samples to MTGase treatments and the hole score was decreased during storage time. Whey cheese consistency affected by the addition of the enzyme significantly ($P \leq 0.05$), and the highest score for consistency was recorded for 8 U gm^{-1} protein, compared with the control sample, the same result was achieved by Mahmood and Sebo (2008) for soft cheese consistency and the significant difference ($P \leq 0.05$) had occurred in treated samples as compared to untreated samples. Sensory analysis performed by Aloğlu and Öner (2013) showed that significant differences ($P \leq 0.05$) were observed among labneh samples (treated with 2 and 4 U and untreated) for consistency.

Effect of MTGse addition on texture

Sensory evaluation for texture of control and MTGase treated whey cheese samples were investigated and the results are presented in Table 2. Significant differences ($P \leq 0.05$) could be seen among all treatments with storage period, and the maximum texture score (9.66) was detected in 8U gm^{-1} protein after one day of storage, while control sample recorded the minimum score (7.33) after the same storage period. Sensory evaluation for soft cheese texture attained by Mahmood and Sebo (2009) differs from present result and they illustrated that the texture of soft cheese was not significantly affected by MTGase treatments. On the other hand, Aloğlu and Öner (2013) showed that the highest score (4.86 from 5 points) for texture of labneh samples was recorded for maximum MTGase unit used (4 units) and it was significantly differ from control samples.

Effect of MTGase addition on flavor

The effect of MTGase treatment on flavor property is illustrated in Table 2. Results showed that the control sample got a lower score of 9.33 than treated samples (9.50 and 9.83) after one week of storage. Similar result was obtained by Mahmood and Sebo (2009) on the effect of MTGase on sensory evaluation of white soft cheese. Aloğlu and Öner (2013) obtained significant differences ($P \leq 0.05$) results of sensorial evaluation among labneh samples for odor and flavor, and the lowest overall score of 12.45 was detected for control labneh sample, while the highest score of 27.35 was detected for 2 U TGase treated sample. However, Şanli *et al.* (2011) reported that MTGase treatment did not affect aroma and odor values of yogurt samples.

One of the important properties of the MTGase-treated whey cheese which had noticed by the panelists was the absence of drained whey during storage period. On the contrary, synersis was occurred in the untreated whey cheese sample. This may due to the formation of a protein network with high molecular weight which enclosed water and other components. The other observation was the creamy taste and improved mouth feel of the product as compared to the control sample. The same observation was stated by Mahmood and Sebo (2009). In spite of some differences that occurred during storage period, the produced cheeses did not show deterioration signs. Also, no bitter taste was detected in cheese during all storage time, this made product to be desirable and accepted by panelists. The overall scores for sensory evaluation of whey cheese did not affected by the enzyme addition (Table 2), whereas there is no significant increasing in the total score with increasing the enzyme units and the using of 8U gm^{-1} protein had led to get the highest total score.

Conclusion

The use of MTGase enzyme has led to a marked increase in the goat whey cheese yield, as well as improves some of the physicochemical and sensory properties of it. The panelists were noticed that the treated samples had a creamy taste when compared to untreated.

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