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EFFECT OF ADDING TRANSGLUTAMINASE ON THE PROPERTIES OF FERMENTED MILK

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ABSTRACT

Rheological, chemical and organoleptic properties of yoghurt made from (buffaloes', cows' and goats' milk) were studied by adding Microbial Transglutaminase (MTGase) at different ratios. Adding MTGase at different concentrations decrease the development of acidity, syneresis and acetaldehyde content. Also, increased the values of firmness, apparent viscosity and organoleptic properties. Buffaloes' milk yoghurt showed the highest firmness, apparent viscosity and organoleptic properties. Meanwhile it had the lowest syneresis and acidity development. Cows' milk yoghurt showed intermediate values between (buffaloes' and goats' milk yoghurt) for rheological, chemical and organoleptic properties. On the other hand, it had the lowest value of acetaldehyde (aroma compound). Goats' milk yoghurt showed the highest values of acetaldehyde, syneresis and acidity development but it had the lowest firmness and apparent viscosity.

Keywords : Microbial Transglutaminase, Yoghurt .

INTRODUCTION

Yoghurt is the most popular fermented dairy products in Egypt and world wide. Many health benefits have been attributed to yoghurt such as improved lactose tolerance, protection against gastrointestinal infection, effective treatment for specific types of diarrhea and cholesterol reduction (Kurmann, 1992). The Egyptian markets prefer the set style yoghurt and attempts to produce set flavoured yoghurt was not cited. Preparing zabady, popular fermented milk in Egypt that is free from defects in body, consistency and syneresis continues to be a problem in the local dairy industry. The texture of yoghurt is highly dependent on the amount and functionality of the individual components comprising the yoghurt gel, on the interactions between the components as well as on the technological steps in the production process.

The gel structure of set style yoghurt is influenced by many factors including milk composition and the processing conditions during yoghurt manufacture. The superior yoghurt is made from buffaloes' milk. It is well known that buffaloes' milk is richer in fat, protein and TS content compared to cows' and goats' milk.

Protein structure can be modified in a number of ways, chemically, physically or enzymatically. The advantage of using enzymatic modification instead of chemical modification is the higher specificity of enzymatic reactions and thus avoidance of possible toxic side products. Food industries worldwide are looking forward to develop new ingredient or dairy products with novel physical and functional characteristics. One of the means of altering the properties of dairy products is via modification of milk proteins using transglutaminase (Motoki & Seguro, 1998)

Enzymatic cross-linking has been investigated in some detail using the enzyme transglutaminase which is an enzyme naturally present in most animal tissues and body fluids (Sharma, et al. 2001) and catalyses an acyl group transfer reaction between γ – carboxyamide groups of peptide-bound glutamine residues (acyl donor) and the primary amino groups in a variety of amine compounds (acyl acceptor), including peptide-bound ε-amino groups of lysine residues in protein molecules (Lorenzen & Schlimme, 1998).

The caseins are good substrate for TGase due to their open structure, while the globular whey proteins, unless denatured, are poor substrates (Traorè & Meunier, 1992; Sakamoto, et al. 1994; Christensen et al., 1996; Schorsch, et al. 2000) protein polymerization triggered by TGase increases linearly with increasing degree of whey protein denaturation by heat (Lorenzen, 2000). Food treated with microbial transglutaminase appeared to have an improved flavour, appearance and texture. In addition, this enzyme can increase shelf-life and reduce allergenicity of certain foods Zhu, et al., 1995).

Lorenzen & Schlimme, (1998) described the properties and potential fields of application of the enzyme transglutaminase (EC.2.3.2.13) in dairying. They concluded that transglutaminase may be used in stabilizing products such as yoghurt, whipping cream, fresh cheese and novel milk products, which is seemingly the most interesting field .

Sharma, et al. (2001) investigated the reaction of transglutaminase on the casein micelle and whey proteins in their natural states in milk, and distinguish between the crosslinking and other reactions catalysed by transglutaminase. They stated that transglutaminase in unheated milk had a small effect on proteins whereas in the preheated milk, considerable crosslinking, deamidation and/or amine incorporation occurred. In both unheated and preheated milk, major reduction in the monomeric forms of κ-casein and β-casein occurred due to the reaction with transglutaminase. Also, they reported that preheated β-lactoglobulin was susceptible to transglutaminase action, while α-lactalbumin was crosslinked with or without preheating to the same extent. Abou El-Nour, et al. (2004) followed the changes in the rheological, chemical and organoleptic characteristics of the cow's milk yoghurt treated with TGase during cold storage. They observed that yoghurt made from milk treated with 0.4 – 0.5 g / L is consider preferable treatments to obtain yoghurt of acceptable texture, good keeping quality, and less post acidification changes during storage.

The goal of this study is to investigate the effect of MTGase catalyzed cross-linking in set style yoghurt of three species of milk (buffaloes', cows', and goats' milk) on their chemical, rheological and organoleptic properties.

MATERIALS AND METHODS

Materials :

Fresh cows' , buffaloes' and goats' milk were obtained from the herd of the Faculty of Agriculture, Suez Canal University. The analysis of milk were (12.16 % T.S., 3.95 % Fat, 3.11 % Protein, 0.7 % Ash and 4.4 % Lactose) for cows' ; (14.91 % T.S., 5.8 % Fat, 3.48 % Protein, 0.82 % Ash and 4.81 % Lactose) for buffaloes' and (12.36 % T.S., 4 % Fat, 2.78 % Protein, 0.67 % Ash, 4.91 % Lactose) for goats' milk respectively.

Skim milk powder (T.S 96.2 %) which produced in USA was obtained from the local market.

A pure culture of (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) were obtained from (Chr. Hansen's Laboratories Denmark A /S).

Microbial transglutaminase preparation ACTIVA MP contains 1% Transglutaminase was gift from Ajinomoto Europe Sales (Stubbenhuk 3, D-20459, Hamburg, Germany); the declared activity of the preparation was approximately 100 units / g. The enzyme preparation is later on referred to as MTGase.

Methods :

Manufacture of yoghurt:

Yoghurt was manufactured according to the protocol proposed by Tamime & Robinson (1999). Fresh buffaloes' and cows' milk were divided into seven batches, while goats' milk was divided into eight batches. Three batches from each group were reserved without enzyme addition as (T1, T2 and T3). (T1) was reserved as control treated exactly like (T4:T8) except the addition of enzyme, (T2) was manufactured from high heated milk (80 ^{o}C/10 min.) and (T3) contained 4% skim milk powder and pasteurized at 72 ^{0}C/15 s. the rest four batches (T4, T5, T6 and T7) for (cows' and buffaloes') milk and The rest five batches (T4, T5, T6, T7 and T8) for goats' milk were heated at 72^{o} C for 15 s and cooled to 40^{o} C and incubated at 40^{o} C for 2 h after addition of MTGase at a rate of 0.2g L^{-1} milk (T4), 0.3 g L^{-1} milk (T5), 0.4 g L^{-1} milk (T6), 0.5 g L^{-1} milk (T7) and 0.75 g L^{-1} milk (T8). The enzyme was inactivated by heat treatment at 80^{o}C for 1 min before adding starter culture . All milks were cooled down to 43^{o}C and inoculated with yoghurt starter culture at rate 0.04 g starter L^{-1} milk. Incubation was carried out until pH (4.8, 5.0 and 4.6) for buffaloes', cows' and goats' milk yoghurt, respectively at 43 ^{o}C. Then, the set yoghurt samples were placed into cold room (6 ^{o}C) and stored for 10 days. The samples were analyzed with 4-day intervals during cold storage.

Analytical methods :

Total solids; Ash; Protein and Lactose content of milk used in yoghurt manufacture were determined according to Marshall (1992); Fat content by Gerber butyrometer according to Ling (1963). Yoghurt was analyzed for pH using Jenway pH meter with Jenway spear electrode No: 29010(Jenway limited, Gransmore Green, Felsted, Dunmow, England) according to ling (1963), Acetaldehyde was determined as described by Kosikowski (1982), synersis was evaluated by measurement of whey separation by using centrifugation method as following: 50 g of set-style yoghurt was centrifuged using IEC PR-700 Centrifuge, USA at 2500 Xg for 5 min. The weights of supernatant whey was determined and expressed in percent. Firmness of yoghurt was determined at 7 ^{O}C using Brabender Structograph, OHG, Duisburg, Germany, Model 8603 with spindle No: 449644 and force 500 cm g^{-1} the heights of resultant curve express the firmness. Three replicates were done for each sample. Apparent viscosity was measured with a Brookfield rotational viscometer; model RV III (Brookfield Engineering Laboratories Inc., MA, USA). A cylindrical spindle (Spindle No. SC4-14) was used in the determination of viscosity. All viscosity readings were taken at 25 ^{o}C in shear rate ranging from (0.8 to 8.0 s^{-1}). All rheological parameters were performed in quadruplicates. Flow curves for yoghurt were drawn from measured values of apparent dynamic viscosity & shear rate, plastic viscosity & treatments and consistency index & treatments. The whole experiment was repeated three replicates for each treatment.

Organoleptic properties:

The yoghurt samples organoleptically scored for flavour (50 points), body & texture (40 points) and appearance (10 points) according to score card suggested by Pappas et al, (1996). Samples were judged by the stuff members of Dairy Department, Faculty of Agriculture . Suez Canal University.

RESULTS AND DISCUSSION

Chemical properties:

Variation in pH during fermentation :

The pH values of yoghurt treatments gradually decreased during incubation due to the developed acidity by the yoghurt culture Table (1) and Fig. (1).

Table (1): Development of yoghurt pH during fermentation at 43 ^{o}C.

Treatments								
Time (h)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
				a. Buffaloes' milk				
1	6.3	6.35	6.24	6.45	6.50	6.53	6.54	-
2	5.4	5.45	5.55	6.1	6.15	6.25	6.32	-
3	5.3	5.6	5.65	5.85	5.9	6.09	6.12	-
4	5.2	5.25	5.35	5.4	5.45	5.4	5.47	-
4.5	-	-	5	5.25	5.35	5.3	5.35	-
5	-	-	-	-	-	5	5	
				b. Cows' milk				
1	6.33	6.37	6.26	6.49	6.51	6.55	6.60	-
2	5.46	5.58	5.60	5.70	5.85	6.00	6.05	-
3	5	5	5	5.30	5.40	5.50	5.61	-
4	4.8	4.8	4.8	4.80	4.80	5.21	5.32	-
5	-	-	-	-	-	5.00	5.00	-
5.5	-	-	-	-	-	4.80	4.80	-
				c. Goats' milk				
1	6.05	6.10	5.97	6.20	6.24	6.26	6.29	6.32
2	5.45	5.50	5.30	5.73	5.80	5.85	5.88	5.96
3	4.73	4.85	4.79	4.90	4.97	4.98	4.99	5.19
4	4.62	4.63	4.6	4.78	4.80	4.84	4.90	4.99
5	-	-	-	4.60	4.60	4.60	4.70	4.80
6	-	-	-	-	-	-	4.60	4.60

T1 = (0 g L^{-1} MTGase at 72 ^{o}C for 15 s., control). T2 = (0 g L^{-1} MTGase at 80 ^{o}C for 10 min.).
T3 = (0 g L^{-1} MTGase at 72 ^{o}C for 15 s. with 4% skim milk powder).
T4 = (0.2 g L^{-1} MTGase at 72 ^{o}C for 15 s.). T5 = (0.3 g L^{-1} MTGase at 72 ^{o}C for 15 s.).
T6 = (0.4 g L^{-1} MTGase at 72 ^{o}C for 15 s.). T7 = (0.5 g L^{-1} MTGase at 72 ^{o}C for 15 s.).
T8 = (0.75 g L^{-1} MTGase at 72 ^{o}C for 15 s.).

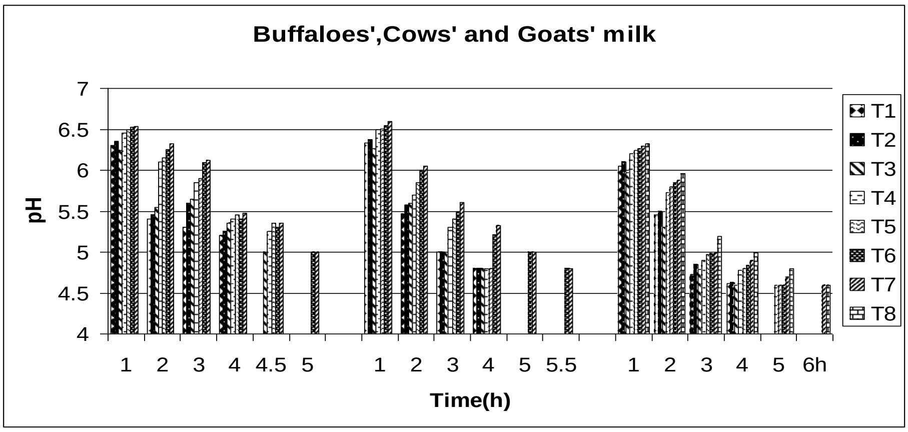

Fig. (1) pH values of yoghurt made with MTGase during fermentation at 43ºC.

T1 = (0 g L^{-1} MTGase at 72 ºC for 15 s, control).
T2 = (0 g L^{-1} MTGase at 80 ºC for 10 min, control).
T3 = (0 g L^{-1} MTGase at 72 ºC for 15 s with 4% skim milk powder, control).
T4 = (0.2 g L^{-1} MTGase at 72 ºC for 15 s).
T5 = (0.3 g L^{-1} MTGase at 72 ºC for 15 s).
T6 = (0.4 g L^{-1} MTGase at 72 ºC for 15 s).
T7 = (0.5 g L^{-1} MTGase at 72 ºC for 15 s).
T8 = (0.75 g L^{-1} MTGase at 72 ºC for 15 s).

This decrease occurs at a slower rate in MTGase treated milk than control. The higher the MTGase doses added to milk, the longer the fermentation time.These results were in agreement with (Færgemand et al., 1999) who found an increased in fermentation time for yoghurt cross-linked simultaneously with the fermentation. Also, (Lorenzen & Schlimme 1997; Neve et al., 2001) demonstrated that the enzymatic cross-linking step led to a minor imbalance of the associative growth of yoghurt starter culture. It was noticed that the greatest coagulation time was found in goats' milk (6h) then that of cows' milk (5.5h) which displays mediated time of fermentation while the lowest time was found in buffaloes' milk (5h). This may be due to the lower levels of casein in total proteins of goats' milk. These results are in agreement with Abou El-Nour & El-Kholy (2003). They found that the coagulation time of goats' milk treated with three different coagulants was greater than that of cows' and buffaloes' milk. The heat treatment of milk i.e. 70 ºC / 15 s. or 80 ºC / 10 min. had no effect on the development of pH during incubation time for all milks. It is clear from the data in Table (1) that buffaloes' and cows' milk took (5h) to reach pH 5 in comparison with goats' milk (3 - 3.5h) this is may be due to the buffering capacity of goats' milk (the ability of the product to be acidified or alkalinized) is lower than buffaloes' and cows' milk. Also, yoghurt made from goats' milk reached to the lowest pH (4.6) than cows' milk pH (4.8 - 5) and buffaloes' pH (5). It may due to lower casein content of goats' milk than buffaloes' and cows' milk (Laurent et al., 1992).

Variation in pH during cold storage :

Table (2) and Fig. (2) showed the decrease in pH values of yoghurt treatments during storage for 10 days at 6 ºC. Whereas the development of acidity in MTGase treated samples was slower than yoghurt made without enzyme for the three types of milk, and, overall, the higher the MTGase concentration added into milk, the slower the development of acidity in the yoghurt upon storage.

Table (2): Development of yoghurt pH during cold storage at 6 ºC.

Treatments								
Time(days)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
a. Buffaloes' milk								
1	4.73	4.75	4.80	4.82	4.84	4.90	4.95	-
4	4.53	4.59	4.74	4.70	4.73	4.80	4.85	-
7	4.35	4.40	4.63	4.57	4.61	4.70	4.76	-
10	4.25	4.35	4.53	4.45	4.49	4.62	4.69	-
b. Cows' milk								
1	4.60	4.65	4.68	4.70	4.75	4.77	4.82	-
4	4.50	4.54	4.59	4.60	4.63	4.66	4.72	-
7	4.30	4.36	4.45	4.43	4.5	4.55	4.62	-
10	4.10	4.20	4.39	4.32	4.42	4.47	4.53	-
c. Goats' milk								
1	4.40	4.43	4.53	4.48	4.51	4.55	4.57	4.6
4	4.18	4.23	4.36	4.28	4.31	4.34	4.38	4.43
7	4.05	4.10	4.32	4.17	4.23	4.28	4.33	4.39
10	3.88	3.98	4.20	4.03	4.10	4.18	4.25	4.31

See Table 2 for treatments designation.

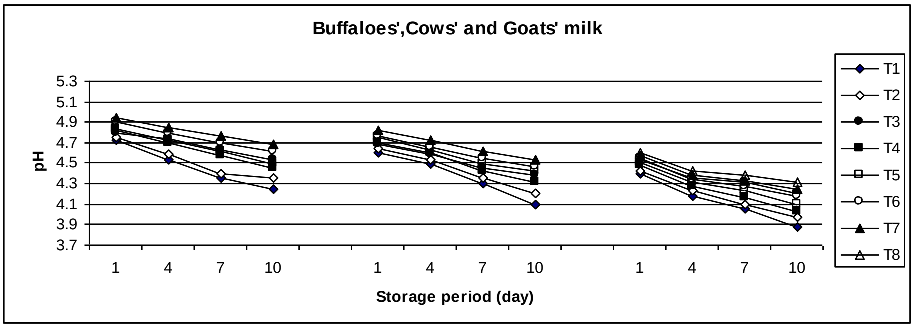

Fig. (2) pH values of yoghurt made with MTGase during cold storage:(See Fig.1 for treatments designation).

These results is in agreement with (Lorenzen et al., 2002) they found that MTGase treatment of milk led to a lower post acidification of yoghurt products during three weeks of storage. The decreased availability of low molecular weight peptides needed for the growth and activity of bacteria (Tamime & Robinson 1985) due to cross-linking which may explain the slow growth and activity of yoghurt starter.

Acetaldehyde content of yoghurt :

Fig. (3) showed the changes of the acetaldehyde contents of yoghurt made from three types of milk during cold storage. The typical flavour of yoghurt is due mainly to various carbonyl compounds, i.e. acetaldehyde and diacetyl, produced by *Str. thermophilus* and *Lb. delbrueckii* subsp. *bulgaricus*. Acetaldehyde is considered as the major flavour component of yoghurt. The average acetaldehyde contents of yoghurt varied between milks according to their acetaldehyde concentration. The results showed that the highest acetaldehyde content was found in yoghurt made from goats'; buffaloes' and cows' milk in order. On the other hand, the results showed a negative correlation between MTGase concentration and the formation of acetaldehyde in the three types of yoghurt milk, this was due to increasing in MTGase concentrations which decreased acetaldehyde formation. It is highly unlikely that the acetaldehyde formed during fermentation was transformed and / or degraded into another chemical form(s) by MTGase .

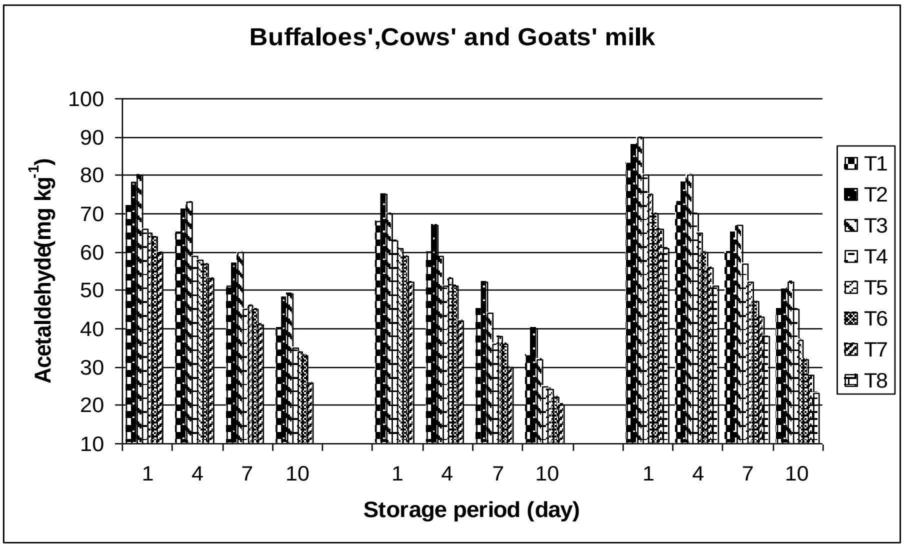

Fig. (3) Acetaldehyde content of yoghurt made with MTGase: (See Fig.1 for treatments designation).

Therefore, the lower level of acetaldehyde in the MTGase-containing samples may be due to the slow bacterial growth and, naturally, slow metabolic activity of the starter bacteria in the presence of MTGase. Another possibility could be that MTGase might have interfered with the mechanism of the acetaldehyde synthesis from amino acids (i.e. methionine or threonine) which is one of the routes of acetaldehyde production during fermentation (Chaves et al., 2002). The level of acetaldehyde in all samples gradually decreased during storage periods. These results are agreement with (Tamime & Robinson1999). They found that during cold storage the level of acetaldehyde gradually decreased and this was independent with MTGase concentration. Much of the acetaldehyde is formed during fermentation and the stability of acetaldehyde is pH-dependent whereas at lower pH values, acetaldehyde can easily be oxidized to acetate and, therefore, during storage, the level of acetaldehyde decreases.

Rheological parameters:
Syneresis (whey separation) properties:

The texture of yoghurt, as other food, is a function of their composition. Therefore, any variation in the formula of the yoghurt can be produce marked change in the texture of the resultant products.

The whey separated (syneresis) values for yoghurt of the three types of milk were showed in Fig. (4). Syneresis is factor affecting consumers' acceptance of yoghurt (Tamime & Robinson 1999). The results showed that yoghurt made from heat treated milk without adding MTGase (T1) exhibited higher syneresis than that either made from high heat treated milk (T2) or that made with 4% skim milk powder (T3). Using of 4% skim milk powder in the production of yoghurt resulted in a product with firm body and low syneresis. Using high heat treated milk (80 °C / 10 min) produced yoghurt with better body and low syneresis than low heat treated milk (72 °C / 15 s).

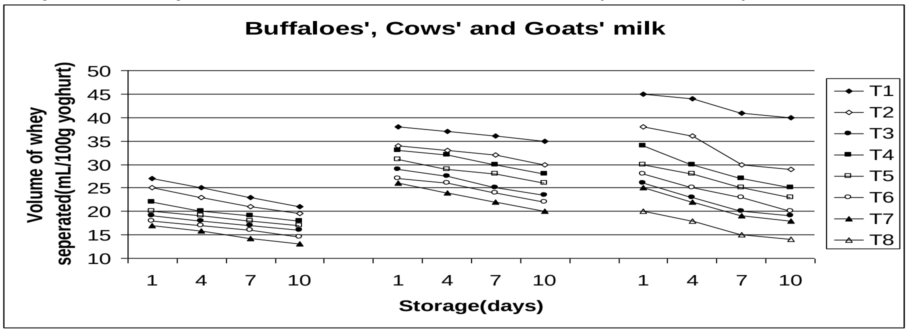

Fig. (4) Whey separation during cold storage of yoghurt made with MTGase: (See Fig.1 for treatments designation).

The data indicated that the syneresis of curd resulted from goats' milk was the highest while the lowest level of syneresis was for yoghurt made from buffaloes' milk than that of yoghurt made from cows' milk which displays mediated level of syneresis. This may be due to the lower levels of casein in total proteins of goats' milk about (68 to 70% of the total proteins in goats' milk are caseins); this is low compared with the percentage obtained for cows' milk, in which 78 to 80% of the total proteins are caseins, as a rule. Goats' milk therefore contains more serum proteins but less casein than cows' milk. These results are in a good agreement with (Abou El-Nour & El-Kholy 2003).

The volume of whey separated from the yoghurt samples of three types of milk decreased with increasing MTGase dose. This was a result of permanent cross-links between the milk proteins triggered by the MTGase, leading to a decrease in gel permeability (Faergemand et al., 1999; Lauber et al., 2000;). Decrease in gel permeability causes a more compact and stable microstructure with smaller compartments in yoghurt, and, hence, more free water is entrapped in the yoghurt gel network (Moon & Hong 2003). Additionally, MTGase improves the water holding capacity of yogurt gel network (Motoki & Seguro 1998). During cold-storage, the quantities of the whey separated from yoghurt decreased slightly and this decrease was found to be independent from the enzyme treatment.

The firmness properties:

Firmness is stress (force) necessary to attain a given deformation (Sherman, 1969). Fig. (5) showed that using high heated milk (80 ^{o}C for 10 min) in yoghurt manufacture increases its firmness due to increasing of total solids of used milk, denaturation of whey protein and then interaction with casein micelles. The denatured whey proteins interact with casein micelles to cross-link casein particles in the gel network and increasing the rigidity of yoghurt gels (Lucey et al., 1998).

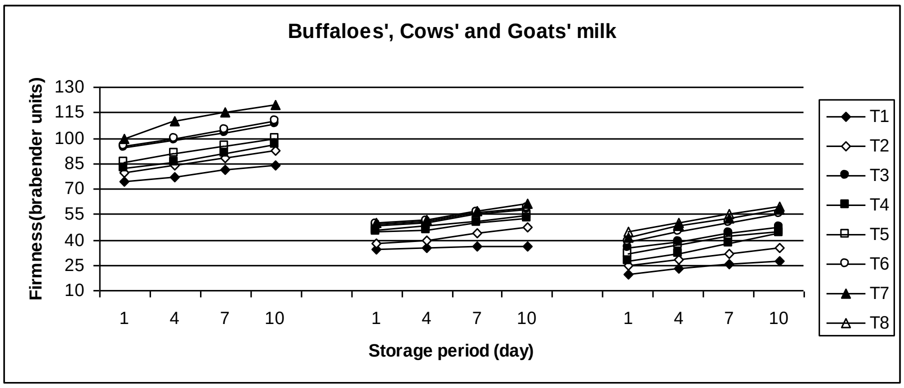

Fig. (5) Firmness during cold storage of yoghurt made with MTGase: (See Fig.1 for treatments designation).

The results showed that yoghurt made from three types of milk appeared differences in firmness values whereas the lowest firmness values were that of goats' yoghurt, the highest firmness values were that of buffaloes' yoghurt while cows' yoghurt showed intermediate firmness values. These obtained results are attributed to the differences of casein content in total protein among the three types of milk whereas the lowest casein content was found in goats' milk then that of cows' milk which displays mediated value of casein content while the highest casein content was found in buffaloes' milk. Using of MTGase treated milk in yoghurt manufacture improved its firmness e.g. using of 0.2 g MTGase L^{-1} milk produced yoghurt similar in all types of milk in firmness to those yoghurt sample made from high heated milk (T2). Using 0.4 g MTGase L^{-1} milk produced yoghurt similar in firmness to those made with 4% skim milk powder. Increasing the MTGase level to 0.75 g L^{-1} milk caused firmness which are about two times higher than yoghurt made from heated milk (T1). These results are in agreement with those reported by (Faergemand et al., 1999; Lorenzen & Neve 2002;). The reason for the enzyme-induced increase of the gel strength is due to a reduction of mish sizes of the protein network and to a more regular distribution of the protein chains in the product (Lorenzen & Neve 2002). The firmness was found to increase slightly with cold storage for all yoghurt samples.

Apparent viscosity properties:

From Fig. (6), it was noticed that apparent viscosity of the MTGase treated samples was much higher than control (T1) and control (T2). However, increasing MTGase dosage increased the yoghurt viscosity in all samples (T4:T8). These results coincided with those of (Han et al., 2003) who stated that as MTGase dosage increased, more extensive cross-linkage of proteins increased and the viscosity of the products increased. The apparent viscosity values of yoghurt made from three types of milk were different. Whereas, it was higher for buffaloes' milk than that of cows' or/and goats' milk. On the other hand, yoghurt from goats' milk had the lowest levels of apparent viscosity as the results of the positive relationship between protein content and viscosity. These results are in agreement with those of (Tamime & Robinson 1985; Han et al., 2003) they stated that more extensive cross-linkage in proteins increased the viscosity of the products.

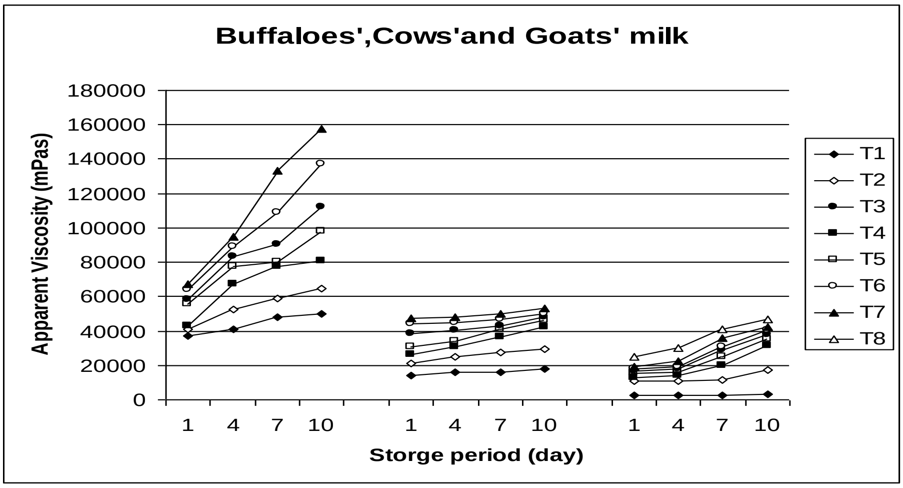

Fig. (6) Variations in the apparent viscosity during cold storage of yoghurt made with MTGase: (See Fig.1 for treatments designation).

Apparent viscosity - Shear rate properties:

Fig. (7) showed the relation between apparent viscosity and shear rate of yoghurt made from three types of milk during cold storage. It was noticed that there were different values between the three types of yoghurt milk. In general, the apparent viscosity – shear rate values tended to gradually increase along the storage period up to 10 days. The apparent viscosity – shear rate values were the highest in yoghurt made from buffaloes' milk than that cows' milk which displayed mediated values while the lowest values were recorded for goats' milk. This is may be due to higher content of protein of buffaloes' milk than other types of milk. The control sample (T1) showed lower values of apparent viscosity than that made from high heated milk (T2) or other treatments. Addition MTGase increased the yoghurt apparent viscosity whereas, the apparent viscosity values of yoghurt sample continuously decreases with increasing shear rate.

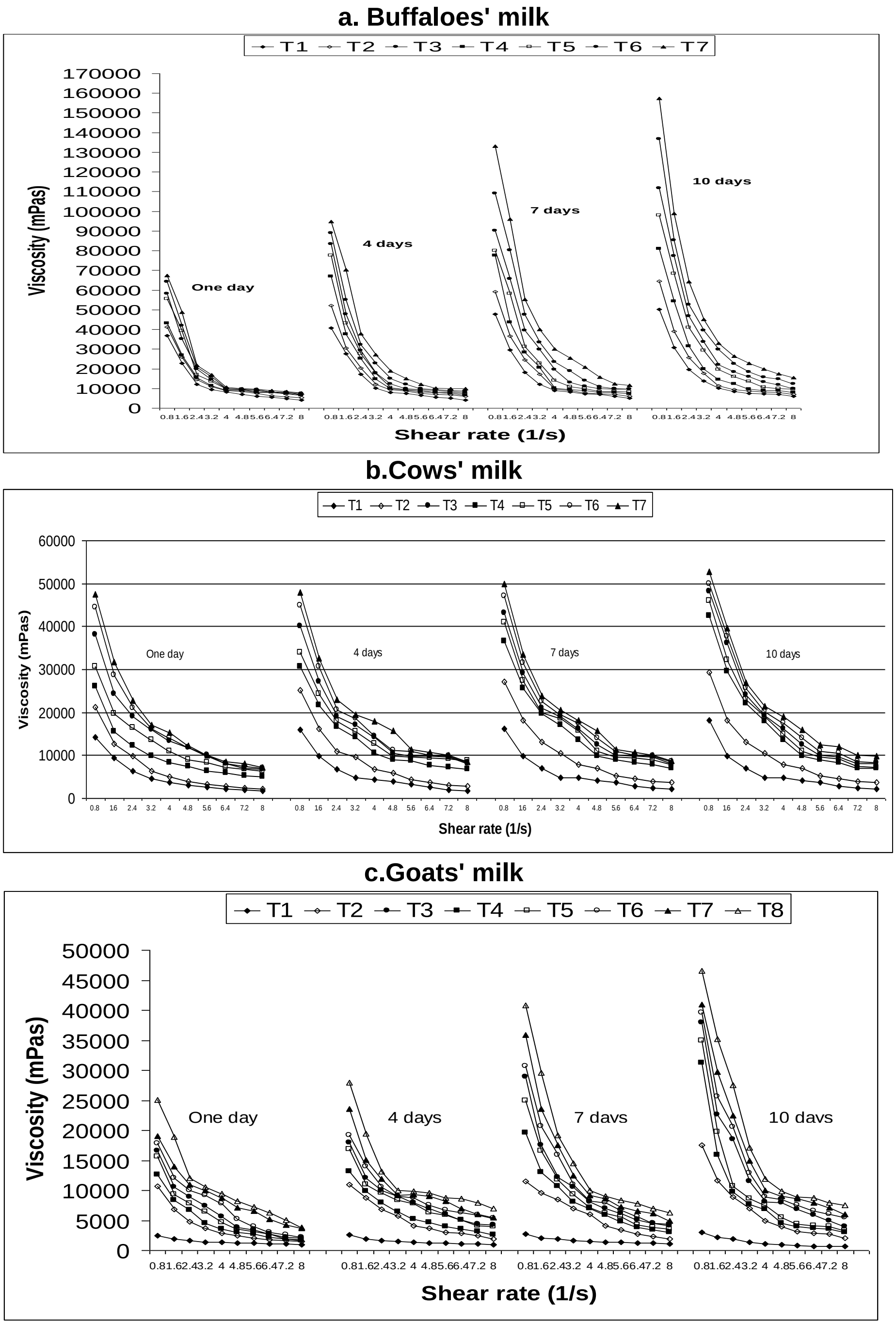

Fig. (7) Apparent viscosity- Shear rate of yoghurts made with MTGase during cold storage: (see Fig. 1 for treatments designation).

In general, the higher the MTGase doses added into milk, the higher the apparent viscosity values in the yoghurts. These results were, to some extent, expected since the basic function of MTGase is to cross-link milk proteins covalently which results, eventually, in a finer and stronger gel network in the yoghurt, compared with acid-induced gels. Introduction of new covalent bonds leads to gels which are, therefore, different in structure (Schorsch et al., 2000).

Plastic viscosity and consistency index properties :

Figs. (8, 9) showed that plastic viscosity and consistency index increased with increasing MTGase levels. Moreover, results showed wide differences in plastic viscosity and consistency index between yoghurt from heated milk (T1) that gave lowest values of plastic viscosity and consistency index than that made from other treatments (T2 : T8).

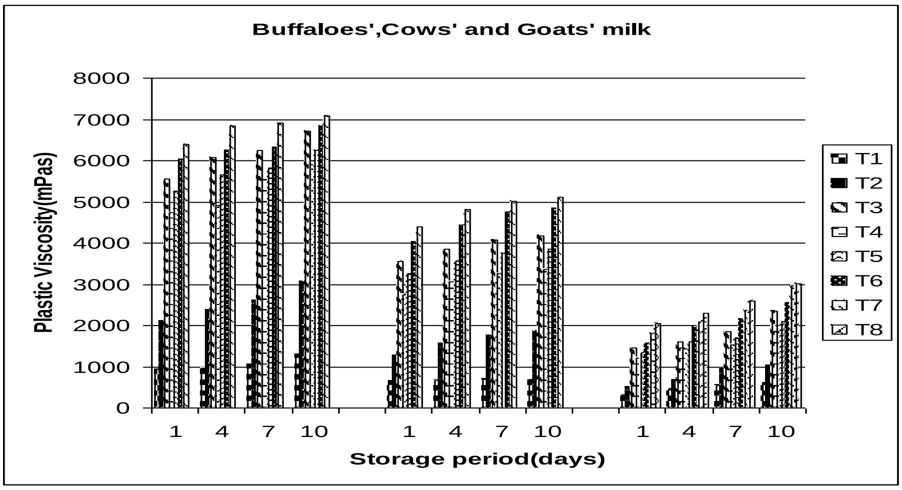

Fig. (8) Plastic viscosity (mPas) of yoghurt made with MTGase during cold storage: (see Fig. 1 for treatments designation).

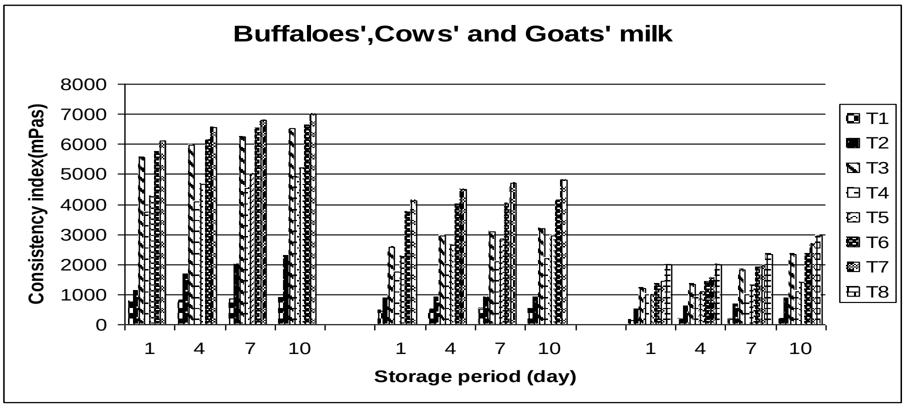

Fig. (9) Consistency index (mPas) from power low model of yoghurt made with MTGase during cold storage: (See Fig. 1 for treatments designation).

It was also noticed that the control (T3) that made with 4% skim milk powder appeared higher plastic viscosity and consistency index than those of low enzyme concentration. However treatments with high enzyme levels produced higher values of plastic viscosity and consistency index. In general, the higher the MTGase doses, the higher the plastic viscosity and consistency index. Plastic viscosity and consistency index results of yoghurt samples made from three types of milk (buffalo, cow and goat) appeared the highest values for yoghurt made from buffaloes' milk than those made from cows' or goats' milk. The explanation of the above behavior can be attributed to the increase in apparent viscosity values of corresponding milk types in the same orders as follows : buffaloes' milk greater than cows' milk and cows' milk greater than goats' milk.

Organoleptic properties:

The average values of the organoleptic assessment of different types of yoghurts in terms of flavour, body, texture and appearance are shown in Tables (3). After one day of storage, yoghurt made from heated buffaloes', cows' and goats' milk (T1) received the lowest score for appearance compared to other treatments. Scores of yoghurt made from three types of milk treated with 0.3 g L^{-1} MTGase resemble scores that obtained from yoghurt made from high heated milk (T2) of buffaloes', cows' and goats' yoghurt. Using 0.4 and 0.5 g L^{-1} MTGase (T6 and T7) for yoghurt made from buffaloes' and cows' milk and using 0.5 and 0.75 g L^{-1} MTGase (T7 and T8) for yoghurt made from goats' milk gave yoghurt with highest score for appearance, compared to other treatments as were revealed a dry, smooth and whiter shining surface. On the other hand, samples made from milk containing 0.2 and 0.3 g MTGase L^{-1} milk (T4 and T5) received in between scores. Milk with 4% skim milk powder (T3) resulted in yoghurt of similar appearance to yoghurt made from 0.4 and 0.5 g MTGase L^{-1} (T6 and T7). The surface fat layer that made naturally was lower in yoghurt made from goats' milk than other types; this is due to the fat globules in goats' milk are smaller than buffalos' and cows' milk. The apparent reason for creaming is the lack of a specific protein (agglutenating euglobulins) which made individual fat globules to cluster and rise. So, this milk naturally homogenized. This protein found only in cows' milk. Yoghurt made from buffaloes' milk had clear and fatty flavour while yoghurt made from goats' milk had intensive flavour and odor. Yoghurt made from cows' milk had less flavour and odor than yoghurt made from goats' milk. It is may be due to a major difference between the milk fat of the goat and the cow is the percentage distribution among specific short chain fatty acids. Goats' milk have an appreciably higher proportion of capric, caprylic and caproic acids. The high amounts of these specific fatty acids (short and medium chain fatty acids) are responsible for the characteristic flavor and odor associated with goat's milk. The flavour scores of yoghurt treatments respectively (T3, T2 and T1) were marked higher scores than other treatments after 1 and 4 days of cold storage for treatments (T1, T2) and after 1, 4 and 7 days of storage for treatment (T3) then scores decreased.

Table (3): Organoleptic properties of yoghurt (from buffaloes', cows' and goats' milk) during storage in cold condition.

Treat.	Flavour (50)			Body & Texture (40)			Appearance (10)			Total (100)		
One day												
	b	c	g	b	c	g	b	c	g	b	c	g
T1	47	45	42	28	26	20	7	6	3	82	77	65
T2	47	45	45	31	28	28	7	7	5	85	80	78
T3	48	47	47	38	38	38	9	9	9	95	94	94
T4	43	40	38	35	30	30	7	7	5	85	77	73
T5	40	40	38	37	34	34	8	7	7	85	81	79
T6	41	40	38	39	38	37	9	9	8	89	87	83
T7	40	40	38	39	39	39	10	10	9	89	89	86
T8	-	-	38	-	-	39	-	-	9	-	-	86
4 days												
T1	47	43	42	28	24	20	7	5	2	82	72	64
T2	47	45	45	31	28	28	7	7	4	85	80	77
T3	48	47	48	38	38	38	9	9	9	95	94	95
T4	43	43	42	36	33	36	8	7	7	87	83	85
T5	43	43	41	37	35	37	9	8	8	89	86	86
T6	42	43	41	39	38	39	9	9	9	90	90	89
T7	42	42	41	40	39	40	10	10	10	90	91	91
T8	-	-	41	-	-	40	-	-	10	-	-	91
7 days												
T1	45	43	39	23	24	17	4	5	2	72	72	58
T2	46	43	42	25	24	24	5	5	4	76	72	70
T3	47	46	46	37	36	36	8	8	8	92	90	90
T4	45	46	44	36	34	35	9	8	8	90	88	87
T5	45	46	44	39	36	36	9	8	9	93	90	89
T6	44	46	44	43	38	40	9	9	9	96	93	93
T7	44	46	44	43	39	40	10	10	9	97	95	93
T8	-	-	44	-	-	40	-	-	9	-	-	93
10 days												
T1	36	35	34	20	20	16	4	3	1	20	58	51
T2	41	40	39	21	21	20	5	5	4	28	66	63
T3	45	45	44	35	33	34	7	8	8	38	86	86
T4	45	46	44	36	33	35	9	8	8	30	87	87
T5	45	46	44	39	35	36	9	8	9	34	89	89
T6	44	46	44	43	38	39	9	9	9	37	93	92
T7	44	46	44	43	39	40	10	10	9	39	95	93
T8	-	-	44	-	-	40	-	-	9	-	-	93

b= buffalo, c= cow, g=goat.

However prolonged storage to 7 and 10 days for (T1, T2) decreased the flavour score. This was due mainly to post acidification of yoghurt. On the other hand, yoghurt made from MTGase received slightly lower scores for flavour in the early storage period and these scores increased then after. The acidity development of yoghurt made from MTGase was slower than yoghurt made without MTGase (Table 3). Yoghurt made from MTGase treated milk were consider to be flat and less intense in yoghurt specific flavour particularly for odour attributes (Lorenzen et al., 2002).

The effect of milk preheating to 72 ^{o}C for 15 s and addition of MTGase were more apparent on body and texture. Thus treatments T3, T6, T7and T8 received higher score for firmness and wheying off (less whey formation).

Furthermore when the level of MTGase added was increased smooth-textured yoghurt were obtained. Yoghurt made from heated milk only received poor scores for body and texture.

From the foregoing, it is recommended to make yoghurt from buffaloes', cows' and goats' milk treated with 0.4, 0.5 g and (0.75 g of goats' milk) MTGase L^{-1} in order to obtain yoghurt of acceptable texture, good keeping quality and less post acidification changes during storage.

Conclusion

Adding microbial transglutaminase to milk (buffaloes', cows' and goats' milk) which are using in the manufacture of yoghurt has a pronounced effect on improving the quality (chemical, rheological and organoleptic properties) of the product. The yoghurt made from goats' milk treated with MTGase was improved in an observed way by such enzyme in their quality. This observed improvement is attributed to enzyme which cross-links milk protein. It is recommended to make yoghurt from buffaloes', cows' and goats' milk heated at 72˚C/ 15s and treated with 0.4, 0.5 and 0.75 g MTGase L^{-1} respectively in order to obtain yoghurt of acceptable texture, good keeping quality and less post acidification changes during storage.

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تأثير إضافة ترانس جلوتامينيز على خواص الألبان المتخمرة

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وجد إنزيم الترانس جلوتامينيز (Transglutaminase) على نطاق واسع في كثير من الكائنات الحية حيث يوجد في الإنسان والحيوان والأسماك وكذلك في بعض النباتات. وأيضا تم تخليقه بواسطة بعض الميكروبات حتى يكون متاح على نطاق تجارى (Microbial Transglutaminase). ولهذا الأنزيم مقدرة على ربط السلاسل البروتينية بعضها البعض عن طريق روابط معينة.

وفى هذه الدراسة تم تتبع و دراسة تأثير إضافة أنزيم الترانس جلوتامينيز الميكروبي MTGase بتركيزات مختلفة إلى زبادي مصنع من ثلاثة أنواع من الألبان (الجاموس والبقر والماعز) على الخواص الريولوجية والكيميائية والحسية.وأوضحت النتائج ما يلي:

١- أدى إضافة أنزيم MTGase بتركيزاته المختلفة إلى حدوث بطيء في تطور الحموضة وانخفاض كمية الشرش المنفصل والأسيتالدهيد مع زيادة ثبات الخثرة واللزوجة الظاهرية وكذلك الخواص الحسية.
٢- أظهر زبادي اللبن الجاموسى أفضل نتائج من حيث ثبات الخثرة وارتفاع قيم اللزوجة الظاهرية و الخواص الحسية وانخفاض كلا من كمية الشرش المنفصل وسرعة تطور الحموضة.
٣- أظهرت نتائج زبادي اللبن البقرى قيم متوسطة للخواص الريولوجية والحسية وأقل قيم للاسيتالدهيد.
٤- أظهر زبادي اللبن الماعزأعلى قيم للأسيتالدهيد و الشرش المنفصل وسرعة تطور الحموضة وأقل قيم لثبات الخثرة و اللزوجة الظاهرية.

المجالات التطبيقية: يمكن الاستفادة من نتائج هذا البحث في تحسين صفات وكمية الزبادي المصنع من الأنواع المختلفة للبن في جمهورية مصر العربية.

الكلمات المرشدة: الترانس جلوتامينيز- زبادي.