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IMPROVEMENT OF PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF KOMBUCHA FERMENTED MILK PRODUCTS BY ADDITION OF TRANSGLUTAMINASE AND WHEY PROTEIN CONCENTRATE

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The objective of this work was to investigate the effect of addition of transglutaminase (TG-0.02%, w/w) and whey protein concentrate (WPC-0.03%, w/w), on quality of kombucha fermented milk product. Samples were prepared from pasteurized semi-skim milk (0.9%, w/w fat) and kombucha inoculum (10%, v/v). The pH values were measured during the fermentation of milk (lasted until reached 4.5). Syneresis, water holding capacity and the product texture (firmness and consistency,) were assessed after production. Rheological properties of kombucha fermented milk samples were measured during ten days of storage.

The sample containing TG had the lowest syneresis (21 ml), the highest water holding capacity (62%) and the highest textural characteristics (firmness - 23.99g, consistency - 626.54gs) after production. The addition of WPC to milk improved the rheological properties, while the addition of TG improved it even to a significantly greater extent after the production and during 10 days of the storage.

KEY WORDS: fermented milk products, kombucha, transglutaminase, whey protein concentrate, rheology

INTRODUCTION

The use of kombucha as non-conventional starter culture for fermented dairy product, have been a topic of research in the resent years (1-5)**.** Kombucha is a sweetened tea beverage which is fermented by a symbiosis of bacteria and yeast embedded within cellulosic pelliculs. It has been shown that the major bacterial genus was *Gluconobacter* present <85% in the most samples, *Acetobacter* detected <2%. A prominent *Lactobacillus* population was also identified up to 30%. The yeast population was found to be dominated by *Zygosaccharomides* at 95% in kombucha (6). Some authors found that different starter cultures (yoghurt starter, probiotic or kombucha) had a significant effect on milk fermentation, textural characteristics and rheological properties of the final product. Milk

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fermentation with kombucha as starter is slower compared to the production of probiotic fermented milk beverages (4, 5, 7).

Many studies were aimed to analyse the textural characteristics and rheological changes in fermented milk products with addition of transglutaminase (8-10). The enzyme transglutaminase (TG) is a transferase which catalyses both inter and intra-molecular isopeptide bonds between proteins by cross-linking of the amino-acid residues of glutamine and lysine (8). Activity of TG and degree of polymerisation of product in milk depends on different factors during milk fermentation: type and composition of milk, temperature and time of fermentation, pH value. Neve et al. (2001) investigated the effect of TG treatment on microbiological and rheological characteristics of set skim milk yoghurt (9). They concluded that yoghurt starter culture requires longer fermentation time and gel strength of the enzyme treated yoghurt was increasing during the first 5 weeks of storage. They assumed that the TG is still active during this period.

Besides, other ingredients such as whey protein concentrate (WPC) are commonly used for fermented milk production with the aim to change the ratio of casein to whey proteins, which affects the mesh size and structure of protein network (10).

The main objective of this study was to determine the influence of TG and WPC on milk acidification and quality of kombucha fermented milk products (syneresis, water holding capacity, texture and rheological properties).

EXPERIMENTAL

Materials

Homogenized and pasteurised cow milk from AD Imlek, Division Novi Sad Dairy, was used for the production of kombucha fermented milk beverages. The composition of milk was as follows: fat content – 0.9 g/100g, total solids – 10.23 g/100g, total proteins – 3.15 g/100g and lactose – 4.74 g/100g.

Kombucha was cultivated on a black tea (*Camellia sinensis* – oxidized, 1.5 g/L) with saccharose concentration of 70 g/L. The tea was cooled to room temperature, after which inoculum from a previous fermentation was added in concentration of 100 mL/L. The incubation was performed at 29^{o}C for 7 days (2). The obtained kombucha tea was used as non-conventional starter culture for inoculating the milk.

The TG of microbial origin (*Streptoverticillium mobaraense*) preparation Activa MP, with declared activity of 100 U/g, was purchased from Ajinomoto Co. Inc. (Hamburg, Germany), and it was added to milk to the concentration of 0.02% (w/w).

WPC (Lactomin 80S, LACTOPROT, Aplenandisch Milchindustrie und Handels-GMBH, Germany**)** contained: 78.5% (w/w) proteins, 4.5% (w/w) ash, 4.0% (w/w) fat and 5.0% (w/w) moisture, was applied in a concentration of 0.3% (w/w).

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Methods

Kombucha fermented milk beverage manufacturing. Three kombucha fermented milk samples were manufactured: first from milk with TG, second from milk with the WPC and third control sample. TG was added to milk and activated at 40°C for two hours. After mixing, the sample with activated TG was treated at 80°C for 1 min, cooled to 43°C, and inoculated with the kombucha inoculum - 10% (v/v) (sample 10K, TG). The sample with WPC (10K, WPC) was treated at 80°C for 15 min, followed by cooling (43°C), was also inoculated with the kombucha inoculum. The control sample (10K) did not contain any of the two ingredients. In all cases, the fermentation was stopped when the pH 4.5 was reached. Finally, the gels were cooled to 8°C, mixed and packed in plastic containers. Each trial was repeated three times.

The following chemical properties: total solids (TS) (11), total protein (TP) by Kjeldahl method (12), fat by Gerber method (13) were determined in all samples after production. The pH was measured on a pH-meter (EcoScan pH 6 Eutech Instruments, Netherlands). Syneresis was measured by whey separation and it was expressed in mL of whey separated during filtration of 50 mL sample for 3 hours, at room temperature (14). Water-holding capacity (WHC) of the samples was determined according to a procedure introduced by Guzman-Gonzalez *et al.* (15).

Textural properties (firmness and consistency) of the kombucha fermented milk samples were analysed by Texture Analyser TA.HD.plus (Stable Micro System, Godalming, England) through a single compression test, using a back extrusion cell (A/BE) disc (diameter 35 mm; distance 30 mm; speed 0.001 m/s) and an extension bar, using 5 kg load cell at 5ºC. Using the options Return to Start, a trigger force of 10 g was applied.

Rheological measurement. Rheological properties of fermented milk samples were measured at 5°C using a viscometer HAAKE RheoStress 600HP (Karlsruhe, Germany), fitted with a PP60Ti sensor (gap 1 mm). The samples of kombucha fermented milk products were taken with a spatula and placed on the rheometer plate and were allowed to achieve temperature of 5°C. For each sample, replicate measurements were done independently, and data processing was performed using a RheoWin Pro software package (Version 2.94, Thermo Haake, Karlsruhe, Germany). The flow curves were obtained by registering shear stress at the shear rate which was increased from 0 to 200 s^{-1} in 180 s, held constant at 200 s^{-1} until the total system destruction, and decreased from 200 to 0 s^{-1} in 180 s. The other rheological parameters considered were the area under the upward (A_{up}) flow curve, the area difference under upward and downward (A_{down}) flow curves (ΔA or hysteresis loop area) (5). The magnitude of gels thixotropy was estimated as the coefficient of thixotropic breakdown, Kd, which is defined as the ratio of the hysteresis area to the area beneath the ascending shear curve $Kd=\Delta A/A_{up}$ (16). The rheological characteristics of samples were measured after production and during ten days of storage.

Statistical analysis. The data were expressed as means and standard deviation using the software program STATISTICA version 6 (2001) (StatSoft Inc, Tulsa, OK, USA) (17).

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RESULTS AND DISCUSSION

The effect of TG and WPC on physicochemical and textural characteristics of samples

The pH values of the samples (4.5) were uniform in all fermented milks after production. The fermentation time of control sample (10K) lasted 9.3 hours (Table 1). In the sample with TG the fermentation lasted 40 min longer and 10 min shorter compared to the fermentation in the control sample and sample with the WPC, respectively. These results are in accordance with the literature data (6, 18, 19) showing increased fermentation times for fermented milk samples with addition of TG. Chemical analyses showed that total solids of kombucha fermented milk products varied from 10.92% (sample 10K) to 11.17 4g/100g (sample 10K, WPC) (Table 1). The protein content in the samples ranged from 2.98% (10K) to 3.25% 10K, WPC).

The syneresis of the sample containing TG was 21 ml. In the other samples, the syneresis was higher by 4 ml (10K, WPC) and by 8 ml (control sample). A larger quantity of separated whey was the result of formation of the bonds in the protein matrix to give a non-homogenous gel. The sample with addition of TG had the highest value of WHC (62.5%) and the highest values of textural characteristics (firmness -23.99g, consistency 626.53 gs). It was the result of a stronger structure due to the addition of TG, which was also shown by the lower syneresis. These results are in accordance with the literature data (10, 20), where the authors found that WHC and textural characteristics of the control probiotic yoghurt produced from milk of 0.1% fat content significantly were lower compared to the same characteristics of the sample containing TG.

Table 1. Physicochemical and textural characteristics of kombucha fermented milk products manufactured using TG and WPC

Sample	Time (hour)	TS (%)	TP (%)	Syneresis (ml)	WHC (%)	Firmness (g)	Consistency (gs)
10K	9.3	10.92	2.98	29	35.5	13.60	383.88
10K, TG	10.0	10.97	3.20	21	62.5	23.99	626.53
10K, WPC	10.2	11.17	3.25	25	58.0	14.89	416.03

Rheological characteristics

The rheological characteristics of samples during 10 days of storage are presented in Fig. 1. The appearance of hysteresis area in the plot of shear rate meant that all samples exhibited typical shear thinning flow behaviour. The Kombucha fermented milk product containing TG had significantly higher yield stress (65.3Pa) than samples without TG addition – control sample (1.14 Pa).

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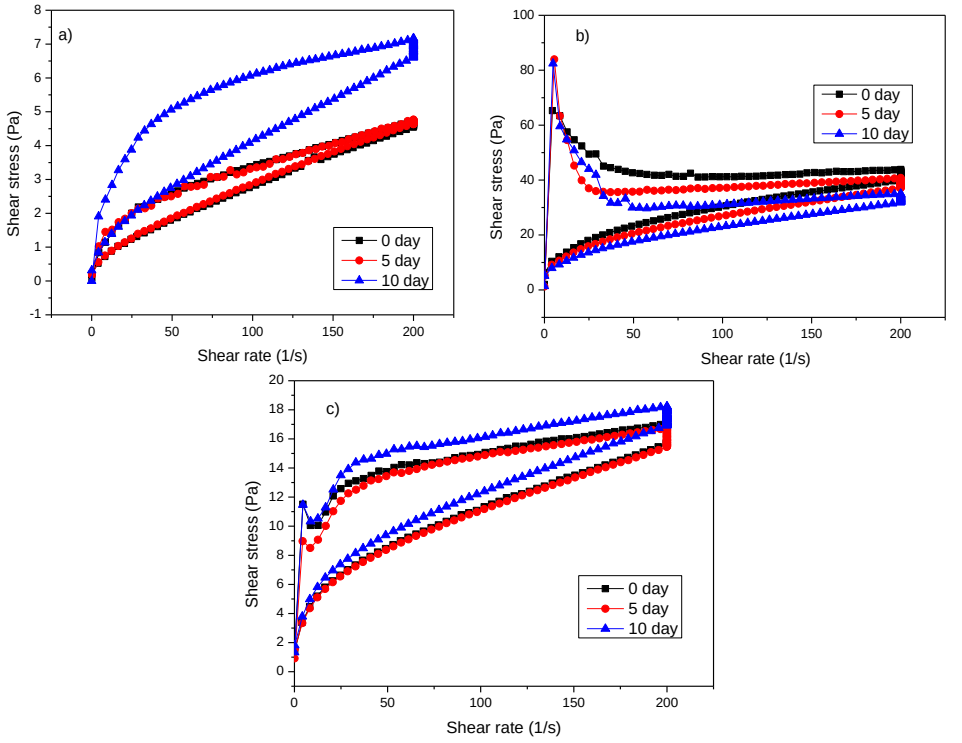

Figure 1. Flow curves of kombucha fermented milk products: a) Control sample (10K); b) Sample containing TG (10K, TG); c) Sample with addition of WPC

The hysteresis loop area (ΔA) is the indicator of the yoghurt structural breakdown and rebuilding (a degree of thixotropy) during shearing (5, 22). Fermented milk product containing TG produced with kombucha inoculum showed the highest hysteresis loop area (3000 Pa/s). The hysteresis loop area of kombucha fermented milk with WPC and control sample were lower compared with HLA of the sample containing TG (Fig. 2). The differences in hysteresis area between the samples were results of higher total proteins in sample with WPC (10K, WPC), as well as of the formation of strong intermolecular bonds in the protein matrix with addition of TG to the kombucha fermented milk product (10K, TG). The obtained results for the hysteresis loop area of fermented milk samples with TG with kombucha inoculum were higher compared with the literature data (4), where the values of hysteresis area of stirred probiotic yoghurt produced from milk of 0.9% fat with 0.02% TG addition varied from 1400 to 1500 Pa/s during ten days of storage. The magnitude of gels thixotropy was estimated also as the coefficient of thixotropic breakdown (Kd) or the relative hysteresis area (16). The coefficient of thixotropic breakdown is an index of the energy needed to destroy the structure of the system. The highest value of the coefficient of thixotropic breakdown was noticed for the samples

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produced with addition of TG (0.4). So, the sample with TG needed the highest energy to breakdown structure. The coefficient of thixotropic for the sample with addition of WPC was similar during ten days of storage. The experimental data indicated that the sample with TG and WPC during storage showed a structure which was more stable compared to the control sample.

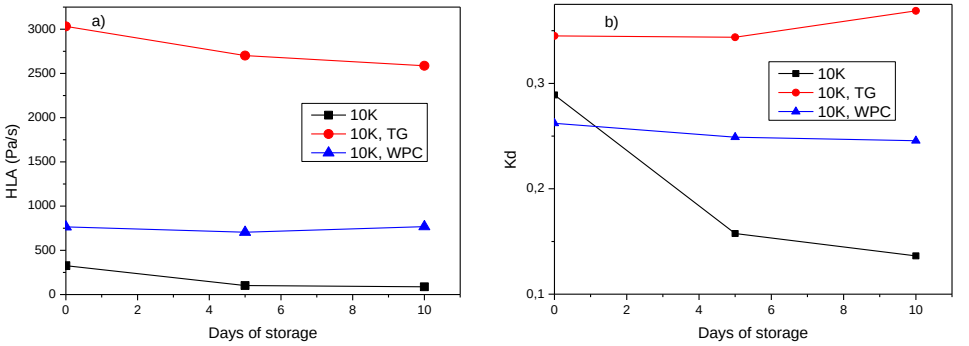

Figure 2. Rheological properties of kombucha fermented milk products during 10 days of storage**:** hysteresis loop area and b) coefficient of thixotropy

CONCLUSION

The fermentation time for kombucha fermented milk sample with addition of transglutaminase and whey protein concentrate lasted longer compared to the control sample. The addition of WPC to milk at a level of 0.3% contributed to the formation of kombucha fermented milk product with improved physical characteristics and better textural properties, while the minimal concentration of TG (0.02%, w/w) produced even more favourable effects. The addition of TG to the kombucha fermented milk sample improved significantly the rheological characteristics. During ten days of storage, the structure of the samples containing TG and WPC was more stable compared to the control fermented milk product.

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ПОБОЉШАЊЕ ФИЗИЧКОХЕМИЈСКИХ И РЕОЛОШКИХ КАРАКТЕРИСТИКА КОМБУХА ФЕРМЕНТИСАНОГ МЛЕЧНОГ ПРОИЗВОДА СА ДОДАТКОМ ТРАНСГЛУТАМИНАЗЕ И КОНЦЕНТРАТА ПРОТЕИНА СУРУТКЕ

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У раду је испитан утицај додатка ензима трансглутаминазе и концентрата протеина сурутке на физичкохемијске и текстуралне карактеристике, и реолошка својства комбуха ферментисаног млечног производа добијеног из млека са 0,9% млечне масти. Ензим трансглутаминаза коришћен је у концентрацији од 0,02% (w/w), док је концентрат протеина сурутке додат у млеко у концентрацији од 0,3%(w/w). Контролни узорак је произведен уз додатак 10% комбуха инокулума (без додатка наведених ингредијената). Ферментација млека је прекинута при pH вредности 4,5. Синерезис, способност везивања воде и текстурална својства варијанти комбуха ферментисаних млечних производа анализиране су након производње, а реолошке карактеристике су испитане током 10 дана складиштења.

Резултати анализе показују да. узорак који садржи трансглутаминазу има најмању вредност синерезиса (21mL). Додатак концентрата протеина сурутке и трансглутаминазе има значајан утицај на физичкохемијске и текстуралне карактеристике испитаних узорака након производње. Реолошки параметри квалитета показују, да је гел комбуха ферментисаног млечног производа са додатком трансглутаминазе значајно стабилнији током 10 дана складиштења у односу на контролни узорак и узорак произведен применом концентрата протеина сурутке.

Кључне речи: ферментисани млечни производи, комбуха, трансглутаминаза, концентрат протеина сурутке, реологија

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