Journal

J. Biol. Chem.
Environ. Sci., 2009,
Vol. 4(1): 333-344
www.acepsag.org

EFFECT OF MICROBIAL TRANSGLUTAMINASE AND MILK PROTEIN FORTIFICATION ON THE QUALITY OF FERMENTED GOATS MILK

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ABSTRACT

This work was done to produce fermented milk like yoghurt from goat' milk fortified using different milk protein sources (skim milk powder, sodium sodium caseinate and whey protein) to enhance the sensory characteristics and the shelf life. At the same time transglutaminase was used for the same reason. Fermented milk was manufactured using the different protein sources with different concentrations (0.5, 1.0, 1.5%). Results obtained showed a decrease in titratable acidity and acetaldehyde during storage in the protein fortified samples. In contrary the soluble nitrogen indexes were increased with protein fortification. Using transglutaminase showed better sensory evaluation (appearance and body and texture). Thus it can be recommended the use of transglutaminase in goat's fermented milk production to produce healthy and safe product with good sensory properties.

Key words: Fermented milk, goat's milk, Microbial Transglutaminase, protein fortification

INTRODUCTION

Goats constitute an import animal resource under arid and semi-arid conditions. The total number of goats in Egypt is about 5 millions heads. Average daily milk yield of goat in Egypt is varied and ranged from 0.2 to 1.2 kg /head/day according to different location, breeds and stage of lactation. Moreover, the average lactation period ranged from 120 to 180 days. Goats constitute the majority of animal population in Sinai. They account for an average of about 61% of the total number of the animals' population.

Transglutaminases (EC 2.3.2.13) are aminoacyltransferases that catalyse acyl transfer reactions from glutamine residues in proteins to primary amines. When the ε -amino group of a lysine residue acts as an acyl acceptor, the two residues are covalently linked through an ε - (γ-glutamyl) lysine bond. A prerequisite for the cross-linking reaction with transglutaminase is the availability of sufficient lysines and glutamines for the formation of additional covalent bonds. While most substrate proteins have adequate quantities of these amino acids, their structures can dictate their availability or accessability for reaction.

Milk proteins, especially S.C., have the potential to be good substrates for cross-linking by transglutaminase (Sharma *et al.*, 2001). Cross-linking of proteins results in the formation of dimers, trimers and larger protein polymers. Without any interference, crosslinking will continue until glutamines or lysines become unavailable to the enzyme. Under the appropriate conditions of pH, protein concentration and substrate/enzyme ratio, gelation of the cross-linked protein is often the main effect. Partial cross-linking of proteins requires control of time, enzyme concentration, protein concentration and pH. In addition, the degree of aggregation of the protein substrate prior to incubation with the enzyme may have significant effects on any eventual functionality. Schorsch *et al.* (2000) showed that when transglutaminase was added to cow skimmed milk at pH 6.7, no gel formation was observed. This inability to gel was attributed to electrostatic and/ or steric repulsion between the protein particles, caused by a layer of κ-S.C. on the surface. It was further suggested that this repulsive layer had to be neutralised (through acidification) before gelation became evident. In contrast, Nonaka *et al.* (1992) showed that cow skim milk suspensions could be turned into self-supporting gels by using appropriate concentrations of transglutaminase and that the breaking strength and hardness of the gels were remarkably enhanced by employing higher enzyme concentrations. O'Sullivan *et al.* (2001) showed that incubation of skimmed milk or concentrated skimmed milk with Transglutaminase increased heat stability at pH values of 6.6 or greater. Results indicated that treatment with Transglutaminase prevented the dissociation of κ-S.C. from the S.C. micelles. Sodium S.C.ate is a functional milk protein ingredient with good emulsifying properties and water binding properties (Kinsella, 1984). Improving the water binding capacity of sodium S.C.ate dispersions could lead to a more efficient or cost effective use of this food ingredient.

Transglutaminase can be added to the S.C. at any stage of the production process and the degree and type of covalent linkages formed should be influenced by the concentration of S.C., its degree of aggregation and the pH. Since cross-linking of S.C. through its glutamine and lysine residues is an irreversible process, it is important to control the type (inter/intra-protein aggregate linkages) and degree of bond formation. Moreover, transglutaminase increases the water-holding capacity and emulsifying properties of milk proteins (Faergemand *et al.* 1999, Oezrenk, 2006).

On the other hand, one of the most important criteria for consumer acceptance of foods is flavor. Food matrix components, such as proteins known to interact with flavor compounds (Gianelli *et al.* 2005). Proteins are added to foods primarily because of their functional properties, such as emulsifying and stabilizing capacities, and because of their nutritional value. However, interactions between proteins and flavors are known to influence the perceived flavor of a food product (Land 1996). Protein ingredients not only reduce the perceived impact of desirable flavors but also may transmit undesirable off-flavors to foods (Semenova *et al.* 2002). In addition, proteins may change the texture of a food that is gelling, and thus decrease the flavor perception due to inhibition of mass transfer (Wilson and Brown 1997).

This paper investigates the effect of transglutaminase or milk protein fortification with different protein sources and levels on some fermented milk (like yoghurt) quality and shelf life.

MATERIALS AND METHODS

Materials

Fresh Shami goat's milk (3..20% fat , 3.17% protein ,4.24% lactose , 0.84% ash and 11.45% total solids) was obtained from El Arish city. Skim milk powder (SM) and dried whey protein concentrate (WPC) were obtained from the local market at Cairo. Sodium caseinate (S.C.) was obtained from Lactovit Co., Germany. Active yoghurt culture (Yo- Flex Culture) from Chr. Hansen was used. Microbial transglutaminase enzyme derived from

Streptovertierllium sp. was obtained from Gewuerzrmueller GmbH, Salzburg, Bergheim, Germany (100 units / g protein).

Preparation of milk protein fortified fermented milk in the presence of transglutaminase:

Goat milk was firstly fortified with 0.5, 1.0 or 1.5% protein from the different protein sources (SM, WPC, S.C.). The fermented milk bases were heat treated at 85°C for 5 min. then cooled to 4°C as described by Tamime and Robinson (1999). Thereafter, MTG was added at a level of 5 unit enzyme / g protein to the control treatment milk. After 12 h incubation at 4°C, milk mixes were heat treated again at 80°C for 1 min. for enzyme and vegetative cells inactivation then cooled to 42°C , inoculated with 2% of activate starter culture , filled into 100 ml polystyrene containers, covered, and incubated until complete coagulation (through *ca.* 3 h.). Thereafter, the containers were transferred to the refrigerator (5±1°C), where they were kept for the periodical analyses.

Analytical methods

Dry matter (DM), fat, protein, soluble nitrogen and titratable acidity (TA) contents were determined according to AOAC (2007). Acetaldehyde was estimated as described by Lees and Jago (1969).

The count of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were carried out using in order M17 and MRS agar media respectively as described by Gueimonde *et al.*, (2003).

Organoleptic evaluation of fermented milk samples was applied for storage period by regular score panels including the staff members of Food Science Department, Faculty of Agriculture, Ain Shams University according to Tamime and Robinson (1999) using the yoghurt evaluation scheme III approved by the American Dairy Science Association

The data obtained were exposed to proper statistical ANOVA analysis according to statistical analyses system user's guide (SPSS, 1998).

RESULTS AND DISCUSSION

The gross composition of goat fermented milk fortified with different milk protein sources or transglutaminase(MTG) are shown in Table (1). The obtained results reveal that, the DM content raised as well as the fat content, as the protein fortification level increased in fermented milk.

Table (1) Gross composition of yoghurt as affected by the level and source of milk protein fortification and the presence of transglutaminase.

%	Enzyme treated	Level and source of protein fortification								
		0.5			1.0			1.5		
		SM	S.C.	WPC	SM	S.C.	WPC	SM	S.C.	WPC
Total solids	11.45	13.23	12.07	12.10	14.95	12.77	12.67	16.6	13.2	13.15
Fat	3.20	3.22	3.25	3.22	3.24	3.25	3.24	3.24	3.25	3.24
Protein	3.17	3.70	3.72	3.72	4.2	4.25	4.23	4.7	4.69	4.72

SM:Skim milk, S.C.: Sodium S.C.ate, WPC: Whey protein

Table (2) reveal that titratable acidity raised significantly as the protein level increased and the presence of MTG. SM imparted the resultant fermented milk the highest titratable acidity followed by WPC and S.C.. Inversely, the protein source that caused the highest titratable acidity imparted the lowest pH value (Data not shown). As the storage of fermented milk prolonged, the acidity increased. Similar observations were reported by Neve *et al.,* (2001); Lorenzen *et al.,* (2002),Abou El- Nour *et al.,* (2004) and Hussein et al. (2006). The effect of MTG treatment on TA% was not related to the protein level ($P>0.05$), but its effect was correlated to the kind of protein source as well as the duration of storage periods as indicated.

Table (3) shows the acetaldehyde content of fermented goat"milk during storage period as affected by the level and source of fortifying protein or the presence of MTG. Data confirmed that, gradual increase in acetaldehyde content was associated with the proportional increase in the protein level of the fermented milk($P<0.001$). Moreover, SM as protein source gave the highest acetaldehyde value followed by WPC and S.C. respectively. During storage period, the acetaldehyde increased until the 7^{th} day gradually.

Then decreased till the end of storage period. The MTG treatment of milk led to delay the formation of acetaldehyde. This agreed with those reported by Abo-El Nour *et al.,* (2004) and Hussein *et al.* (2006). Moreover, the statistical interactions between the MTG treatment and the level as well as the kind of protein source were significant ($P<0.001$), while that between it and the storage period was not significant ($P>0.05$).

Table (2) Titratable acidity (TA) of fermented goat milk during storage period as affected by the level and source of milk protein fortification and the presence of transglutaminase.

Storage period (day)	Enzyme treated	Level and source of protein fortification								
		0.5%			1.0%			1.5%		
		SM	S.C.	WPC	SM	S.C.	WPC	SM	S.C.	WPC
0	0.7	0.80	0.68	0.70	0.84	0.66	0.70	0.85	0.68	0.70
7	0.85	0.94	0.73	0.78	0.94	0.72	0.74	0.94	0.74	0.78
14	0.93	1.10	0.81	0.83	1.20	0.78	0.85	1.23	0.82	0.88
21	1.23	1.30	0.87	0.90	1.40	0.84	0.92	1.46	0.88	0.94
28	1.25	1.50	0.93	0.95	1.65	0.95	0.98	1.65	0.95	0.99

SM:Skim milk , S.C.: Sodium S.C.ate , WPC: Whey protein

Table (3) Acetaldehyde (µml/100 g) content of goat's fermented milk during storage as affected by the level and source of protein fortification and the presence of transglutaminase.

Storage period (day)	Enzyme treated	Level and source of protein fortification								
		0.5%			1.0%			1.5%		
		SM	S.C.	WPC	SM	S.C.	WPC	SM	S.C.	WPC
0	120.72	220.25	123.45	140.32	240.34	112.48	159.37	260.32	98.25	187.32
7	150.35	260.32	135.82	170.22	272.42	129.36	181.12	287.45	115.27	164.23
14	114.32	222.34	112.03	145.30	230.45	105.46	150.23	242.73	89.45	148.12
21	105.41	190.23	102.04	125.33	210.00	91.03	132.06	223.65	82.45	125.46
28	93.42	180.43	87.35	102.87	200.25	82.31	118.34	208.31	79.23	112.89

SM:Skim milk , S.C.: Sodium S.C.ate , WPC: Whey protein

Table (4) shows the soluble nitrogen / total nitrogen of fermented goat's milk during storage period as affected by the level and source of fortifying protein or the presence of MTG. Data confirmed that, gradual increase in soluble nitrogen was associated with the increase of storage period. Moreover, WPC as protein source gave the highest soluble nitrogen value followed by SM and S.C. respectively. The MTG treatment of fermented milk led to more protein lysis.

Table 4: Soluble nitrogen /total nitrogen content of goat's fermented milk during storage as affected by the level and source of protein fortification and the presence of transglutaminase

Storage period (day)	Enzyme treated	Level and source of protein fortification								
		0.5%			1.0%			1.5%		
		SM	S.C.	WPC	SM	S.C.	WPC	SM	S.C.	WPC
0	4.9	5.52	5.66	5.13	5.56	5.32	5.26	5.46	5.15	5.32
7	5.21	6.45	6.01	6.42	6.07	5.95	6.36	6.02	5.93	6.42
14	6.83	6.85	7.42	8.10	7.78	6.37	7.95	7.65	6.52	8.08
21	7.95	8.45	7.05	9.05	8.81	7.01	9.36	8.86	6.85	9.46
28	8.12	9.12	7.09	9.85	9.03	7.25	9.88	9.13	6.89	10.05

SM:Skim milk , S.C.: Sodium S.C.ate , WPC: Whey protein

Tables (5) and (6) show the lactic acid bacterial counts in the fermented goat's milk during storage period. The counts of *Lb. delbrueckii* subsp. *bulgaricus* were gradually increased as the protein level increased, the count of *Str. thermophilus* raised significantly only when the protein level heightened to 1.5%. With regard to the protein source in relation to the lactic acid bacterial count, SM offered the best condition for growth of both strains enumerated, followed by WPC and S.C. respectively. This can be due to the higher concentration of lactose in SM. Duration of storage of fermented milk was associated with gradual decline in the count of both strains.

Table (5) *Streptococcus thermophilus* count (log cfu/g) of goat's fermented milk during storage as affected by the level and source of protein fortification and the presence of transglutaminase

Storage period (day)	Enzyme treated	Level and source of protein fortification								
		0.5%			1.0%			1.5%		
		SM	S.C.	WPC	SM	S.C.	WPC	SM	S.C.	WPC
0	9.34	9.65	9.43	9.52	9.45	9.48	9.52	9.62	9.24	9.46
7	8.92	8.87	8.65	8.96	9.11	8.76	9.24	9.22	8.62	8.94
14	8.12	8.23	8.12	8.12	8.76	8.25	8.62	8.72	8.11	8.42
21	7.88	8.02	7.43	7.68	8.18	7.98	8.07	8.31	7.24	7.83
28	7.05	7.38	7.25	7.42	7.88	7.65	7.05	7.45	6.95	7.29

SM:Skim milk , S.C.: Sodium S.C.ate , WPC: Whey protein

Table 6: *Lactobacillus delbrueckii* subsp.*bulgaricus* count (log cfu/g) of goat's fermented milk during storage as affected by the level and source of protein fortification and the presence of transglutaminase

Storage period (day)	Enzyme treated	Level and source of protein fortification								
		0.5%			1.0%			1.5%		
		SM	S.C.	WPC	SM	S.C.	WPC	SM	S.C.	WPC
0	8.92	8.63	8.74	8.64	8.79	8.78	8.57	8.78	8.97	8.65
7	8.21	8.45	8.25	8.36	8.65	8.15	8.35	8.47	8.11	8.34
14	7.94	8.01	7.87	7.97	8.12	7.64	7.93	8.33	7.56	7.94
21	7.53	7.63	7.02	7.67	7.58	7.32	7.54	7.84	7.12	7.34
28	7.02	7.02	56.49	6.94	7.22	7.11	7.08	7.28	6.83	7.11

SM:Skim milk , S.C.: Sodium S.C.ate , WPC: Whey protein

Similar findings were described by Neve *et al.,* (2001) and Hussein *et al.* (2006). The comparison effect between MTG and other fortification was significantly depended on the level and source of fortification protein (P<0.05) as well as the storage period (P<0.001).

Tables (7) illustrate the organoleptic evaluation of the treated samples in terms of appearance, consistency and flavour.

Concerning the appearance criterion, that was significant affected among the protein fortification level (P<0.001). The fermented milk supplemented with 0.5% protein led to obtain the highest score, followed by 1.0, 1.5 without any significant differences between SM, and S.C. as milk protein sources. While WPC caused some yellowness leading to significant reduction in the appearance score.

The enzymatic treatment of milk with MTG improved significantly (P<0.001) the appearance of fermented milk. The highest score for each one was as revealed a dry, smooth and whiter shining surface. Similar observations were reported by Lorenzen *et al.*, (2002) , Abo-El Nour *et al.,* (2004) and Hussein *et al.* (2006).

The body and texture score of goat's fermented milk was increased by adding 0.5 or 1.0% protein more than that done when the level raised to 1.5% (P<0.001), especially when SM was applied, followed by WPC and S.C. (P<0.001). The body and texture score of MTG treated fermented milk varied slightly significantly (P<0.05) from those untreated, where some cheesy body was observed in the

Table (7) Organoleptic evaluation scores of of goat's fermented milk during storage as affected by the level and source of protein fortification and the presence of transglutaminase

Storage period (day)	Enzyme treated	Level and source of protein fortification								
		0.5%			1.0%			1.5%		
		SM	S.C.	WPC	SM	S.C.	WPC	SM	S.C.	WPC
Appearance (5)										
0	5	5	5	5	5	5	5	5	5	5
7	4.5	5	5	5	5	5	5	5	5	5
14	4.5	4.5	4.5	4.5	4.5	5	5	4.5	5	5
21	4.5	4.5	4.5	4.5	4.5	5	5	4.5	5	5
28	4.0	4.0	4.0	4.0	4.0	4.5	4.5	4.0	4.5	4.5
Body and Texture(5)										
0	4.5	5	4.5	4.5	5	4.5	4.5	5	4.0	4.5
7	4.5	5	4.5	4.5	5	4.0	4.5	5	4.0	5.0
14	4.5	5	4.5	5	5	4.0	4.5	4.5	3.5	4.5
21	4.5	5	4.5	4.5	4.5	3.5	4.5	4.5	3.0	4.0
28	4.5	4.5	4.5	4.5	4.5	3.5	4.0	4.0	3.0	4.0
Flavour (10)										
0	9	9	8	9	9	8	9	8	8	9
7	9	9	8	9	9	8	8	8	7	8
14	8	8	7	8	8	7	8	8	6	7
21	8	7	7	7	7	7	7	7	6	7
28	7	7	6	7	7	6	7	7	5	7
Total scores (20)										
0	18	19	17.5	18.5	19	17.5	18.5	18	17	18.5
7	18	19	17.5	18.5	19	17	17.5	18	16	18.
14	16.5	17.5	16	17.5	17.5	16	17.5	17	14.5	16.5
21	16.5	16.5	16	16	16	15.5	16.5	16	14	16
28	15.5	15.5	14.5	15.5	15.5	14.0	15.5	15	12.5	15.5

SM: Skim milk, S.C.: Sodium S.C.ate, WPC: Whey protein

former especially when protein was added at the highest level (1.5%) and supplied from S.C. ($P<0.001$). There was no significant relationship ($P>0.05$) between the MTG treatment and the storage period in body and texture. Furthermore, gradual increase in the flavour score was recorded, when the protein level of milk raised until 1.0%. The highest level (1.5%) suffered from flavour. The score lower than even the control. The adding of WPC was the better protein enrichment procedure toward the flavour, followed by SM and S.C.

Fermented milk made with MTG was considered to be flat and less intense in flavour particularly for aroma attributes. Similar observations were reported by Lorenzen *et al.*, (2002), Abo-El Nour *et al.,* (2004) and Hussein *et al.* (2006).

Thus, it can be concluded that treatment of goat milk with transglutaminase could be considered as alternative of protein elevation with a level of 0.5% and improve the organoleptic quality as well as the shelf life of .fermented goat's milk like yoghurt.

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تأثير ترانس جلوتامينيز وإستخدام مصادر مختلفه من بروتينات اللبن على بعض خواص لبن المعز المتخمر

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[2]- قسم بحوث تكنولوجيا الألبان ، معهد بحوث الإنتاج الحيوانى ، مركز البحوث الزراعية ، الجيزه.

تم هذا البحث بغرض دراسة انتاج لبن متخمر (يوجهورت) من البان المعزيتميز بخواص حسيه وقوة حفظ مرتفعه.. ونتيجه لإنخفاض محتوى البان المعز من الجوامد الكليه فتكون الحاجه شديده لرفع الجوامد لتحسين القوام والتركيب للمنتج النهائى ، لذلك تم التفكير فى تدعيم لبن المعز بمصادر مختلفه من بروتين اللبن وبنسب مختلفه من كل مصدر والتى شملت اللبن الفرز المجفف ، كازينات الصوديوم وبروتينات الشرش المجففه بنسب 0.5% ، 1.0% ، 1.5% إضافة انزيم الترانس جلوتامينيزكعامل مساعد لتحسين القوام.. وقد تم معاملة اللبن حراريا ثم اضيف البادىء بعد تثبيط الإنزيم حراريا وخزن الناتج النهائى لمدة 28 يوم فى الثلاجهو تقدير الحموضة واعداد بكتريا حمض اللاكتيك الكرويه والعصويه، المحتوى من الاسيتالدهيد، والتغير فى النيتروجين الذائب بالنسبه للنيتروجين الكلى ، بالاضافة للصفات الحسية اسبوعيا طوال فترة التخزين..

ولقد أوضحت النتائج انخفاض نسبة الحموضة والاسيتالدهيد و الاعداد البكتيرية للبادئ اثناء التخزين للعينات المضاف لها بروتينات. كما أظهرت العينات المضاف لها بروتينات ارتفاعاً في قيم النيتروجين الذائب مقارنة بالعينات غير المعاملة كما اظهرت اختبارات التقييم الحسى ان العينات المعاملة بالأنزيم كانت الأفضل من حيث المظهر والقوام مقارنة بالعينات غيرالمضاف لها بروتينات ، بينما حصلت العينات غير المضاف لها بروتينات على الأفضلية من حيث النكهة ودرجات التقييم الحسية الكلية.وقد كانت العينات المدعمة بنسبة 0.5% بروتين هى الأفضل من حيث المظهر بينما حصلت العينات المدعمة بنسبة 1.0% بروتين على الأفضلية من حيث القوام ومجموع درجات التقييم الحسي. وبناء على ذلك فيمكن استخدام انزيم الترانس جلوتامينيز لإنتاج لبن متخمر من البان المعز ذو جوده تركيبيه وحسيه جيده وآمنه .