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A TRIAL FOR IMPROVING QUALITY OF BIO-YOGHURT USING TRANSGLUTAMINASE

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ABSTRACT

The aim of the present study was to investigate impact of adding transglutaminase (TGase) under different conditions on making bio-yoghurt. The TGase was added before (B) and after (A) heat treatment of milk (92ºC/10min), followed by pre-incubation at 40ºC (B1, A1) or 50ºC (B2, A2) for 1h and inactivation at 80ºC/2min. Bio-yoghurt was prepared by inoculation the milk with yoghurt starter and *B. infantis* (1:1). Chemical properties of the bio-yoghurt were affected by both heat treatment of milk and pre-incubation temperatures of the enzyme. TGase mostly increased values of pH, NPN/TN and TVFA and decreased the acidity values. Viscosity and water holding capacity (WHC) were significantly increased when TGase was added, especially, after heat treatment of milk, followed by pre-incubation at 50ºC/1h (A2). The highest WHC values were correlated with minimum susceptibility to syneresis (STS). The control bio-yoghurt had the lowest WHC and the highest STS.

The maximum *L. delbrueckii subsp. bulgaricus* and *B. infantis* counts were found in fresh and stored bio-yoghurt made with TGase when added after heat treatment of milk with pre-incubation at 50ºC/1h (A2). This increased counts of *B. infantis* throughout storage period and their populations remained above 10^7cfu/g.

The higher scores for all sensorial properties were recorded for bio-yoghurt made with TGase added after heat treatment of milk (A1, A2), while the lowest scores were given for the control. Generally, bio-yoghurt of acceptable quality can be made from heated milk (92ºC/10min) followed by treatment with TGase (0.07%) with activation of the enzyme at 50ºC/1h before fermentation.

Keywords: Transglutaminase, Bifidobacteria, Bio-yoghurt.

INTRODUCTION

The term ‘probiotic’ generally refers to a definition given by Fuller in 1989 that stated probiotic as friendly live microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance. Production and consumption of such probiotic food products including bio-yoghurt have increased dramatically in the past two decades.

Different methods were followed commercially to improve texture of yoghurt. These include addition of stabilizers and various types of milk-derived ingredients, or the use of various types of membrane concentrate or fractions or specific cultures, or via enzymatic cross-linking of milk proteins, using transglutaminase, which catalyses the cross-linking reaction between protein molecules aiming to production of gelled products, with improved quality (Özrenk, 2006).

Transglutaminase (TGase; EC 2.3.2.13) is an enzyme of which its action with proteins was previously explained in the literature to get new functions (Motoki & Seguro, 1998; Özrenk, 2006). Such functions, beside the optimum conditions for activation of the enzyme and their impacts on improving textural properties and biological value of foods and yoghurt were

given by Yokoyama, *et al.,* (2004), Bŏnisch, *et al.* (2007a) and Mosoud, *et al.* (2008). On the other hand, yogurt made from enzyme-treated milk was characterized by reduced syneresis and improved viscosity. Also TGase – cross linked casein resulted in an increased gel firmness, lower permeability and finer protein networks (Faergemand *et al.,* 1999; Özer *et al.* 2007; Shanda, *et al.,* 2008). However, milk products -in general- prepared with m-TGase were shown to display increased gel strength, less syneresis and more creamy consistency (Lorenzen, *et al.* 2002; Gauche, *et al.* 2009).

There are some studies on the effect of different heat treatments of milk before incubation with TGase as well as some other treatments on cross-linking behavior and functional properties and quality of yoghurt (Abou El-Nour, *et al.,* 2004; Rodriguez-Nogales, 2006 and Farrag, *et al.,* 2010).

On the other hand, to achieve therapeutic value of the treated product, it was suggested that bio-yoghurt should be consumed as more than 100 g per day, that containing viable probiotic cells of more than 10^6-10^7cfu/ml (Lourens-Hattingh and Viljoen, 2001). In the present study, *B. infantis* (Bb-02) was used with the traditional yoghurt culture hoping to achieve the optimum conditions for their survival and activity via using TGase.

Thus, the aim of the current study was to investigate the impact transglutaminase (at level 0.07%) on the quality of bio-yoghurt, since TGase was added prior to or after heat treatment of cow's milk with pre-incubation at 40 and 50^{o}C for 1 h before the fermentation process.

MATERIALS AND METHODS

Fresh bulk cow's milk was obtained from the herd of Sakha Experimental Station, Anim. Prod. Res. Inst., Min. of Agric. Biobond TG-YG transglutaminase was a gift from Shanghai Kinry Food Ingredients Co., Ltd, China; the declared activity of the preparation was approximately 100 units/g. Yoghurt starter (YC-380, DVS) consisting of *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii subsp. bulgaricus* (*L. bulgaricus*), as well as *Bifidobacterium infantis,*Bb-02 *(B. infantis*) were obtained from Chr. Hansen's Lab., Denmark.

For the preparation of the fermented milk, cow's milk (3.1%fat; 2.9% protein and 10.9% total solids; Ling, 1963) was divided into 5 portions. First portion was served as control without enzyme. Second and third portions were treated with TGase before (B) the heat treatment of milk with pre-incubation at 40^{o}C (B1) and 50^{o}C (B2), followed by heat treatment at 92^{o}C for 10min. Fourth and fifth portions were treated with TGase after (A) heat treatment at 92^{o}C for 10min with pre-incubation at 40^{o}C (A1) and 50^{o}C (A2) for 1h, followed by inactivation of TGase by heating at 80C^o/2min.TGase was added to milk at level of 0.07% (w/w) according to the recommendation given by the supplier. Yoghurt was manufactured according to the method described by Tamime and Robinson (1999) using yoghurt starter (YC-380, DVS) and *B. infantis* (1:1). The resultant product was stored for 7 days at 5 ±1^{o}C and analyzed at zero time (after overnight cold storage) and after 7days for some chemical, some rheological properties, viability of starter microorganisms and organoleptic properties.

Titratable acidity, pH value, non protein nitrogen (NPN) and total nitrogen (TN) were measured according to Ling (1963), whereas total volatile fatty acid (TVFA) was measured according to Kosikowski (1982), and the result were expressed as ml 0.1N NaOH/100g sample.

Viscosity was measured in triplicates at controlled temperature of 25^{0}C using a digital rotational Brookfield viscometer (Brookfield Engineering Laboratories, Middleboro, USA, Model RV DV– E). The readings were taken per samples at speed 60 rpm with spindle #5 was used for all measurements (Radomir, *et al.,* 2009). Viscometer reading was recorded in centipoises (mPa•s). Water holding capacity (WHC) was determined by the method of Harte *et al.,* (2003). Susceptibility to syneresis (STS) after 2h at 25^{o}C was determined using the drainage method (Hassan *et al.,* 1996).

Enumeration of *S. thermophilus* was carried out using M-17 agar (Merck, Germany) and aerobically incubated at 37°C for 48h (Tharmaraj and Shah, 2003). MRS agar adjusted to pH 5.2 and incubated at 40^{o}C for 72 h was used for the enumeration of *L. delbrueckii ssp. bulgaricus*. The viable numbers of bifidobacteria were enumerated according to the method of Tharmaraji and Shah (2003) using MRS-L (lithium chloride and L-cystein chloride) agar. The inoculated plates were incubated anaerobically at 37^{o}C for 72 h.

The sensory evaluation was assessed by 10 panelists from the staff members of Dairy Department, Animal Production Research Institute using scoring card recommended by El-Shibiny, *et al.* (1979).

Statistical analysis was carried out using SPSS program Inc. software (version 10.0; SPSS Inc., Chicago, IL) and statistically different treatments were determined by the DUNCAN's Multiple Rage tests (SPSS, 1998). All data are presented as average ± standard error

RESULTS AND DISCUSSION

Changes in acidity and pH during cold storage of the resultant bio-yoghurt are shown in Table (1). In both fresh and stored bio-yoghurt, the control samples had significant higher acidity values than those recorded for the treated samples. The differences in acidity due to the applied heat treatment before or after the addition of the enzyme were almost insignificant, whereas the pre-incubation at 50^{o}C decreased acidity of the resultant fresh yoghurt (B2 vs B1) and (A2 vs A1). The pH values of fresh bio-yoghurt made from all treatments were higher when compared with the control bio-yoghurt. The acidity increased while pH values decreased during storage period in all samples (Table 1). Different trends of results were recorded in the literature concerning impact of TGase on the fermentation process and development of acidity and changes of pH during making and storage of yoghurt. Faergemand *et al.,* (1999) and Neve *et al.* (2001) demonstrated that the enzymatic cross-linking step led to a minor imbalance of the associative growth of the yoghurt starter culture. Schey (2003) observed no interference of TGase with starter bacteria during fermentation of yoghurt. However, Özer *et al.* (2007) found that the acidity values of the experimental yoghurt samples were affected (P<0.05) by the TGase treatment. Also, at day 1 of

storage, the highest pH value was obtained with TGase- treated yoghurt, and the effect of the enzymatic treatment on pH was significant, but post acidification developed slowly in all the samples after 7 days of storage, whereas the pH decreased continuously throughout storage period in a similar way for all treated and non-treated samples. We believe that such different trends of data might be due to differences in type of yoghurt milk, different sources of the enzyme used and differences in the processing and sequence of addition, degree of activation and inactivation of the enzyme.

Table (1): Some chemical changes in fresh* and stored bio-yoghurt made without (control) and with TGase added prior to (B1, B2) or after (A1, A2) the heat treatment of milk with different pre-incubation temperatures

Samples	Control	Treatments			
		B1	B2	A1	A2
	Acidity, %				
Fresh yoghurt	0.68 ±0.02^{c}	0.62 ±0.01^{b}	0.61 ±0.00^{b}	0.61 ±0.00^{b}	0.57 ±0.01^{a}
Stored yoghurt	0.76 ±0.02^{c}	0.65 ±0.02^{ab}	0.69 ±0.01^{b}	0.65 ±0.01^{ab}	0.63 ±0.01^{a}
	pH				
Fresh yoghurt	4.47 ±0.02^{a}	4.58 ±0.03^{b}	4.57 ±0.03^{b}	4.62 ±0.02^{b}	4.70 ±0.03^{c}
Stored yoghurt	4.30 ±0.03^{a}	4.33 ±0.03^{a}	4.33 ±0.02^{a}	4.42 ±0.02^{b}	4.44 ±0.01^{b}
	NPN/TN, %				
Fresh yoghurt	6.07 ±0.0.17^{a}	6.74 ±0.4^{ab}	7.78 ±0.52^{bc}	8.58 ±0.33^{cd}	9.67 ±0.31^{d}
Stored yoghurt	7.10 ±0.16^{a}	7.85 ±0.53^{ab}	9.32 ±0.34^{b}	8.68 ±0.33^{b}	11.02 ±0.75^{c}
	Total volatile fatty acids, ml 0.1N NaOH/100g				
Fresh yoghurt	1.27 ±0.07^{a}	1.40 ±0.12^{a}	1.53 ±0.07^{a}	1.40 ±0.20^{a}	1.47 ±0.03^{a}
Stored yoghurt	1.67 ±0.13^{ab}	1.57 ±0.07^{a}	1.87 ±0.13^{b}	1.73 ±0.07^{b}	1.80 ±0.20^{b}

*** After overnight cooling. Data are Means ±SE for three replicates**

$^{a, b, c}$ Means within the same row with unlike superscripts are significantly different (P<0.05).

Treatments code: Control = samples without TGase; B1 and B2= TGase added before heat treatment of milk with pre-incubation at 40ºC (B1) and 50 ºC (B2) for 1h; A1 and A2= TGase added after heat treatment of milk with pre-incubation at 40ºC (A1) and 50 ºC (A2) for 1h.

As shown in Table (1) the averages of non protein nitrogen / total nitrogen (NPN/ TN, %) were 6.74 and 7.78%, respectively, for bio-yoghurt made with TGase added prior to heat treatment of milk with pre-incubation at 40^{o}C and 50^{o}C (B1, B2) and 8.58 and 9.67%, respectively in samples of A1 and A2 bio-yoghurt which was pre-incubated at 40^{o}C (A1) and 50^{o}C (A2) for 1h. Such values were higher than the average value given for the control yoghurt. This was true in case of the fresh and stored product, and could be due to the increased proteolytic activity of the used starter culture in the presence of TGase enzyme. This impact was also noticed during storage of all samples indicating that the proteolytic impact still even at low storage temperature. On the other hand, the role of the used enzyme in this respect was more pronounced since the values of NPN/ TN were always higher when the enzyme was added after the heat treatment applied. The results of proteolysis given by Yüksel and Erden (2010) and expressed as tyrosine content and areas of peptide peaks from RP-HPLC chromatograms showed

that proteolytic activity significantly decreased when TGase was applied in making yoghurt, and the prementioned indices values increased during storage period of 30days.

Changes in total volatile fatty acids (TVFA) content in the bio-yoghurt made with TGase added prior to (B) and after (A) the heat treatment of milk and then pre-incubated at 40ºC(B1, A1) and 50ºC (B2, A2) are shown in Table (1). The TVFA content of fresh bio-yoghurt seems to be not significantly affected by treatment with TGase. The data showed pronounced increase in TVFA in all bio-yoghurt samples during storage. However, the increase of TVFA might be a result of the lipolytic impact of the starter culture added which was continued during storage. The role of bifidobacteria in this respect is unique since it was reported that it produces 1.5 mole of acetic acid as well as 1.0 mole of lactic acid as the end product of the fermentation process of 1 mole of glucose (Tamime, *et al.,* 1995).

Viscosity is an important property of yoghurt that affects the mouth fell and texture. Changes of viscosity of bio-yoghurt samples are shown in Figure (1).

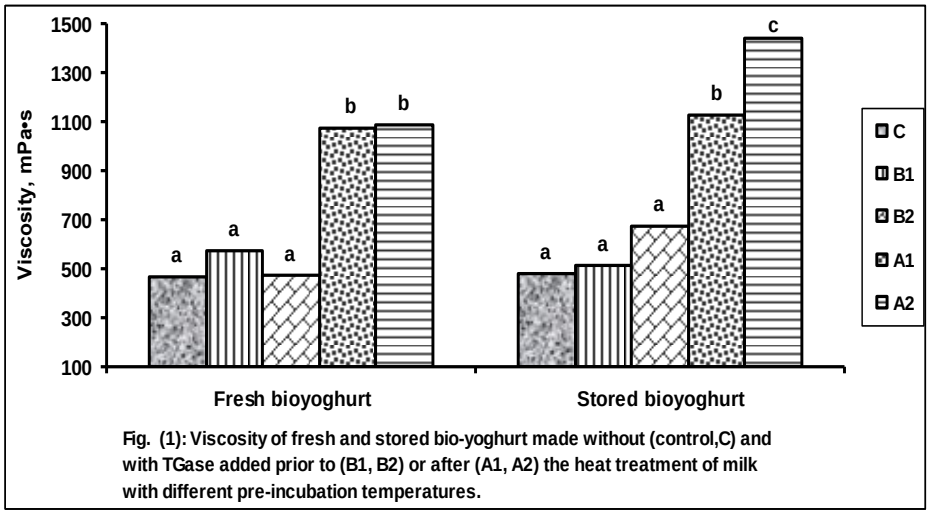

Fig. (1): Viscosity of fresh and stored bio-yoghurt made without (control,C) and with TGase added prior to (B1, B2) or after (A1, A2) the heat treatment of milk with different pre-incubation temperatures.

The apparent viscosity of TGase treated bio-yoghurt was significantly higher than that of the control. This agrees with the results given by Abou El-Nour, *et al.,* (2004) and Özer, *et al.* (2007), who found that TGase treated yoghurt samples had significantly higher viscosity values than the untreated yoghurt and this could be attributed to the basic function of TGase is to cross-link milk proteins covalently causing a finer and stronger gel network in the yoghurt, compared with acid-induced gels. Suitability of caseins to be good substances for TGase was reported by Özrenk (2006), who explained that this is probably due to their low degree of tertiary structure, flexible, random-coil arrangement and the absence of any disulphide bonds in the α_{s1}- and β-casein leaving the active groups exposed to the enzyme. The present results showed also that bio-yoghurt produced with adding TGase after heat treatment had a significantly higher viscosity than that made with adding

TGase before heat treatment of milk. However, treated of heated milk with TGase with pre-incubation at 50ºC for 1h increased significantly viscosity of the resultant bio-yoghurt samples. The effect of heat treatment is related to protein interactions as well as to the cross-linking reaction of TGase. The heat treatment of the yoghurt milk causes denaturation of whey proteins and interactions of them with the casein micelles surface, and therefore leads to an integration of denatured whey proteins into the gel network, thus improving the gel firmness of yoghurt (Lucey, *et al.,* 1998). Denaturation of whey proteins, however, also enhances their susceptibility towards cross-linking by TG (Bŏnisch, *et al.* (2007a,b).

After 7 days of storage, the bio-yoghurt gel made with TGase added after heat treatment of milk (A1, A2) was characterized by higher viscosity than the control product. Similar trend of results was given by Özer, *et al.* (2007), who mentioned that minor increases in the viscosity values of TGase treated and untreated yoghurt samples were noted during cold storage with slightly more pronounced in case of treatment with higher dose of the enzyme. This effect was assumed to be resulted from the enzyme activity not being constant during the gelation process, but gradually decreased with time as the enzyme becomes trapped within the formed network (Özer, *et al.* 2007). However, the differences in pH values may led also to differences in viscosity values of yoghurt examined. Our results are also in agreement with the results given by Lorenzen *et al.* (2002), Farnsworth *et al.* (2006) and Iličić, *et al.,* (2008), who used TGase in making yoghurt from different types of milk.

Water holding capacity values of (B1, B2) or (A1, A2) samples found to be significantly more than that of control (Figure 2). Also, that occurrence of activation at 50ºC (A2) showed more different variation for fresh bio-yoghurt in WHC values than the other samples (Figure 2). At the end of storage period, WHC of all samples decreased. It was assumed -in general- that modifications of milk proteins (especially caseins and denatured whey proteins) via cross- linking might be lead to increase of WHC. According to Lorenzen *et al*. (2002) and Farnsworth *et al.* (2006), gels formed with cross-linking agent ε-(γ-glutaminyl) Lysine exhibited a better water retention capacity. The set-type yoghurt made from TGase- treated milk had a greater capacity for holding water, and whey separation was prevented (Yüksel and Erden, 2010). This confirmed our results. Bio-yoghurt made from heated milk treated by TGase with pre-incubation at 50ºC (A2) markedly showed higher WHC compared to control yoghurt.

This agrees with the results given by Milanovic, *et al.* (2007) and Farrag, *et al.,* (2010). The decrease in gel permeability causes a more compact entrapped in the yoghurt gel network (Moon and Hong, 2003). Figure (3) shows that susceptibility to syneresis (STS) of bio-yoghurt samples treated with TGase was significantly less compared to the untreated samples. Less STS (%) was recorded for bio-yoghurt made with TGase added after heat treatment of milk with pre-incubation at 50ºC (A2). STS values showed decreased rate with advancing storage period. TGase addition contributed to a decrease in syneresis in all samples (B1, B2, A1 and A2). This agrees with the trend recorded in the literature. The cross linking of protein chains can stabilize the three-dimensional network of yoghurt gel and prevent yoghurt

whey expulsion as a result of a decrease in gel porosity; thus reducing syneresis (Lorenzen, *et al.,* 2002; Abou El-Nour, *et al.,* 2004; Farnsworth, *et al.,* 2006 and Gauche, *et al.,* 2009). The set-yoghurt made from TGase treated milk can overcome the problem of serum separation upon change of temperature or physical impact (Farrag *et al.,* 2010). On the other hand, reduction of syneresis may be caused by effect of TGase on the pore size of milk gels. As pore size reduces, the protein network will result in less syneresis (Lorenzen, *et al.,* 2002).

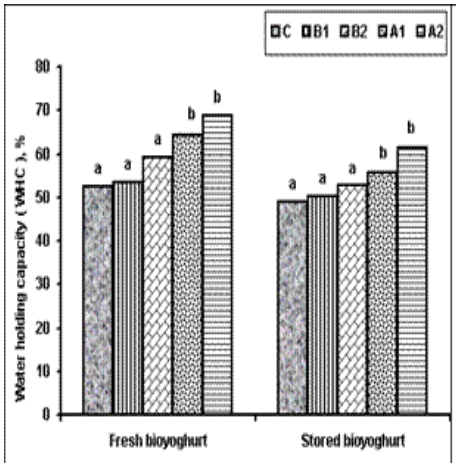

Fig.(2): Water holding capacity for fresh and stored bioyoghurt made without (control, C) and with TGase added before (B1, B2) and after(A1, A2) the heat treatment of milk with different pre-incubation temperatures.

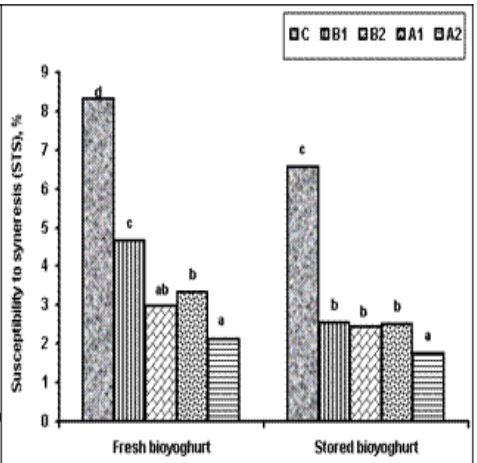

Fig. (3): Susceptibility to syneresis (STS) for fresh and stored bioyoghurt made without (control, C) and with TGase added before (B1, B2) and after(A1, A2) the heat treatment of milk with different pre-incubation temperatures.

The changes in the counts of *S. thermophilus, L. bulgaricus and B. infantis* in the control and treated bio-yoghurt are presented in Table (2). All bio-yoghurt samples contained high numbers (log cfu/g) of *S. thermophilus* (Table 2) when fresh (10.36-10.69) that decreased (9.67-9.12) at the end of storage period. No significant differences were found between the TGase treated bio-yoghurt and the control samples. It was reported that using cross-linking enzyme in bio-yoghurt production had no promoting effect on growth and viability of *S. thermophilus* (Neve, *et al.* 2001).

The counts (log cfu/g) of *L. bulgaricus* in fresh treated bio-yoghurt (A1, A2) were significantly higher (P<0.05) (9.02-10.04) than those in the control (8.59). Pre-incubation at 50^{o}C /1h (A2) of TGase added after heat treatment of milk promoted effectively higher bacterial growth and viability compared to the control or the other treatments. However, *L.bulgaricus* showed a more marked decrease than *S. thermophilus* during storage (Table 2). In this respect, Neve, *et al.* (2001) found that the preliminary incubation of cow's milk with m-TGase resulted mainly in disturbance of yoghurt culture growth during fermentation process, whereas the addition of enzyme and simultaneous fermentation without enzyme inactivation stabilized the growth of *L. bulgaricus*.

Table (2): Counts of starter culture *(log cfu/g)* of fresh and stored bio-yoghurt made with out (control) and with TGase added prior to (B1, B2) or after (A1, A2) the heat treatment of milk with different pre-incubation temperatures

Samples	Control	Treatments			
		B1	B2	A1	A2
	S. thermophilus				
Fresh yoghurt	10.67 ±0.25 [a]	10.54 ±0.51[a]	10.53±0.42 [a]	10.36±0.19 [a]	10.69 ±0.18 [a]
Stored yoghurt	9.76 ±0.38 [a]	9.15 ±0.17 [a]	9.44 ±0.27 [a]	9.67 ±0.26 [a]	9.12 ±0.16 [a]
	L. delbruckii ssp. bulgaricus				
Fresh yoghurt	8.59 ±0.28[a]	8.74 ±0.19[a]	9.36 ±0.22[b]	9.02 ±0.24[ab]	10.04 ±0.16[c]
Stored yoghurt	7.38 ±0.32[a]	8.13 ±0.19[a]	8.96 ±0.18[b]	9.08 ±0.21[ab]	9.58 ±0.17[b]
	B. infantis				
Fresh yoghurt	7.56 ±0.14[a]	8.37 ±0.25[b]	8.85 ±0.18[bc]	8.87 ±0.14[bc]	9.28 ±0.12[c]
Stored yoghurt	6.23 ±0.16[a]	7.60 ±0.50[a]	8.57 ±0.26[b]	8.54 ±0.23[b]	9.10 ±0.17[b]

See legend to Table (1) for details.

The count (log cfu/g) and viability of *B. infantis* in the bio-yoghurt samples are shown also in Table (2). The fresh TGase treated bio-yoghurt made with TGase addition after heat treatment of milk (A1, A2) had significantly higher numbers (p<0.05) of *B. infantis* (8.87-9.28) than the control (7.56). However, the pre-incubation temperature (40 or 50^{o}C) had significant effect on the growth and viability of *B. infantis*. The log number of *B. infantis* decreased slightly in all samples during storage. The enhanced growth and viability of bifidobacteria in the present study probably due to protective role of cross-linking microcapsules which allows bidirectional diffusion meaning entrance of the nutrients essential for the cell growth and exit of the produced metabolites (e.g. lactic acid) into the environment (Heidebach, *et al.,* 2009). Also, *L. bulgaricus* are proteolytic in nature, causing liberation of free amino acids which act as growth promoters for the weakly proteolytic bifidobacteria. Pavunc, *et al.* (2010) found that transglutaminase did not influence the probiotic cell viability. This could be due to inactivation of the enzyme by pasteurization before addition of probiotic bacteria. On the contrary, Neve *et al.* (2001) reported decline in the growth rate of yoghurt cultures in samples where TGase was used concomitantly with cultures. Farnsworth *et al.* (2006) reported no significant differences in the rate of probiotic cultures count (*L.acidophilus, Bifidobacteria ssp and L. casei*) between control and TGase treated samples.

The average results of the organoleptic assessment of all bio-yoghurt samples are shown in Table (3). Bio-yoghurt made without TGase (Control) received the lowest scores for all the organoleptic properties, compared to the other treatments. Addition of TGase after heat treatment of milk (A1, A2) resulted in bio-yoghurt with more firmness and smoothness as compared to bio-yoghurt made with TGase added before heat treatment of milk (B1, B2). Using pre-incubation at 50^{o}C/1h for TGase addition after heat treatment of milk (A2) gave a fresh and stored bio-yoghurt with highest scores for smoothness, wheying-off compared to other treatments. The scores given for A1 and A2 treatments were the highest, followed by those of B1 and B2

treatments, whereas the control yoghurt ranked the lowest scores in this respect. The results showed also that the TGase treatment with pre-incubation at 50°C had a significant effect on the overall acceptability of the fresh and stored bio-yoghurt, however different of pre-incubation temperature of TGase treated milk had a little effect on the organoleptic properties. Farrag *et al.* (2010) found that yoghurt from TGase treated milks was firmer with more grainy and creamy texture than yoghurt samples from untreated milk.

Table (3): Organoleptic properties of fresh and stored bio-yoghurt made without (control) and with TGase added prior to (B1, B2) or after (A1, A2) the heat treatment of milk with different pre-incubation temperatures

Property	Control	Treatments			
		B1	B2	A1	A2
Fresh yoghurt					
Appearance (10)	8.33 ±0.42^{a}	8.33 ±0.21^{a}	8.83 ±0.17^{ab}	9.50 ±0.22^{b}	9.00 ±0.37^{ab}
Firmness (10)	7.50 ±0.22^{a}	8.50 ±0.56^{ab}	8.67 ±0.49^{ab}	9.50 ±0.34^{b}	9.00 ±0.37^{b}
Smoothness (10)	7.33 ±0.33^{a}	8.00 ±0.58^{ab}	8.33 ±0.42^{ab}	8.83 ±0.40^{ab}	9.17 ±0.17^{b}
Wheying- off (10)	7.83 ±0.31^{a}	8.00 ±0.26^{a}	8.50 ±0.22^{a}	8.67 ±0.33^{ab}	9.33 ±0.21^{b}
Flavour (60)	52.00±0.37^{a}	54.17±0.98^{ab}	54.33 ±1.84^{ab}	56.17±0.54^{bc}	57.67 ±0.61^{c}
Total (100)	83.00±0.97^{a}	87.00 ±1.18^{b}	88.67 ±1.87^{b}	92.67 ±0.71^{c}	94.17 ±0.54^{c}
Stored yoghurt					
Appearance (10)	4.00 ±0.63^{a}	7.00 ±0.58^{b}	7.83 ±0.31^{b}	8.00 ±0.26^{bc}	9.17 ±0.17^{c}
Firmness (10)	3.60 ±0.40^{a}	6.50 ±0.22^{b}	7.50 ±0.34^{c}	7.83 ±0.17^{c}	9.17 ±0.17^{d}
Smoothness (10)	5.00 ±0.55^{a}	6.50 ±0.43^{bc}	7.33 ±0.33^{b}	8.33 ±0.42^{cd}	9.17 ±0.17^{d}
Wheying- off (10)	3.60 ±0.40^{a}	5.50 ±0.22^{b}	6.83 ±0.31^{c}	7.33 ±0.42^{c}	8.67 ±0.21^{d}
Flavour (60)	43.00±0.55^{a}	42.17 ±0.98^{a}	46.50 ±0.72^{b}	49.33 ±1.15^{c}	54.17 ±0.31^{d}
Total (100)	59.20±1.07^{a}	67.67 ±1.26^{b}	76.00 ±0.73^{c}	80.83 ±1.38^{d}	90.3 ±0.67^{e}

See legend to Table (1) for details.

Generally, TGase addition after heat treatment of milk in the bio-yoghurt production significantly improved the organoleptic properties of final production. Our results are in agreement with the results reported by Lorenzen *et al.* (2002), Milanovic *et al.* (2007) and Farrag *et al.* (2010).

In conclusion, addition of TGase after heat treatment of milk (92°C/10min) with pre-incubation at 50°C for 1h in the production of bio-yoghurt caused a significant decrease of syneresis and improved water holding capacity and viscosity which reflected on improving the sensory characteristics of the final product.

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REFERENCES

Abou El-Nour, A. M.; El-Kholy, A. M. and Abd El-Salam, M.H. (2004). Rheological properties of cow's milk yoghurt treated by transglutaminase (TGase). Egyptian J. Dairy Sci., 32: 73.

Bönisch, M. P.; Huss M.; Lauber, S. and Kulozik, U. (2007a). Yoghurt gel formation by means of enzymatic protein cross-linking during microbial fermentation. Food Hydrocoll., 21:585.

Bönisch, M. P.; Huss, M.; Weilt, K. and Kulozik, U. (2007b). Transglutaminase cross-linking of milk proteins and impact on yoghurt gel properties. Int. Dairy J., 17: 1360.

El-Shibiny, S.; El-Dien, H. F. and Hofi, A. A. (1979). Effect of storage on the chemical composition of zabadi. Egyptian J. Dairy Sci. 7: 1.

Faergemand, M.; Sorensen, M. V.; Jorgensen, U.; Budolfsen, G. and Qvist, K. B.(1999). Transglutaminase: Effect on instrumental and sensory texture of set style yoghurt. Milchwissenschaft, 54: 563.

Farnsworth, J.; Lia, P J; Hendricks,G.M. and Guo, M.R. (2006). Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. Small Ruminant Res., 65:113.

Farrag, N. S; Hussein, A. M; Gaafar, A. M and Haggag, H. F. (2010). Effects of enzymatic cross-linking of buffaloe's milk proteins by transglutaminase on functional properties of set-style yoghurt. Proc. 11th Egyptian Conf. Dairy Sci. & Techn. 423.

Fuller, R. (1989). Probiotics in man and animals. J Appl. Bacteriol. 66:365.

Gauche, C.; Tomazi, T.; Barreto, P.L.M.; Ogliari, P.J. and Bordignon-Luiz,M.T. (2009). Physical properties of yoghurt manufactured with milk whey and transglutaminase. LWT - Food Technol., 42: 239.

Harte, F.; Luedecke, L.;Swanson, B. and Barbosa-Canovas, G. V.(2003).Low-fat set yoghurt made from milk subjected to combinations of high hydrostatic pressure and thermal processing. J. Dairy Sci., 86: 1074.

Hassan, A. N.; Frank, J. F. and Schmidt, K. A.(1996). Textural properties of yoghurt made with encapsulated non-ropy lactic cultures. J. Dairy Sci., 79: 2098.

Heidebach, T.; Fŏrst, P. and Kulozik, U.(2009). Transglutaminase-induced caseinate gelation for the microencapsulation of probiotic cells. Int. Dairy J., 19: 77.

Iličić, M. D.; Carić, M. Đ.; Milanović, S. D; Dokić,L. P.; Đurić,M.S.; Bošnjak, G. S. and Duraković, K G. (2008). Viscosity changes of probiotic yoghurt with transglutaminase during storage. APTEFF, 39, 11.

Kosikowski, F.V. (1982). Cheese and Fermented Milk Food. 2nd Ed. Published and Distributed by the author and Associates, New York.

Ling, E.R. (1963). A Text Book of Dairy Chemistry. 3rd Ed. Vol. 2. Practical, Chapman and Hall, Ltd. London.

Lorenzen, P C.; Neve, H.; Mauther, A. and Schlimme, E.(2002). Effect of enzymatic cross-linking of milk proteins on functional properties of set-style yoghurt. Int. J. Dairy Technology, 55: 152.

Lourens-Hattingh, A., and Viljoen, B.C. (2001). Yogurt as probiotic carrier food. Int. Dairy J., 11: 1.

Lucey, J. A., Tamehana, M., Singh, H. and Munro, P. A.(1998). Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological properties of acid skim milk gels. J. Dairy Res., 65: 555.

Masoud, M. S.; Youssef, M, S.; Hussein G. A. M. and Fayed, A. E. (2008). Effect of cow's milk treatment with transglutaminase on the composition and quality of yoghurt with particular reference to its biological value. Annals Agric. Sci. Ain Shams Univ., 53:221.

Milanović, S.; Carić, M.; Đurić, M.; Iličić, M. and Duraković, K. (2007). Physico-chemical properties of probiotic yoghurt produced with transglutaminase. Acta Periodica Technol., 38 :45.

Moon, J.H. and Hong, Y. H. (2003). Electron microscopic property of transglutaminase added milk. Korean J. Food Sci. Animal Resources, 23: 350.

Motoki M, and Seguro K (1998) Transglutaminase and its use for food processing. Trends Food Sci Technol., 9:204

Neve,H.; Lorenzen, P. Chr.; Mautner, A.; Schlimme, E. and Heller, K. (2001). Effects of transglutaminase treatment on the production of set skim milk yoghurt: Microbiological aspects. Kieler Milchwirtschaftliche Forschungsberichte, 53: 347.

Őzer, B.; Kirmaci, H. A; Oztekin, S.; Hayaloglu, A.and Atamer, M.(2007). Incorporation of microbial transglutaminase into non-fat yoghurt production. Int. Dairy J., 17: 199.

Özrenk, E. (2006). The use of transglutaminase in dairy products. Int. J. Dairy Technol.,1: 1.

Pavunc, A.L.; Beganović,J.; Kos, B.; Buneta, A.; Beluhan, S. and Šušković, J. (2010). Influence of microencapsulation and transglutaminase on viability of probiotic strain *Lactobacillus helveticus* M_{92} and consistency of set yoghurt. Int. J. Dairy Technol.,63: 1.

Radomir, V.M; Spasenija, D.M; Eva S.L.; Mirjana, S.D; Marijana, D.C; Mirela D.I and Ljiljana, K.(2009) Milk-based beverages obtained by kombucha application. Food Chem. 112:178.

Rodriguez-Nogales, J. M. (2006). Enhancement of transglutaminase induced protein cross- linking by preheat treatment of cow's milk: A statistical approach. Int. Dairy J., 16: 26.

Schey, A. (2003). Texture improvement of fermented dairy products by enzymatic cross-linking with transglutaminase. IDF seminar on aroma and texture of fermented milk. Denmark, June (2003), pp 371-375. CF. Őzer et al., (2007)

Shanda, P.J.; Ya, H.; Pietrasik, Z. and Wanasundar, P.K.J.P.D. (2008). Transglutaminase treatment of pea proteins: Effect on physicochemical and rheological properties of heat-induced protein gels. Food Chemistry, 107: 692.

SPSS. (1998). Statistical Package for Social Science. SPSS Inc., Illions, USA.

Tamime, A. Y. and Robinson, R. K. (1999). Yoghurt: Science and Technology. London, UK: Woodhead Publishing.

Tamime, A. Y.; Marshall, V. M. and Robinson, R. K.(1995).Microbiological and technology aspects of milks fermented by bifidobacteria. J. Dairy Res., 62: 151.

Tharmaraj, N., and Shah, N.P. (2003). Selective enumeration of *Lactobacillus delbrueckii ssp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, bifidobacteria, *Lactobacillus casei*, *Lactobacillus rhamnosus* and propionibacteria. J. Dairy Sci., 86: 2288.

Yokoyama, K.; Nio, N. and Kikuchi, Y. (2004). Properties and applications of microbial transglutaminase. Appl. Microbiol. Biotechnol. 64: 447.

Yúksel, Z. and Erden, UK. (2010). The influence of transglutaminase treatment on functional properties of set yoghurt. Int. J. Dairy Technol., 63: 86.

استخدام انزيم الترانس جلوتامينيز فى محاولة لتحسين جودة اليوجورت الداعم للحيوية

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أثبتت الابحاث الحديثة ان البكتيريا الداعمة للحيوية لها فوائد صحية متعددة وهذة البكتيريا تتأثر بعدة عوامل لذلك يهدف هذا البحث الى تصنيع يوجورت داعم للحيوية من اللبن البقرى بإضافة أنزيم الترانس جلوتامينيز لتحسين جودة المنتج و تهيئة الظروف المثلى للمحافظة على بقاء اعداد البكتريا الداعمة للحيوية التى تعطى التأثير الصحى للمستهلك وقد استخدام الانزيم بنسبة ٠,٠٧% من وزن اللبن وتم ذلك بإجراء خمس معاملات كالاتى: أضافة الانزيم الى اللبن البقرى قبل المعاملة الحرارية (ب) أو بعد المعاملة الحرارية (أ) على ٩٢ °م / ١٠ق ثم التحضين الاولى لكل منهما على ٤٠ °م (ب١،أ١) أو ٥٠ °م (ب٢ ،أ٢) لمدة ساعة قبل اضافة البادئ و الذى اشتمل على بادئ اليوجورت التقليدى (*S. thermophilus* and *L. delbrueckii subsp. bulgaricu*) مضافاً له البكتريا الداعمة للحيوية *B. infantis* بنسبة (١:١) اما الكنترول فكان بدون اضافة انزيم. تم تقييم جميع المعاملات السابقة من حيث بعض الخواص الكيماوية والريولوجية و الحسية وكذلك حيوية البادئ المستخدم لليوجورت الطازج والمخزن لمدة اسبوع .

أوضحت النتائج المتحصل عليها ما يلى: أدت المعاملة بالانزيم الى تناقص الحموضة و زيادة ارقام pH مع زيادة التحلل البروتينى و التحلل الدهنى وتحسن القدرة على الاحتفاظ بالماء (Water Holding Capacity) و صاحب ذلك تناقص انفصال الشرش وزيادة اللزوجة ، يبدو هذا التأثير واضح معنوياً عند المقارنة بالمنتج للمعاملة (أ٢) حيث يضاف الانزيم الى اللبن بعد المعامل الحرارية (٩٢ °م/ ١٠ق) و التحضين الاولى على ٥٠ °م/ساعة. أما بالنسبة لحيوية و اعداد *B. infantis* , *L. bulgaricus* فكانت تزيد فى اليوجورت الطازج و المخزن بإضافة الانزيم و التحضين الاولى على ٥٠ °م (ب٢ ، أ٢) لمدة ساعة مقارنة بالكنترول (بدون أنزيم) لكن انخفضت الاعداد اللوغارتمية قليلا فى اليوجورت الحيوى المخزن الا انها احتفظت بالمستوى الحيوى المؤثر صحيا للعائل (١٠ ٧). أظهرت نتائج التحكيم الحسى للمنتج ان اضافة انزيم الترانس جلوتامينيز للبن المعامل حراريا حسنت القبول لدى المحكمين وذلك من خلال التغلب على مشاكل تصنيع اليوجورت من اللبن البقرى و الراجعة الى انخفاض الجوامد الصلبة الكلية وانفصال الشرش خلال التخزين و التداول.

هذا وفى ضوء النتائج المتحصل عليها ينصح عند تصنيع اليوجورت الداعم للحيوية والجيد الصفات من اللبن البقرى استخدام انزيم الترانس جلوتامينز بنسبة ٠,٠٧% من وزن اللبن المستخدم ويتم ذلك باضافة الانزيم بعد المعاملة الحراريه (٩٢ °م/ ١٠ق) والتى تجرى على اللبن ويجب ان يتبع ذلك فترة تحضين على ٥٠ °م/ساعة لتنشيط الانزيم وذلك قبل اضافة البادئ.

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