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Changes in textural parameters and microstructure of kombucha fresh cheese initiated by the addition of wild thyme

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Abstract

Using kombucha as a starter culture led to the successful production of fresh cheese in earlier research. Innovations in the dairy industry, the growing concept of circular economy and consumer demand for functional foods with extended shelf life led to the improvement of kombucha fresh cheese by adding wild thyme as a natural preservative. The aim of this study was to investigate the effects of wild thyme in the form of ground supercritical fluid extract and dry extract on the physicochemical and textural properties as well as the microstructure of fresh kombucha cheese during a thirty-day storage period. In terms of physicochemical properties, the greatest change was observed in the total protein content of the kombucha fresh cheese sample with the dry extract of wild thyme (from 23.21±0.15 to 23.77±0.24 %). In terms of textural properties, the greatest change was observed as a decrease in firmness in the control sample (from 5137.93±43.20 to 1955±16.39 g). PCA analysis showed that the pH values, which were determined for all samples after production, had the greatest influence on their firmness and work of shear. A slight disintegration of the kombucha fresh cheese structure was observed, due to the influence of storage time, proteolysis and the activity of the bioactive components of wild thyme.

Keywords: kombucha fresh cheese; wild thyme; texture; extracts; microstructure

Introduction

Fresh cheese represents an unripened, particulate and acidic cheese whose production begins with the pasteurisation of standardised milk and subsequent acidification with mesophilic lactic acid bacteria (Miloradović et al., 2018). During fermentation, milk protein aggregates form a continuous protein network commonly referred to as an acidified milk gel (Repajić et al., 2019). Milk proteins and milk protein gelation have been extensively studied due to their ability to impart structure, texture, flavour and functionality (Remondetto and Subirade, 2003; Van Vliet et al., 2004). In cheese matrices, aggregated casein micelles form a microstructural network with entrapped solid fat globules and serum and their digestion is directly related to their physical properties, especially their textural and structural properties (Van der Sman, 2012; Lamothe et al., 2012). Since it exhibits a soft texture, a slightly acidic taste, a high moisture content and a low price, fresh cheese belongs to the group of highly accepted dairy products (Deolindo et al., 2019).

The use of non-conventional, functional starter cultures could offer desirable technological and nutritional advantages in the production of various dairy products. Among them, kombucha offers a great opportunity to be used as an innovative starter culture in the production of fermented dairy products as well as fresh cheese (Hrnjez et al., 2014; Kanurić et al., 2018; Vukić et al., 2021; Bjekić et al., 2021). Kombucha represents a slightly sweet and sour beverage traditionally produced from the fermentation of sweetened black or green tea (*Camellia sinensis*) by a biofilm of cellulose containing a symbiotic culture of acetic and lactic acid bacteria and osmophilic yeasts, also known as "tea fungus" (Kapp and Sumner, 2019; Dutta and Paul, 2019). According to Cotton et al. (2017) and Marsh et al. (2014), because a significant amount of lactic acid bacteria has been detected, kombucha inoculum is suitable for initiating the lactic fermentation process.

Supplementation of a variety of dairy products with natural ingredients, including plants, has been extensively studied to increase bioactivity for human health (Villarreal-Soto et al., 2018). The purpose of adding plants and plant extracts to cheese is to impart a different taste and flavor, improve shelf life and promote cheese consumption (Yerlikaya et al., 2020). Also, various herbs can be added to fresh cheese to increase the antioxidant activity and improve the colour parameters (Akan et al., 2021). Essential oils and extracts of certain medicinal herbs, e.g. from the genus *Thymus*, represent additives that provide protection against pathogenic microorganisms and have a significant impact on the sensory and antioxidant properties of food, including cheese. Wild thyme (*Thymus serpyllum* L.) is an example that lends itself to such use. The main reason for this is its long list of pharmacological properties, such as antiseptic, antibacterial, anthelmintic, diaphoretic, antispasmodic, expectorant, cough suppressant, analgesic, carminative and diuretic properties (Rasooli and Mirmostafa, 2002; Mihailović-Stanojević er al., 2013). *T. serpyllum* L. has also been extensively studied for its antioxidant activity, which directly correlates with a high content of polyphenolic compounds (Jovanović et al., 2017; Kulišić et al., 2006). Since large amounts of so-called herbal dust remain after the production of wild thyme filter tea, it has the potential to be valorized as a suitable raw material for the production of various extracts that can also be used in the food industry. From the aspect of circular economy, it is important to mention that the reuse of this by-product could be an important step towards achieving sustainability in novel food production (Vukić et al., 2022; Vukić et al., 2023).

Since our previous study kombucha fresh cheese enriched with wild thyme showed very good antioxidant activity and sensory acceptability (Degenek et al., 2023a), this study represents a continuation of development-oriented research aimed at obtaining an innovative functional dairy product. Referring to that fact, the main focus of this study is to investigate the influence of physicochemical properties on the texture and microstructure of fresh cheese produced with the kombucha inoculum as a non-conventional starter culture and the addition of wild thyme as a natural additive in the form of ground, supercritical fluid extract and dry extract.

Materials and methods

Materials

Milk with 8.59 % of dry matter content, 2.95 % of milk fat, 3.21 % of total proteins, 0.71 % of ash, pH value of 6.69 and titratable acidity of 6.6 °SH was donated by the dairy "Mlekoprodukt" D.O.O., Zrenjanin, Serbia. Black tea (*C. sinensis*) and sucrose were purchased from retailers. The kombucha drink was donated from ongoing research at the Faculty of Technology in Novi Sad. The coagulating enzyme CHY-MAX®Powder Extra NB was purchased from Chr. Hansen Holding A/S (Hoersholm, Denmark). The plant material of wild thyme was donated by Macval D.O.O. Novi Sad (Serbia). It was separated as a by-product of filter tea production, as its dust fraction had a particle diameter of less than 0.315 mm, which is the limit for filter tea production. The herbal material was collected and used as raw material for further extractions and for direct use as herbal grounds (G). Maltodextrin (DE 19.7) was purchased from Brenntag (Muelheim, Germany). Commercial carbon dioxide (Messer, Novi Sad, Serbia), purity > 99.98 %, was used for laboratory supercritical fluid extraction and critical point drying.

A by-product of wild thyme (*T. serpyllum* L.) from Serbia collected after the filter tea production process was kindly donated by Macval Tea D.O.O. (Novi Sad, Serbia). The fractions with small particle size of the wild thyme herbal material left after vibration sieving and fractionation during the filer tea production were used as a ground material and raw material for obtaining the supercritical fluid extract and dry extract.

Methods

Wild thyme extracts production

Supercritical fluid extraction was performed using a high-pressure extraction system (NOVA, Swiss, Effretikon, Switzerland) consisting of a gas cylinder containing CO_2, a diaphragm compressor (with a pressure of 1000 bar), an extractor (with an internal volume of 200 mL), a separator, a temperature control system and pressure control valves. Each sample was placed in the extraction vessel, whereupon the extraction process was carried out for 3 hours at a temperature of 50 °C, a pressure of 350 bar and a flow rate of 0.3 kg CO_2/h. The separation conditions were set at 15 bar and 25 °C. The obtained extract was collected in plastic bottles and stored at a temperature of 4 °C.

The liquid extract used for spray drying was prepared by ultrasound-assisted extraction under optimal conditions, described in detail by Mrkonjić et al. (2021). A spray drying system (APV Anhydro AS, Denmark) was used to convert the liquid extract into a dry form. The liquid feed was prepared by adding 20 % maltodextrin as a carrier and continuously mixing the contents with a magnetic stirrer at a temperature of 40 °C. A laboratory peristaltic pump (FH100 series, Thermo Scientific, USA) delivered the liquid to the drying chamber at a flow rate of 0.91 L/h. The liquid was sprayed into the drying chamber. The inlet temperature of the spray drying process was set at 140-150 °C and the outlet temperature at 80 °C. After drying the droplets, the dry extract was separated from the heating medium in the cyclone and collected in a plastic container. The dry extract obtained was collected in a glass bottle, sealed and stored at room temperature protected from the influence of air and moisture.

Kombucha fresh cheese production

Kombucha fresh cheese was made from pasteurised milk, with characteristics described in the *Materials* section. Kombucha inoculum was used as a non-conventional starter culture. It was cultivated on oxidised black tea (*C. sinensis*, at a concentration of 1.5 g/L) with the addition of 70 g/L of sucrose. When the tea was cooled to room temperature, an inoculum from a previous fermentation was added at a concentration of 100 mL/L. After that, the kombucha inoculum was incubated at 25±2 °C for 7 days until its pH reached the value of 3.25 (Malbaša et al., 2009). Prepared in this way, kombucha inoculum was ready to be used for lactic fermentation at 35 °C, exactly as in the study of Kanurić et al. (2011). The following step was the addition of coagulating enzyme at a concentration of 5 mL/L at 35 °C. The fermentation process was maintained until a pH of 4.5-4.6 was reached, and it was monitored using a Seven Excellence pH meter by Mettler Toledo, Greifensee, Switzerland. When the coagulation process was finished, the obtained coagulum was cut off, subjected to heat treatment under gentle stirring at 60 °C for 5 min, then cooled to 25 °C and drained. The obtained kombucha fresh cheese was divided into four parts, where one was predicted to be used as a control sample and the other three for the addition of wild thyme. Wild thyme herbal dust was used in three different forms: ground, supercritical fluid extract, and dry extract. Since wild thyme ground, which comes directly from the filter tea industry, is a suitable substrate for the development of a variety of microorganisms that can lead to subsequent contamination of the product, it is lightly pasteurized at a temperature of 55 °C for 1 minute before being added to kombucha fresh cheese.

Each of the prepared forms of wild thyme was added separately at concentrations of 2.1 g/100 g for ground, 0.025 g/100 g for supercritical fluid extract, and 0.62 g/100 g for dry extract. Then, all kombucha fresh cheese samples were mixed, packed in cups with lids, and stored in the refrigerator at 4 °C. Finally, four kombucha cheese samples were produced: kombucha cheese control (KCC), kombucha cheese with ground (KCG), kombucha cheese with supercritical fluid extract (KCSF) and kombucha cheese with dry extract (KCD).

Physicochemical analysis and number of lactic acid bacteria

The chemical quality of the kombucha fresh cheese samples was analysed using the following methods for contents of: dry matter (SRPS EN ISO Standard No. 5534: 2007); milk fat by the Van Gulik method (SRPS EN ISO Standard No. 3343: 2013); total proteins (SRPS EN ISO Standard No. 8968-1: 2016) and ash (ISO Standard No. 5545: 2008). The pH values were determined at a temperature of 25 °C using a pH meter - pH Spear, OAKTON Instruments (Vernon Hills, Illinois, USA), while titratable acidity was determined using the Soxhlet-Henkel method (Carić et al., 2000). The content of total lactic acid (D- and L- lactic acid) in fresh cheese samples was determined using an enzymatic test (K-DLATE 08/18, Megazyme, Wicklow, Ireland).

Determination of the total number of lactic acid bacteria

The total number of LAB was determined according to ISO Standard No. 15214: 1998 and Downes and Ito (2001). A base and a series of decimal dilutions up to 10^{-6} were prepared. 1 ml of the prepared decimal dilutions was placed in Petri plates and covered with Mann Rogosa Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany). The colonized plates were incubated for 72 hours at 30 °C under microaerophilic conditions. The result is expressed as log CFU/g.

Textural analysis

Textural properties such as firmness, work of shear, stickiness, and work of adhesion were determined by a wedge fracture test using the Texture Analyser TA.HD.plus (Stable Micro System, Godalming, United Kingdom). A 5 kg load cell A/WEG at 5 °C (with a pre-test velocity of 1.00 mm/s, a test velocity of 3.0 mm/s and a post-test velocity of 10 mm/s) with a nominal spacing of 15 mm was used. The option ''Return to start'' was used with a trigger force of 5 g.

Microstructure of kombucha fresh cheese samples

The microstructure of the kombucha fresh cheese samples was analysed by scanning electron microscopy (SEM) using the JEOL JSM 6460 LV scanning electron microscope (Oxford Instruments, Abingdon, United Kingdom). Sample preparation involved the fixation of kombucha fresh cheese particles (approximately 10 mm in diameter) in 2.8 % glutaraldehyde solution at 20 °C for 24 h; dehydration in different percentages of ethanol solutions (30, 50, 70, 90 and 100 %); dehydration in absolute ethanol for 24 h; drying the samples with CPD 030 ''Critical Point Dryer'' (BAL-TEC, Balzers, Liechtenstein) and coating the samples with gold using BAL-TEC, SCD 005, Sputter Coater (Kaláb, 1993; Sandoval-Castilla et al., 2004). The voltage used for the SEM analysis was 24 kV.

Statistical analysis

All analyses were performed in triplicate for each kombucha fresh cheese sample and the values obtained were expressed as mean ± standard deviation. For the unmodified treatment of the obtained data, analysis of variance (ANOVA) was performed in the software "Statistica 13.5.0.17" (Palo Alto, USA). The Duncan multiple range test was used to detect significant differences between the analysed parameters. The differences between the analysed parameters of the samples were considered statistically significant when $p< 0.05$.

PCA analysis was used to identify the main factors controlling the textural properties during the storage period and the distribution of the analysed parameters. The eigenvalue of the variance was greater than one. The loadings (factor values) corresponding to the principal components were calculated using the correlation matrix.

Results and discussion

Physicochemical characteristics

The physicochemical properties of the fresh cheese prepared using kombucha as a non-conventional starter culture and adding wild thyme in the form of ground, supercritical fluid extract and dry extract are shown in Table 1.

A statistically significant difference ($p<0.05$) between the samples was observed in the dry matter content. It ranged from 52.33±0.03 to 53.14±0.01 % after production. Thirty days later, there was an increase in dry matter content.

For fat content, all samples except the KCG sample showed approximate values after thirty days of storage. It is noticeable that the fat content in this sample decreased more compared to the others, from 26.75±0.25 to 25.25±0.25 %. The fat content in dry matter differed significantly between the samples ($p<0.05$) both after production and after thirty days of storage.

The highest protein content (24.21±0.06 %) was found in the control sample. Thirty days later, a slight decrease in protein content was present in all samples. The same trend was observed in the content of total proteins in dry matter. According to Abdalla et al. (1993), the decrease in protein content during storage might happen due to protein degradation, confirmed by Degenek et al. (2023b). In this study, the decrease of protein content in kombucha fresh cheese after thirty days of storage was accompanied by an increase in the content of free amino acids, mostly phenylalanine.

The ash content of the kombucha fresh cheese samples increased during the storage period, with a significant statistical difference ($p<0.05$). The KCG sample showed the highest ash content after production and after thirty days of storage (1.55±0.01 and 1.62±0.01 %, respectively). These results agree with the study by Abdalla and Mohamed (2009), according to which the ash content in cheese increases with the advancement of storage time from zero to the forty-fifth day.

After production, statistically significant differences in lactic acid content were found in all samples tested ($p<0.05$). The control sample had the highest lactic acid content (0.69±0.07 %). After thirty days of storage, there was a significant decrease in all samples, except KCG (there was an increase from 0.44±0.01 to 0.48±0.02 %). The lactic acid content can be related to the number of lactic acid bacteria responsible for production. Figure 1 shows that the number of lactic acid bacteria decreased after thirty days of storage for all samples, which certainly reflects almost the same trend in lactic acid content. In Figure 1 it is shown that the KCG sample is characterized by a more pronounced decrease in the number of LAB during storage period, when compared to the other samples. This can be explained by the fact that additives acting as absorbers or antioxidants can be successfully used to reduce the adverse impact

Table 1. Physicochemical properties of kombucha fresh cheese with the addition of wild thyme after production (Degenek et al., 2023a) and after thirty days of storage

Sample	KCC	KCG	KCSF	KCD	KCC	KCG	KCSF	KCD
Day of storage	0				30			
Dry matter (%)	52.89±0.01^{a}	53.14±0.0^{1}b	52.33±0.03^{c}	52.36±0.02^{c}	53.36±0.1^{d}	53.35±0.08^{d}	53.54±0.04^{e}	54.05±0.02^{f}
Fat (%)	26.75±0.25^{a}	26.75±0.25^{a}	27.25±0.25^{a}	26.75±0.25^{a}	27.00±0.00^{a}	25.25±0.25^{b}	26.75±0.25^{a}	26.75±0.25^{a}
Fat in dry matter (%)	50.57±0.47^{a}	50.33±0.47^{a}	52.07±0.44^{b}	51.08±0.49^{b}	50.99±0.09^{b}	47.33±0.54^{c}	49.96±0.49^{a}	49.49±0.48^{a}
Total proteins (%)	24.21±0.06^{a}	23.32±0.18^{b}	23.54±0.15^{abc}	23.21±0.15^{b}	24.05±0.01^{ac}	23.08±0.01^{b}	23.34±0.10^{bc}	23.52±0.49^{abc}
Total proteins in dry matter (%)	45.76±0.12^{a}	43.88±0.33^{bc}	44.99±0.32^{ac}	44.32±0.29^{abc}	45.08±0.06^{ac}	43.26±0.05^{b}	43.61±0.22^{b}	43.52±0.89^{b}
Ash (%)	1.43±0.00^{a}	1.55±0.01^{b}	1.37±0.00^{c}	1.46±0.01^{d}	1.54±0.00^{b}	1.62±0.0^{1}e	1.50±0.01^{f}	1.59±0.01^{g}
Lactic acid (%)	0.69±0.07^{a}	0.44±0.01^{b}	0.63±0.01^{ac}	0.53±0.01^{bcd}	0.60±0.02^{acd}	0.48±0.02^{bd}	0.47±0.00^{b}	0.47±0.06^{b}
pH	4.98±0.01^{a}	4.89±0.01^{b}	4.95±0.01^{a}	4.95±0.00^{a}	4.67±0.01^{c}	4.71±0.02^{cd}	4.75±0.01^{d}	4.82±0.03^{e}
Titratable acidity (°SH)	52.40±0.40^{ab}	56.80±0.80^{c}	47.60±0.40^{d}	51.20±0.00^{a}	78.40±0.80^{e}	81.20±0.40^{e}	75.60±0.40^{b}	70.40±0.80^{f}

**KCC - kombucha fresh cheese control sample; KCG- kombucha fresh cheese with the addition of wild thyme ground; KCSF- kombucha fresh cheese with the addition of wild thyme supercritical fluid extract; KCD- kombucha fresh cheese with the addition of wild thyme dry extract. Different letters (a, b, c, d, e, f, g) within the same row indicate a statistically significant difference at the significance level p<0.05*

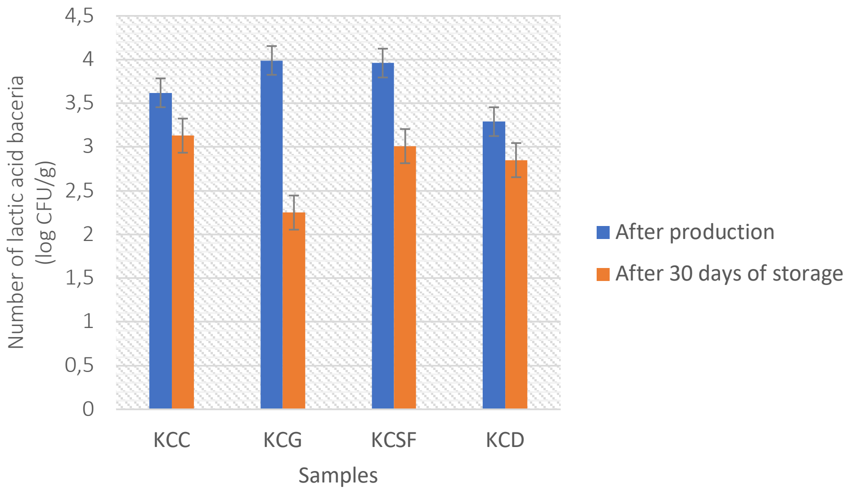

Figure 1. Number of lactic acid bacteria in kombucha fresh cheese with the addition of wild thyme after production and after thirty days of storage

of oxygen on the lifespan of LAB. Such substances increase the survival rate of LAB in food products, as exemplified by the results of the *L. acidophilus* survival study in yogurt with the addition of ascorbic acid (Sionek et al., 2024). In this study, wild thyme in the forms of ground, supercritical fluid extract and dry extract is added to the fresh kombucha cheese as a natural additive with a significant antioxidative activity. Therefore, in comparison with the other samples, a more pronounced decrease in the number of LAB in the KCG sample can be the result of a weaker simultaneous effect of compounds with antioxidant activity (from milk, kombucha and wild thyme ground) on the survival of the present LAB. The opposite trend was observed in the study by Akpinar et al. (2022), where after 30 days of storage an increase in the total number of *Lactobacillus* spp. in Turkish whey (Lor) cheese, especially in the sample to which the combination of black cumin and thyme was added (from 3.02 log CFU/g to 8.64 log CFU/g).

After production, the pH of the KCG sample was different from that of the other samples. However, after thirty days of storage, there was a marked decrease in pH for all samples, especially for the control sample (from 4.98±0.01 to 4.67±0.01). A similar trend was observed in the study by Glušac et al. (2015), where a decrease in the pH of yoghurt samples fortified with honey and whey protein concentrate was observed during storage. The pH fluctuation is related to the change in H^+ ion concentration due to the production of organic acid by the lactic acid bacteria. Since the measured pH values decreased during storage, it could be assumed that the presence of added herbs did not negatively affect the activities of the metabolic bacteria during the ripening period (Yerlikaya, 2020).

The titratable acidity in the samples studied differed significantly ($p<0.05$), which may be related to the forms of wild thyme added. In comparison with the ground form of wild thyme, supercritical and dry extracts represent concentrated forms as they contain higher levels of bioactive components and therefore show increased antimicrobial activity. It is precisely for this reason that the samples fortified with the aforementioned extracts have lower titratable acidity values than the KC and KCG samples. Of course, after thirty days of storage, growth was observed in all samples as the activity of the microflora present increased. The lowest intensity of increase in titratable acidity was observed in the KCSF and KCD samples (from 46.60±0.40 to 75.60±0.40 °SH and from 51.20±0.00 to 70.40±0.80 °SH, respectively).

Textural properties

Textural properties of fresh cheese produced with kombucha inoculum as a non-conventional starter culture and the addition of wild thyme after production and thirty days of storage are shown in Figure 2. All samples showed a significant decrease in firmness after 30 days of storage, especially the control sample (from 5114.60±12.47 to 1955.27±16.38 g). The addition of wild thyme in the form of ground, supercritical and dry extract affected the lower firmness values of kombucha fresh cheese after production compared to the control sample. The chemical composition of the kombucha fresh cheese samples showed some influence on their firmness, as the correlation between dry matter and firmness was moderate ($R^2=0.61$). According to Koca and Metin (2004), there is a positive correlation between protein content and the firmness of cheese, as protein content increases the force required for compression. The results of our study confirm this claim, as can be seen in Figure 2a, where the control sample with the highest protein content had the highest firmness, right after production. More pores in the cheese structure led to a greater reduction in moisture content and also increased the firmness of fresh cheese (Delgado et al., 2011). On the other hand, according to Cunha et al. (2012), the trend of decreasing firmness can be related to fat particle size distribution in all samples. In the aforementioned study, an increase in fat particle size was observed with decreasing strength, which led to a lower number of protein-protein interactions, thus contributing to an easier compression and dissolution of the formed protein network. On the other hand, pH showed some influence on the firmness of the samples studied, as the firmness of the kombucha fresh cheese samples decreased significantly with decreasing pH ($R^2=0.77$). In ripened cheese, proteolysis

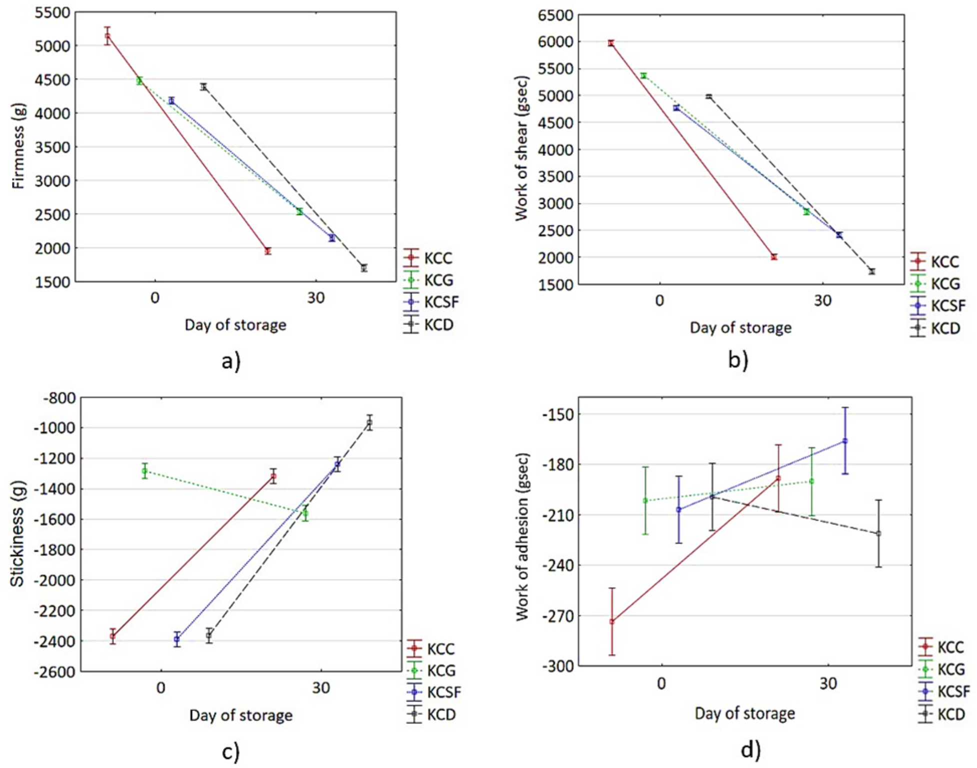

Figure 2. *Textural properties of kombucha fresh cheese with the addition of wild thyme after production and after thirty days of storage, in a) firmness; b) work of shear; c) stickiness; d) work of adhesion*

leads to a change in cheese texture as the protein network disintegrates, the a_w value decreases due to the water binding of the released carboxyl and amino groups and the pH value increases (Sousa et al., 2001). However, fresh cheese made from milk with a lower fat content (2.5 % in Panić's study (2004)) experienced a drop in pH during storage, which was certainly confirmed in our study.

A similar trend was observed for the work of shear (Figure 2b), again with the most drastic decrease in value after thirty days of storage for the control sample (from 5970.18±16.35 to 2014.85±16.30 gsec). There was also a strong correlation between the work of shear and pH determined in the kombucha fresh cheese samples (R^2=0.75).

Figure 2c shows that after thirty days of production, there was an increase in stickiness in KCC, KCSF and especially in sample KCD (from -2366.14±16.39 to -967.24±16.34 g). The stickiness observed in sample KCG decreased during storage, probably due to the nature of ground wild thyme as an additive and its distribution in the sample. The influence of the chemical composition of kombucha fresh cheese samples on their stickiness was also high, as dry matter content and stickiness were strongly correlated (R^2=0.82). Among the dry matter components, ash content showed the greatest influence on stickiness (R^2=0.65).

Work of adhesion represents the force required to remove the material adhering to a given surface (lips, mouth, teeth, etc.) and is a measure of the stiffness of the material (Guiné et al., 2015). The values obtained of this parameter showed a tendency to increase during the storage period for all samples of kombucha fresh cheese, except for the KCD sample, which may be related to the very good mixing and then solubility of the bioactive components of the wild thyme dry extract in kombucha fresh cheese (Figure 2d). The strongest growth was observed in the control sample, where the work of adhesion changed from -273.89±16.37 to -188.41±16.32 gsec. As in the study by Tenreiro (2014), where the work of adhesion varied between -26.30 and -2.2 Nsec, the values obtained in our study were characteristic of fresh cheese.

PCA analysis

Figure 3 shows the PCA loadings and PCA scores formed using the physicochemical and textural variables. This PCA analysis shows a comparison of the multidimensional physicochemical/textural quality of kombucha fresh cheese samples produced with the addition of wild thyme in different forms projected on a two-dimensional surface and described by two orthogonal factors used as dimension - principal components 1 and 2 (PCA1 and PCA2). These two principal components accounted for 77.73 % of the total variance (64.11 % for PC1 and 13.62 % for PC2). Therefore, they were considered significant based on the eigenvalues (> 1). Another obtained dimension was explained by a small proportion of the variance (<5 %) and was therefore not considered significant.

In Figure 3a it can be seen that different physicochemical features were grouped with different textural parameters, which can describe their mutual influence. In the first quadrant, fat and lactic acid contents were grouped without any textural parameter. This can be explained by the fact that these physicochemical characteristics did not influence the textural

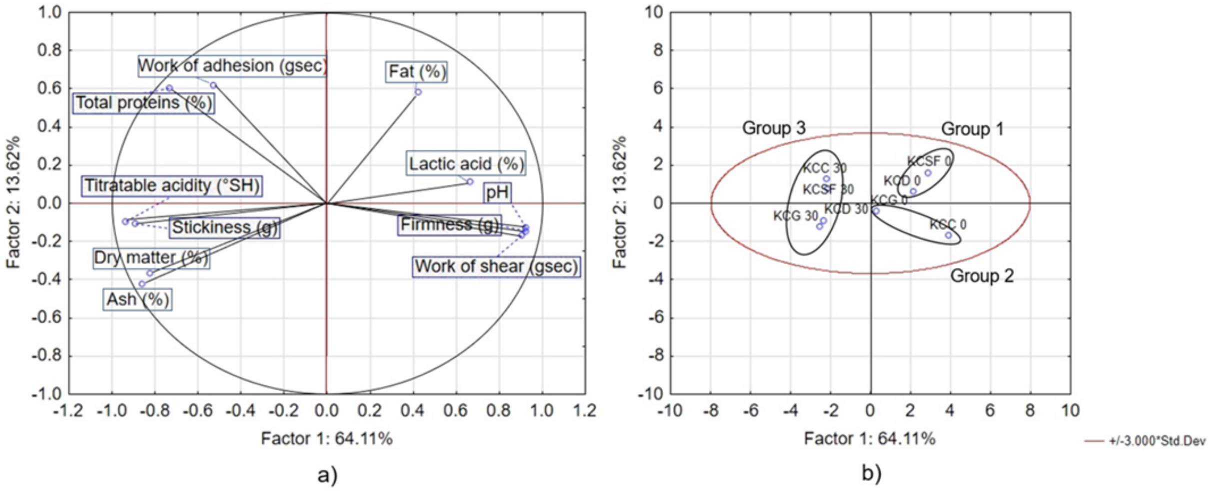

Figure 3. *PCA analysis of textural properties of kombucha fresh cheese with the addition of wild thyme, in a) correlation circle as a projection of the initial variables in the factors space; b) position of examined kombucha fresh cheese samples in factorial plane in coordinates of PCA factors 1 and 2*

properties of the kombucha fresh cheese samples produced for this study. In the second quadrant, pH, firmness and work of shear were very closely grouped. It is obvious that pH had a strong influence on the mentioned textural parameters, which was also confirmed in the section *Textural properties*. In the third quadrant, the dry matter and ash contents, the titratable acidity and the stickiness of kombucha fresh cheese samples were grouped. This confirms the significant influence of the aforementioned physicochemical properties on the respective textural parameter (section *Textural properties*). Finally, in the fourth quadrant, the protein content and the work of adhesion of the kombucha fresh cheese samples were grouped.

Figure 3b shows that samples were grouped mostly by the day of storage rather than the form of added wild thyme. Kombucha fresh cheese samples with the addition of wild thyme in the form of supercritical fluid extract and dry extract after production (KCSF 0 and KCD 0) were grouped for positive values of the first dimension and characterised by chemical parameters such as fat and lactic acid content (Group 1). Differences in pH, firmness and work of shear were mainly responsible for the dispersion of samples in Group 2 along PCA factor 1, i.e., correlation values with PCA factor 1: 0.92, 0.92 and 0.91, respectively. With increasing storage time, the difference between the groups became smaller. Kombucha fresh cheese samples stored for 30 days were grouped at the negative values of the second dimension and were more characterised by other physicochemical and textural parameters such as ash and dry matter content, titratable acidity, total protein content, and stickiness and work of adhesion (Group 3). Samples KCC 30 and KCD 30 were particularly characterised by total protein content and work of adhesion, as they had higher values for these parameters compared to samples KCG 30 and KCD 30 (Table 1). On the other hand, the samples KCG 30 and KCD 30 were characterised mainly by ash and dry matter content as well as stickiness and titratable acidity.

Microstructure

According to El-Salam (2015), the microstructure of fresh cheese produced with the traditional starter culture had an open protein matrix containing voids with milk fat globules of different sizes and shapes. The holes in the protein matrix indicate the spaces occupied by the fat globules. The pasteurisation process suggests that the cheese curd particles have a skin of dehydrated protein. The outside of the curd has been shown to have a denser protein matrix, but this is not so thick as to prevent the outflow of whey from inside the curd particles (Kaláb, 1979; Kaláb, 1993). A somewhat looser structure is observed in the control sample of the fresh cheese produced with kombucha as a starter culture (Figure 4a). It is known that certain lactic acid bacteria synthesize carbohydrates (oligosaccharides and polysaccharides), which are often seen in the form of thin filaments in the microstructure of fermented milk products and certain cheeses (Bottazzi, 2002). A specific feature of the acetic fermentation bacteria, which are part of the kombucha inoculum along with the lactic acid bacteria, is the ability to synthesise extracellular polysaccharides such as cellulose. This synthesis has been demonstrated for *Acetobacter pasteurianus* (a species formerly classified as *A. aceti* subsp. *xylinum*) and *Gluconobacter oxydans* (Divies and Cachon, 1998). The aforementioned cellulose threads are visible on the examined samples of kombucha fresh cheese after production (Figure 4). The control sample had a more continuous protein matrix, which can be explained by its higher hardness compared to the other samples. The influence of the added supercritical fluid extract of wild thyme on the microstructure of the kombucha fresh cheese was low, as there was a high similarity with the microstructure of the control sample (Figure 4c and 4a). In contrast, the addition of the ground wild thyme and its dry extract had a greater effect on changing the microstructure of kombucha fresh cheese. As

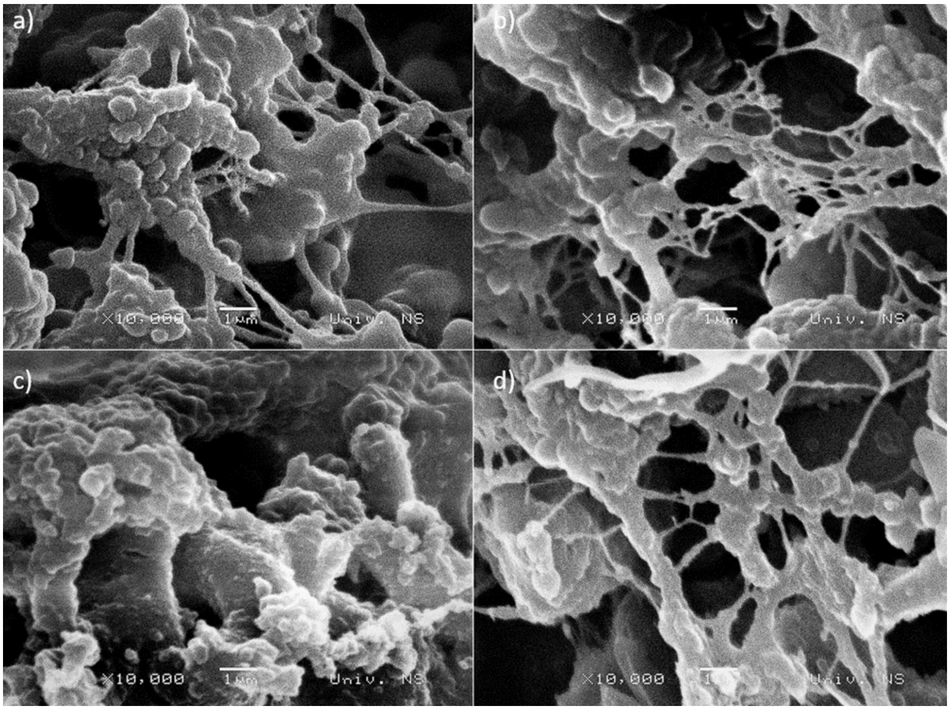

Figure 4. Microstructure of kombucha fresh cheese with the addition of wild thyme after production, with a magnification of 10 000 x, where is a) KC sample; b) KCG sample; c) KCSF sample; d) KCD sample

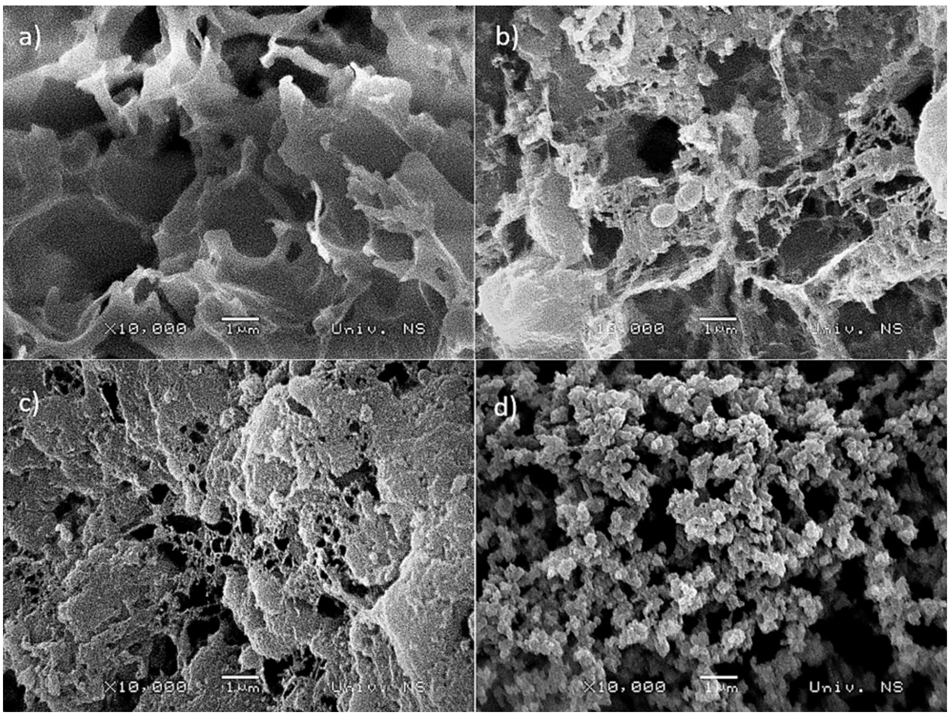

Figure 5. Microstructure of kombucha fresh cheese with the addition of wild thyme after thirty days of storage, with a magnification of 10 000 x, where is a) KC sample; b) KCG sample; c) KCSF sample; d) KCD sample

can be seen in Fig. 4b and 4d, the KCG and KCD samples are characterised by a disintegrated protein network, which can be attributed to the intensive mixing of the aforementioned additives and the interaction of their bioactive components with the kombucha fresh cheese medium.

After thirty days of storage, all four kombucha fresh cheese samples showed significant changes in microstructure (Fig. 5). It can be observed that the protein network is dissolved and thus the compactness it had after production due to proteolysis is lost. Larger changes were again observed in the KCG and KCDE samples (Fig. 5 b and 5 c), which is a consequence of the simultaneous proteolysis and interaction of proteins and fats with the polyphenolic compounds dissolved in the fresh cheese medium (Yerlikaya et al., 2020).

Conclusions

Based on the results obtained, it can be concluded that the addition of wild thyme to kombucha fresh cheese has a significant effect on the physicochemical and textural properties as well as on the microstructure. During storage, the greatest changes in physicochemical properties were observed: an increase in dry matter, protein and ash content in all samples, with the sample of kombucha fresh cheese with the addition of wild thyme dry extract standing out; a decrease in lactic acid content and pH. Considering that the textural characteristics of kombucha fresh cheese are closely related to its physicochemical properties, but also to the action of the bioactive components present in the added wild thyme, it can be concluded from the results obtained that firmness and work of shear were reduced. Changes in the stickiness and work of adhesion values were observed mainly in the samples with the addition of wild thyme in the form of ground and dry extract. PCA analysis showed that the samples studied were grouped more by the day of storage than by the form of wild thyme added. It also showed the influence of physicochemical properties on the textural parameters, with the influence of pH being the strongest on the decrease of firmness and work of shear was the most prominent. As the firmness of the samples studied decreased during storage, their microstructure changed due to the simultaneous effect of proteolysis and the interaction of a considerable number of polyphenolic compounds, both those already present in kombucha fresh cheese and those from wild thyme. In the samples to which thyme was added in the form of ground plant material and dry extract, a more pronounced dissolution of the protein network structure was again observed. Thus, the dry extract of wild thyme had the greatest influence on the desired changes, which can be linked to the potentially good consumer acceptance of this type of fresh cheese.

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Promjene u teksturalnim svojstvima i mikrostrukturi svježeg kombucha sira uzrokovane dodatkom majčine dušice

Sažetak

Korištenje kombuche kao starter kulture dovelo je do uspješne proizvodnje svježeg sira u ranijim istraživanjima. Inovacije u mliječnoj industriji, rastući koncept cirkularne ekonomije i potražnja potrošača za funkcionalnom hranom s produženim rokom trajanja doveli su do poboljšanja svojstava svježeg kombucha sira uz dodatak majčine dušice kao prirodnog konzervansa. Cilj ovog istraživanja bio je ispitati utjecaj majčine dušice u obliku praha, superkritičnog ekstrakta i suhog ekstrakta na fizikalno-kemijska i teksturalna svojstva, kao i na mikrostrukturu svježeg kombucha sira tijekom 30 dana skladištenja. U pogledu fizikalno-kemijskih svojstava, najveća promjena uočena je u ukupnom sadržaju proteina u uzorku svježeg kombucha sira s dodatkom suhog ekstrakta majčine dušice (s 23,21±0,15 na 23,77±0,24 %). U pogledu teksturalnih svojstava, najveća promjena uočena je kao smanjenje čvrstoće u kontrolnom uzorku (s 5137,93±43,20 na 1955±16,39 g). PCA analiza pokazala je da je pH vrijednost imala najveći utjecaj na čvrstoću i rad smicanja svih uzoraka, posebno nakon proizvodnje. Uočena je blaga dezintegracija strukture svježeg kombucha sira, uslijed utjecaja vremena skladištenja, proteolize i djelovanja bioaktivnih komponenata majčine dušice.

Ključne riječi: svježi kombucha sir; majčina dušica; ekstrakti; teksturalna svojstva; mikrostruktura

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