Effect of plant extracts of *Kitaibelia vitifolia* on antioxidant activity, chemical characteristics, microbiological status and sensory properties of Pirotski Kachkaval cheese

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Abstract

The aim of our study was to evaluate the impact of cheese (Pirotski Kachkaval) fortification by polyphenols attributed to *Kitaibelia vitifolia* ethanol herb extract, applied in two different manners (added to the cheese curd after texturizing or sprayed on surface of cheese). Investigation of the used antioxidant effects of polyphenols, physic-chemical composition, microbiological quality and sensory properties of Pirotski Kachkaval was undertaken. Antioxidant activity of conventional and fortified cheese was evaluated by five contemporary and compatible methods, and revealed a slight emphasis on phenol-linked antioxidant activity of fortified samples of cheese in comparison to samples of the control group. Fortified Pirotski Kachkaval had higher sensory evaluation scores than the controls. Statistically significant ($P < 0.05$) changes were observed in moisture content and total solids of control and modified series of cheese, but other parameters did not differ significantly ($P > 0.05$).

Keywords: Pirotski Kachkaval, *Kitaibelia vitifolia*, polyphenols, antioxidant activity, microbiological quality, sensory properties.

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Nowadays, there is a strong trend of investing into research and testing of natural antioxidants (preservatives) and their biological activity for use in the food industry, because many of the substances of synthetic origin have proven to be carcinogens [1]. Application of polyphenols from natural sources through diet may prevent oxidative stress and its deleterious effects and thereby improve the quality and nutritional value of food [2]. Polyphenols are complex group of secondary metabolites, widely distributed in plants and food of plant origin, divided into several classes [3]. The most represented classes of phenol compounds in human diet are phenolic acids and flavonoids [4]. There are numerous other biological processes affected by polyphenols, such as antioxidant activity, protection against cancer [5], cardiovascular diseases [6], inflammatory, allergic, diarrheic and ulcerous disorders [7] and antihypertensive effects [8]. Polyphenols may be detrimental when taken in larger doses and found in dietary supplements and fortified foods, despite the numerous health benefits [9]. In an applied work of the Phenol-Explorer database, which contained information of 502 polyphenols in 452 foods, the richest sources of polyphenols were identified as various spices and dried herbs [10]. The fat in cheese can be degraded by lipolysis due to lipase activity (somatic cells and microorganisms) or oxidation. The extent of lipolysis in Cheddar cheeses has a major impact on its sensory characteristics and excessive lipolysis is associated with downgrading due to oxidative rancidity [11]. One of the studies shows a potential use of rosemary oleoresin as an antioxidant to increase the shelf life of aged Cheddar [12]. Lipolysis and proteolysis are higher in ripened than in fresh cheese for all cheese varieties [13]. The use of the wild garlic herb (*Allium* sp.) in herby pickled cheese production revealed a significant increase in total free fatty acids (TFFAs) contents due to an increase in the herb level [14]. The increasing of mendi (*Chaerophyllum* sp.) amount in cheese samples had a significant ($P < 0.05$) effect on the lipolysis level at 2 and 90 days [15]. In one recent review authors confirmed the benefits of using of antimicrobial herb and spice compounds in food [16].

Design of our study is supported by the results obtained by HPLC/DAD analysis on the phenol component of *Kitaibelia vitifolia* ethanol herb extract [17]. Rosmarinic acid (2.937 mg/g of extract) was determined as a dominant compound. Total phenolics, flavo-

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noids, condensed tannins, and gallotannins were 85.25±0.69 mg of GA/g, 45.32±0.55 mg of RU/g, 54.25±0.75 mg of GA/g, and 41.74±0.55 mg of GA/g, respectively. Extract of *K. vitifolia* possesses total antioxidant capacity of 75.45±0.68 μg of AA/g. Antimicrobial activity of the *K. vitifolia* extract was determined by the dilution method, with Minimal Inhibitory Concentrations from 15.62 to 62.50 μg/mL [17].

The main aim of fortification of Pirotski Kachkaval using identified and quantified polyphenolic compounds which contain ethanol extract of the *K. vitifolia* was to get healthier and more sustainable delicacies. The purpose of the research within this study was to investigate the antioxidant effects of polyphenols in the complex food matrix such as the cheese, physico-chemical composition, microbiological quality, sensory properties of Pirotski Kachkaval, using the herb extract applied in two different manners.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl hydrate (DPPH), Folin–Ciocalteu, ascorbic acid and butylated hydroxytoluene (BHT) were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Hydrochloric acid, formaldehyde, anhydrous sodium carbonate, methanol, ethanol, acetone and petroleum ether were purchased from Centrohem (Centrohem, Stara Pazova, Serbia). All of the other chemicals and reagents were of analytical reagent grade.

Plant material

Kitaibelia vitifolia is a member of the *Malvaceae* family. The above-ground part of the test plant was collected in Central Serbia, at the flowering stage. The species was identified and the voucher specimen was deposited at the Department of Botany, Faculty of Biology, University of Belgrade (16350 BEOU).

Preparation of herb extract

Samples prepared from over ground part of the plant *K. vitifolia* (10.0 g) were extracted by 96% ethanol (100.0 mL) as a solvent. The extraction process was carried out using an ultrasonic bath (Branson and Smith-Kline Company, model B-220, Danbury, CN, USA) at room temperature for 1 h. The goal was the highest extraction yield of phenol acids. After filtration, 5 mL of the liquid extract was used for extraction yield determination. The solvent was removed by a rotary evaporator (Devarot, Elektromedicina, Ljubljana, Slovenia) under vacuum, and was dried at 60 °C to constant weight. The dried extracts were stored in glass bottles at 4 °C to prevent oxidative damage until analysis. Spectrophotometric measurements were performed using a UV–Vis spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia).

Cheese formulation and processing

Pirotski Kachkaval (one of the cheese variety with unique technological process which included texturizing of ripe curd, categorized in the group of Pasta-Filata-Cheese), produced in the laboratory of "Obren Pejić" Dairy School in Pirot, Republic of Serbia by followed procedure: mixture of cow, sheep and goat raw milk (82:16:6 by volume) was heated in cheese vat at the temperature of 32 °C. Heated milk blend was coagulated with rennet for 40–45 min. Processing of cheese curd consists of cutting and stirring the curd in order to achieve forming of grain and it is done using special cheese making harps. Attrition of curd lasts 10 min, subsequently the process of grain formation is stopped for 10 min in order for grains to settle and squeeze whey as much as possible. Second heating-scalding was performed at the temperature of 38 to 42 °C. The dynamics and intensity of heat required to raise the temperature of curd for 1 to 2 °C every 2–3 min. Drying takes 30 to 40 min with constant stirring. Pressing was carried out at a pressure of 5 to 10 kg of cheese per kg of weight in a time of 30 to 45 min, to separate whey and then cut. The resulting fresh curd is cut into pieces the size of 5 to 10 kg and transferred to the ripening chamber and left on the draining table until the next day for acidification (cheddaring). Maturation of the fresh curd, so-called "baskija", aims to change its proteins. Ripe, acidified curd, shows ability for stretching and kneading by soaking in hot water. Maturation of the "baskija" performed by the lactic bacteria acquired in milk during milking and cooling in the chambers at a temperature of 30 °C and it lasts from 4 to 12 h. After completing the ripening, "baskija" is cut into slices with a thickness of 0.5 cm using a special macerator. "Baskija" is afterwards thinly placed into weaved baskets of hazel brushwood on texturizing, in the quantity needed for a one ball of Kachkaval, and immersed in hot water at a temperature of 75 °C. Water for texturizing consisted of one third of used water that was used the day before and two thirds of fresh water. Texturizing in a basket takes 5 to 8 min. On a separate cheese-making table, the textured curd is streched, twisted and dry salted. Obtained texturized curd was divided into three experimental groups and 3 balls for each group (nine balls in total). The extract of *K. vitifolia*, in the active concentration of 3.0 mL/100 g of cheese was added to the curd of the three balls during their formation (experimental group I – EG I). In addition to the 3 balls that belong to EG I, six more cheese extracts were formed into balls, and all nine balls were placed in molds where remains until the next day. Ripening of Kachkaval performed in the room for cheese ripening at the temperature of 15–18 °C.

Once the cheese is removed from the mold, we separated three cheese samples belonging to the EG I. Surface of the next three balls of cheese was treated with a spray solution (10 mL per ball) of the *Kitaibelia vitifolia* extract and formed experimentally group II – EG II. The remaining 3 samples of cheese were the control group C, produced in conventional manner, without any modifications. Samples of EG II were packaged after a 20-min period of herb extract spray solution exposure. At the same time, samples of control and EG I of ripened Kachkaval was packaged in the same manner (under vacuum in polyethylene bags), labeled and declared as Pirotski Kachkaval.

Preparation of the cheese aqueous extract for antioxidant activity testing

Kachkaval cheese was chopped and then homogenized in the blender. Ten grams of homogenized cheese sample was taken and dissolved in 10 mL of distilled water to obtain a concentration of 1 mg per 1 mL of distilled water.

Determination of total antioxidant capacity

The total antioxidant activity was evaluated by the phosphor-molybdenum method [18]. The assay is based on the reduction of Mo (VI)–Mo (V) by antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A total of 0.3 mL of cheese sample was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. Then, the absorbance of the solution was measured at 695 nm using spectrophotometer against the blank after cooling to room temperature. Methanol (0.3 mL) was used as the blank. Ascorbic acid was used as the standard and total antioxidant capacity was expressed as mg of ascorbic acid per g of prepared cheese extract.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The described method used by group of authors [19] was adopted with suitable modifications [20]. DPPH (8 mg) was dissolved in MeOH (100 mL) to obtain a concentration of 80 μg/mL. Serial dilutions were carried out with the stock solution (1 mg/mL) of the cheese extract. Solutions (2 mL each) were then mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur, and the absorbance was measured at 517 nm. Ascorbic acid, gallic acid and butylated hydroxytoluene were used as referential standards and dissolved in methanol to make the stock solution with the same concentration (1 mg/mL). Control sample was prepared containing the same volume without test compounds or reference antioxidants. Ninety-five % of methanol cheese extract was used as a blank. The DPPH free radical scavenging activity (%) was calculated using the following equation:

$$\text{Inhibition} = 100\frac{A_c - A_s}{A_c} \quad (1)$$

The percentage inhibition values were calculated from the absorbance of the control (A_c) and of the sample (A_s), where the controls contained all the reaction reagents except the extract or positive control substance (Eqs. (1)–(3)). The IC_{50} value, defined as the concentration of the test material that leads to 50% reduction in the free radical concentration, was calculated as μg/mL through a sigmoid dose-response curve.

Determination of inhibitory activity against lipid peroxidation

Antioxidant activity was determined by the thiocyanate method [21]. Serial dilutions were carried out with the stock solution (1 mg/mL) of the cheese extract, and 0.5 mL of each solution was added to linoleic acid emulsion (2.5 mL, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g linoleic acid, 0.2804 g Tween-20 as emulsifier in 50 mL 40 mM phosphate buffer and the mixture was then homogenized. The final volume was adjusted to 5 mL with 40 mM phosphate buffer, pH 7.0. After incubation at 37 °C in the dark for 72 h, a 0.1 mL aliquot of the reaction solution was mixed with 4.7 mL of ethanol (75%), 0.1 mL $FeCl_2$ (20 mM) and 0.1 mL ammonium thiocyanate (30%). The absorbance of the mixture was measured at 500 nm and the mixture was stirred for 3 min. Ascorbic acid, gallic acid, α-tocopherol and butylated hydroxytoluene were used as reference compounds. To eliminate the solvent effect, the control sample, which contained the same amount of solvent added to the linoleic acid emulsion in the test sample and reference compound, was used. Inhibition percent of linoleic acid peroxidation was calculated using Eq. (1).

Measurement of ferrous ion chelating ability

The ferrous ion chelating ability was measured by the decrease in absorbance at 562 nm of the iron (II)––ferrozine complex [22,23]. One milliliter of 0.125 mM $FeSO_4$ was added to 1.0 mL sample (with different dilutions), followed by 1.0 mL 0.3125 mM ferrozine. The mixture was allowed to equilibrate for 10 min before the absorbance was measured. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the expression of the right-hand side of Eq. (1).

Determination of hydroxyl radical scavenging activity

The ability of *K. vitifolia* to inhibit non site-specific hydroxyl radical-mediated peroxidation was carried out

according to the described method [24]. The reaction mixture contained 100 μL of cheese extract dissolved in water, 500 μL of 5.6 mM 2-deoxy-D-ribose in KH_2PO_4–NaOH buffer (50 mM, pH 7.4), 200 μL of premixed 100 μM $FeCl_3$ and 104 mM EDTA (1:1, *V*/*V*) solution, 100 μL of 1.0 mM H_2O_2 and 100 μL of 1.0 mM aqueous ascorbic acid. Tubes were vortexed and incubated at 50 °C for 30 min. Thereafter, 1 mL of 2.8% TCA and 1 mL of 1.0 % TBA were added to each tube. The samples were vortexed and heated in a water bath at 50 °C for 30 min. The extent of oxidation of 2-deoxyribose was estimated from the absorbance of the solution at 532 nm. The values are presented as the means of triplicate analyses.

Proximate composition of Pirotski Kachkaval

Moisture content, total solids, fat content, fat in total solids and proteins were determined by standard methods [25].

Determination of NaCl content

Sodium chloride content was determined by a modified Volhard method [26]. After acidification, the sample was added to the solution of silver nitrate in excess, and this excess was titrated standard volumetric solution of ammonium thiocyanate, to the emergence of a stable pink-brown coloration.

Determination of pH values

The pH values of the samples were measured using a laboratory pH-meter, model Cyber Scan 510 pH Meter, EUTECH Instruments, Landsmeer, The Netherlands. pH is measured in the homogenized Kachkaval sample by the procedure that corresponds to the used pH-meter. When a constant value is reached, the pH is read directly from the instrument with an accuracy of 0.01 pH units [27].

Determination of water activity (a_w value)

a_w Value was obtained according to the manufacturer's instructions, FA-st/1 User Manuel (GBX Scientific Instruments, Bourg de Peage, France). Container unit of device FA-st/1 is filled with prepared Kachkaval sample to the ¾ and read off a_w value. The device is calibrated by adjusting the values of a_w corresponding value for the selected reference salts.

Determination of total free fatty acids (TFFA) content

The total free fatty acids (TFFA) content of cheese samples was determined by the extraction titration method [28]. Three grams of grated cheese (W) were made into a paste with 5 mL of distilled water. Ten mL of extraction mixture containing isopropanol, petroleum ether and 4 N sulphuric acid in the proportion of 40:10:1 by volume was added to the paste. Six mL of petroleum ether was then added thorough mixing. The test tubes were kept at 40 °C for 10 min. The contents were vigorously shaken for 20 s. The two layers were allowed to separate (5–10 min) and an aliquot of the upper layer (V = 5 mL) was withdrawn and transferred to a 50 mL conical flask. After adding six drops of 1% methanolic phenolphthalein indicator, the contents were titrated with 0.02 N methanolic potassium hydroxide solution (V). Designation N in Eq. (2) is used for normality of methanolic potassium hydroxide solution, and its value is constant (0.02 N). The reagent blank was used to obtain background titration. The TFFA content of cheese was calculated by the following formula:

$$TFFA = 1000\frac{V\mathrm{N}}{VW}\ [\mu g/g \text{ of fat}] \quad (2)$$

Microbiological analysis

Processed cheese samples were analyzed for colony count of aerobic bacteria according to ISO 6610:2002 [29], coagulase-positive staphylococci according to ISO 6888-1:2005+A1:2005 [30] and anaerobic sulfite-reducing bacteria according to ISO 15213:2003 [31] at the first day of the storage.

Sensory analysis

Sensory analyses of Pirotski Kachkaval samples were performed by five members of the expert committee applying scoring system including the following characteristics: appearance (max. 2 points), colour (max. 1 points), cross section (max. 3 points), odour (max. 2 points) and taste (max. 10 points). The total number of points was a maximum of 18.

Statistical analysis

The results of examinations are presented as mean ± standard deviations of three determinations. Statistical analyses were performed using analysis of variance. Multiple comparisons of means were done by least significant difference (LSD) test. All computations were made by employing the SPSS software, version 15.0 (SPSS, Chicago, IL, USA). IC_{50} values were calculated by nonlinear regression analysis from the sigmoid dose-response inhibition curve.

RESULTS AND DISCUSSION

Antioxidant effects

Results of antioxidant activity testing obtained are shown in Table 1.

The levels of phenolic-linked antioxidant activity revealed a slight emphasis of phenol-linked antioxidant activity of fortified samples of cheese in comparison to samples of the control group. Samples of cheese from EG II (treated with a spray solution of the *Kitaibelia vitifolia* extract – 10 mL per ball) shown the best antioxidant activity. Plant extracts were added to balls of

Table 1. The antioxidant activities of Pirotski Kachkaval samples after 45 d of ripening; mean values ± standard error of three trials; EG I – experimental group I; EG II – experimental group II; C – control group

Sample	IC_{50}[a] / μg mL^{-1}				Total antioxidant capacity μg AA/g
	DPPH scavenging activity	Inhibitory activity against lipid peroxidation	Metal chelating activity	Hydroxyl radical scavenging activity	
EG I	67.45±1.45	94.46±1.23	55.01±0.18	109.41±0.32	58.85±0.80
EG II	63.48±1.44	92.90±0.91	53.15±0.95	101.25±0.43	59.34±0.47
C	66.20±0.89	93.23±1.22	55.89±0.86	108.47±0.52	59.15±0.65
Gallic acid (GA)	3.79±0.69	255.43±11.68	–	59.14±1.10	–
Ascorbic acid (AA)	6.05±0.34	> 1000	–	160.55±2.31	–
Butylated hydroxytoluene (BHT)	15.61±1.26	1.00±0.23	–	33.92±0.79	–
α-Tocopherol	–	0.48±0.05	–	–	–

[a]Values were determined by nonlinear regression analysis

EG I before the heat treatment, but in EG II after heat treatment, on the surface like spray. Our results showed that the temperature of 75 °C during heat treatment inhibited the activity of used plant extracts and thereby reduce the antioxidant activity of the same samples in EG I. Lower IC_{50} value indicates stronger antioxidant activity, and the first four test methods demonstrated the strongest antioxidant activity in samples of EG II. Total antioxidant capacity method revealed the highest capacity (expressed as– μg ascorbic acid (AA)/g) in samples of cheese from the EG II. Herbal extract (applied in different manners) as expected emphasized the antioxidant activity in comparison to samples of the control group. Direct interactions between polyphenols and food proteins and polysaccharides may affect their absorption. The presence of phenol hydroxyl groups of phenol acids is partly responsible for the antioxidant potency of modified cheese. Unsignificantly different level of total antioxidant capacity was found in comparison of the samples of Pirotski Kachkaval from the experimental group EG I to the experimental group EG II. Comparing the level of activity was obtained by addition of phenolic and flavonoid substances identified from above-ground parts of the *K. vitifolia* and activity of BHT, it was observed that the synthetic oxidant with a significantly stronger effect. From the Table 1, the antioxidant activity of modified EG I and EG II experimentally groups of Pirotski Kachkaval cheese was lower than synthetic antioxidant BHT which could be due to the chemical composition of the cheese (fat and protein contents) and the heat treatment of cheese (75 °C) which makes the cheese more sensitive to the oxidation. DPPH is a steady radical, often used to estimate the antioxidant activity of certain natural products and tracklements as well. The degree of the decolorization of purple color of DDPH radicals and reduction of absorbance to 517 nm imply to "scavenger" potential of the samples used in the research of Pirotski Kachkaval. The results of the DPPH scavenging activity with IC_{50} values from 67.45 (EG I), 63.48 (EG II) and 66.20 μg/mL (C) show an average high antioxidant potential in the examined samples. In comparison with the antioxidants that we used as a control group for the level of antioxidant activity, the samples of Pirotski Kachkaval from the control groups EG I, EG II and C have higher IC_{50} values, thus resulting in a lower antioxidant activity.

The examined samples of Pirotski Kachkaval from the experimental series EG I and EG II, as well as those from the control group C show an incredibly high level of inhibitory activity against lipid peroxidation compared to gallic acid and ascorbic acid as the control antioxidants, while the same groups show a rather low level of inhibitory activity against lipid peroxidation when compared to the other two synthetic antioxidants (BHT and α-tocopherol). This result is probably the consequence of the forming of oxidant products and the creation of evaporable substances. Malondialdehyde and the other short chain products are not steady and they are reduced to alcohols and acids which cannot be defined using the method of inhibitory activity against lipid peroxidation.

Hydroxyl radical is a highly aggressive oxidant, capable of oxidizing the majority of biomolecules at a very high speed. Hydroxyl radicals can oxidize polyunsaturated fatty acids and start the natural lipid peroxidation. IC_{50} value of the samples of Pirotski Kachkaval of the control groups EG I, EG II and the control group C, using the method hydroxyl radical scavenging activity show a high level of activity compared to ascorbic acid, and a significantly lower degree of activity compared to gallic acid and BHT. Moreover, since all the antioxidants act synergistically against the noxious effects of oxidative stress [32], the assessment of the total amount of electron-donating antioxidants in foods including cheese may be an interesting approach as a supplement to the measurement of individual dietary antioxidant contents [33].

Physicochemical analyses of Pirotski Kachkaval

Statistically significant ($P < 0.05$) changes were recorded in moisture content and total solids of control (C) and experimental series (EG I and EG II) of cheeses. Fat content, milk fat in dry matter, protein content, pH and a_w values did not differ significantly ($P > 0.05$) between samples of control and experimental groups (Table 2). Lipolysis results in the formation of free fatty acids (FFAs), and statistically significant ($P < 0.05$) changes were recorded between control and cheese samples belonging EG I, where herb extract added in cheese body. Two groups of authors reported very similar results for 20 traditional Turkish ripened herby cheese [13,34]. By comparing the results of our testing with the results that are obtained in specific characteristics of Pirotski Kachkaval study [35,36] minor differences were observed in mean values of chemical parameters (lower total solids and fat content and higher content of fat in total solids), and protein content is very similar. These differences were probably caused by the use of different raw materials compared to those that were used in the previous studies as well as due to application of herb extract and minor differences in the cheese manufacturing process.

The results of microbiological tests

Microbiological analysis revealed that values for colony count of aerobic bacteria, *Coagulase-positive staphylococci* and *Staphylococcus aureus* and anaerobic sulfite-reducing bacteria did not differ significantly ($P > 0.05$) between samples of control and experimental groups (Table 3). The samples from the control and experimental groups of Pirotski Kachkaval were of a high microbiological quality immediately after production, due to the steaming heath treatment of cheese balls on pasteurization temperatures (75 °C for 5 to 8 min). That is very important because it is known that the total aerobic bacteria counts and moulds yeasts were higher in dry-salted and raw milk cheeses [37].

The results of sensory analysis

The sensory evaluation of the fortified Pirotski Kachkaval cheese of EG I and EG II and conventionally produced cheese originating from control group (C) was conducted using 5-point hedonic scale and the mean scores obtained are summarised in Fig. 1. The panellist could not find any difference in appearance, color, cheese body and cross section up to 45 days of ripening for both control and fortified groups; Assesors gave slightly higher scores for the odour of Pirotski Kachkaval samples from EG I compared with C and EG II samples, up to 45 days of ripening. The quality of the cheese body was high and uniformed (in all 3 groups ranked from 1.5 out of a maximum of 2 points). Pirotski Kachkaval was slightly softer consistency as expected, because the assessed after 45 days of ripening, so that the period of ripening after vacuuming was relatively short, it can take up to 3 months). The cross section of samples was very high and uniformed (2.5 out of a maximum of 3 points), with a note that the cheese was of a closed structure with a small number of technological tiny holes.

The odour of the Pirotski Kachkaval cheese was typical, pleasant, stronger in the EG I, under-expressed

Table 2. Physicochemical properties of Pirotski Kachkaval samples after 45 d of ripening; mean values ± standard error of three trials; EG I – experimental group I; EG II – experimental group II; C – control group; a and b indicate differences (P < 0.05) between rows, LSD test

Physicochemical property	C	EG I	EG II
Moisture content, %	47.85±0.06[a]	47.07±0.04[b]	47.20±0.05[b]
Total solids, %	52.15±0.06[b]	52.93±0.04[a]	52.80±0.05[a]
Fat content, %	26.6±0.33[a]	26.67±0.33[a]	26.50±0.29[a]
Fat in total solids, %	51.14±0.66[a]	50.38±0.65[a]	50.19±0.51[a]
Proteins, %	21.39±0.10[a]	21.62±0.69[a]	21.71±0.05[a]
NaCl, %	1.99±0.03[a]	2.03±0.02[a]	2.06±0.01[a]
pH	4.88±0.00[a]	4.90±0.00[a]	4.88±0.00[a]
Water activity (a_w value)	0.94±0.00[a]	0.95±0.00[a]	0.94±0.00[a]
Total free fatty acids (TFFA) content, μg/g	2.18±0.00[a]	2.00±0.00[b]	2.18±0.00[a]

Table 3. Microbiological quality of Pirotski Kachkaval samples; EG I – experimental group I; EG II – experimental group II; C – control group

Experimental group	Aerobic colony count, cfu/g	Coagulase-positive *Staphylococci* and *Staphylococcus aureus*, cfu/g	Anaerobic sulfite-reducing bacteria, cfu/g
C	< 3.000.000	< 100	< 100
EG I	< 3.000.000	< 100	< 100
EG II	< 3.000.000	< 100	< 100

in samples from the control group and the samples collected from the EG II, so that the maximum number of points (2). Commission awarded evaluated samples from the EG I, and the samples evaluated for the samples from the control and EG II at 1.5 points (high score). In judging the taste, predominant flavor was the one of unmaturated cheese, not enough for the type of cheese to be expressed as the salty taste, and the experimental group specific and more prominent than in EG II. The best taste was found in the samples from the EG I (8.5 point out of a maximum 10 points). Generally, the highest sensory quality of the cheese was noted in the evaluation of EG I (17.5 out of a maximum 18 points), followed by samples of the EG II (16.5 points) and control group (16.0 points). Modification of the cheese by treatment with the extract of *K. vitifolia* has beneficial effects on the sensory quality regardless on the way of application, where the effects were more noticeable when adding the extract to the cheese body after texturizing of ripened curd in relation to the spraying of the cheese surface with herb extract.

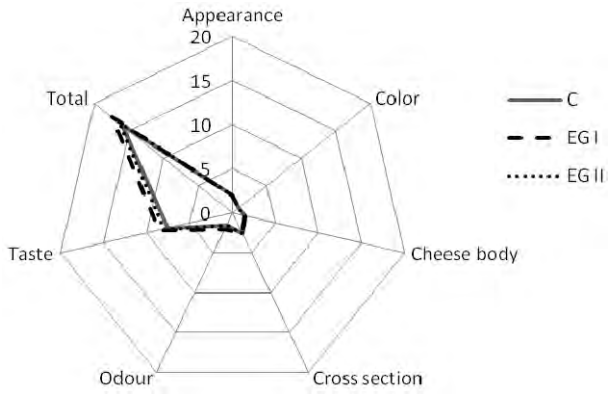

Figure 1. Sensory properties of cheese samples after 45 d of ripening.

CONCLUSIONS

The Pirotski Kachkaval samples were produced in a mixture of cow, sheep and goat raw milk in traditional technological process under industrial conditions, applying the appropriate extract or a spray solution of ethanol extract of the *Kitaibelia vitifolia*. Significant differences were found in moisture content and total solids of control (C) and experimental series (EG I and EG II) of cheeses. The application of the sprayed ethanol extract of the *K. vitifolia* on the surface of the cheese led to a stronger level of antioxidant activity and the highest total antioxidant capacity in the samples of EG II. Plant extract (applied in different manners) as expected fortified the antioxidant activity in comparison to samples of the control cheese. The fortification of Pirotski Kachkaval with *K. vitifolia* plant extract didn't interfere with the sensory perception of traditional cheese. The modified cheese samples (EG I and EG II) had higher sensory evaluation scores than the control group samples.

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IZVOD

EFEKAT EKSTRAKTA BILJKE *Kitaibelia vitifolia* NA ANTIOKSIDATIVNU AKTIVNOST, HEMIJSKI SASTAV, MIKROBIOLOŠKI STATUS I SENZORNA SVOJSTVA PIROTSKOG KAČKAVALJA

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(Naučni rad)

Cilj ispitivanja je da se odredi uticaj fortifikovanja sira (Pirotski kačkavalj) polifenolima poreklom iz etanolnog ekstrakta biljke *Kitaibelia vitifolia*, primenjenog na dva različita načina (dodavanjem u grudu pre uobličavanja ili prskanjem površine sira). Sproveli smo ispitivanja antioksidativnog efekta polifenola, fizičko–hemijskog sastava, mikrobiološkog kvaliteta i senzornih svojstava Pirotskog kačkavalja. Antioksidativna aktivnost konvencionalno proizvedenih i fortifikovanih sireva je ocenjivana korišćenjem pet savremenih i kompatibilnih metoda, i utvrđen je umereni rast antioksidativne aktivnosti poreklom od fenola u uzorcima fortifikovanog sira u odnosu na uzorke kontrolne grupe. Fortifikovani Pirotski kačkavalj je imao više ocene pri senzornom ispitivanju u odnosu na uzorke kontrolne grupe. Statistički značajne razlike ($P < 0.05$) su zapažene u sadržaju vlage i suve materije između uzoraka kontrolne grupe i uzoraka modifikovanih oglednih grupa sira, a ostali parametri se nisu značajno razlikovali ($P > 0.05$).

Ključne reči: Pirotski kačkavalj • *Kitaibelia vitifolia* • Polifenoli • Antioksidativna aktivnost • Mikrobiološki kvalitet • Senzorna svojstva