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Effect of red paprika on physicochemical, antioxidant, microbiological and sensory properties of grilling cheese

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Abstract

This study investigated the impact of red paprika (*Capsicum annuum* L.) in powdered, crushed and fresh (sweet or hot) forms on the physicochemical, sensory, antioxidant and microbiological properties of grilling cheese (Queso Blanco-type). Seven cheese variants were produced: a control (C) and six paprika-enriched formulations (P1-P6), with paprika added at concentrations ranging from 0.5 to 4.0 %. Paprika incorporation significantly ($p<0.05$) increased moisture content (54.60-57.18 % vs. 54.09 %) while decreasing protein content and the protein-to-fat ratio. Enriched samples showed significantly higher phosphorus (59.47-117.42 vs. 50.30 mg/100 g) and magnesium levels compared to the control. The variant enriched with 4.0 % fresh sweet red paprika (P4) exhibited a favorable lipid profile, with 25.15 % MUFA and 3.17 % PUFA, and a significantly reduced thrombogenic index (TI = 3.54) compared to the control (3.88), while the atherogenic index (AI) showed a slight, non-significant decrease. Total phenolic content was significantly higher in enriched samples (612.13-938.37 vs. 577.78 µg GAE/g), with enhanced radical scavenging activity (ABTS: 531.90-595.42 µmol TE/g; DPPH: up to 46.40 µmol TE/g in crushed paprika variants). Antimicrobial activity, assessed by the disk diffusion method, was observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with no activity detected against *Escherichia coli*, *Bacillus cereus* or *Candida albicans*. Sensory evaluation identified P4 as the most promising formulation, showing no statistically significant differences from the control in any evaluated attribute ($p>0.05$). The results obtained indicate that red paprika can be used as a functional, natural ingredient in the formulation of nutritionally enriched dairy products.

Keywords: grilling cheese; red paprika; antioxidant activity; fatty acid profile; sensory quality

Introduction

The growing consumer demand for minimally processed, nutritionally enhanced dairy products has garnered significant attention in the development of functional cheese varieties enriched with natural plant-based ingredients (Salehi, 2021). Among these, grilling cheeses represent a distinct and increasingly popular category, valued for their unique textural properties and versatility in culinary applications. Within this group, grilling cheeses with high melting points commonly include acid-heat coagulated varieties, including globally recognized types such as Ricotta, Latin American Queso Blanco and Indian Paneer (Popović-Vranješ, 2015; Farkye, 2017). These cheeses resist melting due to acid-heat coagulation, a process in which high temperatures (82-90 °C) induce the denaturation of whey proteins and their subsequent interaction with casein micelles, contributing to the formation of a stable protein network that remains firm when reheated (Lucey, 2002; Farkye, 2017). While other grilling cheeses, such as Halloumi, achieve their heat-stable structure through rennet coagulation followed by cooking the curd in hot whey (Deshwal et al., 2020), Queso Blanco-type cheese was selected as the model system for this study. This choice is justified by its standardized production process and its characteristic mild, neutral flavor profile, which makes it an ideal matrix for evaluating the impact of functional additives without the interference of strong baseline flavors or complex ripening microbial cultures (Farkye, 2017).

To achieve such nutritional enhancement, modern dairy approaches increasingly focus on improving nutritional and sensory quality by incorporating natural additives (Tarakcı and Deveci, 2019; Ritota and Manzi, 2020). Driven by the preference for preservative-free foods enriched with bioactive compounds, plant-based additives, including spices and herbs, have gained attention for their role in natural preservation and sensory enhancement (Ritota and Manzi, 2020; Gamage et al., 2023; Indu et al., 2023). These ingredients are rich in bioactive compounds such as phenolic acids and flavonoids, which exhibit antioxidant and antimicrobial activities (Indu et al., 2023). In fresh cheese matrices, where microbial growth and proteolysis are the primary causes of spoilage, the antimicrobial properties of these compounds are particularly important for extending shelf life. However, incorporating plant materials into dairy systems can induce complex interactions within the protein matrix, potentially altering the textural properties and water-holding capacity of the final product (Olmedo et al., 2013).

In this context, *Capsicum annuum* L., commonly known as red paprika, emerges as a highly suitable candidate for enrichment. It is a widely cultivated vegetable valued for its intense color, aroma and functional properties (Loizzo et al., 2016; Brezeanu et al., 2022). It is a rich source of bioactive compounds, including capsaicin, carotenoids and polyphenols, which exhibit strong antioxidant, antimicrobial and anti-inflammatory activities (Loizzo et al., 2016; Kolašinac et al., 2025). While research has shown that adding red pepper and its bioactive components to products such as yogurt or processed cheese can enhance their antioxidant capacity and nutritional profile (Šeregelj et al., 2019; Atwaa et al., 2020; Okur, 2022; Koçak and Tarakçı, 2025), its application in fresh grilling cheese remains largely unexplored. Specifically, the comparative effects of different physical forms of red paprika on the overall quality of grilling cheese have not been fully elucidated, representing a notable gap in the existing literature. In line with current trends in developing innovative dairy products that combine traditional gastronomic appeal with enhanced functional benefits (Ritota and Manzi, 2020), this study aimed to evaluate the impact of red paprika (*Capsicum annuum* L.) added in powdered, crushed and fresh forms on the physicochemical, antioxidant, antimicrobial and sensory properties of Queso Blanco-type grilling cheese.

Materials and methods

Cheese production

Cheese was produced from cow's milk obtained from a local dairy farm. The raw milk used in this study had an average composition of 3.90 % fat, 3.40 % protein and 13.30 % dry matter, with a pH of 6.65. The milk was filtered and heat-treated at 85 °C for 10 min. Coagulation was induced at the same temperature using 0.3 % citric acid, and the mixture was left undisturbed for 10 min to form the curd. The curd was drained through cheesecloth to separate the whey, weighed and salted at 2.0 % of curd weight. In addition to the control sample (C), six experimental variants (P1-P6) were produced by incorporating different forms and concentrations (0.5-4.0 %) of red paprika (*Capsicum annuum* L.), either individually or in combination (P6), as detailed in Table 1. All paprika samples were obtained from a local supplier to ensure commercial representativeness. Fresh paprika varieties (sweet and hot) were washed, deseeded and cut into approximately 5 mm pieces prior to incorporation. To ensure uniform distribution, all additives were mixed into the curd immediately before molding. The samples were then pressed for 1 h using a load-to-cheese mass ratio of 2:1, cooled to 4 °C and stored under identical conditions until analysis. Three batches of cheese samples were prepared for each cheese type under identical processing conditions, then combined and homogenized to obtain a composite sample representative of each treatment.

Table 1. *Types of red paprika and their concentrations in grilling cheese*

Sample	Addition	Concentration (%)
C	No addition	-
P1	Sweet red paprika powder	0.50
P2	Hot red paprika powder	0.50
P3	Crushed hot paprika flakes ("sudžuk" variety)	1.00
P4	Fresh sweet red paprika	4.00
P5	Fresh hot red paprika	4.00
P6	Crushed hot paprika flakes ("sudžuk" variety) and fresh sweet red paprika	0.50 and 2.00, respectively

**All forms of red paprika used in this study belong to Capsicum annuum L.*

Analysis of composition and physicochemical properties

Before analysis, cheese samples were finely ground using a laboratory blender to ensure homogeneity. Cheese chemical quality parameters were analyzed using standard methods. Moisture content and dry matter were determined by oven drying at 103±2 °C to constant weight (BAS EN ISO 5534:2006). Ash content was measured gravimetrically after incineration at 550 °C in a muffle furnace (VIMS Electric LPŽ 75, Tršić, Republic of Serbia). Protein content was calculated from total nitrogen determined by the Kjeldahl method, following BAS EN ISO 8968-1:2015. Fat content was determined using the acid-butyrometric method (BAS ISO 11870:2012). Moisture in non-fat matter (MNFM) and fat in dry matter (FDM) were subsequently calculated (Popović-Vranješ, 2015). Chloride content was measured by potentiometric titration (BAS EN ISO 5943:2015), while titratable acidity was determined using the Soxhlet-Henkel method, with the results expressed in degrees Soxhlet–Henkel (°SH). The pH was measured in a homogenized 1:1 suspension of cheese and distilled water using an HI 2211 pH meter (Hanna Instruments, Smithfield, USA). Water activity (aw) was assessed using a LabMaster-AW 1119971 (Novasina, Lachen, Switzerland). All analyses were performed in triplicate.

Mineral composition analysis

Cheese samples were digested using a mixture of nitric and perchloric acids for mineral determination. Both macro- and microelements were quantified using an Optima 8000 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Shelton, USA), with calibration performed using certified reference materials (CRM, 100 µg/mL) (Savanović et al., 2023). All analyses were conducted in triplicate.

Fatty acid analysis

Total lipids were extracted using the Folch method (chloroform: methanol, 2:1). Fatty acid methyl esters (FAME) were prepared by transesterification with saturated KOH/methanol. Gas chromatography (GC) analysis was performed on a Clarus 680 gas chromatograph (Perkin Elmer, Shelton, USA) equipped with a flame ionization detector (FID) and an Elite-Wax L column (60 m × 0.32 mm × 0.5 µm). Injector and detector temperatures were set at 250 °C, and the injection volume was 1 µL. The oven temperature program was as follows: 60 °C for 2 min, then increased at 10 °C/min to 200 °C and at 5 °C/min to 240 °C, held for 30 min. Nitrogen was used as the carrier gas (1.5 mL/min). Fatty acids were identified by comparing their retention times with a standard FAME mixture. Fatty acid composition was expressed as a percentage of total identified fatty acids. All analyses were performed in triplicate. The atherogenicity index (AI) and the thrombogenicity index (TI) were determined using the following equations (Barać et al., 2025):

$$AI=[\sum C12:0+(4\times C14:0)+C16:0]/\sum UFA \quad /1/$$

$$TI=(C14:0 + C16:0 + C18:0)/[(0.5\times\Sigma MUFA) + (0.5 \times\Sigma n\text{-}6 PUFA) + (3\times\Sigma n\text{-}3 PUFA) + (n\text{-}3/n\text{-}6)] \quad /2/$$

Instrumental color and texture analysis

Instrumental color was measured using a CM-2600d spectrophotometer (Konica Minolta, Osaka, Japan) according to the CIE L*a*b* system. Measurements were taken on both the surface and the cross-section of the cheese samples. Cheese hardness was determined by measuring shear force using a TA.XT plus Texture Analyser (Stable Micro Systems, Godalming, UK) equipped with a Warner-Bratzler shear blade (HDP/BS), with the results expressed in newtons (N). Color and texture measurements were conducted ten times per sample.

Analysis of antioxidant activity

Cheese samples were extracted with 80 % ethanol using 10 min of ultrasonic treatment (ultrasonic bath WUC-A03H, Witeg Labortechnik, Wertheim, Germany), followed by two additional reflux extractions of 10 min each. The combined extracts were filtered, and the final concentration was adjusted to 100 g/L. Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method as described by Wolfe et al. (2003). Non-flavonoid content (TNF) was measured using the formaldehyde precipitation method according to Alberto et al. (2006), while the flavonoid content (TF) was calculated as the difference between total phenolics and non-flavonoids. The results for TPC, TNF and TF were expressed as micrograms of gallic acid equivalents per gram of cheese (µg GAE/g). Antioxidant capacity was evaluated using three complementary assays: DPPH, ABTS and FRAP. DPPH radical scavenging activity was measured according to the procedure described by Liyana-Pathirana and Shahidi (2005). The ABTS radical cation decolorization assay was performed following the method of Re et al. (1999). Results for both DPPH and ABTS were expressed as micromoles of Trolox equivalents per gram of cheese (µmol TE/g). Ferric reducing antioxidant power (FRAP) was determined according to Benzie and Strain (1996), and the results were expressed as micromoles of ferrous iron equivalents per gram of cheese (µmol Fe^{2+}/g). All antioxidant assays were performed in triplicate.

Antimicrobial activity

Antimicrobial activity of cheese extracts was determined using the disk diffusion and agar dilution methods (Ortez, 2005; Ivanović et al., 2022). Four bacterial cultures, *Escherichia coli* WDCM 00013, *Staphylococcus aureus* WDCM 00034, *Pseudomonas aeruginosa* WDCM 00024 and *Bacillus cereus* WDCM 00151, and two yeasts, *Candida albicans* WDCM 00054 and *Candida albicans* isolate (clinical isolate), were used. Bacterial and yeast cultures were prepared from the

logarithmic phase and by direct colony suspension according to the method of Ivanović et al. (2022).

Sensory evaluation

Sensory evaluation of the cheese samples was performed using descriptive sensory analysis by a panel of ten trained evaluators with prior experience in cheese sensory evaluation, in accordance with BAS ISO 22935-2:2011. To promote consistent and reproducible scoring, panelists attended an introductory session before the evaluation on the scoring method and the specific attributes to be assessed (appearance, color, odor, flavor and consistency), together with training on reference cheese samples to ensure consistent interpretation of the evaluation criteria. The samples were coded and presented in random order at room temperature. Evaluators assessed appearance, color, odor, flavor and consistency using a 5-point scale (1 = significant deviation from expected quality, 5 = no deviation). Between samples, evaluators rinsed their mouths with water.

Statistical analysis

Data were statistically analyzed using Microsoft Excel 2013 and IBM SPSS Statistics 22.0 (Armonk, USA). Results are presented as mean ± standard deviation (SD). Differences between means were evaluated by one-way ANOVA followed by Tukey's HSD multiple comparison test, with significance set at $p<0.05$.

Results and discussion

Physicochemical properties of cheese

The chemical composition of the cheese samples was significantly influenced ($p<0.05$) by the type and form of paprika added (Table 2). Overall, many of the observed compositional changes can be attributed to the dilution effect associated with the water content of the added paprika. Samples enriched with fresh red paprika (P4 and P5) exhibited the highest moisture and lowest dry matter contents. Moisture in non-fat matter (MNFM) increased in the majority of enriched variants (72.46-73.05 %) compared to the control (69.12 %), while sample P3 (69.68 %) did not differ significantly. This suggests that paprika particles, even in powdered or crushed forms, influence the protein matrix structure and the water-holding capacity of the cheese (Salehi, 2021; Tarakcı and Deveci, 2019). Regarding the lipid and protein fractions, paprika incorporation led to a significant decrease in protein content ($p<0.05$), with the lowest value observed in sample P4 (15.80 %). The fat content of the cheese samples differed significantly ($p<0.05$) among the variants, ranging from 21.40 % in sample P5 to 23.20 % in sample P6. Lower fat contents were found in samples with fresh paprika (P4, P5), while adding dried forms (P1, P2) resulted in slightly higher fat percentages compared with the control. This resulted in a significant reduction in the protein-to-fat (P/F) ratio across all enriched samples, a trend consistent with observations in other studies where modifications in cheese composition affect the P/F ratio (Lepesioti et al., 2021). The ash content increased significantly ($p<0.05$) in all paprika-enriched samples compared to the control, reaching a maximum of 2.91 % in sample P4, highlighting the mineral enrichment provided by the plant material (Atwaa et al., 2020; Savanović et al., 2023). Water activity (a_w) remained stable ($p>0.05$) across all cheese variants, while chloride content showed statistically significant differences ($p<0.05$) among some enriched samples. Nevertheless, the absolute changes in both parameters were small and are unlikely to have a meaningful impact on microbial growth. pH values decreased slightly in most enriched samples (P1, P4, P5 and P6), which can be attributed to the presence of natural organic acids in paprika (Brezeanu et al., 2022). In contrast, titratable acidity was lower in most of the enriched samples (P1, P3, P4 and P5) compared with the control. This apparent discrepancy between pH and titratable acidity may reflect differences in the buffering capacity of the cheese matrix, potentially influenced by mineral and protein content.

Table 2. Physicochemical properties of the tested cheese samples

Parameter	C	P1	P2	P3	P4	P5	P6
Moisture (%)	54.09±0.25^{a}	56.42±0.11^{d}	56.80±0.14^{de}	54.60±0.10^{b}	57.01±0.12^{e}	57.18±0.17^{e}	55.64±0.14^{c}
Dry matter (%)	45.91±0.25^{e}	43.58±0.11^{b}	43.20±0.14^{ab}	45.40±0.10^{d}	42.99±0.12^{a}	42.82±0.17^{a}	44.36±0.14^{c}
MNFM (%)	69.12±0.40^{a}	72.51±0.16^{b}	73.05±0.23^{b}	69.68±0.13^{a}	72.62±0.39^{b}	72.75±0.22^{b}	72.46±0.23^{b}
Fat (%)	21.75±0.10^{b}	22.20±0.05^{c}	22.25±0.05^{c}	21.65±0.10^{ab}	21.50±0.25^{ab}	21.40±0.10^{a}	23.20±0.05^{d}
FDM (%)	47.37±0.47^{a}	50.94±0.24^{bc}	51.50±0.29^{cd}	47.68±0.11^{a}	50.01±0.72^{b}	49.98±0.20^{b}	52.30±0.28^{d}
Proteins (%)	17.49±0.06^{e}	16.34±0.04^{bc}	16.07±0.05^{ab}	16.90±0.11^{d}	15.80±0.20^{a}	16.54±0.13^{c}	16.32±0.09^{bc}
P/F ratio	0.80±0.01^{e}	0.74±0.00^{c}	0.72±0.01^{b}	0.78±0.01^{d}	0.73±0.01^{bc}	0.77±0.01^{d}	0.70±0.01^{a}
Chlorides (%)	2.61±0.01^{b}	2.65±0.01^{c}	2.62±0.01^{b}	2.53±0.02^{a}	2.67±0.01^{cd}	2.69±0.01^{d}	2.60±0.01^{b}
Ash (%)	2.67±0.01^{a}	2.76±0.02^{b}	2.87±0.01^{d}	2.75±0.01^{b}	2.91±0.01^{e}	2.80±0.01^{c}	2.85±0.01^{d}
a_w	0.939±0.001	0.937±0.005	0.938±0.001	0.939±0.00	0.937±0.002	0.938±0.001	0.936±0.001
pH	5.16±0.01^{d}	4.88±0.01^{a}	5.18±0.00^{e}	5.19±0.01^{a}	5.03±0.01^{c}	4.87±0.01^{a}	4.94±0.01^{b}
Acidity (°SH)	52.42±0.03^{e}	51.60±0.10^{d}	51.83±0.67^{de}	48.15±0.05^{a}	50.60±0.10^{c}	49.82±0.03^{b}	52.20±0.05^{de}

$^{a-e}$*Means within the same row with different letters differ significantly ($p<0.05$)*

Mineral composition

The mineral profile of the grilling cheese was significantly influenced by the type and form of added red paprika ($p<0.05$; Table 3). Sodium (Na) was the predominant macroelement in all samples, ranging from 522.41 mg/100 g in sample P4 to 796.93 mg/100 g in sample P1. Similar to the lipid and protein fractions, these variations largely reflect a dilution effect driven by the differing water contributions of the fresh versus dried paprika forms. Calcium (Ca) content was significantly highest in the control (C) (150.28 mg/100 g) and lower in paprika-enriched variants (117.28 to 138.82 mg/100 g; $p<0.05$), consistent with the previously mentioned trend. Potassium (K) content showed a response similar to sodium. Fresh paprika samples (P4, P5) showed lower K levels, whereas higher values were observed in paprika-enriched formulations (P1, P3 and P6), with the highest values recorded in P1 and P6 ($p<0.05$). Adding paprika significantly increased phosphorus (P) content in all enriched samples compared with the control (50.30 mg/100 g), reaching peak levels in samples P6 (117.42 mg/100 g) and P3 (113.29 mg/100 g). Magnesium (Mg) content was significantly higher in samples P2, P3 and P6 (22.28-22.52 mg/100 g; $p<0.05$). This increase appears independent of the drying process (as evidenced by the low Mg levels in dried P1 and fresh P5) and is more likely attributable to the specific mineral composition of the hot "sudžuk" paprika variety. Among microelements, zinc (Zn) was the most abundant, reaching its maximum in sample P6 (2.11 mg/100 g), followed by iron (Fe), which peaked in sample P6 (0.05 mg/100 g). Copper (Cu) was detected in all tested samples and showed no statistically significant variation ($p>0.05$), with values ranging from 0.06 to 0.09 mg/100 g. Overall, these findings support the use of red paprika as a natural mineral fortifier in dairy products, consistent with previous studies on spice-enriched cheeses (Atwaa et al., 2020; Savanović et al., 2023).

Fatty acid composition

Saturated fatty acids (SFA) were the dominant fatty acid group in all cheese samples, comprising 69.37–70.75 % of total fatty acids (Table 4), with no significant differences in the major SFA fractions between the control and enriched samples ($p>0.05$). These values are consistent with the typical fatty acid composition of dairy fat reported in the literature (Barać et al., 2018; Savanović et al., 2020). Palmitic acid (C16:0) was the most abundant fatty acid, followed by stearic acid (C18:0) and myristic acid (C14:0). The observed variation in SFA content may be attributed to differences in raw milk composition and processing conditions (Hrković-Porobija et al., 2018). Adding red paprika primarily affected the unsaturated fatty acid fraction, particularly polyunsaturated fatty acids (PUFA). In general, PUFA content showed a slight increase in the enriched samples, although the differences were not statistically significant ($p>0.05$), while MUFA remained relatively stable (≈25 %), suggesting that paprika supplementation had little effect on this nutritionally favorable fatty acid fraction (Schwingshackl et al., 2011). Specifically, linoleic acid (C18:2 n-6) reached its highest value in sample P4 (2.10 %) compared with the control (1.75 %), while alpha-linolenic acid (C18:3 n-3) reached its highest concentration in sample P6 (0.68 %). Nutritional quality was further evaluated through fatty acid ratios and lipid health indices. Although the PUFA/SFA ratio remained below the commonly suggested threshold of 0.40 in all samples, reflecting the inherently high saturation of milk fat, the n-6/n-3 ratio ranged from 3.01 to 4.00, remaining within the generally desirable range of 2:1 to 5:1 (Simopoulos, 2002). Adding paprika also reduced the thrombogenicity index (TI), with significantly lower values in P4 (3.54) and P6 (3.47) than in the control (3.88) ($p<0.05$), whereas the atherogenic index (AI) showed a slight, non-significant decrease. Overall, these results indicate a modest change in the fatty acid profile of the cheese, mainly through a small increase in PUFA content and a reduction in TI (Mensink et al., 2003; Schwingshackl et al., 2011). Although these changes are of limited practical nutritional significance, they may contribute to slight improvements in selected lipid quality indices.

Instrumental color and texture analysis

Instrumental color analysis revealed that adding red paprika significantly influenced all parameters (L*, a*, b*) on both the surface and cross-section of the cheese samples ($p<0.05$). While the control sample exhibited the highest lightness (L*), enriched variants showed a marked decrease in L* and a shift

Table 3. *Macro- and microelement content in the tested cheese samples*

Elements	C	P1	P2	P3	P4	P5	P6
Macroelements (mg/100 g)							
Na	673.47±3.47^{d}	796.93±2.53^{f}	677.14±2.43^{d}	604.29±2.28^{b}	522.41±2.31^{a}	636.47±1.19^{c}	754.71±3.34^{e}
Ca	150.28±5.01^{e}	134.69±3.42^{cd}	127.48±1.50^{bc}	134.20±3.76^{cd}	117.28±1.15^{a}	121.47±0.80^{ab}	138.82±0.85^{d}
K	75.92±3.66^{b}	96.10±1.98^{d}	76.70±1.44^{b}	90.07±0.10^{c}	64.33±0.54^{a}	66.06±0.12^{a}	91.49±0.51^{cd}
P	50.30±0.42^{a}	85.56±1.50^{c}	59.47±0.91^{b}	113.29±1.69^{e}	98.64±0.80^{d}	111.19±0.70^{e}	117.42±2.47^{f}
Mg	10.63±0.53^{a}	12.70±0.20^{b}	22.28±0.07^{c}	22.52±0.29^{c}	10.74±0.14^{a}	10.80±0.10^{a}	22.40±0.10^{c}
Microelements (mg/100 g)							
Zn	1.89±0.09^{cd}	1.93±0.01^{d}	1.63±0.01^{b}	1.63±0.08^{b}	1.77±0.04^{bc}	1.46±0.07^{a}	2.11±0.03^{e}
Fe	0.02±0.00^{ab}	0.03±0.00^{b}	0.02±0.00^{ab}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.05±0.01^{c}
Cu	0.06±0.02	0.07±0.01	0.08±0.01	0.08±0.01	0.08±0.02	0.09±0.01	0.09±0.02

$^{a-f}$Means within the same row with different letters differ significantly ($p<0.05$)

Table 4. Fatty acid content in the tested cheese samples

Fatty acid	C	P1	P2	P3	P4	P5	P6
C4:0	2.65±0.11	2.80±0.18	2.58±0.15	2.75±0.10	2.58±0.14	2.55±0.17	2.54±0.13
C6:0	2.35±0.10	2.20±0.07	2.45±0.12	2.30±0.16	2.41±0.13	2.16±0.19	2.25±0.12
C8:0	1.40±0.05^{ab}	1.56±0.08^{b}	1.35±0.07^{ab}	1.45±0.10^{ab}	1.37±0.10^{ab}	1.30±0.08^{a}	1.32±0.04^{a}
C10:0	3.30±0.13	3.45±0.22	3.50±0.12	3.20±0.09	3.18±0.27	3.25±0.16	3.17±0.07
C12:0	3.90±0.18	3.70±0.14	3.85±0.11	3.85±0.16	3.88±0.12	3.55±0.15	3.78±0.13
C14:0	12.10±0.25	11.80±0.24	11.93±0.28	11.90±0.18	12.41±0.22	12.14±0.25	11.98±0.19
C16:0	30.20±0.54	29.80±0.55	29.93±0.58	30.01±0.35	29.76±0.55	29.90±0.42	29.96±0.50
C18:0	13.60±0.35	13.50±0.40	13.51±0.32	13.46±0.38	13.55±0.45	13.40±0.35	13.66±0.42
Other SFA	1.25±0.14	1.05±0.19	1.35±0.17	1.10±0.14	1.22±0.22	1.12±0.16	1.32±0.19
C14:1	0.87±0.04^{ab}	0.85±0.03^{ab}	0.83±0.05^{a}	0.88±0.04^{ab}	0.99±0.10^{b}	0.82±0.03^{a}	0.87±0.05^{ab}
C16:1	1.60±0.07	1.55±0.06	1.62±0.08	1.58±0.05	1.65±0.09	1.48±0.07	1.61±0.06
C18:1 (n-9)	21.80±0.60	22.40±0.55	21.99±0.65	22.10±0.50	21.93±0.70	22.05±0.58	21.84±0.62
Other MUFA	0.55±0.11	0.60±0.14	0.75±0.10	0.55±0.14	0.58±0.18	0.65±0.12	0.64±0.16
C18:2 (n-6)	1.75±0.09^{a}	1.90±0.12^{ab}	1.95±0.08^{ab}	2.08±0.14^{ab}	2.10±0.11^{b}	2.02±0.13^{ab}	2.05±0.15^{ab}
C18:3 n-3	0.50±0.05^{ab}	0.48±0.06^{a}	0.58±0.04^{abc}	0.52±0.07^{ab}	0.65±0.05^{bc}	0.60±0.06^{abc}	0.68±0.05^{c}
Other PUFA	0.40±0.09	0.43±0.11	0.49±0.08	0.38±0.10	0.42±0.12	0.51±0.09	0.54±0.11
Σ SFA	70.75±0.76	69.86±0.82	70.45±0.80	70.02±0.66	70.36±0.84	69.37±0.71	69.98±0.75
Σ MUFA	24.82±0.62	25.40±0.57	25.19±0.67	25.11±0.52	25.15±0.73	25.00±0.60	24.96±0.65
Σ PUFA	2.65±0.14	2.81±0.17	3.02±0.12	2.98±0.19	3.17±0.17	3.13±0.17	3.27±0.19
SFA/UFA	2.58±0.07	2.48±0.06	2.50±0.07	2.49±0.06	2.49±0.07	2.47±0.06	2.48±0.06
PUFA/SFA	0.037±0.002	0.040±0.003	0.043±0.002	0.043±0.003	0.045±0.003	0.045±0.002	0.047±0.003
MUFA/SFA	0.351±0.009	0.364±0.011	0.358±0.010	0.359±0.008	0.357±0.011	0.360±0.009	0.357±0.010
MUFA/PUFA	9.37±0.54	9.04±0.59	8.34±0.39	8.43±0.55	7.93±0.48	7.99±0.47	7.63±0.48
n-6/n-3	3.50±0.39	3.96±0.55	3.36±0.28	4.00±0.60	3.23±0.30	3.37±0.40	3.01±0.31
AI	2.99±0.08	2.87±0.09	2.92±0.07	2.88±0.06	2.91±0.08	2.92±0.10	2.89±0.07
TI	3.88±0.11^{b}	3.71±0.13^{ab}	3.60±0.09^{ab}	3.74±0.12^{ab}	3.54±0.11^{a}	3.59±0.13^{ab}	3.47±0.10^{a}

$^{a-c}$Means within the same row with different letters differ significantly ($p<0.05$)

from slightly negative a* values (greenish tendency) to positive redness (a*) and increased yellowness (b*). Samples enriched with powdered (P1, P2) and crushed (P3) paprika produced more intense coloration than those enriched with fresh paprika (P4, P5), likely due to higher pigment concentration and more efficient dispersion within the cheese matrix. This characteristic orange-red hue is primarily attributed to the fat-soluble carotenoids capsanthin and capsorubin, which effectively partition into the milk fat phase (Sharma et al., 2019). A similar decrease in lightness and increase in yellowness with increasing pepper content has been reported for capia pepper-enriched Turkish white cheese (Koçak and Tarakçı, 2025). However, the stability of these pigments must be carefully monitored during storage, as carotenoids are highly susceptible to degradation by light and oxygen (Kolašinac et al., 2025).

Incorporating paprika also significantly modulated cheese hardness ($p<0.05$), with the control sample exhibiting the highest value (2.62 N) due to its undisturbed protein-fat matrix. The most pronounced reduction in hardness occurred in samples P1 and P2 (paprika powder), where fine plant particles disrupted the formation of a cohesive protein network and increased moisture retention within the matrix, consistent with the generally higher moisture contents of paprika-enriched cheeses compared with the control (Table 2). Conversely, samples enriched with fresh paprika (P4, P5) exhibited a smaller decrease in hardness than those enriched with powdered forms; despite their higher moisture content, the larger plant pieces and their non-homogeneous distribution exerted less influence on the continuity of the protein network. Sample P6, which combined both forms, exhibited intermediate hardness, reflecting the competing textural effects of the coarse flakes and fresh tissue. These findings align with reports in the literature suggesting that plant-based additives and polysaccharides modulate cheese texture primarily by interfering with protein-protein interactions and altering the moisture-to-protein ratio (Wang et al., 2023; Zandona et al., 2025).

Phenolic compounds and antioxidant activity

Incorporating red paprika significantly modulated the phenolic profile and antioxidant capacity of grilling cheese ($p<0.05$), as shown in Table 6. Total phenolic content (TPC) generally increased in paprika-enriched samples, with the highest values observed in the powder-enriched variants (938.37 µg GAE/g in sample P1, followed by 881.16 µg GAE/g in sample P2). This indicates that paprika powders enable more efficient transfer and/or extraction of polyphenolic constituents into the dairy matrix compared with samples enriched with fresh paprika (P4, P5), which is consistent with the high antioxidant density reported for dried *Capsicum annuum* tissues (Kolašinac et al., 2025). Total non-flavonoids (TNF) and total flavonoids (TF) followed a similar trend, reinforcing the role of these bioactive

compounds in enhancing the functional quality of the final product.

The antioxidant capacity evaluated by FRAP, ABTS and DPPH assays broadly reflected the observed differences in phenolic composition. FRAP values were higher in most enriched cheeses (0.35-0.66 μmol Fe^{2+}/g) than in the control (0.25 μmol Fe^{2+}/g), indicating improved reducing capacity, except for sample P4, which did not differ significantly from the control despite a higher numerical value. The highest FRAP activity was recorded in sample P3 (crushed hot paprika flakes) (0.66 μmol Fe^{2+}/g), suggesting favorable extraction of redox-active compounds from this paprika form. The ABTS assay identified sample P6 (combined formulation) as the most active (595.42 μmol TE/g), possibly reflecting an additive effect of combining fresh and dried paprika forms. In contrast, DPPH radical scavenging activity was not detected in the control or in cheese samples with fresh paprika (P4 and P5), whereas dried paprika forms showed measurable activity, reaching a maximum in P3 (46.40 μmol TE/g). The absence of detectable DPPH activity in the control and fresh paprika samples (P4, P5) likely reflects the lower sensitivity of this assay rather than a true lack of antioxidant capacity. Unlike DPPH, ABTS and FRAP detect a broader range of antioxidant compounds with different reaction mechanisms and sensitivities (Shahidi and Zhong, 2015), suggesting that the concentration of extractable antioxidants in P4 and P5 remained below the DPPH detection threshold despite measurable activity in the other assays. Overall, enriching grilling cheese with *Capsicum annuum* L. increases its bioactive density and supports its potential as a functional dairy product with enhanced antioxidant capacity, consistent with findings reported for similar food systems (Šeregelj et al., 2019; Okur, 2022).

Antimicrobial activity (disc-diffusion method)

As shown in Table 7, cheese extracts enriched with different types of paprika showed a limited antimicrobial effect using the disk diffusion method, with a very narrow zone of inhibition observed only against *S. aureus* and *P. aeruginosa*. No inhibitory effect was detected against *E. coli*, *B. cereus* or either *C. albicans* strain. One possible explanation for the weak antimicrobial activity is that the 80 % ethanol used to prepare the extracts evaporates rapidly at incubation temperatures, limiting the diffusion of active compounds into the agar medium and resulting in insufficient concentrations to produce measurable inhibition zones. This especially applies to components from peppers that are water-insoluble, such as capsaicin and oleoresins (Molina et al., 2022). Cheese extracts were also tested using the agar dilution method, but they did not show an antimicrobial effect, with MIC values above 5 mg/mL (the results not shown), while higher concentrations were not tested due to the antimicrobial effect of alcohol (Ivanović et al., 2022).

Various laboratory methods can be used to evaluate the *in vitro* antimicrobial activity of food samples. While disk diffusion on solid culture media is commonly used (Ortez, 2005), a major drawback of this method is that it has limitations when testing dense and viscous samples, such as cheese extracts. These physical properties restrict the diffusion of active compounds from the disk into the surrounding medium, often resulting in lower apparent activity than with liquid-based methods (Xu et al., 2017). As noted by Shan et al. (2007), direct comparison of results across studies is difficult due to variations in plant material, test methods and bacterial strains; however, the bioactive compounds in the present

Table 5. *Instrumentally measured color and texture of the analyzed cheeses*

		C	P1	P2	P3	P4	P5	P6
Surface color	*L**	90.59±0.44^{c}	77.69±1.22^{a}	76.65±1.18^{a}	78.56±1.58^{a}	85.25±2.85^{b}	85.86±2.21^{b}	77.41±0.95^{a}
	*a**	−1.19±0.16^{a}	16.78±0.61^{e}	17.29±0.69^{e}	9.20±0.35^{d}	4.07±0.47^{b}	3.59±0.42^{b}	7.28±0.28^{c}
	*b**	15.18±0.15^{a}	26.21±1.01^{d}	27.72±0.93^{e}	23.40±0.88^{c}	15.16±1.22^{a}	15.58±0.89^{a}	22.14±0.79^{b}
Cross section color	*L**	89.97±0.38^{d}	79.51±1.05^{a}	79.02±0.88^{a}	78.10±1.55^{a}	85.00±2.68^{c}	83.90±2.15^{bc}	82.74±0.70^{b}
	*a**	−1.31±0.12^{a}	14.91±0.50^{e}	14.41±0.52^{e}	10.81±0.40^{d}	4.41±0.69^{b}	6.11±0.46^{c}	6.12±0.21^{c}
	*b**	14.53±0.18^{a}	27.48±0.92^{d}	26.88±0.89^{d}	26.98±0.85^{d}	14.83±1.05^{a}	16.78±0.98^{b}	20.58±0.68^{c}
Hardness (N)		2.62±0.02^{d}	1.44±0.07^{ab}	1.33±0.01^{a}	1.78±0.03^{c}	1.81±0.01^{c}	1.73±0.01^{bc}	1.65±0.01^{abc}

$^{a-e}$Means within the same row with different letters differ significantly ($p<0.05$)

Table 6. *Total phenolic (TPC), nonflavonoid (TNF) and flavonoid (TF) contents and antioxidant activity (FRAP, DPPH and ABTS) of the tested cheese samples*

Parameter	C	P1	P2	P3	P4	P5	P6
TPC (μg GAE/g)	577.78±6.05^{a}	938.37±8.49^{g}	881.16±3.46^{f}	612.13±3.7^{b}	704.74±4.94^{d}	670.94±0.67^{c}	790.89±2.46^{e}
TNF (μg GAE/g)	303.01±5.35^{a}	402.17±0.85^{e}	365.24±4.53^{d}	344.79±4.17^{bc}	348.03±4.53^{c}	334.99±3.14^{b}	352.84±2.29^{c}
TF (μg GAE/g)	274.77±6.14^{a}	536.20±3.34^{f}	518.92±2.99^{e}	267.34±2.59^{a}	356.72±3.47^{c}	335.96±3.47^{b}	438.05±4.75^{d}
FRAP (μmol Fe^{2+}/g)	0.25±0.02^{a}	0.56±0.08^{bc}	0.60±0.02^{bc}	0.66±0.09^{c}	0.35±0.04^{a}	0.50±0.01^{b}	0.58±0.01^{bc}
ABTS (μmol TE/g)	510.10±5.64^{a}	552.21±5.87^{c}	573.67±6.09^{d}	570.44±6.93^{d}	531.90±6.10^{b}	565.21±5.53^{cd}	595.42±6.09^{e}
DPPH (μmol TE/g)	n.d.^{a}	27.30±0.34^{b}	40.55±1.02^{d}	46.40±1.34^{e}	n.d.^{a}	n.d.^{a}	31.08±1.98^{c}

$^{a-g}$Means within the same row with different letters differ significantly ($p<0.05$)
**n.d. - not detected*

Table 7. Antimicrobial activity of cheese extracts (disk diffusion method). Results are expressed as the mean inhibition zone diameter (mm)

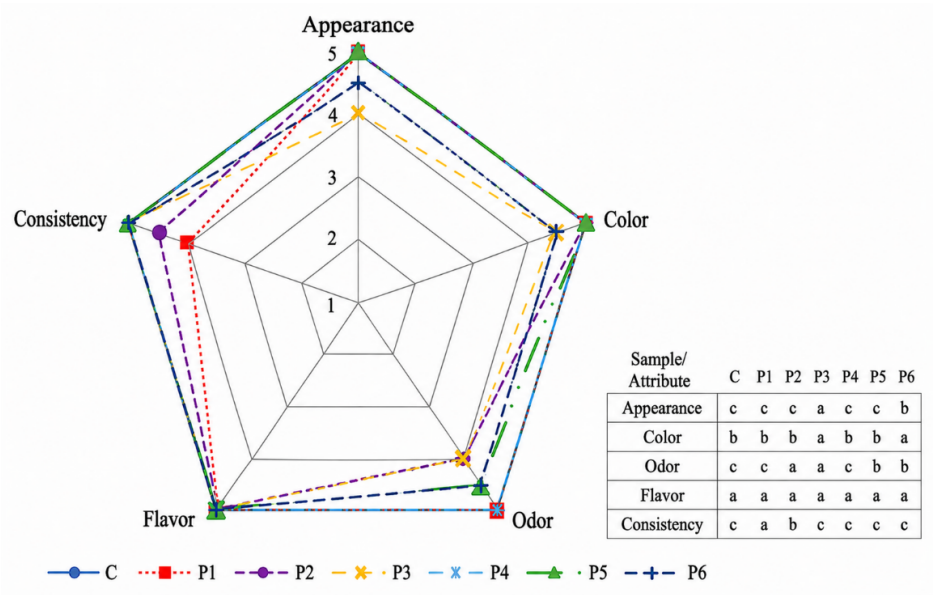
Cheese sample	*E. coli* WDCM 00013	*P. aeruginosa* WDCM 00024	*B. cereus* WDCM 00151	*S. aureus* WDCM 00034	*C. albicans* WDCM 00054	*C. albicans* isolate
	Diameter (mm)	Diameter (mm)	Diameter (mm)	Diameter (mm)	Diameter (mm)	Diameter (mm)
C	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P1	n.d.	n.d.	n.d.	5.58	n.d.	n.d.
P2	n.d.	n.d.	n.d.	4.17	n.d.	n.d.
P3	n.d.	3.21	n.d.	4.70	n.d.	n.d.
P4	n.d.	3.13	n.d.	2.08	n.d.	n.d.
P5	n.d.	n.d.	n.d.	5.58	n.d.	n.d.
P6	n.d.	5.25	n.d.	2.25	n.d.	n.d.

**n.d.- not detected*

cheese extracts were probably present at concentrations too low to yield distinct inhibition zones, despite being detectable by chemical assays. The disk diffusion technique uses higher extract concentrations, resulting in a high extract concentration at the edge of the disk even for a short time and causing a slight reaction in sensitive species, such as *S. aureus* and *P. aeruginosa*. As the solvent evaporates, the active substances, as mentioned, diffuse poorly into the substrate and do not have a visible inhibitory effect on the tested microorganisms. This methodological limitation likely explains why numerous studies reporting strong antimicrobial activity of *C. annuum* extracts against foodborne pathogens often use broth microdilution methods to determine minimum inhibitory concentrations (MIC). For instance, Loizzo et al. (2016) reported that ethanolic extracts from nine *C. annuum* cultivars inhibited *S. aureus* and *Listeria monocytogenes* with MIC values ≤50 mg/mL. When the extract is diluted in broth or agar, lower extract concentrations are used due to the toxicity of the solvent, in this case, ethanol. In addition, when the extract is added to the broth or agar, the hydrophobic extract components undergo microprecipitation due to the change in solvent. This reduces the number of active components in the substrate, and the extract does not exhibit an antimicrobial effect. Adding a surfactant to the substrate, such as Tween 80, could increase the concentration of bioactive components in the substrate. However, using Tween 80 can have unwanted effects, including inhibiting some microorganisms and trapping hydrophobic compounds in micellar structures, thereby reducing their antimicrobial activity. In some cases, Tween 80 can serve as a nutrient source and promote the growth of bacteria such as *S. aureus*, which affects the results of antimicrobial testing (Nielsen et al., 2016). Therefore, working without emulsifiers provides a clearer insight into the intrinsic potential of the matrix without external factors that modify bacterial growth (Nielsen et al., 2016).

Results of sensory analysis

The sensory profile of the grilling cheese was significantly affected ($p<0.05$) by the type and form of red paprika added (Figure 1). The control sample (C) achieved the maximum score (5.0) for all evaluated attributes, while the physical form of paprika was an important factor influencing overall product quality, consistent with previous findings on the impact of plant-based additions on dairy product characteristics (Salehi, 2021). Samples containing crushed paprika flakes (P3 and P6) showed significantly lower visual appearance scores ($p<0.05$), likely due to uneven color distribution and the presence of dark spots. The remaining enriched samples retained high appearance scores comparable to the control. Paprika addition also influenced odor intensity ($p<0.05$). The greatest decrease was observed in samples P2 and P3, followed by moderate reductions in P5 and P6. This may be explained by the masking effect of paprika volatiles on characteristic dairy aroma compounds (Tarakcı and Deveci, 2019). However, flavor scores were not significantly affected ($p>0.05$), as all samples received the maximum score (5.0), suggesting good compatibility of paprika with the cheese matrix. Regarding consistency, samples containing powdered paprika (P1 and P2) had significantly lower consistency scores ($p<0.05$), with values of 4.0 and 4.5, respectively. Sample P1 exhibited a pasty texture that negatively affected slicing, likely due to the disruption of the protein matrix by fine particles, as described in studies on milk protein gel structures (Lucey, 2002). A related textural effect of pepper particles - a granular mouthfeel caused by pepper seeds - has also been reported in capia pepper-enriched Turkish white cheese (Koçak and Tarakçı, 2025). In contrast, the sample with fresh paprika (P4) did not differ significantly from the control, indicating that larger pieces better preserve the structural integrity of the cheese (Gamage et al., 2023). Overall, the sample containing fresh sweet paprika (P4) showed the most favorable results, as it did not differ significantly from the control in any of the evaluated attributes ($p>0.05$). This appearance trend is consistent with previous reports on colorants in cheese (Sharma et al., 2019), while flavor stability aligns with findings for plant-based cheese additives (Olmedo et al., 2013). Consumer preferences are strongly influenced by spice form (Racette and Drake, 2022). Therefore, these findings suggest that using fresh paprika can be recommended for producing value-added grilling cheese with high sensory quality. A limitation of this study is the relatively small trained sensory panel (n=10). Therefore, future studies involving larger trained panels and consumer-based sensory evaluation are needed to improve the generalizability of the sensory findings.



Sample/ Attribute	C	P1	P2	P3	P4	P5	P6
Appearance	c	c	c	a	c	c	b
Color	b	b	b	a	b	b	a
Odor	c	c	a	a	c	b	b
Flavor	a	a	a	a	a	a	a
Consistency	c	a	b	c	c	c	c

Figure 1. Mean sensory scores of grilling cheese samples enriched with different forms of red paprika (Means within the same row with different letters differ significantly ($p<0.05$))

Conclusion

Enriching Queso Blanco-type grilling cheese with different forms of red paprika (*Capsicum annuum* L.) altered its physicochemical composition, fatty acid profile and sensory properties, resulting in acceptable sensory quality that varied with paprika form. Fresh and powdered forms of paprika affected moisture, protein and mineral contents differently, while their impact on sensory attributes also varied with the physical form used. Among all tested variants, the cheese enriched with 4 % fresh sweet red paprika was the most desirable, showing uniform and attractive color, a pronounced fresh-paprika aroma, balanced flavor, as well as a firm and acceptable consistency that contributed to superior sensory quality. Although powdered and crushed paprika variants yielded higher phenolic content and antioxidant activity, the fresh-paprika formulation achieved an optimal balance between functional benefits and sensory appeal, making it particularly suitable for commercial cheese production. These findings highlight the potential of spices in different physical forms not only as flavoring agents but also as functional ingredients for developing health-promoting dairy products without compromising sensory quality. Such applications represent a promising approach for the dairy industry to develop innovative, sensorially acceptable and nutritionally enhanced cheeses. However, their preservative potential and ability to extend shelf life remain to be confirmed through dedicated storage-stability studies.

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

D. Savanović designed the study, supervised all experimental work, performed data analysis, and prepared the manuscript.

A. Velemir performed experimental work and contributed to data collection.

A. Savić and Lj. Topalić-Trivunović contributed to microbiological analyses and the interpretation of the results.

J. Savanović and B. Šojić contributed to experimental design, statistical analysis, and critical revision of the manuscript.

T. Pekić, L. Petković Gajić, T. Lepir and N. Mrzić performed physicochemical and chemical analyses, contributed to sample preparation, analytical measurements, and data processing.

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Utjecaj crvene paprike na fizikalno-kemijska, antioksidacijska, mikrobiološka i senzorska svojstva sira za roštilj

Sažetak

Ovim je istraživanjem ispitan utjecaj crvene paprike (*Capsicum annuum* L.) u obliku praha, tucane i svježe (slatke ili ljute) na fizikalno-kemijska, senzorska, antioksidacijska i mikrobiološka svojstva sira za roštilj (tipa Queso Blanco). Proizvedeno je sedam varijanti sira: kontrolni uzorak (C) i šest formulacija obogaćenih paprikom (P1-P6), s paprikom dodanom u koncentracijama od 0,5 do 4,0 %. Dodatak paprike je značajno ($p<0,05$) povećao sadržaj vlage (54,60-57,18 % u odnosu na 54,09 %), dok je smanjio sadržaj proteina i omjer proteina i masti. Obogaćeni su uzorci pokazali znatno viši sadržaj fosfora (59,47-117,42 u odnosu na 50,30 mg/100 g) i magnezija u odnosu na kontrolni uzorak. Varijanta obogaćena s 4,0 % svježe slatke crvene paprike (P4) pokazala je povoljan profil lipida, s 25,15 % MUFA i 3,17 % PUFA, te značajno smanjen trombogeni indeks (TI = 3,54) u odnosu na kontrolni uzorak (3,88), dok je aterogeni indeks (AI) pokazao blagi, statistički neznačajan pad. Ukupni sadržaj fenola bio je znatno viši u obogaćenim uzorcima (612,13-938,37 u odnosu na 577,78 µg GAE/g), uz pojačanu sposobnost neutralizacije slobodnih radikala (ABTS: 531,90–595,42 µmol TE/g; DPPH: do 46,40 µmol TE/g u varijantama s tucanom paprikom). Antimikrobna aktivnost, procijenjena metodom difuzije diska, uočena je protiv bakterija *Staphylococcus aureus* i *Pseudomonas aeruginosa*, dok aktivnost protiv *Escherichia coli*, *Bacillus cereus* i *Candida albicans* nije zabilježena. Senzorskom je ocjenom formulacija P4 identificirana kao najperspektivnija, budući da nije pokazala statistički značajne razlike u odnosu na kontrolni uzorak ni u jednom ocjenjivanom svojstvu ($p>0,05$). Dobiveni rezultati ukazuju na to da se crvena paprika može koristiti kao funkcionalan, prirodan sastojak u formulaciji nutritivno obogaćenih mliječnih proizvoda.

Ključne riječi: sir za roštilj; crvena paprika; antioksidacijska aktivnost; profil masnih kiselina; senzorska kvaliteta

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