Physico-chemical, antioxidant and microbiological characteristics of bread supplemented with 1 % grape seed micropowder

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Summary

Grape (*Vitis vinifera* L.) seed micropowder (GSMP), produced by a nanotechnology-based process as a rich source of bioactive compounds with potential health effects, was used in our study to enhance the nutritional value of bread. Experimental bread was prepared using 1% addition of GSMP. Mineral composition, total polyphenolics content (*TPC*), total phenolic acids (*TPA*), antioxidant activity, and microbiological as well as technological quality of bread were evaluated. Our results revealed that GSMP addition led to significantly ($P < 0.05$) higher *TPC* (1.44 ± 0.17 g·kg^{-1} expressed as gallic acid equivalents), *TPA* (1.04 ± 0.04 g·kg^{-1} expressed as caffeic acid equivalents) and stronger antioxidant capacity (2.02 ± 0.06 g·kg^{-1} expressed as Trolox equivalents capacity) as compared to the control one (0.97 ± 1.44 g·kg^{-1}, 0.60 ± 1.04 g·kg^{-1} and 1.72 ± 0.08 g·kg^{-1}, respectively). Microbiological investigation revealed a positive effect of GSMP addition on bread quality after the first day of storage ($P < 0.05$). Moreover, data from physical evaluation indicated no considerable impact of GSMP on volume of bread loaves, hardness of the bread crumb and crust, moisture content and water activity. Our results allow for the preliminary suggestion that 1 % GSMP may improve functional properties of wheat bread.

Keywords

bakery products; grape seed micropowder; polyphenol; phenolic acid; antioxidant activity

Current trends in production and consumption of foods together with eating habits have considerable impact on health, social and environmental aspects of human life [1]. Regarding the health concern, there is still increasing demand for development of functional foods with added physiological benefits, which can reduce incidence and prevalence of chronic diseases associated with excessive oxidative stress [2].

Grape seeds are a major industrial by-product of wine and juice processing industries. They represent approximately 5 % of the grape weight [3]. They contain lipids, proteins, carbohydrates, polyphenolic compounds [4] and mineral elements [5], which participate in important biological functions in human body. The antioxidant capacity of grape seeds is attributed to the content of flavanols and proanthocyanidins (condensed tannins), which differ among grape varieties [6] depending on viticultural and environmental factors [7]. The seed tannins consist mainly of epicatechin units together with smaller amounts of catechins, epicatechin gallate and epigallocatechin [8]. In the study by Bagchi et al. [9], the grape seed proanthocyanidin extract exhibited greater protection against free radicals, free radical-

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induced lipid peroxidation and DNA damage than vitamin C, vitamin E and β-carotene in both in vitro and in vivo models. In addition to antioxidant activities, grape polyphenols have also been shown as cardioprotective, anticancer, anti-inflammation, antiaging, antimicrobial [7] and antimutagenic agents [4]. Due to their nutrient profile and diverse health-supporting benefits, grape seeds can be regarded as a functional ingredient for enhancing the nutritional value of bakery products [10], in particular of bread as an essential diet component worldwide. Furthermore, it was found that phenolic compounds may prevent the formation of carcinogens (such as acrylamide) during bread baking [11], which increases the beneficial application of grape seeds in the bakery industry.

Current study was aimed to investigate the impact of white flour substitution with 1% grape seed micropowder (GSMP) on chemical composition, quality, antioxidant activity and microbiological quality of white bread. Grape seed powder addition to bread has been previoulsy extensively studied but not by making use of nanotechnology. Nanosized particles are, for their small size, better bioavailable and highly chemically reactive (as a result of their large surface-to-volume ratio), representing excellent release systems for biologically active molecules [12]. An increase in total phenolics content (TPC) in grape products with a decrease in their particle size was previously revealed [13, 14]. Since evaluation of physico-chemical, antioxidant and microbiological characteristics of bread enriched with GSMP has not been done prior to our experiment, this study provides the first report on improved functional properties of bread enriched with 1% GSMP processed by nanotechnology. Thus, it can give an extended picture of grape seeds as a potential functional ingredient in the bakery industry.

MATERIALS AND METHODS

Sample preparation

Ethanolic extracts were prepared from both raw materials (flour, GSMP) and from experimental bread samples. For each extraction, 0.2 g of GSMP or 0.5 g of bread was extracted by 20 ml (GSMP) or 40 ml (bread) of 80% ethanol for 2 h and centrifuged at 4000 ×*g* for 10 min in Rotofix 32A (Hettich, Spenge, Germany). The supernatants were used for measurement of antioxidant activity using 2,2-difenyl-1-picrylhydrazyl (DPPH method) and detection of total polyphenolics content (*TPC*) and total phenolic acid content (*TPA*).

Chemicals

All chemicals were of analytical grade and were purchased from Reachem (Bratislava, Slovakia) or Sigma Aldrich (Saint-Louis, Missouri, USA).

Antioxidant activity

Radical-scavenging activity (*RSA*) of raw materials (flour, GSMP) and experimental bread samples was measured with DPPH-using previously described procedures [15–17]. Volumes of 0.4 ml flour and GSMP extracts and 1 ml sample extract were added to 3.6 ml (flour, GSMP) or 4 ml (bread) of DPPH solution (0.025 g DPPH in 100 ml ethanol). Absorbance of the reaction mixture at 515 nm was determined using Jenway 6405 UV/Vis spectrophotometer (Cole-Parmer, Stone, United Kingdom). *RSA* was expressed as Trolox equivalent antioxidant capacity (TEAC) in grams per kilogram of dry weight (dw).

Total polyphenolics content

TPC was measured by the method of Ivanišová et al. [15, 16] using Folin-Ciocalteu reagent. In this case, 0.1 ml of flour and GSMP extracts or 0.2 ml bread extract was mixed with 0.1 ml (flour, GSMP) or 0.2 ml (bread) of Folin-Ciocalteu reagent, 1 ml (flour, GSMP) or 2 ml (bread) of 200 $g \cdot l^{-1}$ sodium carbonate and 8.8 ml of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured using Jenway 6405 UV/Vis spectrophotometer. Gallic acid was used as a standard and the results were expressed in grams per kilogram of gallic acid equivalents (GAE) dw.

Total phenolic acids content

TPA was determined using a previously published method [17]. A volume of 0.5 ml of each sample extract was mixed with 0.5 ml of 0.5 $mol \cdot l^{-1}$ HCl, 0.5 ml Arnow reagent (100 $g \cdot l^{-1}$ $NaNO_2$ and 100 $g \cdot l^{-1}$ Na_2MoO_4), 0.5 ml of 1 $mol \cdot l^{-1}$ NaOH and 0.5 ml of water. Absorbance at 490 nm was measured using Jenway 6405 UV/Vis spectrophotometer. Caffeic acid was used as a standard and the results were expressed in grams per kilogram of caffeic acid equivalents (CAE) dw.

Elemental analysis

Experimental sample from GSMP was weighed on analytical balances ABT 120/5-DW (Kern & Sohn, Balingen, Germany) and transferred to polytetrafluoroethylene (PTFE) mineralization tubes. Weight of the experimental sample was 0.3033 g. For experimental bread samples, drying process was carried out at 55 °C in an oven UF110plus (Memmert, Schwabach, Germany) to constant weight. Until processing, the

experimental material was stored in a desiccator at laboratory temperature for 1 h. The basic material for the experiment was bread powder, produced by a homogenization process. Weight of the experimental bread samples ranged from 0.3 g to 0.5 g and was reflected in the measurement. All the chemicals used during the sample preparation were for of purity for trace analysis. The sample was mineralized in the high performance microwave digestion system Ethos UP (Milestone, Sorisole, Italy) in a solution of 5 ml nitric acid ≥69.0% TraceSELECT (Honeywell, Charlotte, North Carolina, USA) and 2 ml of ultrapure water (18.2 MΩ·cm^{-1} at 25 °C) prepared by Synergy UV (Merck, Darmstadt, Germany). Experimental and blank samples were digested according to the method for dry plant tissue developed and recommended by the manufacturer to achieve the most reliable results. The method consisted of heating and cooling phases. During the heating stage, the samples were warmed during 15 min to 200 °C and this temperature was maintained for another 15 min. Afterwards, during the cooling phase, the samples underwent a 15 min phase of active cooling to reach the temperature of 50 °C. The digestates were filtered through quantitative cellulose filter discs (particle retention 12–15 μm; VWR, Radnor, Pennsylvania, USA) into volumetric flasks and filled up with ultrapure water to a volume of 50 ml [18].

Analysis of selected elements (Ca, Cu, Fe, K, Mg, Na and Zn) was carried out using inductively coupled plasma optical emission spectrometer ICP OES 720 (Agilent Technologies, Santa Clara, California, USA) with axial plasma configuration and with auto-sampler SPS-3 (Agilent Technologies). Details of the instrumental operating conditions are listed in Tab. 1. In the experiment, Multielement standard solution V for ICP in 10% nitric acid (Sigma-Aldrich) was used. Detection limits of measured trace elements were 0.01 μg·kg^{-1} for Ca, 0.3 μg·kg^{-1} for Cu, 0.1 μg·kg^{-1} for Fe, 0.3 μg·kg^{-1} for K, 0.01 μg·kg^{-1} for Mg, 0.15 μg·kg^{-1} for Na and 0.2 μg·kg^{-1} for Zn. The legitimacy of the whole method was verified using the certified reference material CRM–ERM CE278 K (Sigma-Aldrich). The GSMP sample was analysed twice and the results were expressed as mean ± standard deviation.

Tab. 1. Inductively coupled plasma optical emission spectrometry operating parameters.

Parameter	Value
Radio frequency power [kW]	1.30
Plasma flow [l·min^{-1}]	15.0
Auxiliary flow [l·min^{-1}]	1.50
Nebulizer flow [l·min^{-1}]	0.85
Replicated read time [s]	5.00
Instrument stabilization [s]	15
Sample uptake delay [s]	25
Pump rate [Hz]	0.25
Rinse time [s]	10.0
Wavelength [nm]	
Ca	315.887
Cu	324.754
Fe	234.350
K	766.491
Mg	383.829
Na	589.592
Zn	206.200

Bread-making process

Wheat flour (T-650), used in our experiment, was obtained from an operating grinding mill (Mlyn Zrno, Veľké Hoste, Slovakia). Other raw materials such as salt (Solné Mlýny, Olomouc, Czech Republic), saccharose (Považský cukor, Trenčianska Teplá, Slovakia) and compressed yeast (Dr. Oetker, Bratislava, Slovakia) were obtained from a market. In experimental bread samples, the wheat flour was replaced by 1 % (w/w) of GSMP. The content of GSMP was selected based on the results of our previous studies [19, 20] in which different contents of GSMP (1 %, 2 %, 5 % and 8 %) were used. In effect, our findings showed a significant decline in the volume of bread loaves enriched with ≥ 2 % GSMP (i.e. 2 %, 5 %, and 8 %) along with the highest scores (even higher than in the control sample) for all quality attributes (overall bread appearance and shape, surface and properties of the crust, overall appearance of the crumb, aroma, taste and overall acceptability of bread samples) assigned by the panel of 15 trained panelists to bread supplemented with 1 % GSMP. In addition, the results of sensory rating were also confirmed instrumentally by e-eye and e-nose. Taking into account the fact that consumer acceptance depends mainly on the physical and sensory properties of the final product, the 1% GSMP addition to wheat flour was selected in the current study. Bread-making method was applied according to Burešová et al. [21] with some modifications. Bread formula consisted of flour blend, water (6 g·kg^{-1}), yeast (0.2 g·kg^{-1}), salt (0.2 g·kg^{-1}) and saccharose (0.1 g·kg^{-1}). Dry yeast was activated in saccha-

rose solution (16.67 g·l^{-1}) at 35 °C for 10 min. To form the dough, all the ingredients were mixed for 6 min in a Diosna SP 12 mixer (Diosna Dierks & Söhne, Osnabrück, Germany). The prepared dough was consequently placed into an aluminium vessel, transferred into a fermentation cabinet (MIWE cube, Pekass, Plzeň, Czech Republic) for 40 min at 32 °C (85% relative humidity) and baked in two phases. Indeed, the loaves were baked first at 180 °C with addition of 160 ml steam for 17 min (phase I) followed by baking at 210 °C for 10 min (phase II) in a laboratory oven MIWE cube. After that, the bread loaves were left to stand at room temperature for 2 h and were prepared for analyses. In total, 6 breads were produced for analysis, i.e. 3 bread loaves per experimental group. The process was carried out in AgroBioTech Research Centre (Slovak University of Agriculture, Nitra, Slovakia).

Loaf volume

The loaf volume was determined with the laser-based scanner VolScan Profiler 300 (Stable Micro Systems, Godalming, United Kingdom).

Water activity

Bread water activity (a_w) was measured using Lab Master aw Standard (Novasina, Lachen, Switzerland). For this purpose, 2.0 g of sample was cut into a cube and placed into a sample pan. The value of a_w was measured automatically at 25 °C for 15–20 min.

Moisture content

Bread moisture content (*MC*) was determined using the moisture analyser DBS 60-3 (Kern & Sohn). In this case, 1.0 g of sample was weighed on the sample plate and measurement was done at 120 °C for 10–15 min.

Microbiological analysis

Bread slices were individually packaged in clear polyethylene (PE) packing bags and stored in laboratory conditions for 4 days. The growth of the microorganisms was measured every day during storage. An amount of 5 g of bread sample was diluted with 45 ml of distilled water, and stirred on a horizontal shaker for 30 min to achieve a tenfold dilution. Plate count agar (PCA; Oxoid, Basingstoke, United Kingdom) for determination of total counts of microorganisms, Violet red bile agar with lactose (VRBL, Oxoid) for coliforms and Malt extract agar base (MEA; Oxoid) supplemented with bromocresol green (0.020 g·l^{-1}) for yeasts and microscopic fungi were inoculated with 0.1 ml of the suspension on surface of plates. For determination of total count of microorganisms, plates were incubated at 30 °C for 48–72 h. For coliforms, plates were incubated at 37 °C for 24–48 h and for yeasts and microscopic fungi, plates were incubated at 25 °C for 5 days. Finally, growing colonies were counted.

Statistical analyses

Data from all analyses, which were performed in triplicates (from 3 independent samples), were statistically evaluated using Prism 8.0.1 (GraphPad Software, San Diego, California, USA). One-way analysis of variance (ANOVA) followed by Tukey's test was used to evaluate significance of differences between analysed groups of samples. The level of significance was set at $P < 0.0001$, $P < 0.001$, $P < 0.01$ and $P < 0.05$.

RESULTS AND DISCUSSION

In the first phase of our study, GSMP as a potential functional ingredient for bread was characterized in terms of nutritional and health-promoting value. Data obtained from the evaluation of antioxidant activity related to the content of *TPC* and *TPA* of GSMP, wheat flour and enriched flour are presented in Tab. 2. From our results it was obvious that GSMP-enriched wheat flour had higher ($P < 0.05$) content of biologically active compounds than the basic wheat flour indicating the potential of GSMP to improve nutritional benefits of white flour. The value for *TPC* was higher than those reported by Shi et al. [22] (15.153–39.246 g·kg^{-1}), Bucić-Kojić et al. [23] (14.72–66.81 g·kg^{-1}) and Dimcheva et al. [24] (2.66 g·kg^{-1}) who similarly used grape seed ethanolic extracts and Folin-Ciocalteu colorimetric method for the *TPC* measurement. On the other hand, the antioxidant activity of our GSMP extract was lower as compared to findings of Babbar et al. [25] (42.23 ± 1.9 g·kg^{-1}) who extracted polyphenols from grape seeds using methanol as a solvent. Among the factors that affect the antioxidant properties of grape by-products, the conditioning and processing methods, extraction protocols, grape variety and climatic conditions (influencing their chemical composition) can be mentioned [26]. Also, the grape seed processing technique could play an important role in the dissimilar observations. The results by Zhao et al. [13] and Beres et al. [14] showed that the phenolics content of grape by-products was influenced by their particle size. In this context, grape by-products with smaller particle size had higher *TPC* as a result of higher extraction yield due to

a better surface contact. All these aspects could participate in different observations between our and aforementioned studies.

Composition of selected macro and micro minerals of GSMP is given in Tab. 3. The general scheme of descending concentrations of the elements in the experimental sample was Ca > K > Mg > Fe > Na > Cu > Zn. In the sample of GSMP, the most abundant elements were macro elements Ca, K and Mg which corresponds to the results by SPANGHERO et al. [5] and MIRONEASA et al. [27]. As a natrophic plant, *Vitis vinifera* accumulates from the soil only small amounts of sodium [28], which was also reflected in our study. The quantities of micro minerals in GSMP ranged from 19.55 mg·kg^{-1} (Zn) to 63.55 mg·kg^{-1} (Fe) which were higher as those analysed in grape seeds by OZCAN [29]. However, elemental composition of grape seeds is known to be affected, in particular, by geographical region and vine varieties [30].

Chemical composition of bread

The addition of 1 % GSMP to bread formula resulted in a significant increase in *TPC* ($P < 0.01$), *TPA* ($P < 0.0001$) and *RSA* ($P < 0.01$) of the bread, as shown in Tab. 2. Our results regarding significantly higher values for *TPC* and *RSA* of grape seed-containing breads as compared to the control ones are similar to previous studies [4, 31]. A positive correlation between *TPC* and antioxidant activity was reported [32]. Thus, *TPC* can be used as an important indicator of free radical-scavenging activity, which in turn can be employed for primary screening of any products intended as a natural source of antioxidants in functional foods [33]. The higher antioxidant activity of our bread supplemented with 1 % GSMP, in comparison with the control sample, suggests that GSMP is a promising ingredient to improve functionality of white bread.

Data on mineral composition (Tab. 3) showed that the replacement of wheat flour by 1 % GSMP resulted in a significant ($P < 0.001$) increase in the content of Ca (1.2-fold increase). Generally, wheat bread is poor in minerals [34] and its consumption contributes by approximately 10 % to the recommended daily intake of minerals [35]. Based on our finding we can conclude that bread with 1 % GSMP may improve the daily intake of Ca for consumers. In terms of dietary reference intake (*DRI*) for calcium (1 500 mg per day) [36], a daily portion of bread (300 g) supplemented with 1 % GSMP can meet 6.5 % of *DRI* for Ca in adult males and females.

Tab. 2. Antioxidant activity and bioactive compounds composition of samples.

	GSMP	Wheat flour	Supplemented flour	Control bread	Supplemented bread
RSA [g·kg^{-1}]	9.77 ± 0.12	0.15 ± 0.08^{a}	0.27 ± 0.02^{b}	1.72 ± 0.12^{a}	2.02 ± 0.06^{b}
TPC [g·kg^{-1}]	71.93 ± 4.11	0.74 ± 0.04^{a}	0.86 ± 0.06^{b}	0.97 ± 0.17^{a}	1.44 ± 0.32^{b}
TPA [g·kg^{-1}]	3.93 ± 0.17	3.09 ± 0.16^{a}	3.58 ± 0.28^{b}	0.60 ± 0.02^{a}	1.04 ± 0.04^{b}

Mean ± standard deviation is presented. Values with different superscripts within the same row are significantly different ($P < 0.05$). Supplement – addition of 1 % (w/w) of grape seed micropowder to the flour.
GSMP – grape seed micropowder, *RSA* – radical-scavenging activity is expressed as grams of Trolox equivalents per kilogram dry weight, *TPC* – total polyphenolics content is expressed as grams of gallic acid equivalents per kilogram dry weight, *TPA* – total phenolic acid content is expressed as grams of caffeic acid equivalents per kilogram dry weight.

Tab. 3. Mineral content of samples.

Elements [mg·kg^{-1}]	GSMP	Control bread	Supplemented bread
Ca	8 460.00 ± 0.24	260.77 ± 12.58^{a}	326.00 ± 9.37^{b}
K	6 240.00 ± 0.05	1 817.37 ± 55.30^{a}	1833.47 ± 45.32^{a}
Mg	2 070.00 ± 0.02	351.00 ± 10.37^{a}	358.93 ± 4.30^{a}
Na	29.77 ± 0.74	6 674.22 ± 199.27^{a}	6 784.83 ± 101.14
Fe	63.55 ± 1.69	13.68 ± 0.81^{a}	13.43 ± 0.39^{a}
Cu	23.01 ± 0.33	2.69 ± 0.20^{a}	2.69 ± 0.04^{a}
Zn	19.55 ± 1.10	13.55 ± 1.08^{a}	13.70 ± 0.46^{a}

Mean ± standard deviation is presented. Values with different superscripts within the same row are significantly different ($P < 0.05$). Supplement – addition of 1 % (w/w) of grape seed micropowder to the flour.
GSMP – grape seed micropowder.

Tab. 4. Quality characteristics of experimental breads.

	Control bread	Supplemented bread
Volume [ml]	764.40 ± 20.70	734.00 ± 10.17
Moisture content [%]	40.54 ± 0.77	41.00 ± 0.32
Water activity	0.93 ± 0.00	0.94 ± 0.00
Crust hardness [g]	4 818.02 ± 806.44	5 490.29 ± 349.78
Crumb hardness [g]	1 222.68 ± 72.20	1 202.68 ± 79.54

Mean ± standard deviation is presented. Values were not statistically different ($P < 0.05$).
Supplement – addition of 1 % (w/w) of grape seed micropowder to the flour.

Volume of bread loaves

The impact of GSMP addition on the loaf volume was examined in this part of the study. From the data shown in Tab. 4 it is clearly evident that wheat flour substitution with 1 % GSMP had no considerable impact on the loaf volume reflecting its good quality. In accordance with our results, no significant effect of wheat flour replacement by 2 % of grape pomace flour on the loaf volume was also observed in previous study [37].

Hardness of bread crust and crumb

Not only the bread volume but also the hardness of the crumb and crust of bread sample was not significantly affected by 1 % GSMP addition (Tab. 4). From the textural characteristics of bread, one of the most frequently determined parameters is its hardness, which predicts the bread durability and its acceptability by consumers [38]. Indeed, our results from this part of the study were previously reflected by the sensory evaluation panel in which trained consumers assigned similar scores for surface and properties of the crust, and overall appearance of the crumb [20].

Moisture content and water activity of bread crumbs

Data presented in Tab. 4 show that no significant differences in the values for *MC* and a_w were found between the control and 1% GSMP-added breads. Bread is considered as intermediate-moisture food with *MC* typically ranging from 35–42 % and a_w approximately above 0.95, which is consistent with results of this study. Both parameters predict the product microbial shelf-life. However, bread a_w is more important than *MC* alone [39]. Concerning the substantial impact on the growth of microorganisms, it was found out that a_w below 0.7 has a preventive effect on microbial spoilage [40]. Based on this fact and on our results, it could be expected that wheat flour replacement with 1 % GSMP would have no impact on bread shelf-life. In agreement with our study, Hayta et al. [37] also revealed non-significant effect of grape pomace on bread a_w.

Microbiological characteristics of bread during storage period

Regarding microbiological characteristics of control bread and bread supplemented with addition of 1 % GSMP during the storage period, no growth of colonies of coliforms, yeasts or microscopic fungi was observed. On the other hand, total counts of microorganisms ranged from 1.36 ± 0.07 log CFU·g^{-1} to 2.27 ± 0.14 log CFU·g^{-1} in the control sample and from 1.16 ± 0.14 log CFU·g^{-1} to 2.44 ± 0.08 log CFU·g^{-1}

Tab. 5. Microbiological characteristics of experimental breads during four days of storage.

Days of storage	Control bread			Supplemented bread		
	Total counts [log CFU·g^{-1}]	Colifom bacteria [log CFU·g^{-1}]	Yeasts and fungi [log CFU·g^{-1}]	Total counts [log CFU·g^{-1}]	Colifom bacteria [log CFU·g^{-1}]	Yeasts and fungi [log CFU·g^{-1}]
Day 0	1.36 ± 0.07^{a}	nd	nd	1.16 ± 0.14^{a}	nd	nd
Day 1	1.57 ± 0.09^{a}	nd	nd	0.89 ± 0.11^{b}	nd	nd
Day 2	2.05 ± 0.10^{a}	nd	nd	2.15 ± 0.14^{a}	nd	nd
Day 3	2.13 ± 0.01^{a}	nd	nd	2.18 ± 0.03^{a}	nd	nd
Day 4	2.27 ± 0.14^{a}	nd	nd	2.44 ± 0.08^{a}	nd	nd

Mean ± standard deviation. Values with different superscripts within the same row are significantly different ($P < 0.05$).
Supplement – addition of 1 % (w/w) of grape seed micropowder to the flour.
nd – less than detection limit.

in the supplemented one (Tab. 5). Among the groups, a statistically significant decrease ($P < 0.001$) in counts was recorded in supplemented bread on the first day of storage indicating a considerable antimicrobial activity of GSMP at 1% ddition. On the contrary, the remaining storage days displayed non-significant differences in counts between the analysed bread samples.

Generally, the microbial spoilage of bakery products is caused by filamentous fungi and bacteria. Its expected shelf life is approximately 3–4 days if they do not contain preservatives [41]. Our results showed that although 1% addition of GSMP had no influence on bread a_w, the total counts of microorganisms on the first storage day was lower in the bread with addition of 1 % GSMP as compared to the control one. In grape by-products, polyphenols have an essential role in antimicrobial effects, thus they can be used to extend the shelf-life of foods [26]. Among them, gallic acid, which was also present in our GSMP [19], is believed to be the most active compound in inhibition of microorganism growth. We speculate that disappearance of its antimicrobial activity in the bread during the remaining days of storage period could be most probably associated with depletion of *TPC* capacity to exert its inhibitory effect on microbial growth.

CONCLUSIONS

Grape seeds are an interesting alternative to traditional antioxidants used in food processing. In this study, we examined their effects on chemical composition and selected qualitative parameters of bread supplemented with 1 % of GSMP. The results of the analyses showed that *TPC*, *TPA* and the content of Ca were significantly increased, and also antioxidant activity of enriched bread was stronger compared to the control one ($P < 0.001$). Analysis of microbial properties of stored bread samples showed positive effects of GSMP addition on bread quality after the first day of storage ($P < 0.05$). Thus, it can be concluded that replacement of wheat flour with 1 % of GSMP improves its nutritional characteristics without adverse impact on bread quality. As a gluten-free ingredient, 1 % GSMP can be considered a valuable material to be successfully used to develop novel formulations of bread with health benefits.

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