THE EFFECT OF PROCESSING PARAMETERS ON THE STRUCTURE OF FERMENTED MILK PRODUCTS WITH TRANSGLUTAMINASE ADDITION

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This study is concerned with the effect of concentration of transglutaminase (TG), content of milk fat and starter culture type (probiotic and kombucha) on the structure of fermented milk products.

The application of TG significantly improved textural characteristics of the fermented milk products. The firmness of the samples produced from milk with 0.1g100g⁻¹ and 0.9g100g⁻¹ fat content with probiotic starter were by 33% and 17.6% higher, respectively, compared to the control samples. During ten days of storage, the value of the hysteresis loop area of all samples produced from milk with 0.9g100g⁻¹ fat content with TG addition, decreased by 14%.

KEY WORDS: fermented milk products, transglutaminase, texture, microstructure

INTRODUCTION

The present trend in dairy technology is the production of "low-fat" (0.5-2% fat) and "non-fat" (less than 0.5% fat) yoghurt. A serious problem is how to achieve the specific properties that are an important indicator of yoghurt quality, homogeneous consistency without syneresis. Rheological properties of yoghurt gel depend on various factors: milk composition, milk heat pretreatment, type and quantity of starter culture, fermentation temperature, ingredients, and storage conditions (1).

To produce yoghurt with low energy value, good physical and chemical properties it is possible to use enzyme transglutaminase (TG), which is a potential tool to increase the formation of covalent cross-links in proteins which as a major component of food.

There are two different ways for TG application: simultaneously with the starter culture or prior to the fermentation. The TG addition prior to the fermentation demands an additional process time as well as thermal inactivation of the enzyme (2, 3). Generally, heat treatment of the milk prior to cross-linking is necessary because the reactivity of TG is very low in unheated or pasteurized milk, despite the high reactivity of the casein (2, 3, 4).
Guyot and Kulozik (2011) found that stirred skim milk yoghurts fortified with TG enzyme and addition of skim milk powder (SMP) have a higher viscosity and lower serum loss compared to yoghurt samples produced with addition of untreated SMP.

Sanli et al. (2011) reported that TG addition did not cause significant changes of chemical properties of yoghurt (6). The microstructure of the samples with TG is characterized by a more regular and dense protein network than of the samples without TG (7).

The aim of this study was to investigate the influence of the TG addition and different starter culture (probiotic and kombucha) on the textural and rheological properties and microstructure of fermented milk products manufactured from milk with 0.1g100g⁻¹ and 0.9g100g⁻¹ fat content after the production and ten days of storage.

**EXPERIMENTAL**

**Milk**

The homogenized (130 bar, 55°C) and pasteurized (82°C, 40 s) milk with 0.1g100g⁻¹ fat (milk 1) and 0.9 g100g⁻¹ fat (milk 2) was obtained from AD Novi Sad Dairy, Novi Sad, Serbia. The milk composition was as follows: milk 1 - 9.08 g100g⁻¹ total solids, 3.18g100g⁻¹ total proteins and 4.95g100g⁻¹ lactose, and milk 2 - 8.85 g100g⁻¹ total solids, 3.06 g100g⁻¹ total proteins and 4.74 g100g⁻¹ lactose.

**Starter culture**

The probiotic starter culture freeze–dried, direct vat set ABT-4-Probio-TecTM (Lactobacillus acidophilus-5, Bifidobacterium BB-12, Streptococcus thermophilus (Chr. Hansen A/S Denmark) was applied in the concentration of 0.005% (w/w).

Kombucha inoculum was prepared according to the procedure by Malbaša et al. (2009) (8). The Kombucha inoculum was used in the concentration of 10% (v/v) (pH = 3.07±0.02).

**Transglutaminase**

The experiments were carried out using the microbiological TG isolated from the Streptoverticillium strains, Activa MP, with a declared activity of 100 units per gram of powder (Ug⁻¹), purchased from Ajinomoto Co. Inc. (Hamburg, Germany).

**Manufacturing of fermented milk products**

The first series was produced from milk 1 by adding TG in the concentrations of 0.02, 0.06 and 0.12% (w/w) at 40°C, which was inactivated after 2 h by heating the milk at 80°C for 1 min. The heating was followed by cooling to 43°C, and probiotic starter was added. The second series was produced from milk 2 and TG addition in the concentration of 0.02% (w/w). After that the probiotic starter or kombucha inoculum was added to the milk, and the further process was the same as in the first series. The control samples (without TG) were produced in both series. The fermentation was stopped when the pH
4.6 was reached. Finally, the gels were cooled to 8°C, homogenized by mixing and packed in plastic containers. Each trial was repeated three times.

**Physicochemical analyses**

All samples were analyzed for total solids (TS) by oven drying, method ISO 6731, IDF 21: 2010; total proteins (TP) by Kjeldahl method SRPS EN ISO 8968-1:2008, and fat by the Gerber method ISO 488:2008. The pH was measured using a pH-meter (Eco-Scan pH 6 Eutech Instruments, Netherlands).

**Textural properties**

The textural properties of the fermented milk products were analyzed by Texture Analyser TA.HD.plus (Stable Micro System, Godalming, U.K.) through a single compression test, using a back extrusion cell (A/BE) disk (diameter 35 mm; distance 30 mm; speed 0.001 ms⁻¹) and an extension bar, using 5 kg load cell at 5°C.

**Viscosity measurement**

The viscosity of the fermented milk products were measured at 5°C using the viscometer HAAKE RheoStress 600HP (Karlsruhe, Germany) fitted with a Z20 DIN cylinder (first series) and with a PP60Ti sensor (gap 1mm) (second series). Replicate measurements were done independently for each sample, and data processing was performed using a RheoWin Pro software package (Version 2.94, Thermo Haake, Karlsruhe, Germany). The thixotropy test was initially applied to characterize the flow behavior of the fermented milk products. Shear stress was recorded at increasing shear rates from 0 to 40 s⁻¹ (upward flow curve), followed by decreasing shear rate from 40 to 0 within 50 s⁻¹ (downward flow curve). The difference in the area under the upward and downward flow curves (ΔA or hysteresis loop area) was calculated and expressed as unit (U) (9).

**Microstructure**

The microstructure of the fermented milk products was examined by SEM technique, using a Joel, JSM-6460LV Scanning Electron Microscope, Oxford Instruments, UK (7).

**Statistical analysis**

All experiments were carried out in triplicate and all data were expressed as mean values and standard deviation. Statistical and graphical analyses of the results were carried out with the computer software program "Statistica 9.1” and "Origin Pro 8.5.1”.
RESULTS AND DISCUSSION

Characteristics of fermented milk products after the production

The milk composition and the type of applied starter culture significantly influenced the textural characteristics of all fermented milk products (Table 1). The obtained values of firmness (17 g), consistency (450 gs), cohesiveness (-11 g) and index of viscosity (-9.91 gs) in control samples (Y1) were the lowest in the series 1. The firmness of the samples with the addition of TG in the concentration of 0.02% (w/w) was approximately by 35% higher compared to that of the control sample. The kombucha fermented milk products manufactured from the milk with 0.9 g100g⁻¹ fat content had significantly lower firmness and consistency than the samples produced by probiotic starter culture. There were no significant differences in the textural characteristics among fermented milk products with probiotic starter produced from the milk with different fat content.

Table 1. Textural characteristics of fermented milk products manufactured from the milk with 0.1 g100 g⁻¹ and 0.9g100 g⁻¹ fat content with addition of TG

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Firmness (g)</th>
<th>Consistency (gs)</th>
<th>Cohesiveness (g)</th>
<th>Index of viscosity (gs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1</td>
<td>17.0±1.19</td>
<td>450±35</td>
<td>-11.0±1.34</td>
<td>-7.91±3.2</td>
</tr>
<tr>
<td>C1TG Y1</td>
<td>25.5±0.30</td>
<td>660±4</td>
<td>-17.0±0.07</td>
<td>-30±0.40</td>
</tr>
<tr>
<td>C2TG Y1</td>
<td>26.0±0.30</td>
<td>662±10</td>
<td>-17.5±0.02</td>
<td>-30.9±0.41</td>
</tr>
<tr>
<td>C3TG Y1</td>
<td>24.0±0.14</td>
<td>630±3</td>
<td>-16.5±0.09</td>
<td>-26±0.13</td>
</tr>
<tr>
<td>Y2</td>
<td>17.8±3.0</td>
<td>485.1±58.1</td>
<td>-12.3±2.4</td>
<td>-9.7±2.0</td>
</tr>
<tr>
<td>C1TG Y2</td>
<td>21.6±2.4</td>
<td>583.1±61.0</td>
<td>-18.9±3.0</td>
<td>-31.9±3.4</td>
</tr>
<tr>
<td>KFM2</td>
<td>14.9±1.7</td>
<td>415.3±52.8</td>
<td>-7.2±1.5</td>
<td>-2.2±0.8</td>
</tr>
<tr>
<td>C1TGBKFM2</td>
<td>23.9±1.1</td>
<td>555.9±45.3</td>
<td>-18.1±1.8</td>
<td>-27.0±1.0</td>
</tr>
</tbody>
</table>

* Fermented milk products manufactured from the milk with 0.1g100g⁻¹ fat with probiotic starter: Y1- control sample 1, C1TGY1, C2TGY1, C3TGY1 - samples produced with TG addition in the concentration of 0.02, 0.06 and 0.12% (w/w), respectively.
Fermented milk products manufactured from the milk with 0.9g100g⁻¹ fat: Y2 - control sample 2 with probiotic starter, C1TG Y2 - sample produced with TG addition in concentration 0.02% (w/w), KFM2 - control sample 2 with kombucha inoculum and C1TGBKFM2 - sample 2 with the kombucha inoculum and TG addition in the concentration 0.02% (w/w).

Characteristics of fermented milk products during the storage

The changes of the firmness of the samples with 0.02% and 0.06% (w/w) TG had the similar trend and decreased during 10 days of storage (Fig. 1a). In the second series, of the fermented milk samples without TG addition (Y2 and KFM2) produced from the milk with the 0.9 g100g⁻¹ fat content had a similar value of the firmness during the storage (Fig. 1b). The addition of TG in the concentration of 0.02% (w/w) significantly improved the firmness of the samples during the storage (Fig. 1b).
Figure 1. The effect of the TG concentration on the value of firmness of fermented milk products from the milk with a) 0.1 g 100g⁻¹ and b) 0.9 g 100g⁻¹ fat content during the storage.

The hysteresis loop area of the sample with TG addition was significantly higher compared to that of the control sample in series 1 (Fig. 2a), and it was the highest for the sample with 0.12% (w/w) TG after 10 days of storage -1991U, while for the sample with the lower concentration of TG decreased by 7% (0.06% (w/w) TG) and by 18% (0.02% (w/w) TG).

Figure 2. Changes of the hysteresis loop area during the storage of different fermented milk products manufactured with addition of TG from the milk of a) 0.1 g 100g⁻¹ and b) 0.9 g 100g⁻¹ fat content.

The HLA value of the kombucha fermented milk product was significantly lower compared to that of the probiotic fermented milk product in both series. The addition of TG in a minimal concentration (0.02%) increased the value of HLA by 20% in probiotic fermented milk product compared to the control sample. The hysteresis loop area of the probiotic and kombucha fermented milk products (with and without TG) slightly decreased during 10 days of storage. The highest value was calculated for the kombucha fermented milk with TG -2500 U, after 10 days of storage (Fig. 2b).
It could be concluded that the fermented milk products with TG addition manufactured from the milk with 0.9g 100g⁻¹ fat content had better stability during 10 days of storage compared to the samples produced from the milk with 0.1g 100g⁻¹ fat content.

**Microstructure of fermented milk products**

Yoghurt is a protein network formed by casein micelles entrapping serum and fat globules (11). Textural properties of fermented milk products are in accordance with microstructure of the samples (Fig. 3). The addition of TG in the concentration of 0.02 % (w/w) significantly improved the gel structure. The gel was more compact in the samples produced with probiotic starter with or without TG, compared with the corresponding kombucha fermented milk products.

**Figure. 3.** Microstructure of fermented milk products with: a) probiotic starter with addition of 0.02 % (w/w) TG, 0.1g 100g⁻¹ fat (C1TGY1) b) probiotic starter, 0.9 g 100g⁻¹ fat (Y2) c) kombucha inoculum, 0.9 g 100g⁻¹ fat (KFM2) and d) kombucha inoculum with addition of 0.02 % (w/w) TG (C1TGKFM2).
CONCLUSION

The application of TG in the manufacture of fermented milk products resulted in a significant improvement of the structural properties. The firmness and hysteresis loop area of the samples produced with the addition of TG changed during the ten days of storage. Even, the minimal concentration of TG of 0.02% improved the firmness and structure of the samples during the storage.

Acknowledgements

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REFERENCES

УТИЦАЈ ПРОЦЕСНИХ ПАРАМЕТРА НА СТРУКТУРУ ФЕРМЕНТИСАНИХ МЛЕЧНИХ ПРОИЗВОДА СА ДОДАТКОМ ТРАНСГЛУТАМИНАЗЕ

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У раду је испитан утицај трансглутаминазе (ТГ), садржаја млечне масти и врсте стартер културе на структурне карактеристике ферментисаних млечних производа. Применом ТГ значајно се побољшавају текстурална својства ферментисаних млечних производа. Чврстоћа узорака произведенih из млека са 0,1g100g⁻¹ масти и 0,9g100g⁻¹ масти са пробиотском културом већа је за 33%, односно 17%, у поређењу са контролним узорком. Током десет дана складиштења вредност површине хистерезисне петље узорака произведенih из млека са 0,9g100g⁻¹ масти опада просечно за 14%.

Кључне речи: ферментисани млечни производи, трансглутаминаза, текстура, микроструктура

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