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Physicochemical Properties of Set-Style Yoghurt as Effect by Microbial Transglutaminase and Milk Solids Contents

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ABSTRACT

The functionality and structure of proteins can be modified with physical, chemical and enzymatic procedures. Enzymatic modification has been recommended as a useful technique owing to high specificity of enzymatic reactions and therefore a little risk of formation of toxic products. In the present study, the effect of addition of microbial transglutaminase (MTGase) at the levels of 0.01, 0.02, 0.03% and milk solid non-fat (MSNF) at the levels of 8 and 9% on physicochemical properties of set yoghurt during 21 days of storage was investigated. The yoghurt sample without enzymatic treatment was considered as the control. Results showed that all physicochemical parameters of the yoghurt samples were affected by the MTGase enzyme, MSNF content and storage time and the differences between the samples were statistically significant ($p<0.05$). While the addition of MTGase showed minor effect on the pH of yoghurt, it significantly decreased the acidity in the enzyme-treated yoghurts. The MTGase-treated samples had significantly lower syneresis and higher viscosity values than the untreated yoghurt/control. Results also revealed that as MSNF content of yoghurts was increased from 8 to 9%, the acidity and viscosity was increased and pH and syneresis was decreased noticeably. The highest syneresis was recorded for control as 6.02 g/100 and the lowest for yoghurts treated with 0.03% MTGase as 3.85 g/100. The highest viscosity was recorded for yoghurts treated with 0.03% MTGase as 20.96 Pa.s., and the lowest value for control as 15.37 Pa.s. The extent of syneresis in yoghurt samples was increased markedly during storage period and the initial mean value of 3.84 g/100 at the first day of storage reached to 5.75 g/100 at the end of storage. The mean value of viscosity at the first day of storage was recorded as 17.49 Pa.s. which reached to 19.98 Pa.s. after 21 days of storage.

KEYWORDS: Physical property, acidity, syneresis, viscosity, storage period.

INTRODUCTION

Yoghurt is a common fermented dairy product wildly produced and consumed in Iran. Yoghurt is defined as a milk product fermented with thermophilic lactic bacteria, usually Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus [1-3]. The most common type of yoghurt mass-produced in Iran is a set type yoghurt fermented in retail containers and no extra stirring or water elimination occur after the fermentation process. Rheological properties are important for foods, such as fermented dairy products, in the design of flow processes, quality control, storage and processing and in predicting the texture of foods [4, 5]. Viscosity and syneresis are two critical factors effecting appearance and texture of the product. These parameters may significantly influenced by the addition of hydrocolloids, milk composition (amount and type of milk ingredients) and processing treatments (heat treatment, homogenization, enzymatic treatment, etc.).

Microbial transglutaminase (MTGase, E.C. 2.3.2.13), catalyze acyl transfer reactions between c-carboxyamide group of peptide bound glutamine residues (acyl donors) and diversity of primary amines (acyl acceptors), comprising the ε-amino group of lysine residues to form a ε-(c-glutamyl) lysine isopeptide bonds [6]. These reactions create new covalent intra- and intermolecular bonds [6-9] and can modify the structure and functionality of proteins [10].
Transglutaminase (TG) are widely distributed in animal tissues and body fluids [11], plants [12, 13], fish and birds [14] and microorganisms [7, 15-17]. Microbial TGases are the most widely used for food modification due to lower costs and high yields involved with their extraction and purification when compared to mammal sources [18]. These enzymes are generally recognized as safe (GRAS) in the food industry [19]. Interdisciplinary efforts have been aimed at producing enzymes synthesised by microorganisms which may have a wider scope of use [20]. Extracellulr MTGases are purified from cultural filtrate of *Streptoverticillium mobarens* [21], *Streptoverticillium ladakanum* [22] and

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Streptoverticillium lydicus [23]. However, some studies have been carried out to produce a recombinant transglutaminase from the *E. coli* strain [24].
Inclusive researches were conducted to study the MTGase catalyzed reactions and their impact on food products. The MTGase treatment was used mostly in case of yoghurt production, indicating the excellent cross-linking properties of casein that result in the increase of gel strength and the decrease of syneresis phenomena [25, 26]. Indeed, the most advanced area of dairy product processing using MTGase is yoghurt manufacturing [27]. MTGase could be incorporated during the fermentation process to reduce the manufacturing time. The enzymatic cross-linking reactions could be prevented by the acidification induced by lactic bacteria and no heating is required to enzyme inactivation [28]. Nowadays, milk' and milk protein powders progressively become more expensive. On the other hand the utilization of stabilizing agents/hydrocolloids in yoghurt in many countries such as Iran are forbidden. Therefore, in the present work, we primarily aimed to investigate the influence of MTGase concentration and total milk solids content on physicochemical of the yoghurt during 21 days of storage.

2. MATERIALS AND METHODS

2.1. Materials

Raw milk of high microbial quality (pH 6.68 and acidity 0.14 g% lactic acid) was supplied from Pegah Dairy Company (Shush, Khuzestan, Iran). A Ca^{2+}-independent MTGase from *Streptoverticillium mobaraense* (ACTIVA YG) was used. The enzyme (declared activity of 100 U/g) was procured from Ajinomoto Paris, France. The composition of enzyme powder was consist of transglutaminase enzyme, lactose, yeast extract, maltodextrin and vegetable oil. Starter culture was a blend of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* (1:1 ratio, code: Express, from Chr.Hansen Company, Denmark. The skim milk powder for adjusting yoghurt total solids content was produced by Pegah factory. All chemicals used were purchased in analytical and purified grade from Sigma Chemical Co. (St. Louis, MO) and Merck (Darmstadt, Germany).

2.2.Methods

2.2.1. Yoghurt production

The set yoghurt samples were produced according to procedure used by Iran Dairy Industry Inc., Pegah Co (Khuzestan, Iran). The standardized and pasteurized milk with 1.5% fat and 8% milk solid non-fat (MSNF) was warmed to 65˚C and homogenized at pressure of 180 bar. In samples containing 9% MSNF, one per cent skim milk powder was added to the milk before homogenization. After heat processing at 90˚C for 10 min, the milk was cooled down to 43˚C and MTGase at the levels 0.01, 0.02 and 0.03% was added. The yoghurt without MTGase considered as a control. After inoculation with 2% (w/w) mixed culture of lactic acid bacteria, the milk was poured into 100 mL polystyrene plastic containers, and incubated at 43 ˚C. The extent of the inoculum used for yoghurt preparation was according to producer recommendations. The acidification progress of the milk was checked by inspection the pH during the entire incubation period. The fermentation was considered accomplished when the pH of 4.6 was reached for the control samples. The yoghurts were afterwards cooled and stored at 4 ˚C for 21 days. Experimental yoghurts were made in three trials on separate days.

2.2.2. Physicho-chemical analysis

Changes in various parameters including pH, titrable acidity, syneresis and viscosity was studied after one day of yoghurt production and after 11 and 21 days of storage. The samples were analyzed in duplicate for mentioned analysis and a mean value was considered. Total solids and acidity were determined according to Iranian Standards Institution Methods [29, 30]. Total solids was measured by oven drying method at 102 ± 2 °C after 2 h. Acidity was determined by titrating with 0.1 N NaOH using phenolphthalein as indicator. Total acidity expressed in Dornic degrees (˚D) which was equal to per cent of lactic acid. The pH of the yoghurt was determined using a digital pH meter (Metrohm Company, Model 827, Switzerland). The milk fat content was determined by Gerber method according to Kleyn et al. [31].

The extent of syneresis of yoghurts was assessed according to the procedure proposed by Hassan et al. [32]. A 100 mL sample of each yoghurt was drained through a 120-mesh stainless screen found on the top of a funnel, which was led in a graduated cylinder to collect the liquid. The liquid extent (mL) per 100 mL of sample was taken as an index of syneresis after 2 h of draining at 6 ± 1 °C.

Viscosity was measured according to Gauche et al. [33] with a Brookfield rotational viscometer, model DV-III ULTRA (Brookfield Engineering Laboratories Inc., 11 Commerce Boulevard, Middleboro, MA 02346, USA). A ULA spindle at 40 rpm was used in the determination of viscosity. Prior to viscosity measurement, the yoghurt was gently

stirred by making five up and down movements of a spoon in the yoghurt cup to ensure homogeneity, as reported by [34]. All viscosity readings were taken at 4°C and readings were recorded after 20 s.

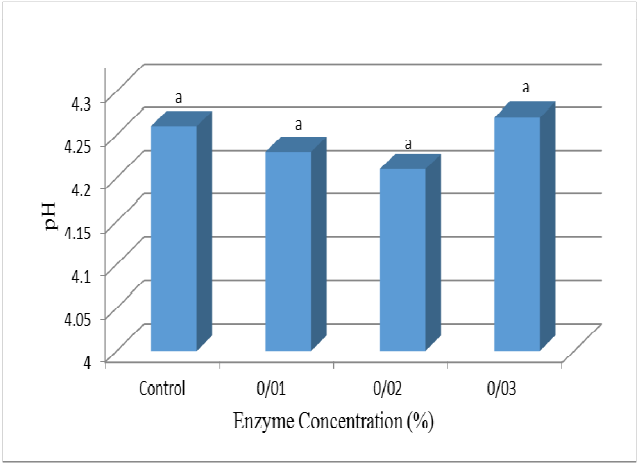
2.2.3. Statistical analysis

The experimental design was completely randomized, with factorial arrangement 4 × 2 × 3 (Enzyme concentration × MSNF content × storage time). The experiment was carried out in triplicate, resulting in 72 experimental units. Data were analyzed and the mean values, standard deviation, variance analysis were calculated with using SPSS software, version 20 [35]. Duncan's multiple-comparison test was used as a guide for pair comparisons of the treatment means. The level of significance for all analysis was done at $p<5\%$. To determine the best level of each variable and to find significant interactions between variables two way analysis and to select the best treatment among 24 treatments base on physical properties one way analysis was applied.

3. RESULTS

3.1. Effect of MTGase treatment and MSNF content on pH and acidity

Results showed that the enzyme treatment had no significant influence ($p= 0.492$) on the pH of yoghurt samples while the effects of two other variable factors, i.e. amount of MSNF and storage time on the pH were considerable ($p<0.001$). Furthermore, all three variables had significant effect on the acidity of yoghurt sample. The variations in the pH and acidity in the enzyme-treated yoghurts is given in Fig. 1. The mean values of pH and acidity as a result of enzymatic treatment and the amount of yoghurt MSNF during 21 days of storage is given in Table 1. Although by increasing the enzyme concentration up to 0.02%, the pH decreased but at higher level of enzyme, i.e. 0.03% concentration, the pH increased. However, these changes were not significant. Except in sample contained with 0.03% enzyme, no significant differences were found between acidity of enzyme-treated yoghurts and control. Sample treated with 0.03 enzyme had considerably lower acidity than yoghurt treated with 0.02% enzyme and the mean value was recorded as 86.2 and 89.28˚D, respectively.



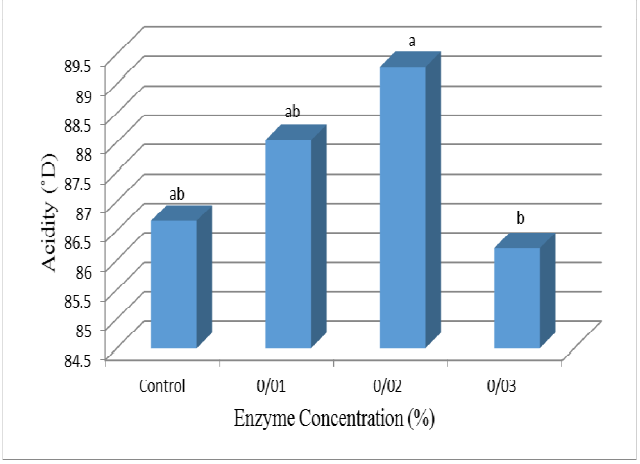


Fig. 1. Effect of different MTGase concentrations on pH and acidity of yoghurts.

Effect of different MTGase concentrations on the pH and acidity of yoghurts during 21 days of storage is shown in Fig. 2. During the period of storage, we noticed a remarkable decrease in pH and increase in acidity for all yoghurt samples. The highest acidity (94 ˚D) and the lowest pH (4.04) were recorded for yoghurt contained 0.02 enzyme at the end of storage. The results also showed noticeable variations in pH and acidity between yoghurt samples contained different amount of MSNF. The mean values for the pH and acidity in samples having 8 and 9% MSNF were 4.19, 85.19 and 4.21, 89.91, respectively.

Table 1. Effect of different MTGase concentrations and MSNF contents on physicochemical properties of yoghurts during 21 days of storage (Mean ±SD)

9% MSNF				8% MSNF				Storage time (Day)	Parameter
Enzyme concentration									
0.03	0.02	0.01	Control	0.03	0.02	0.01	Control		
4.37 ±0.26 ^{a}	4.33 ±0.17 ^{ns}	4.35 ±0.21 ^{ns}	4.40 ±0.17 ^{ns}	4.47 ±0.22 ^{ns}	4.45 ±0.21 ^{ns}	4.45 ±0.20 ^{ns}	4.41 ±0.19 $^{ns\ NS}$	1	
4.15 ±0.10 ^{ab}	4.18 ±0.22	4.09 ±0.12	4.14 ±0.15	4.35 ±0.17	4.22 ±0.16	4.25 ±0.18	4.32 ±0.17 ^{NS}	11	**pH**
4.11 ±0.16 ^{bAB}	4.02 ±0.14 ^{B}	4.07 ±0.13 ^{AB}	4.11 ±0.05 ^{AB}	4.21 ±0.08 ^{A}	4.06 ±0.09 ^{B}	4.15 ±0.08 ^{AB}	4.17 ±0.09 ^{AB}	21	
87.93 ±4.17 ^{bA}	87.11 ±1.15 ^{bA}	86.07 ±2.64 ^{bA}	87.94 ±1.00 ^{nsA}	81.15 ±0.57 ^{bC}	84.45 ±3.78 ^{bAB}	82.91 ±1.19 ^{bBC}	81.05 ±0.58 ^{bC}	1	
88.98 ±1.16 ^{abA}	89.55 ±2.64 ^{bA}	90.37 ±1.13 ^{abA}	89.87 ±1.11A	83.02 ±0.80 ^{abC}	87.35 ±4.17 ^{abAB}	87.23 ±1.17 ^{aAB}	84.35 ±1.52 ^{abBC}	11	Acidity
90.43 ±3.06 ^{aBC}	96.94 ±3.99 ^{aA}	93.14 ±3.03 ^{aAB}	90.59 ±3.08^{BC}	85.71 ±2.65 ^{aD}	90.30 ±4.09 ^{aBC}	88.54 ±3.09 ^{aCD}	86.23 ±3.78 ^{aD}	21	(D)
2.38 ±0.28 ^{aA}	2.47 ±0.25 ^{aA}	4.67 ±0.89 ^{nsD}	5.32 ±0.12 ^{nsE}	3.20 ±0.38 ^{aB}	3.33 ±0.47 ^{aB}	3.68 ±0.39 ^{aC}	5.65 ±0.45 ^{aF}	1	
2.62 ±0.15 ^{bA}	3.11 ±0.16 ^{bB}	4.81 ±0.36 ^{CD}	5.39 ±0.15 ^{E}	5.08 ±0.14 ^{bD}	5.24 ±0.68 ^{bDE}	5.47 ±0.60 ^{bE}	6.65 ±0.52 ^{bF}	11	Syneresis
2.85 ±0.22 ^{bA}	3.75 ±0.37 ^{cB}	4.95 ±0.28 ^{C}	5.45 ±0.19 ^{D}	6.95 ±0.13 ^{cE}	7.15 ±0.17 ^{cEF}	7.25 ±0.14 ^{cF}	7.65 ±0.15 ^{cG}	21	(g/100)
22.11 ±0.85 ^{cA}	19.69 ±2.90 ^{aC}	18.81 ±0.18 ^{bD}	18.25 ±1.11 ^{aE}	21.15 ±1.51 ^{aB}	14.68 ±1.23 ^{cG}	15.56 ±0.79 ^{bF}	13.55 ±1.43 ^{bH}	1	
23.87 ±0.46 ^{aA}	14.94 ±1.75 ^{bF}	19.38 ±1.55 ^{aB}	16.54 ±0.65 ^{bE}	18.73 ±0.34 ^{cBC}	23.95 ±2.72 ^{aA}	17.15 ±0.31 ^{aCD}	16.26 ±2.61 ^{aEF}	11	Viscosity
23.28 ±0.58 ^{bA}	19.90 ±0.26 ^{aB}	15.12 ±1.00 ^{cD}	15.63 ±1.08 ^{cCD}	19.55 ±0.80 ^{bB}	19.44 ±1.71 ^{bB}	16.87 ±0.88 ^{aC}	11.99 ±2.80 ^{cE}	21	(Pa.s.)

Means in the same row (8 values) having different large letters are significantly different and means in the same column (3 values) having different smal letters are significantly different (P □□0.05); n=3; ns/NS, not significant.

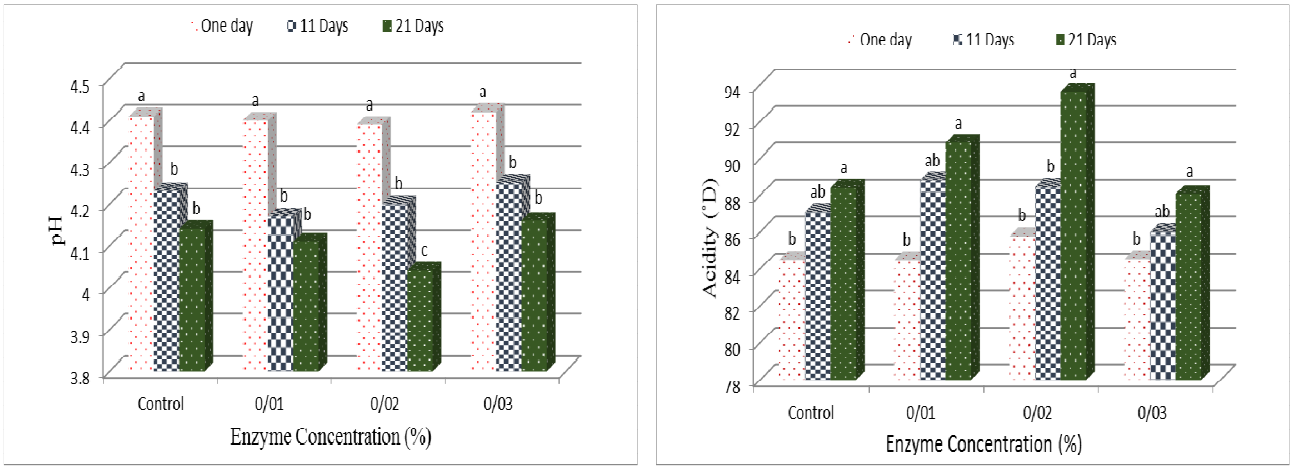


Fig. 2. Effect of different MTGase concentrations on pH and acidity of yoghurts during 21 days of storage.

3.1. Effect of MTGase treatment and MSNF content on syneresis and viscosity

Based on results, we found that all variable factors had significant effects ($p<0.001$) on the syneresis and viscosity of yoghurts (Table 1.). The syneresis and viscosity were related to amount of enzyme; the higher enzyme concentration, the more viscosity and less syneresis (Fig. 3). The amount of syneresis and viscosity for control were recorded 6.02 g per cent and 15.37 Pa.s., respectively. These values for yoghurts treated with MTGase were 3.85 g per cent and 20.96 Pa.s., respectively. Results also revealed that the amount of syneresis and viscosity were significantly affected by the amount of MSNF. As MSNF content of yoghurts was increased from 8 to 9%, the viscosity was increased and syneresis was reduced markedly.

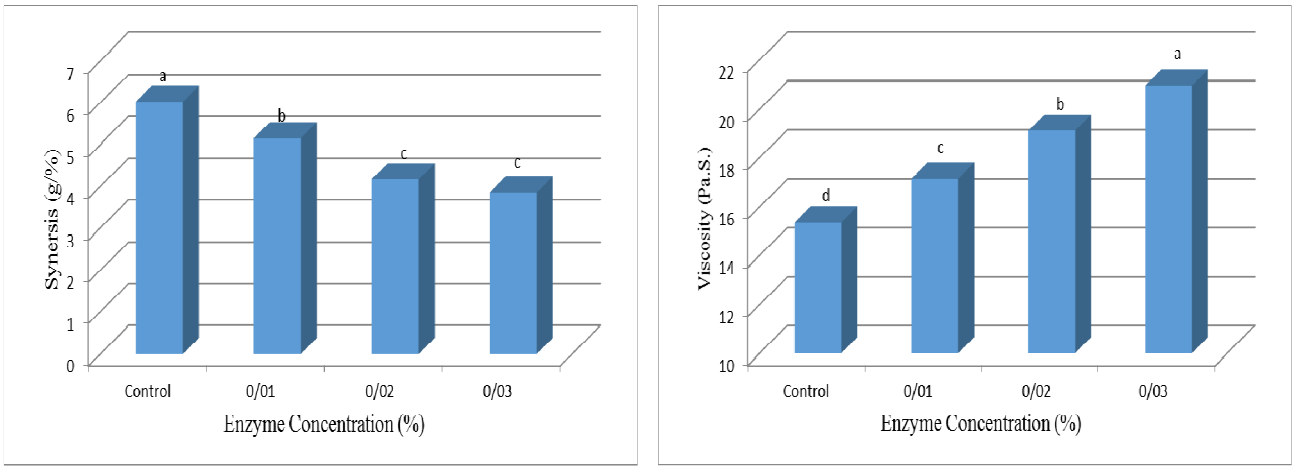


Fig. 3. Effect of different MTGase concentrations on syneresis and viscosity of yoghurts.

Similar to pH and acidity, the period of storage had significant effect on syneresis and viscosity of yoghurts (Table 1). The extent of syneresis in yoghurt samples was increased noticeably during storage period and the initial mean value of 3.84 at the first day of storage reached to 5.75 g/100 at the end of storage. The mean value of viscosity at the first day of storage was recorded as 17.49 Pa.s. and reached to 19.98 Pa.s. after 21 days of storage. As it shown in Fig. 4, the amount of syneresis and viscosity affected by enzymatic treatment were also changed during storage. Higher treatments of the MTGase enzyme decreased the extent of syneresis while with increasing storage time the syneresis was constantly increased. Furthermore, the higher level of enzyme up to 0.02% increased viscosity while afterward the viscosity was noticeably decreased. The mean values for syneresis and viscosity of control at the beginning of storage were recorded 5.48 g/100 and 15.9 Pa.s., respectively. These values changed after

21 days of storage and reached to 6.55 and 13.8 Pa.s. For treated-yoghurt samples with 0.03% enzyme, the initial values 2.79 g/100, 19.65 Pa.s. reached to 4.9 g/100 and 21.42 Pa.s. at the end of storage.

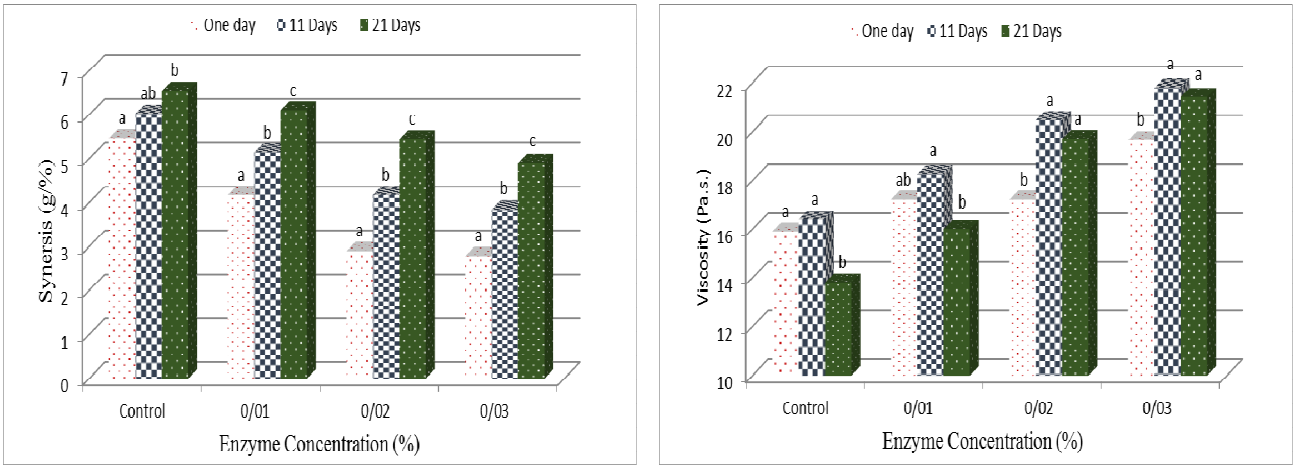


Fig.4. Effect of different MTGase concentrations on syneresis and viscosity of yoghurts during 21 days of storage.

4. DISCUSSION

4.1. Effect on pH and acidity

While the addition of TG showed no significant (p<O.O5) effect on the pH of yoghurt, it significantly (p<O.O5) decreased the acidity in the enzyme-treated yoghurt contained 0.03% enzyme (Table1 and Fig. 1). These findings are in good agreement with Faergemand et al. [36] and Neve et al. [37] who demonstrated that the enzymatic crosslinking cause an imbalance of the associative growth of the yoghurt starter culture. On the contrary, Schey [38] observed no interfering of MTGase with starter bacteria during fermentation of yoghurt. Increasing enzyme concentration may led to a delay in bacterial multiplication and this is the cause of the slower acidity development in the yoghurts [10]. The reason of slow growth of starter bacteria may be due to MTGase crosslinking of low molecular weight peptides and/or amino acids required by yoghurt starter cultures [37].

At day 1, the highest pH value was obtained in all yoghurt samples. The pH of the samples decreased continuously throughout storage period in a similar way for all the samples. Our results are in keeping with those reported by Lorenzen et al. [39], who demonstrated that the pH values of yoghurt diminished during storage period. However, in contrast with our results, Lorenzen and Schlimme [40] observed that there was no pH or acidity differences among enzyme-treated and untreated yoghurts during 14 days of storage. As with pH values, the titratable acidity values of the experimental yoghurts were increased throughout of storage.

Our results also showed the lower pH and higher acidity in yoghurt samples contained higher MSNF content. This may be due to the higher lactic acid production by lactic bacteria caused using higher amount of milk solids [3, 41].

4.2. Effect on the synersis of yoghurt

Whey separation which also called as water holding capacity (WHC), is defined as the appearance of the serum on the gel surface [42]. Traditional methods like the supplementation of dry matter or protein content besides addition of hydrocolloids or stabilizers are common methods of preventing whey syneresis. One of the emerging trends is the enzymatic crosslinking of milk proteins with transglutaminase enzyme [8]. The variations in the quantities of whey separated in the enzyme-treated yoghurts are given in Table 1 and Figs. 3 and 4. In general, the MTGase-treated samples had significantly lower syneresis values than the untreated yoghurt (P<0.05). Also, increasing the doses of added enzyme led to a considerable decrease in the serum separation of the yoghurts.

The effect of TG enzyme on syneresis is comparable with the results published by Aprodu et al. [28] who reported higher WHC in enzyme treated yoghurt contained 0.02 and 0.03% of MTGase. Tsevdou et al. [43] also showed that the amount of separated whey in enzyme-treated yoghurt samples were significantly lower than

untreated ones. Lorenzen et al. [39] also claimed that with the enzymatic treatment the serum separation could be decreased up to 20 percent as compared to control.

Obvious increases in the syneresis values of all yoghurt samples were noted during cold-storage with slightly more pronounced in enzyme-treated samples. The rise of syneresis during yoghurt storage may attributed to increase in yoghurt acidity [39]. However, contradictory to our finding, improvement of WHC during 21 days of cold storage was reported by Aprodu et al. [28]. Tsevdou et al (43) also didn't find significant variations in syneresis between enzyme-treated yoghurts and control during storage period.

4.3. Effect on the viscosity of yoghurt

The acceptability and sensory quality of yoghurt is to a great extent dependent on its physicochemical properties particularly its acidity, syneresis and viscosity. The effect of MTGase on physical property of the yoghurt was also estimated and the results obtained for the yoghurt produced using different enzyme doses were reported as viscosity. Table 1 and figs. 3, 4 shows the impact of TG treatment and addition of MSNF on the viscosity of yoghurts. The viscosity of the yoghurt samples was affected by the TG enzyme, MSNF content and storage time and the differences between the samples were statistically significant ($p<0.05$). As it can be shown from Fig.3, The higher the enzyme treatment the higher was the viscosity. It was well-demonstrated that irreversible casein crosslinking induced by MTGase caused in a reduced gel permeability resulting in the higher gel strength and yoghurt viscosity [44, 45].

Based on our results, storage time had multifaceted effect on yoghurt viscosity. The amount of viscosity in all the yoghurts significantly increased during primary stage of storage and reached to a maximum level after 11 days of storage and afterwards significantly decreased. Initial rise of viscosity at the beginning of storage was probably due to increase of protein chains' cross-linking [28] and formation of more compact gel protein network [3]. Development of acidity at the ending of storage period was likely the reason of decrease in viscosity [39]. This effect may also be due to this fact that the enzyme activity not being constant during the gelation process, and gradually decreasing with time as the enzyme became trapped within the formed network [46].

Conclusions

An enhancement in the doses of MTGase added into yoghurt milk at the time of fermentation decreased the extent of syneresis and increased viscosity. Crosslinking of milk proteins using MTGase seems to be an acceptable alternative instead of adding extra protein or stabilizer in yoghurt. Nevertheless, for a good physicochemical quality yoghurt, the amount of MTGase added into milk should be carefully chosen. Based on our results, from the range of MTGase concentrations examined for the physicochemical properties of the reduced-fat yoghurts, MTGase level of 0.03% (0.3 g L^{-1}) was seen as optimal. However, even yoghurt sample prepared with 0.01% MTGase had a better physical properties as compared to control.

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