Research Article

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Wheat Bread with Grape Seeds Micropowder: Impact on Dough Rheology and Bread Properties

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Abstract: The current study was designed to enhance the functionality of white bread by replacement of wheat flour with different levels (1%, 2%, 5%, and 8%) of grape seeds micropowder (GSMP) with nanosized particles (10 µm). Chemical composition of GSMP, volume and sensory attributes, evaluated with the panel of evaluators and an electronic nose (e-nose) and an electronic eye (e-eye) were investigated in the tested breads. It has been found out that GSMP contained appreciable amounts of flavonoids including catechin, epicatechin, gallic acid and minerals especially, Ca, K and Mg. The data from rheological analysis showed that the addition of GSMP (mainly at 5% and 8% levels) to the wheat flour had a positive effect on dough manifesting with rheology by increased dough stability. The volume of the experimental breads (above 1% concentration) was demonstrably declined (P < 0.0001) in comparison with the control bread. Sensory rating revealed that the bread fortified with 1% GSMP was judged by the consumer panelists as the most acceptable with the highest scores for all quality attributes which was also confirmed by the data of e-nose and e-eye. Our results suggest for the first time that 1% GSMP addition appears to be a promising functional ingredient to improve bread with required qualitative and sensory properties.

Keywords: grape seeds; polyphenols; physical properties; rheological properties; sensory evaluation

Graphical abstract

1 Introduction

Bread is a staple food product consumed by most of the population mainly due to easy consumption, quick digestion,

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low price, nutrient abundance, and assorted taste [1, 2]. This popular food (prepared from a dough formulation consisting of flour, leavening agents and water) is an important source of carbohydrates in the food pyramid that covers energy requirements [3]. Wheat (*Triticum aestivum*) benefits human health in several ways (mainly dietary fiber, vitamins, minerals and unique phytochemicals), especially when it is used in a whole-grain bakery product that consists of bran, germ and endosperm [4]. However, there is a growing interest in the addition of plant compounds rich in phenolic fractions to enhance the antioxidant capacity of cereals [5].

Grape seeds, the major industrial by-product derived from wine and juice processing, contain high amounts of biologically active constituents which benefit health. The most valuable compounds of grape seeds are the monomeric phenolics (*e.g.*, epicatechin, epicatechin-3-O-gallate, and catechin), dimeric, trimeric, and tetrameric procyanidins [6]. According to Spranger [7], the content of phenolic substances in seeds is much higher than in skins and stems of grapes. Besides phenolic compounds, grape seeds also contain 7-20% lipids (fats and oil), 35% fiber, 11% protein, 3% minerals and 7% water, with respect to dry weight [8]. Moreover, grape seeds are suitable primary materials for the production of dietary supplements with antioxidant activity [9] targeted at reducing the risk of atherosclerosis and complications related to diabetes, reducing cholesterol levels, cancer prevention, and facilitation of wound healing [10]. With regard to bread, phenolic compounds were found to have a preventive impact against the generation of carcinogens (such as acrylamide) during baking [11].

Many researchers have focused their research on improving the functional properties of bread through the addition of grape seed powder (GSP). A positive effect of GSP on the content of bioactive substances in bread was reported in several studies [12, 13]. Moreover, the assessment of GSP substitution on dough rheology and bread characteristics was evaluated in depth in the above mentioned studies. For instance, in a study by Meral and Doğan [12], it was found that GSP increased dough development time at concentration of 2.5% and 5%. Dough stability value increased from 6.4 to 12.3 min with the increase of GSP level (from 0% to 75%). Peighambardoust and Aghamirzaei [13] showed that overall acceptability of bread decreased when the amount of GSP added was over 10%. Hoye and Ross [14] found that the replacement of more than 5% of flour with GSP resulted in decreased loaf volume and increased hardness and porosity of bread.

However, when wheat dough is enriched with phenolic antioxidants, formation of complexes with proteins or polysaccharides can occur. The affinity of phenolic antioxidants is influenced by their molecular size, conformational flexibility and water solubility. Moreover, both wheat flour [15, 16] and GSP characteristics [17] could be strongly affected by the milling method and the grinding conditions. Indeed, smaller particle size of grape products has been found having higher content of phenolic compounds due to increase of the extraction yield grace to a better surface contact [18]. Moreover, modern food technologies offer powerful new approaches in the food and nutrition sector because they are able to create new opportunities for achieving intended functionality of products with more enhancement beneficial impacts on human health as compared to GSP produced by conventional methods used in aforementioned studies. To the best of our knowledge, replacement of bread with grape seeds micropowder (GSMP), a product of nanosized particles, and determination of the quality of the resultant product have not yet been reported in the literature. Therefore, the present study evaluated the influence of different concentrations of GSMP, a commercially available product, for the first time for scientific purposes, on the rheological properties of dough and qualitative characteristics of bread. To accomplish our research objectives, the above mentioned properties were studied using instrumental analyses of the rheological properties of dough (Mixolab 2, Chopin Technologies, Cedex, France) and volume of bread (Volscan Profiler, Stable Micro Systems, Godalming, UK), sensory evaluation (a panel of evaluators) and analytical measurements by e-nose and e-eye. As a result, the recommended concentration of GSMP used for bread supplementation that ensures the preservation of acceptable rheological, physical and sensory properties was determined.

### 2 Materials and Methods

#### 2.1 Materials

Wheat flour (T-650) was obtained from a grinding mill (Mlyn Zrno, s.r.o., Velké Hoste, Slovak Republic). A part of wheat flour was replaced with GSMP containing nanosized particles (10 µm) produced by a commercial producer (Slovak Wine, Slovak Republic) in the following quantities (w/w): 0% (control sample), 1%, 2%, 5% and 8%.

Methodology of mechanical grain breakage excluded conventional milling with chemical and heat alterations and, intellectual property. Other raw materials such as salt (Solně Mlýny, a.s., Czech Republic), saccharose (Považský cukor, a.s., Slovak Republic) and compressed yeast (Dr.
Oetker, s.r.o, Slovak Republic) were purchased in a local supermarket.

Single-component standards (epicatechin, gallic acid, chlorogenic acid, 4-hydroxybenzoic acid, trans-caffeic acid, trans-p-coumaric acid, rutin, trans-ferulic acid, resveratrol, quercetin, kaempferol, galloallocatechin, catechin, epigallocatechin gallate, galloallocatechin gallate, epicatechin gallate and catechin gallate) were obtained from Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

2.2 Analysis of GSMP Polyphenolic Composition

The extraction method previously described by Kulig [19] was used in a modified version for the extraction of polyphenolic compounds from GSMP. The polyphenolic compounds were determined with a high-performance liquid chromatograph Agilent 1260 with a diode-array detector (DAD) (Agilent Technologies, Waldbronn, Germany). The quantitative chemical composition of GSMP was determined according to methodologies by Kulig [19] and Gabriele [20]. A sample of GSMP was analyzed in two replicates and the data was expressed as mean ± standard deviation (maximum ± 5%).

2.3 Elemental Analysis

Experimental sample from GSMP was weighed on an analytical balance ABT 120/5-DW (Kern & Sohn, Balingen, Germany) and transferred to polytetrafluorethylene (PTFE) mineralization tubes. The weight of the experimental sample (0.3033 g) was included in the calculations. The sample was mineralized in a high performance microwave digestion system Ethos UP (Milestone, Sorisole, Italy) in a solution of 5 mL HNO₃ ≥ 69.0% TraceSELECT (Honeywell Charlotte, North Carolina, USA) and 2 mL of ultrapure water (18.2 MΩ cm⁻¹; 25°C, Synergy UV; Merck, Darmstadt, Germany). The method for dry plant tissue, developed and recommended by the manufacturer, was applied both for the experimental and blank samples. The method included heating and cooling phases. Throughout the heating stage, the samples were warmed to 200°C over 15 min, then they were kept at this temperature for 15 min. Afterwards, during the cooling phase, the samples underwent active cooling to achieve a temperature of 50°C in 15 min. The digestates were filtered through VWR Quantitative filter discs (size 125 mm, particle retention: 12–15 µm) (VWR, Radnor, Pennsylvania, USA) into volumetric flasks which were then filled with ultrapure water up to a volume of 50 mL [21]. 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 equipped with an SPS-3 auto-sampler (Agilent Technologies, Santa Clara, California, USA). The details of the operating conditions were as follows: RF Power 1.3 [kW]; Plasma flow 15 [L/min]; Auxiliary flow 1.5 [L/min]; Nebulizer flow 0.85 [L/min]; Replicated read time 5 [s]; Instrument stabilization 15 [s]; Sample uptake delay 25 [s]; Pump rate 0.25 [Hz]; Rinse time 10 [s]; Element: Ca 315.887, Cu 324.754, Fe 234.350, K 766.491, Mg 383.829, Na 589.592 and Zn 206.200 [λ/nm]. Multielement standard solution V for ICP in 10% Nitric acid (Sigma-Aldrich, Saint-Louis, Missouri, USA) was used. Detections limits [µg/kg] of measured trace elements were as follows: Ca 0.01; Cu 0.3; Fe 0.1; K 0.3; Mg 0.01; Na 0.15 and Zn 0.2. The method was validated using a certified reference material (CRM–ERM CE278 K, Sigma-Aldrich, Saint-Louis, Missouri, USA). The GSMP sample was analyzed twice and the results were expressed as the mean of two determinations ± standard deviation.

2.4 Mixolab Measurement

The rheological behavior of dough was analyzed with Mixolab Profiler (Chopin, Tripette et Renaud, Paris, France) which records the torque (Nm) produced by mixing the dough between two kneading arms.

The amounts of flour samples required for the analyzes were calculated with Mixolab software based on input values of moisture and water absorption (WA) of flour mixtures. 75 g of total flour mass and distilled water were placed into Mixolab bowl. All the measurements were performed using the standard Chopin+ protocol in accordance to the AACC International Method 54-60.01 [22]. The following parameters were recorded: C1 – maximum consistency obtained in the first 8 min (used to determine WA); C2 – protein weakening related to mechanical work and temperature; C3 – gelatinization of starch; C4 – stability of hot gel; C5 – retrogradation of starch in the cooling phase (final starch paste viscosity); Stability – time until the torque at the point C1 decreases by 11%; and WA – the amount of water absorbed by the tested flour.

2.5 Breadmaking Process

Bread samples were prepared according to Burešová et al. [23] with some modifications. Five bread formulations were prepared by replacing 0%, 1%, 2%, 5% and 8% (w/w) of the
wheat flour with GSMP. Bread formula consisted of flour blend, water (60 g / 100 g), yeast (2 g / 100 g), salt (2 g / 100 g) and saccharose (1 g / 100 g). Dry yeast was reactivated in saccharose solution at 35°C for 10 min. To form the dough, all the ingredients were mixed for 6 min in a Diosna mixer (DIOSNA SP 12, DIOSNA Dierks & Söhne GmbH, Osnabrück, Germany). The obtained doughs were consequently placed in an aluminum vessel, transferred into a fermentation cabinet (MIWE cube, Pekass Ltd, Pilsen, Czech Republic) for a 40-min incubation at 32°C (85% relative humidity), and baked in two phases. The loaves were first baked at 180°C with addition of 160 mL steam (at the same temperature) for 17 min (phase I). This was followed by baking at 210°C for 10 min (phase II). A laboratory oven (MIWE cube, Pekass Ltd, Pilsen, Czech Republic) was used for baking. Finally, the bread loaves were cooled to room temperature for 2h and prepared for analyses. In total, 15 bread samples were produced for the analyses, 3 bread loaves per experimental variants. The experiments were performed in the AgroBioTech Research Centre (SPU, Nitra, Slovak Republic).

2.6 Evaluation of Bread Quality

Bread quality analyses included determination of physical and sensory properties.

2.6.1 Physical Evaluation of Bread Samples

The determination of bread volume was modified from Konitzer et al. [24]. The volume [mL/g] of a bread loaf was measured in triplicate by a laser-based scanner (VolScan Profiler 300, Stable Micro Systems, England).

2.6.2 Sensory Evaluation of Bread Samples

The Panel Evaluation Method

A panel of 15 trained members of both sexes (male and female, 7:8), positioned in partitioned booths, was involved in the sensory rating of bread samples. The sensory laboratory in which the analysis of samples was performed meets the requirements of the ISO 8589:2007 standard [25]. The rating questionnaire was prepared according to the study of Sádecká et al. [26] with minor modifications. The analysis was carried out using semi-structured scales scoring 1 (lowest) to 5 (highest) according to the mentioned authors. The sensory attributes evaluated were shape and overall bread appearance, crust surface and properties, overall appearance of the crumb (related to elasticity, softness and porosity), aroma, taste and overall acceptability of bread. For each of these attributes, the average response of the panelists was calculated by the following coefficients: shape and overall appearance – 1, crust surface and properties – 2, overall appearance of the crumb – 2, aroma – 4, taste – 5, and overall acceptability of bread sample – 6. Finally, sensory properties were scored in the final scale from 1 to 100.

Analytical Analyses by E-nose and E-eye

For the aromatic profile analysis of bread samples, electronic nose (e-nose) based on gas chromatography with two flame-ionization detectors (gas-sensor array; Heracles II, Alpha M.O.S., Toulouse, France) were used according to a previously described methodology by Štefániková et al. [27]. Each sample (15 breads) was weighed and placed in three different vials; each one was analyzed once. The electronic eye (e-eye) Visual Analyzer VA400 (IRIS, Alpha M.O.S.) which ensures standardized conditions in a closed chamber where white light is uniformly dispersed to avoid any shadows, was employed to monitor visual differences between the analyzed bread samples. After calibration of the e-eye with a certified color scale, slices of bread samples were placed into the chamber and photographically documented with a complementary metal-oxide semiconductor (CMOS) camera operating within RGB (red-green-blue) and CIELAB color space – CIE L* (lightness), a* (the red/green coordinate) and b* (yellow/blue coordinate) values. Three pictures of three pieces from one sample (n=9) were analyzed with AlphaSoft software (Alpha M.O.S.). The analysis combined image analysis (4096 colors represented as a percentage on a fixed scale) and advanced multivariate statistics (principal component analysis, PCA; Alpha M.O.S.).

2.7 Statistical Analyses

In addition to PCA, GraphPad Prism 8.0.1 (GraphPad Software Incorporated, San Diego, California, USA), one-way ANOVA was used. In cases of statistically significant results (p < 0.05) a Tukey HSD post hoc test was performed. Statistical significance was accepted at **** (P < 0.0001), *** (P < 0.001), ** (P < 0.01), and * (P < 0.05).
Note: Values are expressed as means of three determinations ± standard derivation. Different letters in the columns indicate significant differences (P < 0.05) between the experimental groups.

Figure 1: Thermo-mechanical characteristics of the experimental flours (C1: maximum consistency obtained in the first 8 min; C2: protein weakening related to mechanical work and temperature; C3: gelatinization of starch, stability of hot gel; C4: retrogradation of starch in the cooling phase; C5: time until the torque at the point C1 decreases in 11%; WA: the amount of water taken up by flour)
Table 1: Polyphenolic composition and mineral content in GSMP experimental sample

| Compounds                        | Concentrations [mg/100g] |
|----------------------------------|--------------------------|
| catechin                         | 212±11.00                |
| epicatechin                      | 40.0±2.00                |
| gallic acid                      | 18.4±0.00                |
| quercetin                        | 3.88±0.30                |
| rutin                            | 2.44±0.10                |
| kaempferol                       | 0.85±0.16                |
| trans-caffeic acid               | 0.65±0.03                |
| trans-p-coumaric acid            | 0.64±0.11                |
| resveratrol                      | 0.52±0.02                |
| 4-hydroxybenzoic acid            | 0.32±0.14                |
| trans-ferulic acid               | 0.31±0.11                |
| Ca                               | 846±24.00                |
| K                                | 624±5.00                 |
| Mg                               | 207±2.00                 |
| Na                               | 2.98±0.07                |
| Fe                               | 6.36±0.17                |
| Cu                               | 2.30±0.03                |
| Zn                               | 1.95±0.11                |
| gallocatechin, chlorogenic acid, rosmarinic acid, epigallocatechin gallate, gallocatechin gallate, epicatechin gallate, catechin gallate | n/d |

Values are expressed as mean of two determinations ± standard deviation; n/d – not detected

3 Results

3.1 Polyphenolic and Elemental Composition of GSMP

The concentrations of eleven compounds, including flavonoids (quercetin, rutin, kaempferol), flavanols (catechin, epicatechin), phenolic acids (caffeic acid, ferulic acid, hydroxybenzoic acid, p-coumaric acid, gallic acid) and stilbenes (resveratrol), were determined using HPLC-DAD analysis (Table 1). The obtained data revealed that the most abundant constituent of GSMP was catechin (212 mg/100g) followed by epicatechin (40 mg/100g) and gallic acid (18.4 mg/100g).

Results pertaining macro and micro minerals are presented in Table 1. Generally, the selected elements could be ordered on the basis of decreasing concentrations according to the following pattern: Ca > K > Mg > Fe > Na > Cu > Zn. The most represented elements were macro elements Ca, K and Mg. Quantity of micro elements ranged from 1.95 mg/100g (Zn) to 6.36 mg/100g (Fe).

3.2 Effect of GSMP on Thermo-Mechanical Behavior of Flour Samples

Changes in dough thermo-mechanical behavior of wheat flours substituted with 0%, 1%, 2%, 5% and 8% GSMP were analyzed with the Mixolab instrument. Data obtained from the measurements are shown in Figure 1. The results clearly show that the flour sample replaced with 1% and 2% of GSMP had significantly higher values of C1, C2 (P < 0.05) and C3 (P < 0.01) as compared to the control. Likewise, the sample with 2% substitution of GSMP displayed considerably higher values (P < 0.001) for C1, C2, and C3 when compared to the control flour. A pronounced increase of C2, C3 (P < 0.0001) and stability (P < 0.001) were recorded in the 5% GSMP-replaced flour as compared to the control. Replacement of flour with GSMP up to a concentration of 8% led to significantly higher values of C1, C2, C3 (P < 0.0001), stability (P < 0.001) and a noticeably lower value for C5 (P < 0.01) with respect to the control.

3.3 Influence of GSMP Addition on Bread Loaves Volume

From the results shown in Figure 2 it can be observed that wheat flour substitution with 1% GSMP had no considerable impact on the loaf volume which indicates its proper quality. On the other hand, the presence of higher (2%, 5%,...
8%) GSMP concentrations in the flour led to a significant decrease ($P < 0.0001$) in the volume of the bread samples in comparison with the control. Moreover, our results revealed that the volumes of the experimental breads were remarkably different from each other whereas the level of statistical significance increased with increasing content of GSMP. An extreme level of significance ($P < 0.0001$) was determined between the following GSMP concentrations: 1% vs. 8%, 1% vs. 5%, and 2% vs. 8%, whilst between 1% vs. 2%, 2% vs. 5%, and 5% vs. 8%, the calculated significance levels showed minor differences.

### 3.4 Sensory Evaluation of Bread Containing GSMP

#### 3.4.1 The Panel of Evaluators

Data from the analysis of sensory attributes (shape and overall appearance, crust surface and properties, overall appearance of crumb, aroma, taste, and overall acceptability) of bread loaves supplemented with different GSMP levels are presented in Figure 3. Given the total number of points from the sensory ratings, the bread with 1% GSMP addition was evaluated by the consumer panelists as the most acceptable. Moreover, statistically significant ($P < 0.05$) differences were found between the taste score of breads supplemented with 0% and 1% of GSMP. This indicates that the substitution of wheat flour with 1% GSMP improves the taste of bread significantly. On the other hand, breads supplemented with 2% and 8% of GSMP showed the lowest scores which differed with a statistical significance ($P < 0.05$) from the control sample. The replacement of wheat flour with the highest amount of GSMP (8%) led to a demonstrable ($P < 0.05$ or $P < 0.01$) decrease in the scores for all the sensory attributes, with the exception of aroma. In the bread samples with 2% GSMP, considerably ($P < 0.01$) declined scores of overall appearance of crumb and of whole bread were reported. Interestingly, no significant changes in all the investigated sensory characteristics of bread were observed in breads containing 5% GSMP. Moreover, the aroma of bread was found to be unaffected by the introduction of GSMP in any amount.

#### 3.4.2 Analytical Analyses by E-nose and E-eye

Principal component analysis plot of aromatic profiles of all the analyzed bread samples is shown in Figure 4. Both components of the PCA plot were able to explain 99.98% of the total variance with the first dimension (PC1) and second dimension (PC2) representing 99.78% and 0.22%, respectively. The PC1 showed a significant separation of the control bread and the bread supplemented with 1% GSMP (positive score) from the other samples (negative score). This finding does not correspond with the sensory rating of bread aromas assessed by the evaluators (Figure 1). Significant divergence in aromatic profile between the bread supplemented with 0% and 5% GSMP obtained from the e-nose analysis was not revealed by the sensory evaluation performed by the consumer panelists.

![Figure 3: Radar plot obtained from sensory evaluation of bread samples with different (0%, 1%, 2%, 5%, 8%) concentrations of GSMP](image)

![Figure 4: PCA analysis of the aromatic profiles of bread samples supplemented with different levels (0%, 1%, 2%, 5%, 8%) of GSMP acquired by e-nose](image)

The e-eye IRIS with CMOS camera was used for the identification of color and shape changes of bread loaves with different concentration of GSMP addition (Figure 5). Various undertones of brown (2169 – 3803 color scale) and darkening of bread as well as deterioration of its shape were
Figure 5: The shape and color of the bread samples replaced with different levels of GSMP (0%, 1%, 2%, 5%, 8%) acquired by the e-eye observed with increasing GSMP content. The color with a discriminant of >0.900 were selected, based on which a semi-qualitative evaluation was performed by PCA (Figure 6).

The results (n=9) have shown pronounced color differences between the bread supplemented with 0% and 1% GSMP addition (negative score of PC1; 98.48%) in comparison with those supplemented with 2%, 5% and 8% GSMP additions (positive score of PC1).

4 Discussion

Grape seeds contain a complex mixture of low molecular weight compounds among which polyphenols are dominant [6]. For instance, GSP from a red grape cultivar of Vitis Vinifera (Carignan; north Tunisia) was found to be composed mainly of gallic acid [28] and epicatechin [29] which was different from our results. On the other hand, the elemental composition of GSMP used in this study was similar to the one described in the study of Mironeasa [30]. Regarding the fact that the concentration of Na was the lowest among the analyzed macro elements in the GSMP used for the purposes of this research, it is known that, being a natrophic plant, vine accumulates little sodium from the soil [31]. Generally, various factors such as the variety of grape, climatic conditions, geographical origin, and the degree of grape maturity influence the composition of grape polyphenols [32]. Also, the particle size of the grape by-product plays an important role in their phenolic composition. Zhao et al. [18] have found significant increase in the contents of total polyphenolics and flavonols in grape pomace powders with decreasing their particle size prepared by superfine grinding method.

The addition of fiber-rich ingredients such as grape by-products generally affects dough rheological properties depending on the addition level, as well as particle size as different particle fractions of grape by-products have different constituents which impact the rheology differently [33]. To determine the influence of various concentrations of GSMP on dough behavior that might be expected during the process of baking, Mixolab 2 by Chopin was used in our study. One of the most fundamental quality parameter of flour is WA which expresses the optimal quantity of water necessary for hydration of flour components in order to yield a dough with an optimal consistency (e.g., non-stickiness for processing) determining appropriate quality of the final product [34, 35]. Since based on this definition WA can be interpreted as the function of the relative amounts of the components capable of being hydrated (starch, proteins, pentosans) and their specific water binding capacity [36], WA capacity of flour is also a function of economic perspectives.

Our results showed that the replacement of wheat flour with different GSMP amounts had no considerable impact on WA of flour indicating that the contents of chemical compounds with hydrophobic (such as fatty acids) and hydrophilic (fiber-rich fractions) properties had to be balanced in all the GSMP containing mixes. Interestingly, Aghamirzaei et al. [37] observed a significant reduction in WA of flour with increasing levels (from 0% to 25%) of flour substitution with GSP. We speculate that the dissimilarity identified among our study and that performed by the above mentioned authors may lie most probably in the differences in chemical composition of the powder/micropowder introduced into white bread (mainly in the content of hydrophobic substances such as fatty acids) which can be changed depending on the particle size.
Our study revealed that increasing the content of GSMP (from 5%) in wheat flour resulted in considerably higher dough stability even in spite of the decreased amounts of gluten in the samples. The reason for the observed changes might be linked to the higher proportion of fatty compounds and polyphenols in 5% and 8% GSMP additions which may interfere with polymeric fraction of gluten which leads to forming of lipoprotein complexes and the softening of dough. This in turn enhances the rheological performance of dough by facilitating the formation of a stronger and more stable gluten network (despite lower amounts of gluten proteins) making the dough more cohesive which is commonly preferred in most baking applications. Regarding the polyphenol content in GSMP it was found that incorporation of polyphenols in bakery products resulted in alterations of secondary and tertiary protein structures (due to the interactions between phenolics and proteins through covalent and non-covalent bonds) [11] which could also participate in improved stability of dough. Also, higher amounts of proteins in wheat flours replaced with 5% and 8% GSMP may contribute to enhanced dough stability. Regarding the proteins of GSMP, enhanced dough stability in the samples with 5% and 8% substitution GSMP might have had occurred because of protein aggregation. A significant increase in the stability of dough was found to be induced by the increased addition of GSP (from 0 to 7.5%) in a study by Meral and Doğan [12] which is in accordance with our findings.

Parameters C1 and C2 are related to the quality and quantity of flour proteins such as gliadin and glutenin (80-90% of flour proteins), and albumin and globulin (7-15% of flour proteins) [38]. In this context, we have found that the substitution of wheat flour with GSMP demonstrably increased both parameters (except for C1 in 5% GSMP addition) compared to the control wheat flour. This fact indicates that the introduction of GSMP decreases weakening in proteins, i.e., it leads to reduced degradation of protein network and higher gluten quality and strength. These findings can be related to complexation of phenolic antioxidants present in GSMP with wheat flour proteins which occurs via hydrogen bonding between hydroxyl groups of phenols and the carbonyl group of peptide residue of proteins [17]. The parameter C3 was also higher in all of the samples with GSMP substitution which indicates better starch performance in terms of increased degree of its gelatinization. We propose that the introduction of GSMP to wheat flour might have intensified the deterioration of starch (amylosis) and increased the quantity of fermentable sugars in the formed dough which leads to a drop of the viscosity of the formed dough gel. Our results also revealed that replacement of wheat flour with 8% GSMP contributed to a significant decrease in C5 torque, i.e., the maximum torque of the cooling stage which reflects the extent of starch retrogradation. Švec and Hrušková [39] stated that lowering the rate of recrystallization of starch could prolong the shelf life of the final product. On the other hand, any concentration of GSMP used in our study had no considerable impact on C4 torque which is the indication of amylase activity. This finding signalizes that GSMP supplementation of wheat flour does not affect the stability of hot-formed gel [40] and physical breakdown of the granules influencing the viscosity of flour samples [41]. Finally, it can be concluded that a partial replacement of wheat flour with GSMP results in improved behavior of dough during thermal treatment and mechanical shearing, leads to a significant increase in stability (5% and 8% substitution) during mixing and a decrease in dough weakening upon heating (demonstrated by increased values for C1 and C2 in all experimental breads), better performance of starch gelatinization (increased C3 in all experimental breads) and a potential to prolong bread shelf-life (decreases C5 in the bread with 8% addition).

When it comes to the adverse effect of grape seeds introduction on the bread volume, the same trend was reported by Høy and Ross [14]. These authors observed a negative impact of GSP on the parameter which increased as the content of the additive rose (0%, 2.5%, 5%, 7.5%, and 10%). A considerable decline in the volume of experimental bread supplemented with different levels of grape pomace from a red variety of Merlot (6%, 10%, and 15%) was reported by Šporin [42]. In contrast to our results, the volume of breads supplemented with the increasing levels of grape pomace flour (2%, 5%, and 10%) was not found to be significantly impacted in the research by Hayta [43]. Additionally, Smith and Yu [40] noted a non-significant impact of white flour substitution with 5% of grape pomace on bread volume. Demonstrably decreased loaf volume was recorded when the content of grape seed was increased to 10%. Based on the differences in grape varieties used in our and the aforementioned studies, we propose that the discrepancies between the results may be variety related. Also, the processing technique used for the preparation of grape seed could have been the reason behind dissimilar observations.

In general, the final loaf volume is strongly connected with dough expansion during the processes of fermentation and baking, as well as with the ability of the matrix to stabilize the retained gas [45]. The study by Koksel [46] showed that Mixolab parameter C3 is significantly negatively correlated with bread volume which suggests the importance of the starch gelatinization phase for obtaining a proper volume of bread. Regarding these findings, we propose that the deleterious effect of GSMP addition (2%, 5%, 8%) on the volume of our bread loaves may be associated with a
weakening of the gluten-starch matrix because of hydrogen bonding of foreign proteins derived from GSMP. The weakening of gluten network might also be attributed to the interaction of thiol groups (SH) of gluten with phenolic acids present in GSMP which consequently resulted in the breakdown of disulfide (SS) bonds in the network. Also, a negative effect of soluble fibers on retention (due to the interaction with the gluten network) and production of gas can be considered in wheat flour substituted with 2%, 5%, and 8% of GSMP. As a result, reduced gas retention in the dough [47] followed by a subsequent decline in the volume of bread [45] and smaller slice area [48] could have been observed. Generally, the technology of bread production is a highly complex phenomena including not only the rheology aspect but also a combination of individual technological, chemical and sensory characteristics.

Bread has attractive sensory characteristics, especially when it is freshly baked. In the production of bread enriched with bioactive compounds, which enhance its nutritional potential, it is indispensable to investigate their effect on the sensory attributes of the product in order to achieve the best quality and meet specific consumer expectations. In our study, the overall acceptability of bread (the measure of how readily the consumer panelists accepted the product with all parameters of sensory evaluation taken into account) was found to be decreased by bread replacement with 2% and 8% of GSMP. Lower consumer acceptance was also reported for breads containing 7.5% GSP in the research by Hoye and Ross [14]. Similarly to our results, no impact of 5% of white flour replacement with GSP on overall acceptability of bread was found by Peighambardoust and Aghamirzaei [13]. Nonetheless, the authors observed a decrease in this sensory attribute of breads when the content of GSP was greater than 10%. Moreover, our findings revealed non-significant effects of higher levels of GSMP (≥ 2%) on aroma and taste (necessary for the market success of novel foods) which is inconsistent with other studies that deal with the issue of bread replacement with GSP [13, 14]. Sensory properties of polyphenolic compounds, particularly flavan-3-ols (such as catechins and epicatechins which were the most abundant in GSMP) can be characterized by two main descriptors, bitterness and astringency, and are well known for evoking negative consumer response when present in a product at high concentrations [49, 50]. Despite the high concentration of catechin and epicatechin in GSMP, no undesirable taste and aroma were detected in our experimental breads. We thus suppose that it might have been the result of the processing methods used for GSMP production. Indeed, the methods of emerging technologies producing nanosized particles can lead to reduction of bitterness of the final products [51]. Significantly declined scores for the overall appearance of crumb (concerning its porosity, elasticity and softness) in bread with 2% and 8% GSMP substitution may be attributed to the changes in dough viscosity induced by phenolic compounds contained in GSMP, most probably by catechin. These bread samples were characterized by high crumb density with less visible air bubbles. As a result of the compact crumb structure, loaf volume was significantly reduced. It was found that a higher content of catechins in green extract might act as a reducing agent which causes the conversion of SS bonds into SH, thereby altering the gluten network viscoelasticity. We assume that catechins can reduce inter-molecular SS bonds of the glutenins and reduce the size of glutenin polymers followed by decreased elasticity and increased viscosity of dough making it softer. Enrichment of dough with green tea extracts resulted in smaller volumes and harder textures of the produced breads [52, 53]. Interestingly, the score for overall appearance, shape, surface and properties of the crust was considerably decreased only in the case of bread with 8% GSMP.

To confirm and improve the sensory perceptiveness of bread, e-nose based on a head-space gas chromatography was applied in our study for the first time to describe the aromatic profiles of bread samples with various concentrations of GSMP. The instrument shows high sensitivity and it can provide precise complementary data to these obtained from the sensory analysis carried out by the consumer panelists. Moreover, it can perform the assessment of aroma profile of samples on a continuous basis and with a reduced cost and time [54]. The perception of a product odor is generally determined by various volatile compounds with low odor thresholds whose relative levels in the headspace determine the dominant perceived odor [55]. Indeed, methods of sensory evaluation require a set of well-trained evaluators and several established features in order to provide dependable results without subjecting to individual breakdown or modification of sensitivity [54]. Mainly in bakery products, the complex matrix and aromatic profile of the analyzed samples can limit the discriminating ability and sense perception of evaluators. Additionally, concentrations of volatile compounds in the samples can be too close or even below the detection limit of assessors. Thus, the results from the sensory evaluation obtained by the panel evaluators can be often inaccurate and distorted [56].

Following the texture, color is the second primary sensory characteristic of bread. The changes of overall appearance and shape and overall appearance of crumb evaluated by panelists were confirmed by the e-eye analysis. However, the results obtained by e-eye are more objective and accurate. Our results are in agreement with the study of Sun-Waterhouse [57] where bread volume, color, and crumb...
structure were demonstrably changed by the incorporation of phenolic extracts. On the contrary, Zhu et al. [58] found that addition of black tea powder (rich in polyphenolic compounds) to Chinese steamed bread in concentrations ranging from 1 to 5% had a little effect on bread texture. Darkness of the bread, however, was increased with the increasing content of black tea powder. The findings by Ning et al. [59] also revealed that bread darkness increased with increasing amounts of green tea powder. The increasing values of color might be attributed to Maillard reaction, non-enzymatic browning, and the transformation of heat-sensitive compounds [60]. According to the results, electronic systems appear as more objective tools for the characterization and quality evaluation of foods in routine analysis. They allow the results to be acquired in a short time and can complement sensory evaluation.

5 Conclusions

Currently, there is a growing demand for bread products containing bioactive compounds (such as grape by-products) which brings some important innovations in the food industry. Since the negative effects of grape by-products on the final bakery product quality (dough rheological properties, bread physical and sensory quality parameters) can be eliminated by reducing their particle size, we used nanosized micropowder of grape seeds (GSMP) in our study. Hereby we report the very first results on the enhancement the functionality of white bread by the substitution of wheat flour with different concentrations of GSMP (1%, 2%, 5%, and 8%). A positive impact of GSMP supplementation on the rheological features of breads was found, especially in the samples that were supplemented with 5% (P < 0.001) and 8% (P < 0.001) of the additive. On the other hand, significantly decreased bread volume was observed in the breads supplemented with ≥ 2% GSMP. In sensory evaluation, the bread supplemented with 1% GSMP had the highest scores for all the quality attributes, whilst the bread with 2% and 8% GSMP additions were characterized by decreased consumer acceptance. These findings were also confirmed instrumentally with the e-eye and e-nose. Since consumer acceptance depends mainly on the physical and sensory properties of the final product, the addition of 1% GSMP is recommended for the production of bread with acceptable rheological, physical and sensory properties.

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