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STRUCTURE PROPERTIES OF STIRRED YOGHURT MADE WITH TRANSGLUTAMINASE AND AMARANTH

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ABSTRACT
The aim of this work was to investigate structure changes in stirred yoghurt manufacture. Stirred yoghurts were made from UHT milk subjected to heat treatment at 90 °C for 5 min. After cooling to 40 °C, amaranth flour and transglutaminase (TG) were added and mixed. Lyophilized concentrated starter culture was added in amount of 1 %. Milk was fermented until pH 4.6 – 4.7. Amaranth flour was added to sour milk as protein additive for nutritive value increasing and structure modification. TG enzyme well-known for its binding ability was added to catalyze protein network formation. It was shown viscosity decreases when amaranth flour concentration increases. Use of TG allows increasing yoghurt viscosity and therefore preventing the negative effect of polysaccharides containing in amaranth flour on the viscosity. TG contributes to a smoother yoghurt surface formation. TG binding effect was confirmed electrophoretically. All samples were judged as organoleptically acceptable. The data obtained indicate the possibility of applying amaranth flour and TG for stirred yoghurt manufacture.

1.Introduction

There are various additives for dairy products functionality. It was studied influence of fruit components on polyphenols and antioxidant activity (Oliveira et al., 2015; Chouchouli et. al, 2013) and on rheological properties (El-Said et al., 2014) of yoghurts. It is reported (Sun-Waterhouse et al., 2013) blackcurrant polyphenols can be added to drinking yoghurt pre- or post-fermentation resulting in higher yoghurt viscosity.

Viscosity is a quality attribute of drinking yoghurts. To alter rheological properties of final product we suggest using milk fortification with plant proteins concomitantly with TG enzyme action to form protein network in yoghurt manufacture (Argymbaeva et al., 2015; Shleikin et al., 2015). Earlier, we have also conducted research aimed at processing of set yogurt with amaranth and transglutaminase (Shleikin and Danilov, 2015). Also we showed possibility of TG utilization for whey protein – gluten combining products manufacture to prevent gluten toxity for celiac patients nutrition (Shleikin et al., 2011).TG (EC 2.3.2.13) is an enzyme widely used in food applications due to ability to cross-link different proteins and impact on products rheological properties (Gaspar and de Góes-Favoni, 2015). TG addition improves quality attributes both set and stirred yoghurt (Cancinoa et al., 2006). TG affects significantly on the viscosity of stirred yoghurt, especially in the low-shear rate region and at small deformations (Jaros et al., 2007). It is stated TG catalyzed formation of casein intramicellar cross-links (Mounsey et al. 2005; O’Sullivan et al. 2002; Vasbinder et al. 2003). It was noted higher viscosity and degree of protein polymerization if use TG for stirred yoghurts (Tabari, 2010). Methods of TG utilization in stirred yoghurt production and their impact on yoghurts shear stress are shown in study (Iličić et al., 2008).

Thermal treatment of milk (95°C for 5 min) allows denaturing the whey proteins and inducing interactions between the κ-casein, β-lactoglobulin and α-lactalbumin, therefore the hydrophilic properties of the coagulum and the stability of the yoghurt gel increase (Tamime and Robinson, 1988). In stirred yoghurt, milk is fermented in a tank, the yoghurt gel being broken up during the stirring, cooling and packaging stages. The rheological properties of stirred yoghurt may be changed due to several factors. These have physical nature related with total solid content, milk composition and type of starter culture, or processing conditions-related, such as homogenisation, thermal pre-treatment of the milk and post-incubation stages including: stirring, pumping, cooling and packaging (Afonso and Maia, 1999).

Food fortification is one of the most important processes for improvement of the nutrients quality and quantity in food. Yoghurt can be fortified with vitamins, calcium, iron, fibers e t.c. (Hadi et al., 2015). We suggest to fortify stirred yoghurt with amaranth flour addition. Amaranth is technically a pseudo-cereal. Amaranth seeds are gluten-free foodstuff important for celiac disease patients nutrition (Mlakar et al., 2009). Amaranth is gaining popularity due to its nutritional excellence and agronomic features. Amaranth contains high protein amounts, up to 35% more than rice, oats and wheat flour. In addition, the protein present in amaranth flour is complete and it contains all the vital amino acids in contrast with other protein sources (Chauhan et al., 2015). Amaranthus seeds were added to bio-yoghurt to increase the levels of starch and dietary fibre (Sady et al., 2005). In addition, we suppose to study milk and amaranth protein binding in our experiments; therefore, amaranth should be added prior to fermentation simultaneously with TG.

2.Materials and methods

UHT milk (Pyatigorsk dairy plant, Pyatigorsk, Russia) with protein content of 2.8 % and fat content of 3.2 % was used. Yoghurt starter culture was AiBi 22.11 R3 (lyophilized concentrated starter culture of Streptococcus thermophilus, Lactobacillus bulgaricus – $5.0*10^{10}$ CFU/g) obtained from Green Lines Ltd, Krasnogorsk, Russia. TGase preparation was presented by "Next Ingredients" company (Moscow, Russia) had the activity of 100 U/g. Amaranth flour (Russian Olive Ltd, Voronezh, Russia) with high protein concentration (30 % of protein, 10 % of fats, 40 % of carbohydrates) was used as vegetable additive.

2.1. Yoghurt preparation

The milk was heated to 90 °C, kept at this temperature for 5 min, then cooled to fermentation temperature of 40 °C, was poured into the samples. Amaranth flour was added in the amount of 1 %, 2 %, 3 % and stirred until the formation of homogeneous mass. Then TG was added into the samples in an amount of 0.5 U/g, 1 U/g, 2 U/g and stirred for 5 min. Starter culture was added in an amount of 1 % and stirred. The prepared samples were put into thermostat at 40 °C for 7 hours until the pH 4.6 to 4.7. The finished product was cooled to 4 °C. Samples were numbered accordingly to table 1.

Table 1. Yoghurt samples labeling.

Amaranth flour concentration, % w/v	TG concentration, U/g			
	0	0.5	1	2
0	1	2	3	4
1	5	6	7	8
2	9	10	11	12
3	13	14	15	16

2.2. Viscosity measurement

The yoghurt samples were heated to a temperature of 25 °C. Viscosity measurements were carried out on a rotational viscometer Rheotest RN4.1 (Rheotest Medingen GmbH, Medingen, Germany) within 30 s, a shear gradient of 100 s^{-1}, used spindle S1. Viscosity – time curves were received and analyzed using "Rheo-42" and "Excel" software.

2.3. Texture Profile Analysis

The yoghurt samples of 0.1 L were cooled to 4 °C. Texture analysis was performed using a TA-XT Plus texture analyzer with a load cell of 5 kg (Stable Micro Systems Ltd., UK). The samples were subjected to a compression test to construct the Texture Profile Analysis graphs, using 5 mm Cylinder Probe. The parameters were as follows: pretest speed of 3 mm/s; test-speed of 1 mm/s and post-test speed of 3 mm/s; distance of 20 mm; temperature of 4 °C; and force of 1 g.

Force–time (g–s) curves were recorded and analyzed using the Texture Exponent Application (Stable Micro Systems Ltd, UK) and softness was calculated. The results were calculated as the average of three measurements taking into account the standard deviation.

2.4. The study of the microstructure of the yoghurt surface

A few drops of yoghurt samples were applied on an object glass, rubbed to a thin layer. The structure changes were estimated with the MC100 microscope (Micros HgmbH, St. Veit/Glan, Austria). Magnification 40x and 100x was used.

2.5. Electrophoretic studies

To yoghurt sample of 100 μl Tris/HCl buffer of 100 μl with pH = 8.7 was added. Then SDS (3 % solution) was added in amount of 150 μl and prepared solution was left for 30 min. at room temperature. Then distilled water of 650 μl was added and mixture was held at 100 °C for 5 min. Bromophenol blue 1 % solution was added in amount of 100 μl. Ready solution of 3 μl was applied into the wells on the gel surface. Gel was made as follows: 5 ml of 0.2 M Tris/HCl buffer mixed with 5 ml of 12.5 % acrylamide solution. TEMED of 14 μl was added. The reaction mixture was purged with argon. SDS was added 10 mg per 10 ml of solution, potassium persulfate in amount of 0.2 ml of solution with a concentration of 7 mg/ml. The resulting solution was applied to the openings between the plates for electrophoresis. The formation of a gel was observed. Electrophoresis was carried out on the Flat Bed Apparatus FBE-3000 (Pharmacia, Uppsala, Sweden). The apparatus was filled with 450 ml of 0.1 M Tris/HCl buffer containing 0.1% SDS. The electric field intensity was 30 V/cm. The voltage U = 300 V, current I = 100 mA, power W = 40 watts. Gels were stained with 0.2 % Coomassie R-250 in mixture of 7.5 % v/v of acetic acid, 25 % v/v of methanol and 67.5 % v/v of water. Gels were photographed and quantified using ImageJ (National Institutes of Health, Bethesda, Maryland, USA) software.

2.6. Sensory analysis

Sensory analysis was performed by a panel of 5 trained assessors. The samples were served at the 12 °C in 50 g of cups. Mean scores for each attributes were used for comparison of the samples. Whey separation ("syneresis"), "color," "firmness", "flavor intensity", "distribution of ingredients", "acidity", and "sweetness," were rated using a 10-point scale with the end values labeled as "weak" and "strong" where 10 was as higher point. The sensory sets of data were subjected to a one-way ANOVA using Minitab 16 (Minitab Ltd., Coventry, UK) software, to establish whether the sensory scores differ. The means were compared using Fisher's least significant difference test and the statistical significance was determined at $P < 0.05$.

3.Results and discussion

The effect of additives on the viscosity of yoghurt

The yoghurt samples were prepared in accordance with p. 2.1. Viscosity analysis was conducted in accordance with p.2.2. The viscosity of yoghurts with the addition of amaranth flour, without the addition of TG, depending on the time of rotation of the rotor is presented in Figure 1.

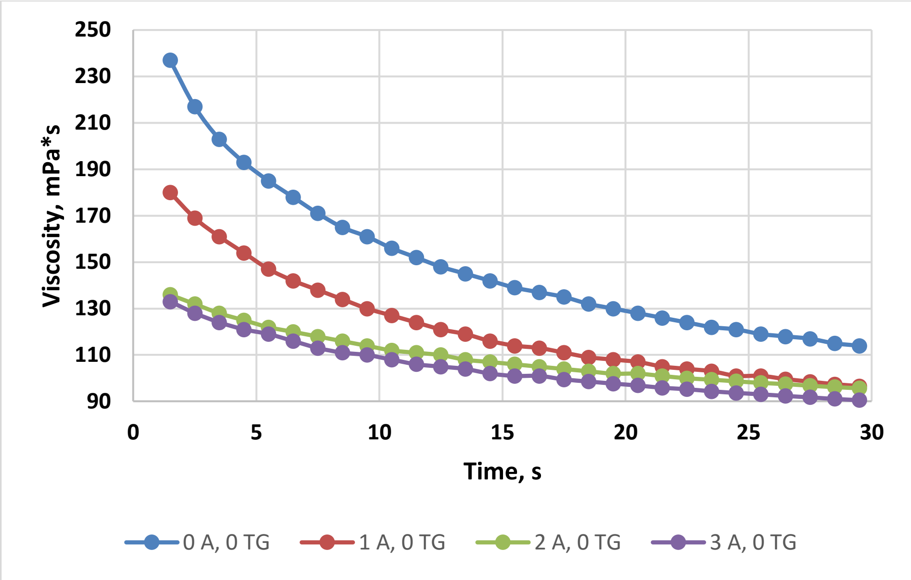

Figure 1. The viscosity of yoghurts with the addition of amaranth flour, without the addition of TG, depending on the time of rotation of the rotor

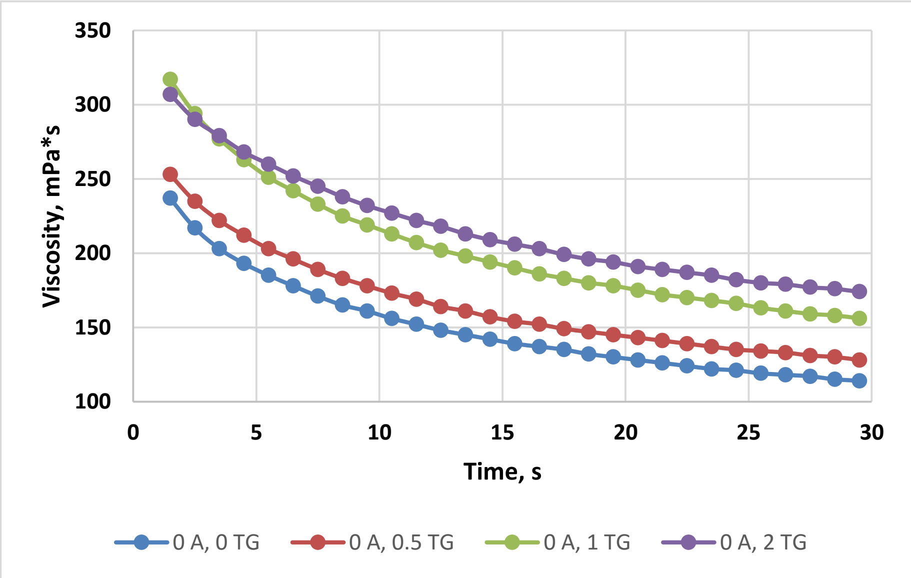

Figure 2. The viscosity of yoghurts with the addition of TG, without the addition of amaranth flour, depending on the time of rotation of the rotor.

Figure 1 shows the decrease of viscosity curves in the plane graph with increasing concentration of amaranth flour. Thus, the increase in the concentration of amaranth flour leads to a decrease of the viscosity of the finished product. In Figure 2 was shown the dependence of the viscosity of yoghurts with the addition of TG, without the addition of amaranth flour, from time of rotation of the rotor.

It can be seen from Figure 2 that the increase in TG concentration shifts the viscosity curves in the upper part of the graph. The use of TG, therefore, allows to increase the viscosity of the final product. The dependence of the viscosity of the yoghurts on the concentration of amaranth flour is presented in Figure 3.

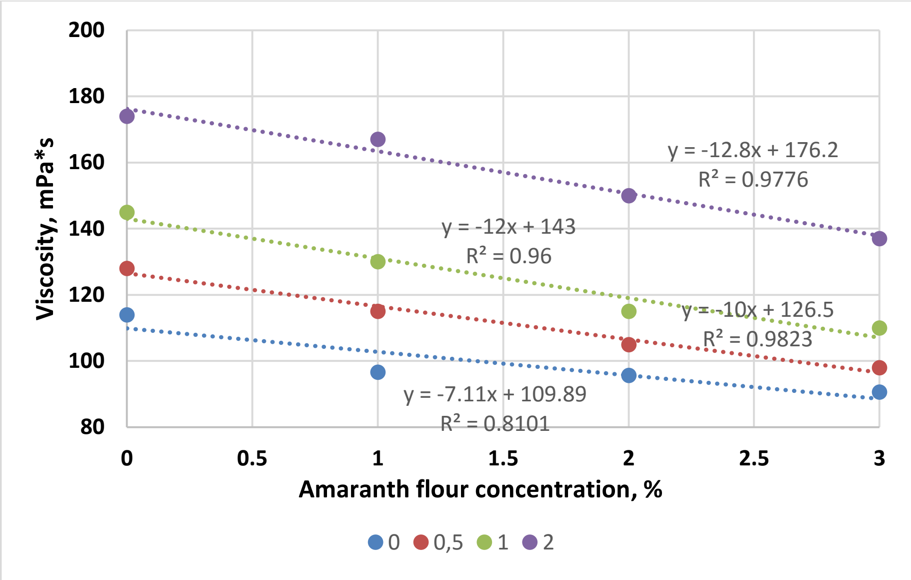

Figure 3. The viscosity of yoghurts with the addition of TG (see legend 0, 0.5, 1, 2 U/g) depending on the concentration of amaranth flour.

As can be seen from Figure 3, the use of amaranth flour reduces the viscosity of yoghurt. The dependencies are described by linear equations of the form $y = kx + b$, where k is the angle of inclination of a straight line, characterizes the rate of change of the dependent variable. As can be seen from the graphs Figure 3, the rate of the yoghurt viscosity change depending on the concentration of amaranth flour ranges from -7 to -12 mPa*s/% at concentrations of amaranth flour 0 – 3% w/v.

The dependence of the viscosity of the yoghurts on the concentration of TG is presented in Figure 4.

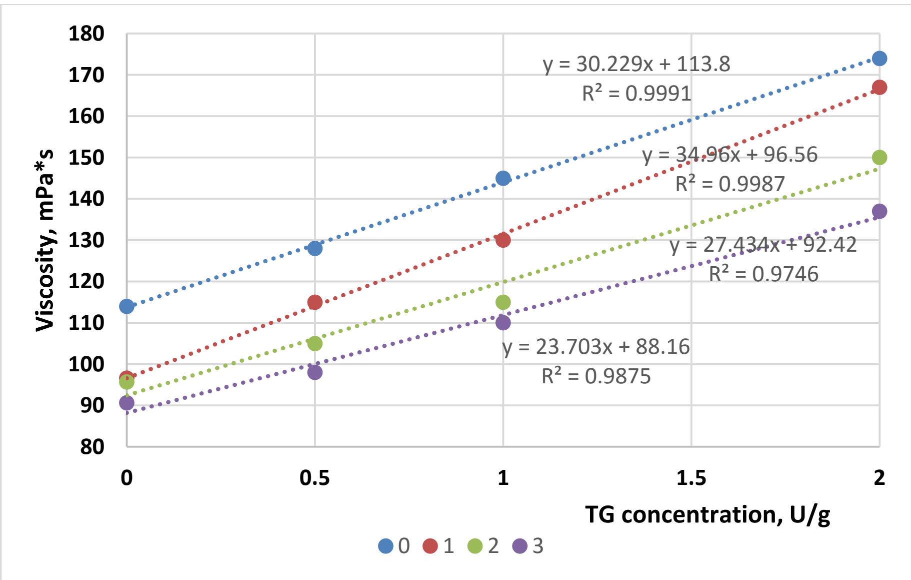

Figure 4. The viscosity of yoghurts with the addition of amaranth flour (see legend 0, 1, 2, 3 % w/v) depending on the concentration of TG.

As can be seen from Figure 4, the use of TG increases the viscosity of yoghurt. The dependencies are described by linear equations of the form $y = kx + b$. As can be seen from the graphs Figure 4, the rate of the yoghurt viscosity change depending on the concentration of TG is between 23 to 30 mPa*s/(U/g) in TG concentration range 0 – 2 U/g. Analyzing the data of Figure 3 and 4, it can be concluded that among the studied samples use of TG gives a higher positive effect on the viscosity of yoghurt due to the protein binding effect. In comparison with this, the negative influence of amaranth flour, associated with the thinning effect of ballast substances of flour, – mainly polysaccharides – was less. In other words, the use of TG allows to override the negative effect of amaranth flour on the viscosity of the final product and get the yoghurt of the required viscous parameters.

The effect of additives on the textural properties of yoghurt

Prepared samples were analyzed in accordance with p. 2.3. The data obtained are presented in table. 2.

Table 2. The dependence of the strength of the yoghurts on the concentration of amaranth flour and TG.

Amaranth flour concentr., %	TG concentration, U/g			
	0	0,5	1	2
0	5.30±0.22	5.15±0.21	5.08±0.24	5.31±0.20
1	4.76±0.18	5.39±0.20	4.97±0.15	5.03±0.18
2	3.82±0.41	4.99±0.21	5.29±0.13	5.76±0.25
3	5.03±0.19	4.83±0.21	5.40±0.22	5.50±0.25

As can be seen from table 2, the addition of TG causes a more pronounced change in the strength of the yoghurt when using amaranth flour in concentrations of 2 % and 3 %. In the control sample without amaranth flour and the yoghurt sample with amaranth flour at a concentration of 1%, this change is virtually nonexistent. The obtained data demonstrate the success of applying TG to increase the strength of yoghurt with amaranth flour in concentrations of 2 % and 3 %.

The effect of additives on the microstructure of yoghurt

Preparation of samples of the yoghurt and the analysis were carried out in accordance with p. 2.4. The data obtained are presented in Figure 5.

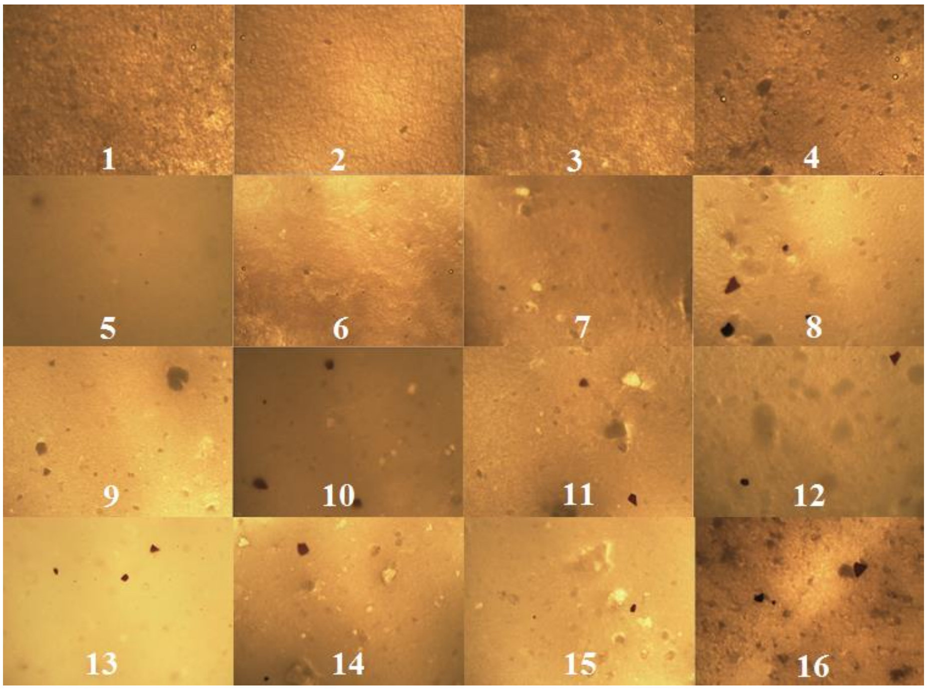

Figure 5. Photomicrographs of yoghurt samples with 40x magnification (see table 1 for yoghurt samples 1 – 16 notation).

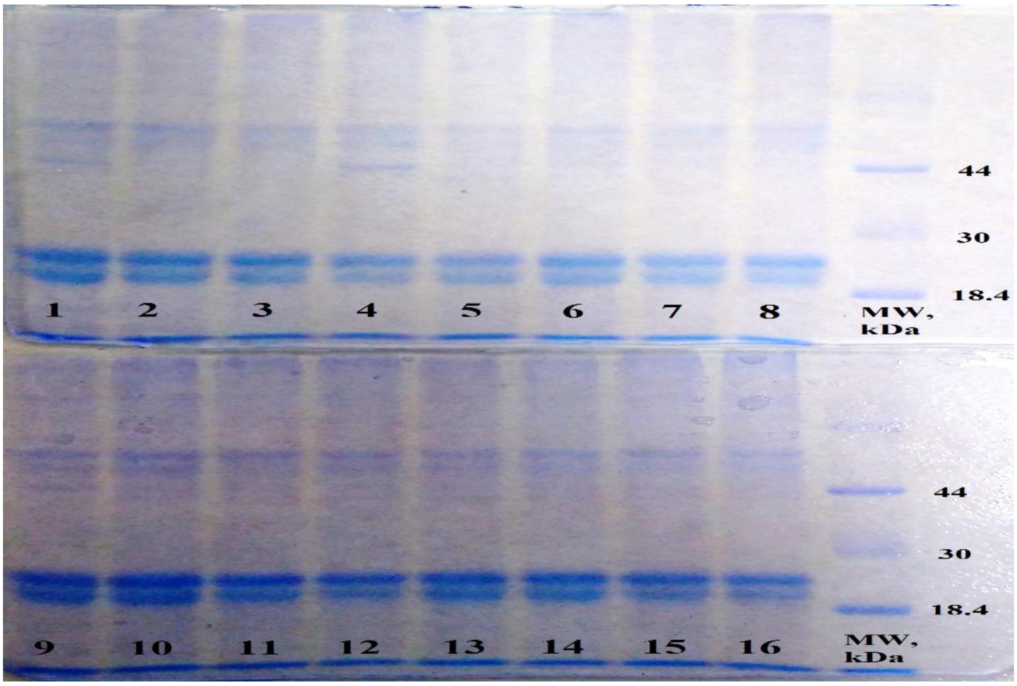

Figure 6. Electrophoregrams of yoghurt samples (see table 1 for yoghurt samples 1 – 16 notation).

3.4. The results of electrophoresis of yoghurt

Electrophoretic studies were carried out in accordance with p. 2.5. The data are presented in Figure 6.

In Figure 6 bands corresponding to casein (20 – 26 kDa) present. The bands corresponding to serum proteins (14 – 18 kDa), are not visible, which indicates the denaturation resulting from thermal treatment of milk prior to fermentation. The data peaks replotted were represented in Figure 7.

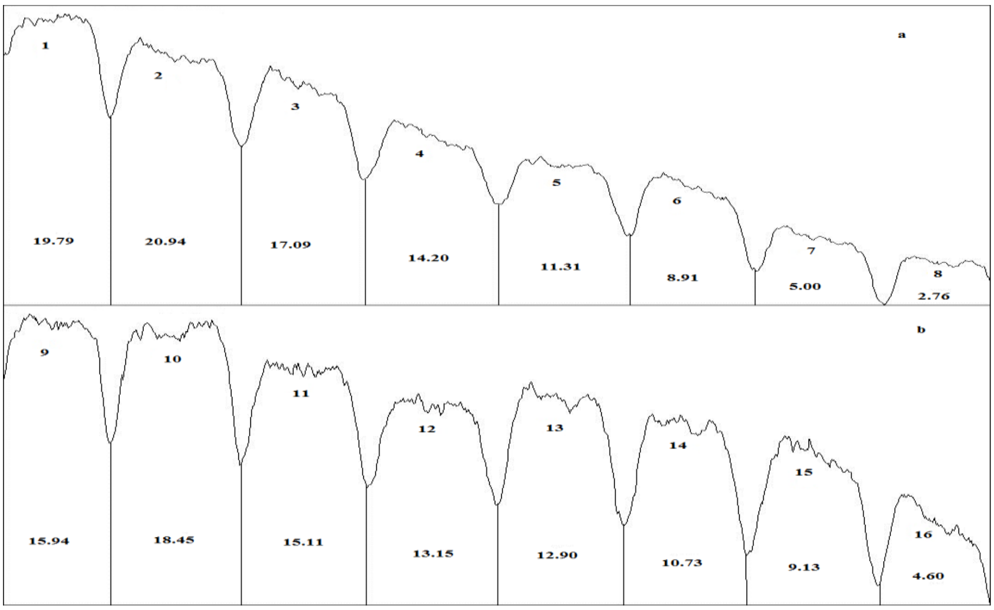

Figure 7. Replotted band peaks percentage of SDS-PAGE analysis of yoghurt samples (see table 1 for yoghurt samples 1 – 16 notation)

It can be seen lower bands intensity when use TG compared to control (bands 3, 4 compared to 1, bands 6 –8 compared to 5, bands 11, 12 compared to 9 and bands 14 – 16 compared to 13), which indicates binding effect.

3.5. Sensory evaluation

Sensory analysis was conducted accordingly to p. 2.6. Scores are presented in table 3.

Table 3. Sensory characteristics of yoghurt samples.

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Syneresis	8.5^{c}	9.5^{a}	9.5^{a}	9.5^{a}	8.4^{c}	9.5^{a}	9.4^{a}	9.3^{a}	8.2^{c}	9.4^{a}	9.3^{a}	9.3^{a}	8.0^{c}	9.3^{a}	9.3^{a}	9.2^{a}
Color	9.4^{a}	9.5^{a}	9.5^{a}	9.3^{a}	9.5^{a}	9.4^{a}	9.4^{a}	9.3^{a}	9.4^{a}	9.5^{a}	9.4^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.2^{a}
Firmness	8.5^{c}	9.5^{a}	9.6^{a}	9.5^{a}	8.4^{c}	9.5^{a}	9.5^{a}	9.4^{a}	8.3^{c}	9.4^{a}	9.5^{a}	9.5^{a}	8.2^{c}	9.3^{a}	9.4^{a}	9.4^{a}
Flavor	9.0^{b}	9.6^{a}	9.5^{a}	9.4^{a}	9.0^{b}	9.5^{a}	9.4^{a}	9.4^{a}	8.9^{b}	9.4^{a}	9.4^{a}	9.4^{a}	8.8^{b}	9.3^{a}	9.3^{a}	9.2^{a}
Distribution of ingredients	9.4^{a}	9.4^{a}	9.3^{a}	9.2^{a}	9.3^{a}	9.3^{a}	9.2^{a}	9.2^{a}	9.2^{a}	9.2^{a}	9.2^{a}	9.1^{a}	9.1^{a}	9.1^{a}	9.1^{a}	9.1^{a}
Acidity	9.5^{a}	9.6^{a}	9.5^{a}	9.5^{a}	9.4^{a}	9.5^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.3^{a}	9.3^{a}	9.4^{a}	9.3^{a}	9.2^{a}
Sweetness	9.6^{a}	9.5^{a}	9.5^{a}	9.4^{a}	9.5^{a}	9.5^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.4^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.3^{a}	9.2^{a}	9.2^{a}

a,b,c Means with different letter differ significantly($P < 0.05$)

All samples were judged as acceptable. No significant changes were found in “color”, “acidity”, “distribution of ingredients”, or “sweetness” for either type of yogurt. Control sample and sample with amaranth and without TG were got almost the same scores at all characteristics. The scores for “syneresis”, “firmness”, and “flavor intensity” showed significant differences for control, samples with amaranth, and samples with TG. Yoghurts with TG were got the best scores at these characteristics.

4. Conclusions

Amaranth flour and TG were added in sour milk concomitantly with starter. The influence of these additives on stirred yoghurt structure was investigated. Rheological measurements show viscosity depends on additives concentration linearly on equation $y = kx + b$,wherein k is the rate of viscosity change. k is between -7…-12 range if use amaranth flour in the investigated range of concentrations 0 – 3 % w/v. The use of TG contributes to yoghurt viscosity increasing: k = 23…30 in the investigated range of TG concentrations 0 – 2 U/g. Thus, use of TG allows overlapping the negative effect of amaranth polysaccharides on yoghurt viscosity and can be recommended in yoghurt with amaranth addition manufacture. Texture measurements show more pronounced effect of TG on yoghurt gel strengthening if use amaranth flour in 2 % and 3 % concentrations compared to control and 1 %. It also gives good opportunity to use TG in combined yoghurt production. Results of optical microscopy shows positive effect of TG in 0.5 U/g and 1 U/g concentrations on the yoghurt surface, it becomes smoother than control. Exceeding of these optimal concentrations leads to protein aggregates formation that break structure homogeneity. Results of electrophoresis clearly indicate TG binding effect: caseins bands become less bright. Whey protein bands are not observed due to whey protein denaturation during milk heat treatment. All yoghurt samples were judged as organoleptically acceptable, moreover samples with TG were got the best values for “syneresis”, “firmness”, and “flavor intensity”. The data obtained indicate the possibility of applying amaranth flour and TG for stirred yoghurt manufacture.

5.References

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