Rheological and sensory properties and bioactive compounds of wheat dough and bread enriched with nettle (*Urtica dioica* L.) flour

Rosen Chochkov[1], Petya Ivanova[1], Denka Zlateva[2], Mariela Bogdanova[1]

1 – University of Food Technologies, Plovdiv, Bulgaria
2 – University of Economics, Varna, Bulgaria

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Corresponding author:

Rosen Chochkov
E-mail: rosen4o4kov@abv.bg

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Abstract

Introduction. The aim of this study was to analyse the effect of nettle (*Urtica dioica* L.) flour on the rheological and sensory properties, as well as the bioactive compound content, of wheat dough and bread.

Materials and methods. The bread was made by type 500 wheat flour with the addition of nettle flour. Wheat dough rheological properties were determined by measuring the water absorption, consistency, dough development time, stability and dough softening on farinograph Brabender. The antioxidant activity of ethanolic extracts was evaluated by FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging methods.

Results and discussion. In the bread formulation, nettle flour was used as a partial substitute for wheat flour at levels of 2, 4, and 6%. The highest dough consistency was observed in the control sample, while all enriched samples showed lower values. However, with increasing substitution levels, consistency values gradually increased. The addition of nettle flour did not affect dough development time. A slight increase (up to 1.3%) in baking loss was observed in breads containing the lowest level of nettle flour compared to the control.

All breads enriched with nettle flour showed a less uniform crumb structure and a more irregular overall appearance relative to the control. The visual quality scores of breads containing nettle flour were lower, and a significant difference in crust coloration was observed. The crumb of the control sample had a typical yellowish hue characteristic of wheat bread, while the addition of nettle flour produced progressively darker coloration. Furthermore, the content of bioactive compounds increased significantly with higher nettle flour content.

Conclusions. As the proportion of nettle flour increased, the dough formation time remained unchanged, while water absorption and dough development time increased compared with the control sample. The incorporation of 2% nettle flour produced the highest bread volume and specific volume. Increasing the nettle flour content enhanced the porosity of the bread crumb but negatively affected its taste quality. The antioxidant activity of the wheat bread increased proportionally with the level of nettle flour substitution.

Introduction

There has been a global trend towards the use of the natural substances present in the food as a source of antioxidant and functional ingredients (Dziki et al., 2014; Kuzmin et al., 2020; Paredes-López et al., 2022; Stabnikova et al., 2021, 2024).

Due to their widespread consumption, cereal-based food products (e.g., bread), which provide more than 50% of the total energy intake in developed societies, are considered excellent vehicles for functional supplementation (Akhtar et al., 2011). Consumption of the wheat-based products, especially bread, is rapidly growing in Europe (Cauvain, 2015). Indeed, bread is consumed every day all over the world. Due to its popularity, bread is often enriched with functional products of natural origin (Ibrahim et al., 2015). To enhance the nutritional value of bread, the upward trend of incorporating plant's seeds, leaves and/or their extracts as a source of phytonutrients is increased (Đurović et al., 2020; Gubsky et al., 2025).

With a history dating back more than 2000 years, stinging nettle has been used as a food and natural medicine for centuries (Said et al., 2015). Due to its balanced protein composition and relatively high content of minerals and vitamins, stinging nettle is often used as a natural supplement that increases the content of biologically active substances in foods. Protein constitutes about 30% of the dry mass and contains numerous amino acids essential for human nutrition. Minerals make up approximately 20% of the dry mass (Said et al., 2015). Nettle is also rich in vitamin C (Guil-Guerrero et al., 2003), chlorophylls, carotenoids, and phenolic compounds, and exhibits significant antioxidant activity (Mannila et al., 2023). Due to its seasonal availability, the consumption of fresh nettle is limited; therefore, it can be utilized in dried form as an additive in various food products.

Nettle leaf flour contains on average about 30% protein, 4% fat, 10% fiber, and 15% ash (Man et al., 2019). Therefore, it can be used as a high-protein supplement and has been incorporated into various food products, including bread (Adhikari et al., 2016). The addition of nettle to bread significantly increases the levels of nutrients such as fiber, calcium, copper, and iron (Maietti et al., 2021). Man et al. (2019) also reported that supplementation of wheat flour with nettle powder enhances the protein, fiber, and mineral content of bread. Sensory evaluation of the enriched breads indicated acceptable organoleptic properties in samples containing up to 4% nettle flour. On the other hand, Saidov et al. (2022) confirmed an increase in protein and fiber content but reported that the specific volume of bread decreased with higher levels of nettle leaf powder. This reduction was attributed to the lower gluten content of the mixture and the interactions between dietary fiber components, water, and gluten. Krawęcka et al. (2021) investigated the effect of *Urtica dioica* L. addition on the nutritional and quality characteristics of wheat pasta. Their results showed that nettle supplementation significantly increased the contents of calcium, iron, potassium, and magnesium. Moreover, as the proportion of nettle increased, a statistically significant ($p \leq 0.05$) rise was observed in total dietary fiber, particularly in the insoluble fraction, as well as in the levels of pigments such as chlorophylls and carotenoids.

Neveen and Shaimaa (2023) found that water absorption gradually increased with the addition of dry nettle leaves in biscuit formulation. Likewise, dough development time increased as compared to the corresponding control. On the other hand, dough stability time was shortened because the existence of fiber particles resulted in a disruption of the starch-gluten network and thus – in a decrease of dough stability time.

Stinging nettle is undoubtedly a raw material worth attention in the context of functional food design. The aim of this study is to investigate the rheological, sensory and antioxidant properties of wheat dough and bread fortified with nettle (*Urtica dioica* L.) flour (added in an amount of 2, 4 and 6% to replace the equal amount of the wheat flour in bread formulation).

Materials and methods

Materials

For the preparation of the bread samples, the following raw materials were used: commercial wheat flour (type 500, Sheri 61 LCOO) with the average chemical composition, g/100 g: fat 0.9 (of which saturated 0.3), carbohydrates 70.3 (of which sugars 3.4), fiber 4.0, protein 10.8, and moisture 10.6; commercial nettle flour (Viki Nuts Ltd) with the average chemical composition, g/100 g: fat 0.85 (of which saturated 0.07), carbohydrates 3.42 (of which sugars 2.56), fiber 40.00, protein 31.66, and moisture 5.40. Additional ingredients included water (ISO 6107-1:2004), compressed yeast (Lesaffre Bulgaria Ltd), and salt compliant with the Codex Standard for Food Grade Salt (CX STAN 150-1985).

Methods

Preparation of dough and bread samples

Kneading was carried out using a one-phase dough preparation process to obtain a homogeneous dough mass at an initial temperature of 26–27 °C. The mixture of flour (wheat and/or nettle), yeast, salt, and water was kneaded in a laboratory kneading machine (Labomix 1000, Hungary).

The control sample (CS) was prepared using only wheat flour (WF), while the experimental bread samples were produced by substituting wheat flour with nettle flour (NF): NF2 (NF 2%: WF 98%), NF4 (NF 4%: WF 96%), and NF6 (NF 6%: WF 94%). For each formulation, the following ingredients were added: water 56%, yeast 2%, and salt 1.5%.

The prepared doughs were allowed to mature for 30 min at 30 °C. Subsequently, the dough was divided into portions of 230 g for flat bread and 440 g for pan bread. After shaping, the dough pieces were subjected to final fermentation at 33–34 °C for 60 min in a fermentation chamber (Tecnopast CRN 45-12, Novacel Rovimpex, Novaledo Trento, Italy).

The doughs were then baked in an electric floor oven Salva E-25 (Salva Industrial S.L.U., Lezo, Spain), preheated to a temperature of 220–230 °C, for 22–24 min. After baking, the breads were allowed to cool for 3 h at room temperature.

Rheological properties of the dough

The following dough properties were determined by a farinograph (Brabender GmbH&Co KG, Duisburg, Germany): water absorption (%), development time (min), stability (min), degree of softening (farinograph units (FU)) and consistency (FU), according AACC Method 54-21.02 (AACC International. AACC Approved Methods of Analysis, 11th ed.; AACC International: St. Paul, MN, USA, 2010).

Physical properties of bread

The quality of the bread samples was assessed as followed: bread loaf volume was determined after baking and cooling for 3 h at room temperature by a rapeseed displacement method (AACC, 2010).

The specific volume was calculated by the ratio between volume (ml) and mass (g) of each sample. Bread height and diameter were measured by a calliper, and the shape stability (Height/Diameter) was calculated (Novotni et al., 2012). Bake loss (%) was determined by weighing each loaf before and after baking (Kim et al., 2015). The bread loaves were wrapped in plastic bags and stored at room temperature (27 ± 2 °C) to determine the storage time (in days) until visible mold growth occurred.

Sensory evaluation

Sensory evaluation of the obtained breads was performed by a descriptive panel consisting of 25 panellists (52% women and 48% men) aged 22 – 60 years, who were familiar with sensory analysis of food but not specifically trained in the evaluation of bread. The analysis was carried out according to ISO 6658:2017. The panellists were asked to score seven parameters, namely appearance, crust color, crumb color, porosity, chewability, aroma and taste. They expressed the intensity of each attribute on a 9-point hedonic scale (9 – extremely good; 1 – extremely bad).

Bioactive compound content

The extraction of bioactive compounds from dry breads was carried out with 70% ethanol as described by Vasileva et al. (2018). Bread samples were sliced (about 1.5 cm thick), dried (40 °C, 24 h), ground in a mill, and sieved (0.5 mm sieve). Ethanol extracts from breads were obtained with 70% ethanol (solid to liquid ratio 1:20) in an ultrasonic bath (VWR, Malaysia; 45 kHz, 30 W) at 45 °C for 15 min. Samples were then centrifuged at 1800xg for 15 min (MPW-251, Med. Instruments, Poland). The supernatants were used for further studies.

Analytical methods

Total polyphenols were quantified by using Folin-Ciocalteu's reagent (Ainsworth and Gillespie, 2007). Gallic acid was employed as calibration standard and the results were expressed as mg equivalents gallic acid (GAE) per gram dry weight (DW).

Total flavonoids were determined using $Al(NO_3)_3$ reagent and measuring the absorbance at 415 nm according to Kivrak et al. (2019). The results were expressed as mg equivalents quercetin (QE) per gram DW.

The antioxidant activity of ethanolic extracts was evaluated by two methods: FRAP (ferric reducing antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging methods.

The FRAP method is based only on single electron transfer mechanism and was measured according to the method of Dimov et al. (2018) with some modification. Three ml freshly prepared FRAP reagent (10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM FeCl3.6H2O in d. H_2O) were mixed with 0.1 ml of investigated ethanolic extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with 70% ethanol. A standard curve was built with $FeSO_4.7H_2O$. The results of FRAP analysis were expressed as µmol Fe^{2+} equivalents per gram DW (Irshad et al., 2012).

The DPPH radical method is based on mixed hydrogen atom transfer and single electron transfer mechanisms. DPPH radical scavenging activity was estimated according to Dimov et al. (2018) with some modification. Briefly, 0.15 ml of ethanolic extract was mixed with 2.85 ml 0.06 mM DPPH fresh solution in 96% ethanol. The mixture was left for 30 min (kept in the dark at room temperature) so that a reaction could take place, and then the absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing 70% ethanol. The results of DPPH analysis were expressed as mmol Trolox equivalents (TE) per gr DW.

Statistical analysis

Results are presented as means of at least three independent determinations±standard deviation (SD). Statistical evaluation was performed by using one-way analysis of variance (ANOVA) of the IBM SPSS Statistics program (Somers, NY, USA). Mean differences were established by Fisher's least significant difference test for paired comparison with a significance level $\alpha = 0.05$.

Results and discussion

Effect of nettle flour on the rheological properties of dough

The dough rheological properties are decisive for its behaviour during technological operations. The results are presented in Table 1.

Table 1

Effect of nettle flour on rheological properties of wheat dough

Bread	Water absorption,%	Consistency, F.U.	Time of dough development, min	Stability, min	Dough softening, F.U.
CS	62.4	520	1.5	7	170
NF2	63.3	490	1.5	10	170
NF4	63.3	480	1.5	8.5	140
NF6	63.3	500	1.5	6.5	140

Note: F.U. - Farinograph Units.

As can be seen, the water absorption of the control and test samples was almost identical. However, the results obtained by other researchers differ from our findings. According to Neveen et al. (2023), the water absorption of enriched samples gradually increased with the addition of dry nettle leaves at 5%, 10%, and 15% substitution levels. This discrepancy is likely due to the different forms of nettle used, as the physical characteristics of nettle flour and dry leaves can influence dough hydration behavior.

The highest consistency score was measured for the control sample. All enriched samples had lower scores, with increasing degree of substitution of wheat flour with nettle flour the values increased. The use of nettle flour did not change dough development time. The stability of the samples with nettle flour for samples NF2 and NF4 increased compared to the control, while for sample NF6 decreased. Compared with the control, dough softening progressively decreased as the proportion of nettle flour increased.

Quality assessment of wheat bread enriched with nettle flour

To assess the baking characteristics of the wheat bread enriched with nettle flour – pan bread was prepared from each formulation (Figure 1). Fermentation and baking of all the samples were carried out under equal conditions, according to the adopted technology.

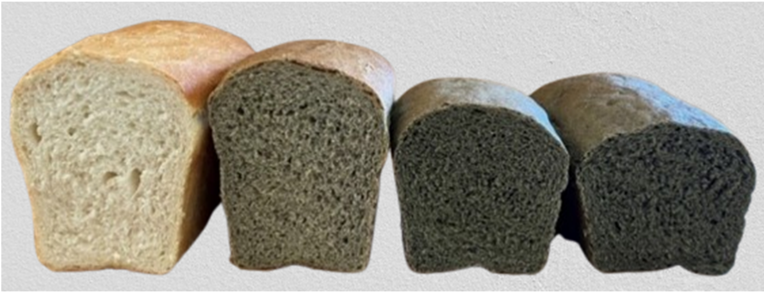

Figure 1. Cross-section of wheat bread enriched with nettle flour (from left to right: CS; NF2; NF4; NF6)

Results from the assessment of the baking characteristics are presented in Table 2.

Table 2

Volume of wheat bread enriched with nettle flour

Bread samples	Volume, ml	
	Floor bread	Pan bread
CS	820	1512
NF2	686	1332
NF4	523	1060
NF6	490	1030

The results show that the volume of wheat bread samples with added nettle flour decreases. With increasing the quantity of nettle flour, respectively, the values decrease. The results obtained for the highest added amount – sample NF6, show a significant decrease in the volume of the floor bread, by 40%, compared to the control sample. This confirms the conclusions drawn from the dough rheological characteristics, that the highest amount of nettle flour leads to a deterioration in the rheology of the dough, and hence the quality of bread. The results for the pan bread are similar to those for the floor bread.

According to the literature, the addition of fiber-rich materials up to 7% leads to a proportional decrease in bread volume, corresponding to the reduction in gluten content within the blend. Laurikainen et al. (1998) reported that when this level is exceeded, bread volume decreases more rapidly than theoretically expected, which they attributed to a reduced gluten protein content.

The specific volume of bread, defined as the ratio of its volume to its weight, provides a more comprehensive characteristic of bread quality. The results for the specific volume of wheat bread supplemented with nettle flour are presented in Figure 2.

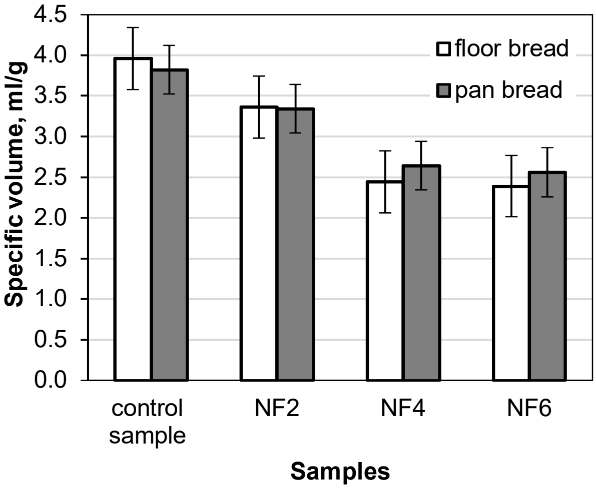

Figure 2. Specific volume of floor and pan wheat bread enriched with nettle flour

Regardless of the method of baking the bread (flat or pan bread), a decrease in the specific volume is observed with increasing the amount of added nettle flour. Man et al. (2019) also reported similar results. They pointed out that the specific volume of the breads decreased as the level of nettle leaves powder increased due the dilution of gluten content in the blend and due to the interactions among fiber components, water and gluten. In this regard, Đurović and co-authors (2020) pointed out that the form in which the nettle is added matters. They added nettle leaves and extract (in equal amounts) to the wheat bread formulation. When using nettle leaves, the reduction in the volume of the loaves of bread was much more pronounced. Some authors, however, do not support this opinion. Hung et al. (2007) argued that the existence of dietary fiber diluted the protein and interfered with the optimal gluten matrix formation during dough mixing.

The shape stability of bread is denoted H/D and is determined by the ratio between the height and diameter of the floor bread. Figure 3 presents the shape stability (H/D) of the control sample and test samples of wheat floor bread supplemented with nettle flour.

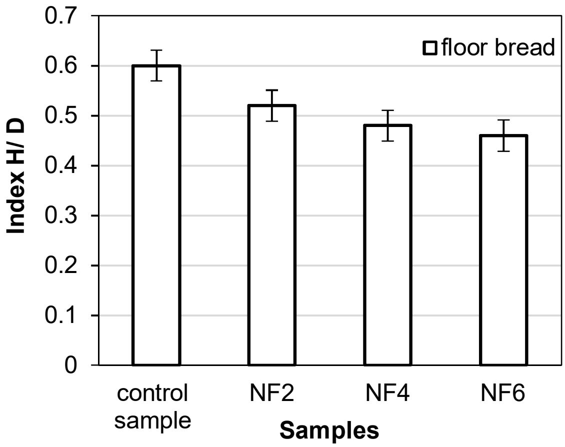

Figure 3. Shape stability of wheat bread enriched with nettle flour

In terms of shape stability (H/D), the samples with nettle flour have lower values compared to the control sample (0.6). The closest to the control sample are the results obtained about sample NF2 – (0.52). The other samples (NF4 and NF6) have almost identical values for H/D much lower than the obtained for the bread prepared from wheat flour.

The baking loss of flour and pan wheat-nettle bread is shown in Table 3.

Table 3

Baking loss of floor and pan wheat bread enriched with nettle flour

Bread samples	Baking loss,%	
	Floor bread	Pan bread
CS	10.00	10.22
NF2	11.30	9.54
NF4	10.40	8.87
NF6	10.86	8.64

With addition of a lowest quantity of nettle flour, baking loss (floor bread) increase by up to 1.3% compared to the control sample. It is interesting to note that the floor bread with nettle flour had lower baking loss compared to the control sample (up to 1.58%). For the pan bread, the difference between control sample and lowest quantity of nettle flour is 0.68%. In the case of pan bread, increasing the amount of added nettle flour has been found to reduce baking loss. Wójcik et al. (2021) conducted similar studies, but they enriched wheat bread with nettle infusion, added in amount of 10, 20, 30 and 40 mg/ml. The authors concluded that the increasing addition of nettle infusion caused a decrease in baking loss and the volume of the obtained loaves. This pattern does not occur in the case of floor bread.

Sensory profile of wheat breads enriched with nettle flour

Overall, the use of nettle flour can change the quality of wheat bread in terms of sensory properties. The results obtained are presented in Figure 4.

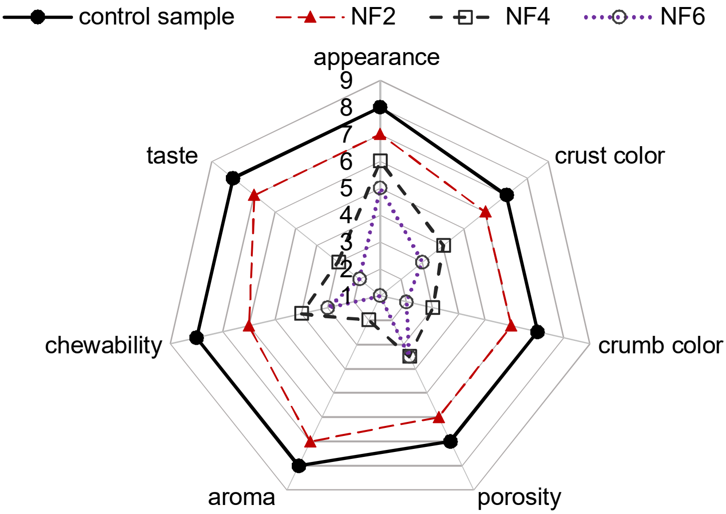

Figure 4. Sensory profile of wheat breads enriched with nettle flour

The present study indicated that the scores for all sensory properties decreased with the increasing the proportion of nettle flour. When cutting, the control sample exhibits a lower tendency to crush, unlike the test samples. All test samples have a more atypical development and a more uneven crumb structure, compared to the control sample. The appearance values of the enriched sample NF2 compared to CS decrease by 12.50%, while for the enriched sample NF6 compared to CS there is a decrease by 37.50%. Significant difference in the coloration of the crust is observed. The color of the bread crumb of the control sample is yellowish, typical for wheat flour bread. In the test samples, the color shade is more saturated and belongs to the green range. The color of the crust and the crumb for CS and NF2 have the higher data values. Diddana et al. (2021) investigated the nutritional composition and sensory acceptability of unleavened flatbread (Kitta) developed from stinging nettle (*Urtica simensis* L.) leaf and maize (*Zea mays* L.) flour. The authors concluded that color acceptability of flatbread was significantly ($p < 0.05$) reduced with increasing the proportion of nettle leaf flour. Sample NF2 exhibits higher porosity compared to samples NF4 and NF6. The results indicated the the control bread and the bread NF2 have the higher values (8 and 6) for chewability compared to the bread NF4 and NF6. For the taste the results were almost similar – the control sample and the sample NF2 have achieved higher scores (8 and 7) compared to the samples NF4 and NF6 (3 and 2). Rădulescu et al. (2024) reported that the addition of nettle powder to the bread dough gives a specific taste and texture to the bread.

The control sample has a well pronounced, typical for wheat bread aroma. In the enriched samples NF4 and NF6, a much more intensive nettle aroma is detected, but it slightly differs from the aroma of fresh nettle. However drying changes the aroma of food products through losses in volatile compounds or the formation of new volatile compounds as a result of oxidation and esterification reactions (Diaz-Maroto et al., 2002; Orphanides et al., 2013). Man et al. (2019) reported almost the same results – bread prepared with 100% of wheat flour scored maximum than the rest of the samples. Evaluating the samples enriched with nettle powder, the highest score was observed in the bread containing 2% NF, while the lowest was recorded for the bread with 6% NF. The enriched breads showed reduced volume and porosity, which resulted in lower overall sensory scores.

Bioactive compounds in wheat bread with nettle flour

The antioxidant activity, dry matter content, total phenols and flavonoids of the bread samples are presented in Table 4.

Table 4

Bioactive compounds in wheat bread with nettle flour

Bread samples	Dry substance,%	Total flavonoids, mg QE/g DM	Total phenols, mg GAE/g DM	DPPH, mM TE/g DM	FRAP, µmol Fe^{2+}/g DM
CS	90.11±0.06^{a}	0.12±0.00^{d}	0.63±0.03^{d}	0.42±0.00^{d}	3.20±0.06^{d}
NF2	90.12±0.12^{a}	0.16±0.00^{c}	0.78±0.03^{c}	0.77±0.05^{c}	4.22±0.05^{c}
NF4	90.12±0.07^{a}	0.21±0.00^{b}	0.96±0.02^{b}	0.86±0.02^{b}	4.68±0.05^{b}
NF6	90.02±0.25^{a}	0.23±0.00^{a}	1.18±0.02^{a}	1.02±0.02^{a}	5.03±0.08^{a}

*DM – dry matter

$^{a\text{-}d}$Means in a column without a common letter differ significantly ($p < 0.05$).

The study found that increasing the proportion of nettle flour (2, 4, and 6%) did not affect the dry matter content of the resulting bread samples. However, the total flavonoid content increased with higher levels of nettle flour, with the most pronounced increase observed in the sample containing 6% nettle flour, where the quercetin equivalent (QE) content was nearly double that of the control. According to Fattahi et al. (2014), stinging nettle is rich in polyphenols and flavonoids and exhibits strong antioxidant activity, contributing to various protective and disease-preventing properties.

The total phenols also increased and the difference between the control and the sample with 6% nettle flour was 0.55 mg, which is 87.30%. Hudec et al. (2007) studied total phenolic content of different nettle parts (root, stalk, and leaves). It was found that the result for nettle leaves was 7.62 mg GAE/g DM. The DPPH radical scavenging and iron-reducing antioxidant capacity increased with increasing of nettle flour. According to the DPPH method, bread with 6% nettle flour showed 2.4 times higher radical scavenging activity, and the iron-reducing ability increased by 1.6 times.

Others studies also demonstrated that the antioxidant activity of wheat bread and bread enriched with nettle flour was higher in the total phenols in nettle flour bread were 0.3 mg more than in wheat bread, which is 36.14%. Lutein in wheat bread is 2.51 mg less than in nettle flour bread, and beta-carotene was 0.31 mg more in nettle flour bread. Maietti et al. (2021) demonstrated that the amount of total phenolic compounds increased from 372 μg GAE/g of fresh weight (FW) in wheat bread to 597 μg GAE/g FW in bread enriched with nettle flour. Furthermore, antioxidant activity was also higher in enriched bread compared to wheat bread (0.83 vs. 0.53 mg TE/g FW, respectively). Đurović et al. (2020) also found that bread prepared with nettle leaf flour showed significant antioxidant activity against the DPPH radical.

In summary, it can be concluded that increasing the amount of nettle flour enhances the antioxidant activity of bread. The results indicate that the NF6 sample exhibits the highest antioxidant levels compared to the control, demonstrating that higher additions of nettle flour significantly increase the content of antioxidant compounds. According to Joshi et al. (2014), stinging nettle (*Urtica dioica*) leaves are rich in phytoconstituents, including polyphenols, flavonoids (kaempferol, isorhamnetin, quercetin, isoquercitrin, and rutin), phenolic acids (caffeic acid and chlorogenic acid), and carotenoids (β-carotene, hydroxyl-β-carotene, luteoxanthin, lutein epoxide, and violaxanthin), as well as essential oils, fatty acids, minerals, and vitamins.

Conclusions

The study found that increasing the amount of nettle (*Urtica dioica*) flour did not affect dough development time, while dough stability increased and dough softening decreased. Incorporating 2% nettle flour resulted in the best bread volume and specific volume compared to the other enriched samples. Sensory analysis showed that higher levels of nettle flour negatively affected the bread's appearance, porosity, and taste. The sample with 2% nettle flour had sensory properties closest to the control. Overall, the study demonstrated that partially replacing wheat flour with nettle flour is an effective strategy to enhance the bread's bioactive potential and boost its antioxidant activity.

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