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ORIGINAL RESEARCH PAPER

THE EFFECT OF TRANSGLUTAMINASE ON THE RHEOLOGICAL PROPERTIES OF YOGURT♦

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Abstract: The aim of this study was to investigate the rheological characteristics of yogurts obtained from milk treated with transglutaminase prior to fermentation with *Streptococus theromophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*. A set of 36 experiments were carried out to test the influence of various enzyme concentrations ranging from 0 to 0.04%, different setting temperatures (35, 40 and 45 ^{o}C), and setting time (60, 90 and 120 min). The cross-linking of milk proteins influenced the post-acidification process as well as the stability of the yogurt samples. The enzymatic treatment of milk allowed avoiding the syneresis phenomena during yogurt storage at 4 ^{o}C; the water holding capacity during centrifugation was also improved. Concerning the rheological properties, the apparent viscosity of yogurt increased by increasing the enzyme concentration and the setting time for the entire tested domain of shear rates. The results indicate that transglutaminase catalyzed cross-linking is an effective tool for improving functional properties of yogurt.

Keywords: *rheological behavior, syneresis, thixotropy, transglutaminase, yogurt*

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INTRODUCTION

Transglutaminases (MTGase) are a family of enzymes (EC 2.3.2.13) that catalyze the acyl transfer reactions between γ-carboxyamide group of glutamine residues and ε-amino groups of lysine residues, generating new covalent intra- and intermolecular bonds [1 – 3]. Current applications of MTGase in food industry involve casein, soy proteins, conalbumin, rabbit and carp myosin, beef actin and myosin, ovomucin as substrates [4 – 9].

Extensive research was conducted to study the MTGase catalyzed reactions and their impact on dairy products processing [3, 10 – 14]. Within milk proteins, sodium caseinate is the most suitable substrate for MTGase [15], whereas the globular whey proteins need to be thermally unfolded prior to cross-linking reaction [16, 17]. The MTGase treatment was used mostly in case of yogurt production, indicating the excellent cross-linking properties of casein that result in the increase of gel strength and the decrease of syneresis phenomena [18, 19]. Different types of experiments were performed to establish the optimal technology to be used for obtaining yogurt with MTG. Some trials were done by adding the MTG prior to the fermentation process [3, 12, 13] or simultaneously [13, 20, 21]. The first procedure offers the advantage that the *p*H remains constant for the entire incubation period but it requires a subsequent thermal inactivation step and longer processing time. In the second case, when the enzyme acts during the fermentation, the cross-linking reaction is stop due to the acidification induces by lactic bacteria. Yogurt texture is highly influenced by the MTGase treatment method.

In addition to yogurt, Kuraishi *et al.* [22] showed that MTGase can be successfully used for cheese making, resulting in the curd yield increase. As reviewed by De Jong and Koppelman [23], the enzyme can be added prior, during or after adding the rennet to the milk.

The objectives of the present paper were to study the effect of MTGase on rheological, physical-chemical and sensorial characteristics of the yogurt. In order to optimize the process of yogurt obtaining, different enzyme concentrations, setting temperatures, and setting time were tested.

MATERIALS AND METHODS

The pasteurized milk with 1.8% fat was purchased from a local supermarket (Galati, Romania).

Activa TG-1 MTGase, provided by Ajinomoto (Inc. Teanec, NJ, USA) was used. The enzymatic product is made up of 99% maltodextrin and 1% transglutaminase with a declared enzymatic activity of about 100 UE/g. The enzyme is active over large ranges of temperature (2 – 60 ^{o}C) and *p*H (5 – 8).

Enzymatic cross-linking of the milk

The milk was tempered to the cross-linking temperature (35, 40 or 45 ^{o}C) in a water bath for 10 min prior to the addition of MTGase. A total of 36 experiments were

conducted by adding MTGase at concentrations of 0.02, 0.03, or 0.04 g MTGase/100 g milk and incubating the samples for 60, 90 and 120 min at 35, 40 or 45 ^{o}C in a laboratory heat chamber. The control samples for each temperature were incubated without enzyme addition. After the incubation phase, the cross-linking reaction was immediately stopped by thermal treatment (the temperature was increased at 1 ^{o}C/ min) to achieve 90 ^{o}C, temperature that was maintained for 10 min. The thermally treated samples were afterwards cooled to 43 ^{o}C using a mixture of water and ice.

Yogurt preparation

The MTGase treated milk tempered at 43 ^{o}C was inoculated with a mixed culture of lactic acid bacteria FD-DVD YF-LB11-Yo-Flex (*Streptococus theromophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (1:1) provided by Christian Hansen, Denmark, poured into 250 mL plastic containers, and incubated at 43 ^{o}C. The size of the inoculum used for yogurt preparation was according to producer recommendations.
The acidification process of the milk was monitored by checking the *p*H during the entire incubation period. The fermentation was considered completed when the *p*H of 4.4 was reached for the control samples. The yogurts were afterwards cooled and stored at 4 ^{o}C for 21 days.

Determination of *p*H and acidity

The *p*H measurements were made directly on the yogurt sample by using a Hanna digital *p*H-meter.
Acidity (expressed as Thorner degree) was determined in duplicate according to Romanian Standard 6353 – 84 by titration with 0.1 N NaOH using phenolphthalein as indicator.

Syneresis

Syneresis of the yogurt was determined through the centrifugation procedure. Approximately 20 g of yogurt was transferred into a 50 mL glass tube and was centrifuged at 3500 rpm for 15 min at 20 °C. The syneresis was estimated as the percentage of the released whey over the initial gel weight and was an average of three determinations:

$$\text{Syneresis}, \% = \frac{\text{weight of supernatant}}{\text{weight of yogurt}} \times 100 \quad (1)$$

Rheological measurements

Yogurt samples were gently stirred before rheological analysis. Rheological measurements were carried out in duplicate by means of a RHEOTEST-2 type rotating viscosimeter manufactured by VEB-MEDINGEN, Germany. Due to the medium viscosity of the samples the coaxial cylinder device S_3 was used and 50 g of sample was tested.

The working frequency was 50 Hz and the shear rate (γ) was varied from 0.1667 to 145.6 s^{-1}. The shear stress (τ) was recorded at increasing shear rates (upward flow curve) followed by decreasing shear rates (downward flow curve). The area under the upward (A_1) and downward (A_2) flow curves were estimates through Rheology Advantage Data Analysis software v5.5.0 (TA Instruments Ltd.) and were used to calculate the thixotropy surface (ΔA) as the difference in area under the upward flow curve and the downward flow curve and the thixotropy index (T) as:

$$T = \left[\frac{S_1 - S_2}{S_1}\right] \cdot 100 \quad (2)$$

The apparent viscosity (η) was calculated as:

$$\eta = \frac{\tau}{\gamma} \quad (3)$$

Sensory properties

The sensory properties of the yogurt samples stored for 21 days at 4 ^{o}C were evaluated by a panel of 12 untrained assessors with previous experience in yogurt evaluation. According to Lorenzen *et al*. [13], a structured scale from 1 (intensity just recognizable) to 5 (very strong), was used to described the following properties: color – tested by visual observation; odor – estimated by smelling the sample immediately after removing the beaker lid; taste and texture – evaluated in the mouth during and after intense chewing.

Statistical analysis

Statistical analysis of the results was performed using Sigma Plot 2001/Statistics Data software. Three experimental batches were performed for each test and the results are reported as mean values. Typical standard deviations are less than 5%.
Rheological data were fit using Rheology Advantage Data Analysis software v5.5.0 (TA Instruments Ltd.).

RESULTS AND DISCUSSION

The efficiency of using MTGase to improve yogurt properties was tested by determining (i) the acidification process, (ii) rheological properties and (iii) the water holding capacity of the gel after centrifugation.

Chemical analysis of the milk

The physical-chemical characteristics of the milk used for preparing the yogurt samples were checked at 15 ^{o}C using Milk-lab Lactostar analyzer and the obtained results are:

dry matter 8.10%, proteins 3.22%, fats 1.28%, lactose content 4.21%, minerals 0.66%, density 1.029 g/cm^3, cryoscopic point – 0.477 ^{o}C.

Variation of acidity

The influence of the MTGase concentration on yogurt properties was estimated by setting the milk with enzyme at different concentrations for 60, 90 and 120 min at 35, 40 or 45 ^{o}C. When incubating the MTGase treated milk with *Streptococus theromophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains, the *p*H decreases as a consequence of bacterial homolactic fermentation when D(-) and/or L(+) lactic acid accumulates into the samples. Milk acidification resulting from starter bacteria activity during yogurt manufacture causes steric stabilization of the micelles that flocculate.
In addition, the presence of some aroma compound such as acetaldehyde, acetone, acetoin and diacetyl were also reported in different yogurt samples [24].
In all studied cases, the yogurt obtained from MTGase treated milk had slightly higher *p*H compared to the control samples prepared without enzyme addition (Figure 1).

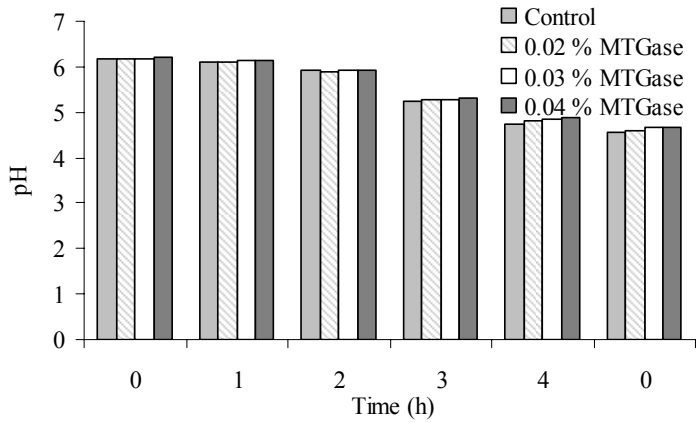

Figure 1. *The evolution of the pH during yogurts fermentation at 43 ^{o}C*
Experimental conditions: setting temperature: 35 ^{o}C; setting time: 1 hour

Therefore the incubation time required for samples with MTGase to reach the final *p*H of 4.4 is longer, and increases with the enzyme doses. These results are in agreement with the findings of Ozer *et al.* [10] and Fæaergemand *et al*. [20] that showed that the cross-linking reaction alters the culture starter growth as a consequence of the reduced availability of the low molecular weight peptides to be used as nitrogen source by the lactic acid bacteria. On the contrary, Schey [25] found no influence of MTGase addition on the total fermentation time.
As with the *p*H, the titratable acidity of the yogurt samples was highly influenced by the MTGase treatment, setting time and temperature (Figure 2). After 24 hours of storage at 4 ^{o}C, the highest titratable acidity was found in case of the control samples and yogurts obtained from milk treated with 0.04%MTGase for 60 min at 40 ^{o}C. The lowest acidity was registered for the yogurt samples incubated for 90 min at 35 ^{o}C and 45 ^{o}C with 0.02% MTGase.

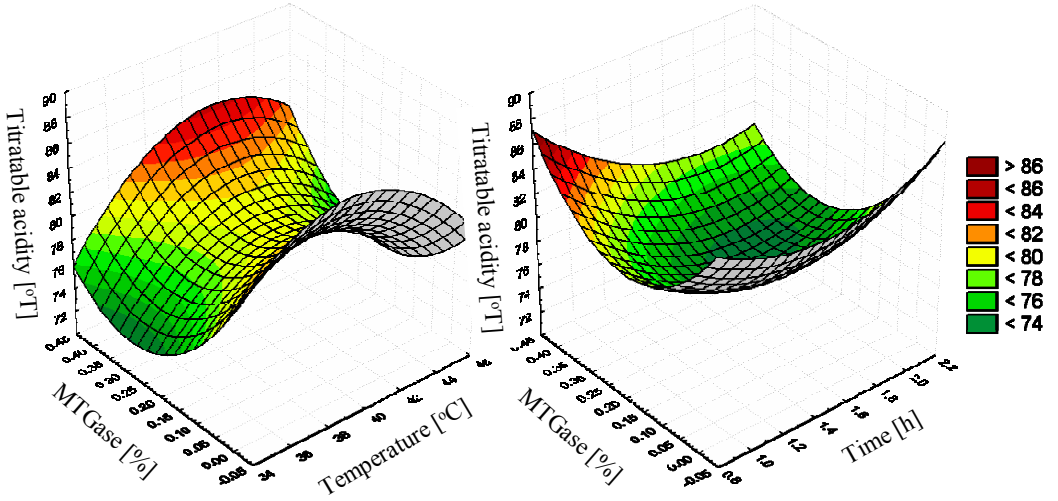

Figure 2. *The influence of MTGase concentration, setting time and temperature on titratable acidity*

The evolution of the *p*H values and titratable acidity was checked during 21 days of storage at 4 °C. According to our results, even at refrigeration temperatures the lactic fermentation occurs at lower rates, when the final titratable acidity reaches values of 103 – 108 °T after three weeks (Figure 3).

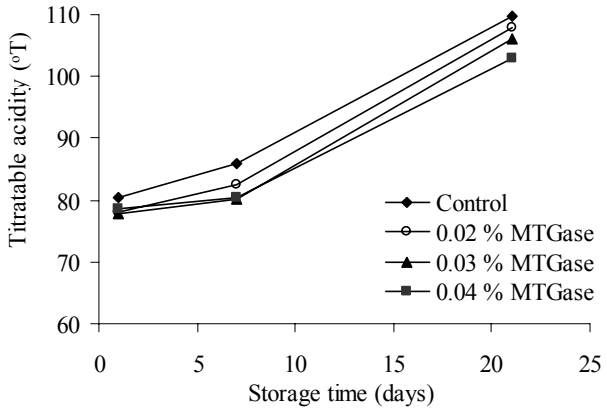

Figure 3. *Variation of titratable acidity during yogurts storage at 4 °C*

Rheological properties

The effect of MTGase on rheological behavior of the yogurt was also estimated and the results obtained for the yogurt prepared using different enzyme concentrations are reported as shear stress and apparent viscosity vs. shear rate in Figure 4 a and b. The MTGase treated samples had higher apparent viscosities compared to the control samples. The enhancement in viscosity was directly correlated with enzyme concentration. The protein chains' cross-linking led to increased apparent viscosity of the yogurt samples over the entire range of tested shear rates.

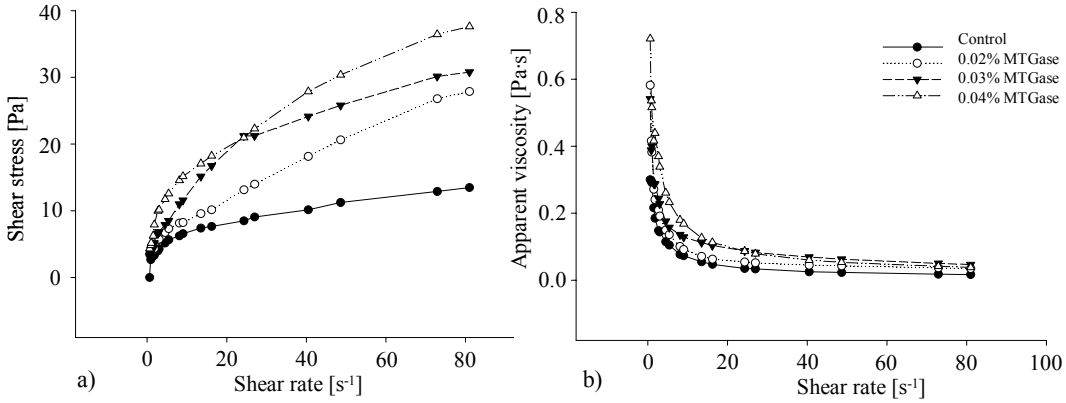

Figure 4. *The influence of MTGase concentration on rheological behavior of yogurt samples set at 45 °C for 120 min: a) Shear stress vs. shear rate rheogram; b) Apparent viscosity vs. shear rate rheogram*

The influence of setting time on the rheological behavior of the yogurt obtained from milk treated with different concentrations of MTGase is presented in Figure 5 a and b. For all shear rates, the shear stress and apparent viscosity of the yogurt obtained from milk treated with 0.04% MTGase, increased with the setting time from 60 to 120 min.

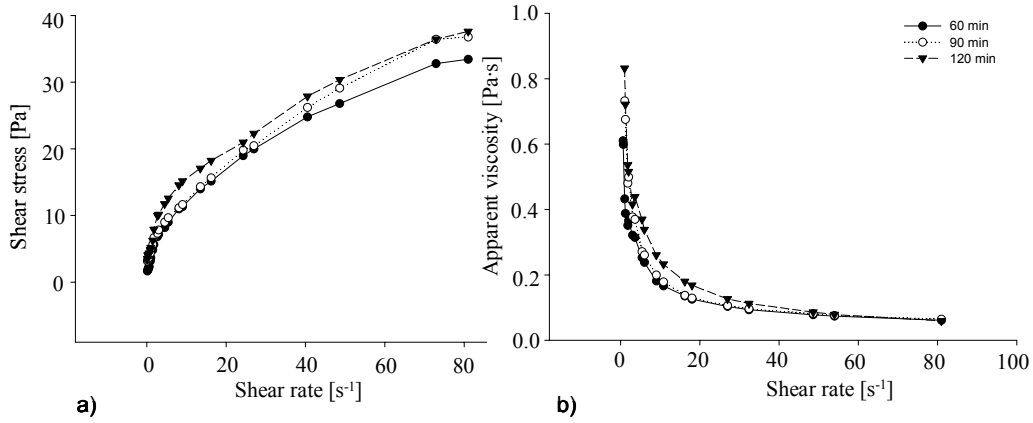

Figure 5. *The influence of setting time on rheological behavior of yogurt samples with 0.04% MTGase (setting temperature 45 °C): a) Shear stress vs. shear rate rheogram; b) Apparent viscosity vs. shear rate rheogram*

When studying the influence of the temperature on the rheological properties of the yogurt, it was shown that both shear stress and apparent viscosity of the samples decreased with the temperature increase (Figure 6 a and b). This effect was more pronounced for the yogurt samples set with enzyme at 45 °C. For a shear rate of 5.4 s^{-1}, the apparent viscosity of the MTGase treated samples at 35 °C was 1.25 and 2.48 times higher compared to the yogurt set with enzyme at 40 and 45 °C, respectively.

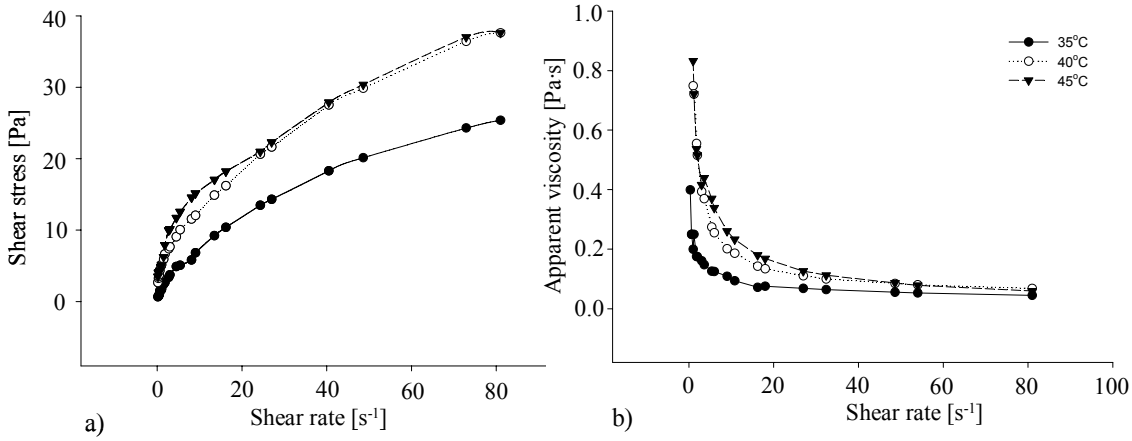

Figure 6. *The influence of setting temperature on rheological behavior of yogurt samples with 0.04% MTGase (setting time 120 min): a) Shear stress vs. shear rate rheogram; b) Apparent viscosity vs. shear rate rheogram*

In all studied cases, the yogurt samples displayed specific behavior to pseudo-plastic fluids, with time-dependent structural viscosity; the apparent viscosity decreased with increasing shear rate up to a constant value, when the destruction rate of the structure match the reformation rate of the protein aggregates [26]. This behavior was mathematically modeled using different rheological flow models such as Herschel-Bulkley, power law, Bingham and Casson, to check the fitting with the shear stress–shear rate results. In addition the apparent viscosity–shear rate models, such as Cross, Carreau, Sisko and Willamson, were tested. The best fit model was selected based on the standard error values. The Herschel-Bulkley model proved to give significantly better fits for the shear stress–shear rate results while Cross model better described the apparent viscosity–shear rate behavior in case of all studied samples ($R^2 > 0.95$).
When performing shear stress measurements for both increasing and decreasing the shear rate, the hysteresis loops could be observed for all samples. In order to quantify the thixotropic behavior of the MTGase treated yogurt, the thixotropy index (Table 1) was calculated with equation 2. According to Gonzalez-Toma *et al.* [27], the thixotropy index gives indications about the energy needed to destroy the structure responsible for flow time dependence.
Analyzing the results in Table 1, one can see that the surface of the hysteresis loop highly depends on the MTGase catalyzed cross-linking reactions since the thixotropy index increases with MTGase concentration and with the setting time.

Syneresis

The water holding capacity of the yogurt was determined by estimating the intensity of the syneresis phenomena after centrifugation. When analyzing the samples stored for 24 hours at 4 ^{o}C, the ability of the yogurt to retain the whey increased with the setting time and temperature. The best water holding capacity was registered for the yogurt samples set with 0.02 and 0.03% MTGase for 2 hours at 45 ^{o}C, when the syneresis was 34.31 and 37.56%, which is ~25% lower compared to the control sample. An improvement of

the water holding capacity of all tested samples was noticed after 21 days of storage at 4 °C (Figure 7). In this case, the syneresis was reduced by 3.4%, 8.8% and 1.3% for all samples set at 35, 40 and 45 °C, respectively.

Table 1. *Thixotropy index (%) of the yogurt samples obtained from milk treated with 0, 0.02, 0.03, 0.04% MTGase for 60, 90 or 120 min at 35, 40 or 45 °C*

MTGase concentration [%]	Thixotropy index [%]			
	Setting time [min]	Setting temperature [°C]		
		35	40	45
0	60	0.8	4	5.1
	90	0.9	4.9	5.8
	120	1.0	5.1	6.0
0.02	60	0.9	5.0	5.6
	90	1.9	5.8	6.2
	120	2.1	6.1	6.3
0.03	60	2.1	5.4	6.0
	90	2.2	6.0	6.5
	120	2.5	6.3	7.0
0.04	60	4.1	6.0	6.4
	90	4.9	6.8	7.3
	120	5.4	7.1	7.5

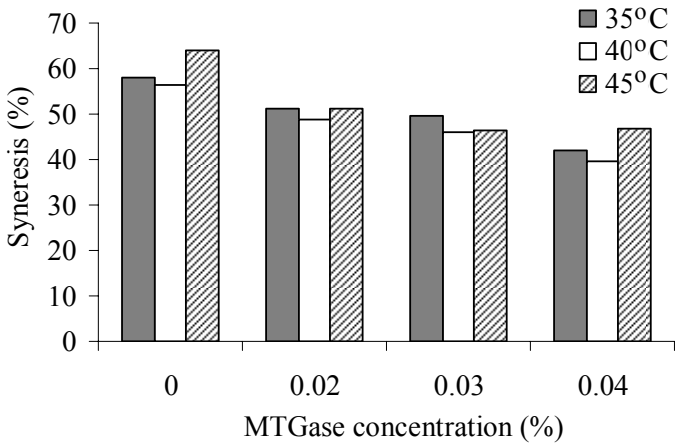

Figure 7. *The influence of MTGase concentration and setting temperature on yogurt syneresis after 21 days of storage at 4 °C (setting time 60 min)*

Our results indicate that proteins cross-linking by means of MTGase catalyzed reactions can be successfully used to stabilize the 3D networks of the yogurt as an alternative to the frequent way of avoiding syneresis problems that consists on the enrichment of the dry matter content by adding proteins or hydrocolloids like gelatin and starch [13].

Sensory evaluations

The results of sensory evaluation are presented as radar plot in Figure 8. All yogurts were considered acceptable by the panelists. Samples prepared with MTGase treated

milk received higher scores compared to the control sample. The panelist preferences did not changes significantly within the cross-linked yogurts; no differences in terms of color, taste, odor, texture and overall acceptability were found between samples with 0.03 and 0.04 % MTGase.

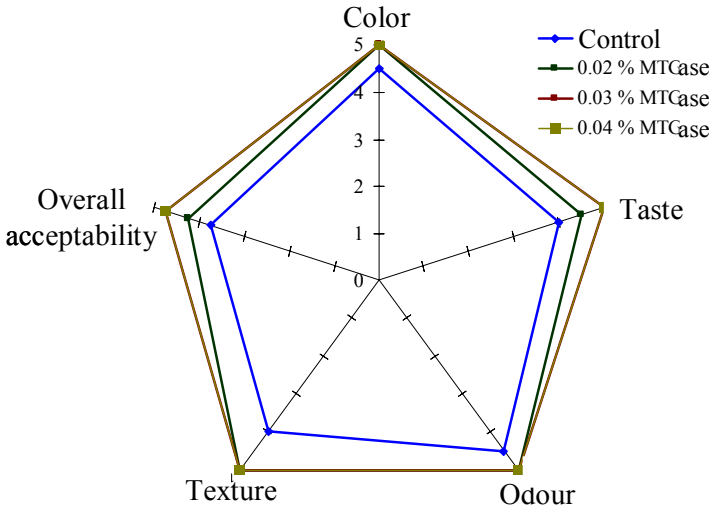

Figure 8. *Sensory profile of the MTGase treated yogurts after 21 days of storage at 4°C*

CONCLUSIONS

Transglutaminase can be successfully used for obtaining yogurt with improved viscosity and higher final *p*H. The best samples in terms of rheological properties and water holding capacity were obtained from milk treated with 0.04% MTGase for 120 min at 45 °C. Yogurt samples present non-newtonian pseudoplastic shear thinning behavior characterized by apparent viscosity decrease with the shear rate increase. The thixotropic behavior was revealed in case of the 36 tested variants of yogurt. The MTGase treated samples were very stable, since no syneresis occurred while stored at 4 °C for three weeks. Sensory analysis showed that the enzymatically treated yogurt present a better quality in terms of texture and aroma, compared to the control sample.

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