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CORRELATION OF THE MICROSTRUCTURE WITH VISCOSITY AND TEXTURAL PROPERTIES DURING MILK FERMENTATION BY KOMBUCHA INOCULUM

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The aim of this study was to examine the changes in the microstructure, textural properties and viscosity of the gel formed during milk fermentation with kombucha inoculum and to establish a relationship between the microstructure and these properties. The values of the analyzed characteristics were measured during the gelation at 42°C at the following pHs: 5.4, 5.1, 4.8 and 4.6. The microstructure analysis revealed disappearance of coarse cluster structure and appearance of finer casein micelles network during fermentation. The obtained results showed significant differences in their viscosity and textural properties during fermentation, which is in accordance with their microstructure. The correlation of the examined properties and microstructure of the gel was established.

KEY WORDS: kombucha, fermented dairy products, microstructure, viscosity, texture

INTRODUCTION

The role of milk and dairy products in recommended daily intake of nutritionally important components is very well known. Fermented milk products such as yoghurt are especially very important as a functional food. Novel studies have investigated the possibility of kombucha application (as non-conventional starter culture) and its interesting technological and nutritional aspects in manufacturing of fermented dairy products (1, 2). Kombucha is a symbiotic association of the yeasts (*Pichia*, *Zygosaccharomyces*, *Saccharomyces*, *Schizosaccharomyces*, *Saccharomycodes*, *Brettanomyces*, *Torulasporea* and *Candida*) and the acetic acid bacteria (*Acetobacter* and *Gluconobacter*), which has been applied for a fermentation of sweetened black or green tea (*Camellia sinensis*) for centuries (3, 4). Furthermore, kombucha can be cultivated on some other type of a tea, a dark beer, red wine, white wine, as well as on lactose and whey (5-7). The result of its metabolic activity is a refreshing beverage with sour taste and many health benefit compounds appears (8-13).

Milk gelation by lactic acid bacteria is the basic process for a number of fermented dairy products. The basic characteristics of yoghurt gel are the appropriate textural properties and viscosity, and inherent microstructure. Major structural changes during the

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yoghurt manufacturing occur on a casein micelle, which are due to a change of the pH value (14, 15). During fermentation, calcium ions are extracted, aggregation takes place (though the exact mechanism of gel forming is still not fully understood) and three-dimensional structure of the gel is formed (16). A maximum protein dissociation occurs at about pH 5.5. Then, as milk is further acidified, the amount of nonsedimentable casein decreases to zero at the pH 4.8, which is the phase when all of the casein is incorporated into the gel network (17).

Scanning electron microscopy (SEM) showed that the gel structure consists of casein micelles interconnected in protein chains and clusters (18-20). Casein micelles are of protein globule size from 50 - 500 nm, with an average diameter of approximately 200 nm, while the least visible particles in milk are casein submicelles, with a diameter of about 10-20 nm (21, 22). However, there are not sufficient data concerning the microstructure of yoghurt gel during fermentation process. McMahan et al. (23) examined the microstructural changes taking place during the milk fermentation using transmission electron microscopy (TEM). Their results showed aggregation of casein micelles and final compact spherical macromolecules at 40°C using glucono- δ -lactone. Harwalker and Kalab (24) reported microstructural differences between yoghurt samples produced from milk with and without previous heat treatment using SEM. Samples with previous heat treatment had a finer network than samples without heat treatment which had coarse network, and also had a firmer liquid phase and lower syneresis values. However, these authors did not examine the microstructure during fermentation. These results indicate opposite results obtained by TEM and SEM techniques, induced by different samples preparations and techniques. A number of authors examined rheological properties of yoghurt gel during fermentation. All these studies showed a significant correlation between the pH value and rheological properties of the yoghurt gel. Small oscillatory deformations testing provide useful information of the gel formation process. As gel forms, the values of G' module increase (25-27). Rheological properties of yoghurt gel depend on various factors such as milk composition, milk heat pretreatment, type and quantity of starter culture, fermentation temperature, ingredients, and storage conditions (28, 29). With a decrease in the milk pH gelation starts and textural properties increase as a result of casein micelles aggregation (30).

Since the relationship between the microstructure and rheological properties during gelation process by applying kombucha has not been investigated, the aim of this study was to examine the changes of viscosity, textural properties and microstructure of gel during milk fermentation with a non-conventional starter – kombucha inoculum and to establish a relationship between the microstructure and these properties.

EXPERIMENTAL

Samples production

Samples were produced from homogenized and pasteurized milk (AD Imlek, Division Novi Sad Dairy). The composition of milk was as follows: fat content – 2.0 g100g⁻¹, total solids – 10.59 g100g⁻¹, total proteins – 3.30 g100g⁻¹ and lactose – 4.60 g100g⁻¹. Kombucha was cultivated on black tea (*Camellia sinensis* – oxidized, 1.5 gL⁻¹) with a sucrose

concentration of 70 gL^{-1} at 29°C for 7 days. The kombucha inoculum in a concentration of 10% was used for milk inoculation (1). Incubation was performed at 42°C . During fermentation process, samples were taken at the pHs: 5.4, 5.1, 4.8 and 4.6 (the end of incubation). All samples were produced in triplicates.

Methods

Viscosity. Viscosity of samples was measured at 5°C using a viscometer HAAKE Rheo-Stress 600HP (Karlsruhe, Germany) with a PP60Ti sensor (gap 1 mm). Modules G' and G'' were measured in a LVT regime, with frequency interval of 1-10 Hz and $\tau=0.015$ Pa. Flow curve was measured by hysteresis loop method at the shear rates from 1 to 40 s^{-1} .

Textural properties. Textural properties: firmness, consistency, cohesiveness and index of viscosity of fermented milk products were analyzed by Texture Analyser TA.HD^{plus} (Stable Micro System, England) 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. It used the options *Return to Start* and the trigger force was 10 g.

Microstructure. Microstructure of fermented milk products was examined by SEM technique, using a Joel, *JSM-6460LV* Scanning Electron Microscope (Oxford, Instruments). The preparation of the samples included their in 2.8% glutaraldehyde, dehydration in ethanol solutions of different concentration, extraction with chloroform, dehydration in absolute ethanol for 24 hours, drying of the samples using CPD 030 "Critical Point Dryer", BAL-TEC, Germany, and their coating with gold using BAL-TEC, SCD 005, Sputter coater (16, 31, 32). The voltage used for the SEM analysis was 25 kV.

Statistical analysis. Statistical analysis of the results was carried out using the computer software program "Statistica 9" and were expressed as average, obtained by three independent experiments. T test was applied for determining differences among the textural properties and viscosity ($P < 0.05$). Regression with correlation coefficient was determined as well. Modeling of textural properties depending on G' and fermentation point were carried out with OriginPro 8.5.1 computer software (Kriging correlation).

RESULTS AND DISCUSION

Changes of viscosity

The obtained results indicate that the produced samples are thixotropic systems (Figure 1). At the beginning shear rate (1.65 s^{-1}) values of viscosity were: 75.53 Pas (pH=4.6), 58.95 Pas (pH=4.8), 32.13 Pas (pH=5.1) and 14.07 Pas (pH=5.4). From a comparison of the same shear rates it is evident that samples with lower pH have higher values of viscosity, which is a consequence of casein micelles networking during the fermentation process. It can be noticed a decrease in the viscosity with the increase in the shear rate, which is due to the distraction of the structure of casein micelles. The highest decrease in the viscosity was observed for the sample of the pH 4.6, due to the completely formed gel.

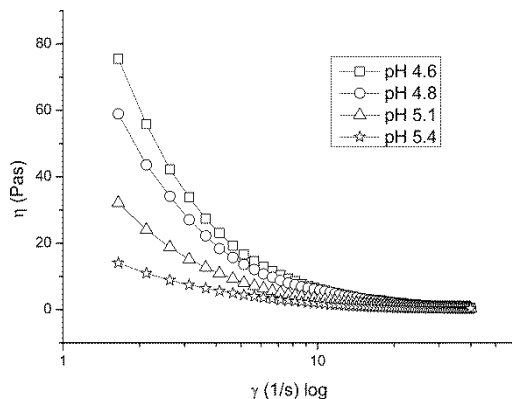


Figure 1. Viscosity changes during milk fermentation with kombucha inoculum as a function of shear rate

The networking of casein micelles increases the storage (G') and loss (G'') modules during fermentation which results in an increase in the complex shear modulus (Figure 2).

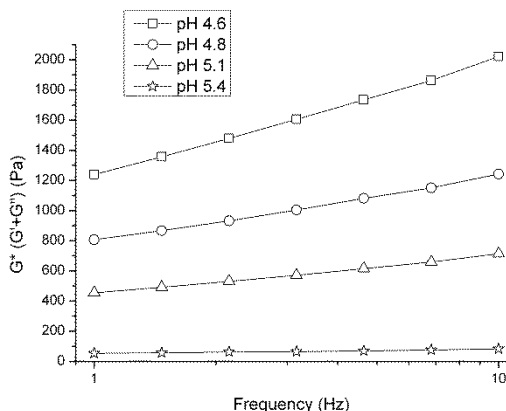


Figure 2. Changes of the complex shear modulus during the milk fermentation with kombucha inoculum as a function of frequency

These results are in accordance with previous studies (26, 27). The average values of the loss tangent ($\tan \delta$) were: 0.2932 (pH=4.6), 0.3004 (pH=4.8), 0.2997 (pH=5.1) and 0.2868 (pH=5.4). The measured values were not significantly different ($P < 0.05$), which indicates formation of a homogenous and compact gel at all examined pH values. On the basis of these results it can be concluded that the major changes in the viscosity and modulus values during milk fermentation using kombucha inoculum occur in the beginning phases of casein micelles networking. As the fermentation is going further, the pH value is decreasing, and the viscosity changes are less pronounced due to the approaching the casein isoelectric point.

Textural properties

The analysis of the textural properties (Figure 3) showed an increase in their values during fermentation and statistically significant differences ($P < 0.05$) among fermentation points, except between the pHs 4.8 and 4.6. Significant differences between these pH values were not due to the fact that the amount of nonsedimentable casein decreased to zero at the pH 4.8, and incorporation of all of the casein into the gelled network, which is in agreement with previous research (17, 30). The biggest changes were recorded between the pHs 5.4 and 5.1, which is in correlation with the viscosity values. The changes of G' values for the samples are correlated with the values of their textural properties. This correlation is linear and clearly visible in Figure 3.

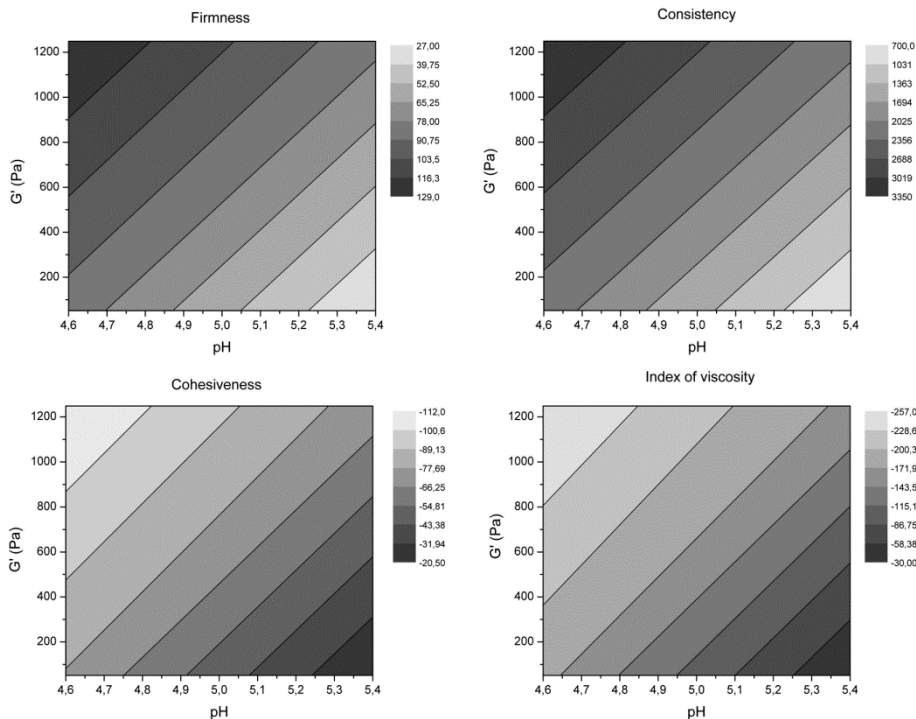


Figure 3. Textural properties and storage modulus (G') as a function of pH values

Microstructure

Microstructural analysis (Figure 4) showed pronounced differences in the gel structure during the fermentation process. During the fermentation, the decrease of the pH and κ -casein destabilization caused casein aggregation, forming clusters. At the pH 5.4 casein micelles are fairly visible as they are packed in the form of coarse clusters and compose a large compartments. At the pH 5.1 coarse clusters are still visible, but casein micelles create smaller compartments, they are more pronounced and begin to create a network in-

side the clusters. Further fermentation (pH 4.8) led to the networkmg of the micelles, which are clearly visible and cavities among them are pronounced. At the pH 4.6 casein micelles form a fine network inside clusters, which although are still present, lost their previous coarse structure and became less visible. The casein micelles diameter is 250 - 400 nm, which is in accordance with previous literature data for yoghurt (21, 22, 29). The microstructure is homogeneous, with a regular arrangement of casein micelles and cavities between them. Although casein micelles are present, their coarse matrix structure at higher pH values induced lower values of textural properties and viscosity. Formation of a finer micelle network (with immobilized liquid phase inside them) improves both, textural properties and viscosity of the gel. The highest changes in microstructure were recorded between the pHs 5.4 and 5.1, while between the pHs 4.8 and 4.6 the differences were the lowest, which is correlated with the viscosity and textural properties of the samples. The insignificant differences between the pHs 4.8 and 4.6 could be explained with the final gel formation prior to the casein isoelectric point.

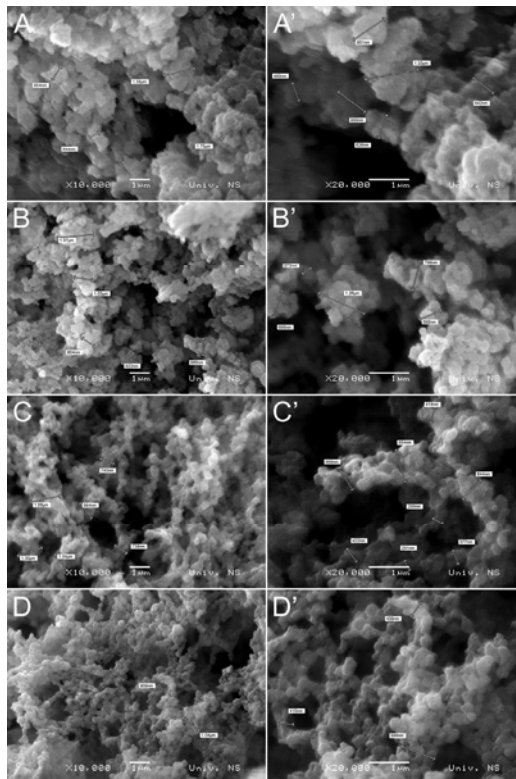


Figure 4. Microstructure of the samples during milk fermentation with kombucha inoculum

pH 5.4 – 10000x, A') pH 5.4 – 20000x; B) pH 5.1 – 10000x, B') pH 5.1 – 20000x; C) pH 4.8 – 10000x, C') pH 4.8 – 20000x; D) pH 4.6 – 10000x, D') pH 4.6 – 20000x

The correlation analysis (Figure 5) of the firmness and thixotropy showed a high correlation between these parameters ($r^2=0.93$, with $p=0.0001$), which is in accordance with their finer networking visible in Fig. 4. On the basis of these results it can be concluded that the microstructural changes are correlated with the changes in textural properties and viscosity of the gel. The viscosity, textural properties and microstructure of the samples can be explained by the changes in casein micelles during fermentation process. It is well known that the formation of lactic acid by activity of starter cultures results in a reduction in surface charge (zeta potential) on a casein micelle from the originally high net negative charge at the pH 6.7 to close to no net charge with the approach of the isoelectric point (4.6) of casein. This change in surface charge allows casein micelles to aggregate through hydrophobic (hydrogen and van der Waals bonds) and electrostatic bonds. Steric repulsion remains from the κ -casein macropeptide hairs, though these may curl up somewhat as the pH drops. Lowering of the pH reduces the repulsive forces and allows for hydrophobic interactions causing the casein micelles to coagulate. Aggregation of the casein particles results in a gel being formed at the pH 5.3 in the heat treated milk, compared with the pH 5.0 in unheated milk (29).

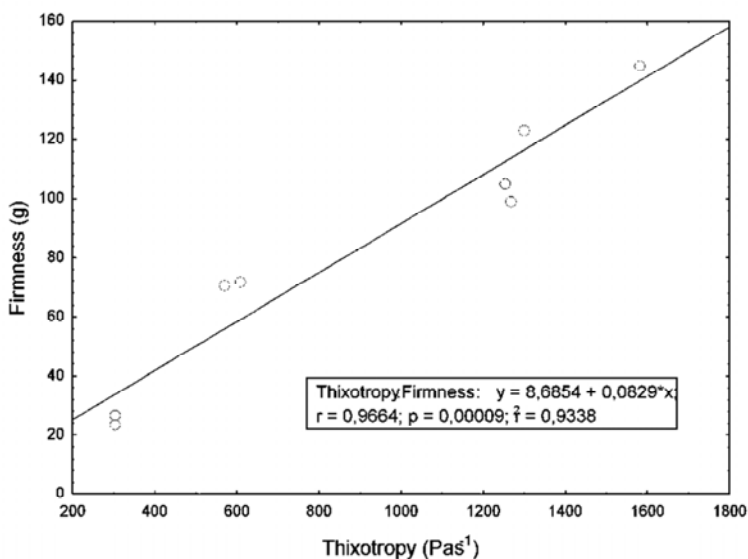


Figure 5. Correlation and regression of the firmness and thixotropy of the samples during milk fermentation with kombucha inoculum

CONCLUSION

The most significant changes in the measured textural properties and viscosity were recorded between the pHs 5.4 and 5.1, which is in correlation with the microstructure. The microstructural analysis revealed disappearing of the coarse cluster structure and appearance of a finer casein micelles network during fermentation. The analysis of the

firmness and thixotropy showed a high correlation between these parameters ($r^2=0.93$, with $p=0.0001$), which is in accordance with the changes of the micelles finer networking. A correlation of the examined properties and microstructure of the gel was established.

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КОРЕЛАЦИЈА МИКРОСТРУКТУРЕ, ВИСКОЗИТЕТА И ТЕКСТУРЕ ТОКОМ ФЕРМЕНТАЦИЈЕ МЛЕКА ДОДАТКОМ ИНОКУЛУМА КОМБУХЕ

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Циљ овог рада био је да се испитају промене микроструктуре, текстуре и вискозитета гела који се формира током ферментације млека додатком инокулума комбухе и да се установи веза између наведених параметара. Анализиране карактеристике мерене су током процеса стварања гела на 42°C на следећим рН вредностима: 5,4; 5,1; 4,8 и 4,6. Анализа микроструктуре током ферментације показала је да груба структура кластера прелази у фину мрежу казеинских мицела. Установљене су значајне разлике у вискозитету и текстуралним карактеристикама узорака између рН=5,4 и рН=5,1 што је у складу са њиховом микроструктуром. Утврђена је корелација микроструктуре, текстуре и вискозитета гела.

Кључне речи: комбуха, ферментисани млечни производи, микроструктура, вискозитет, текстура

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