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Investigation of the Applicability of Stinging Nettle (*Urtica dioica* L.) for the Production of Functional Bakery Products

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ABSTRACT

The demand for health-promoting foods with exceptional nutritional and biological qualities is increasing. Medicinal herbs are rich in antioxidants and polyphenols and often possess favourable organoleptic properties. Therefore, this study aimed to develop a novel functional bakery product enriched with stinging nettle (*Urtica dioica* L.). The functional food consisted of crackers supplemented with nettle leaves and nettle seeds. The objectives were to demonstrate that nettle leaves and seeds are excellent sources of bioactive compounds and that these compounds are retained during food production, and to evaluate consumer acceptance of nettle-enriched crackers through organoleptic testing. The results showed that dried nettle leaves had significantly higher values than nettle seeds for both parameters tested (TAC: 108.1 ± 7.7 mg AAE/g; TPC: 46.6 ± 5.2 mg GAE/g). Among the cracker samples, the baked crackers enriched with nettle seeds exhibited the highest polyphenol content (7.7 ± 0.4 mg GAE/g) and antioxidant content (17.4 ± 1.9 mg AAE/g). These samples were also the most preferred by consumers. From both organoleptic and nutritional perspectives, nettle seeds proved to be the superior choice for enhancing crackers.
Keywords: stinging nettle, functional food, antioxidants, spectrophotometry, consumer acceptance

1. INTRODUCTION

Stinging nettle (*Urtica dioica* L.) is a wild-growing medicinal herb commonly found in temperate regions. It is native to Europe, Asia, North America, and North Africa (Đurović et al., 2020; Kutlu et al., 2020). Although it originated in Eurasia, it is now widely distributed across Europe (especially Northern Europe), northern Africa, Asia, and North and South America (Said et al., 2015).

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The above-ground parts of nettle contain 2.5-3.6 % crude fat, 18-34 % crude protein, 9 % crude fibre, 16 % total ash, and 37 % carbohydrates on a dry matter basis (Tarasevičienė et al., 2023).
In addition, nettle is a rich source of bioactive compounds, including various phenolic compounds (mainly the flavonoids quercetin, kaempferol, and rutin), vitamins (B-group, K, and C), minerals (especially iron), essential amino acids, terpenoids (the main components of nettle essential oil), fatty acids (palmitic, linoleic, and linolenic acids), tannins, carotenoids (such as β-carotene, lutein, and lycopene), chlorophyll, sterols, and isolectins (Đurović et al., 2017; Đurović et al., 2022; Zeković et al., 2017).
Previous studies have shown that the chemical composition, nutritional value, and bioactive properties of nettles and their extracts vary depending on several factors, including plant part used, geographic origin, harvest time, collection site, and extraction method (Devkota et al., 2022; Opačić et al., 2022). Due to its excellent biological properties, nettle has been incorporated into various formulations to develop functional foods with health-promoting effects. Despite the considerable market potential of nettle-enriched foods, most such products remain at the laboratory scale rather than reaching large-scale industrial production. This is mainly attributed to inadequate cultivation and post-harvest processing methods for the plant (Jeannin et al., 2020).
Numerous studies have investigated the incorporation of various plants and plant parts into baked goods, such as cakes and cookies, to enhance their nutritional value and health-promoting properties—an approach that is increasingly demanded by modern consumers (Mitrović et al., 2022). For example, Đurović et al. (2020) reported a significant increase in phenolic content and antioxidant activity in bread when enriched with freeze-dried nettle leaves.
The present study aimed to develop crackers enriched with nettle leaves and nettle seeds and to confirm the positive physiological effects of this enrichment through analytical measurements. In addition, organoleptic (sensory) evaluation was performed to determine whether nettle enrichment improves consumer perception and acceptance of the crackers.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

The seeds and leaves of wild-grown stinging nettle (*Urtica dioica* L.) were collected in early autumn in Hegyeshalom the plain in Northwestern Hungary with coordinates N47°55’28.4”, E17°11’49.1” Latitude 47.924564, Longitude 17.196272. Both the leaves and stems are covered with erect, bristly, glandular hairs containing acetylcholine, formic acid, 5-hydroxytryptamine, and histamine. Therefore, the fresh green plant can cause skin irritation if touched without protective gloves (Asgarpanah & Mohajerani, 2012).
The leaves and seeds were separated from the stems, and the cleaned plant parts were dried in a drying oven at 40 °C until constant weight was reached. The dry matter content of the nettle samples was determined using a gravimetric method. For further analysis, the dried seeds and leaves were ground to a particle size of < 1 mm using a kitchen grinder (Sencor, SCG 2050RD, Budapest, Hungary).

2.2 Chemicals

The chemicals used for the determination of total polyphenol and total antioxidant content were: anhydrous sodium carbonate (99 %, Riedel-de Haën, Seelze, Germany), Folin-Ciocalteu reagent (98 %, Sigma-Aldrich, Budapest, Hungary), 2,4,6-tripyridyl-s-triazine (TPTZ)

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(98 %, Sigma-Aldrich, Budapest, Hungary), acetic acid (96 %, Reanal, Budapest, Hungary), anhydrous iron(III) chloride (98 %, Merck, Budapest, Hungary), ascorbic acid (96 %, Sigma-Aldrich, Budapest, Hungary), and gallic acid (98 %, Sigma-Aldrich, Budapest, Hungary).

2.3 Cracker baking process

The ingredients and their quantities used to prepare the control, nettle seed-enriched, and nettle leaf-enriched crackers are listed in *Table 1*. The total flour base was 250 g (125 g white flour + 125 g whole flour), and the dough was prepared with oil and water as liquid ingredients.

Table 1: Ingredients used to make crackers

Cracker type	Ingredients [g]						
	White flour	Whole flour	Baking soda	Water	Salt	Seed	Leaf
Control	125	125	1.92	120	4	None	None
Enriched with nettle seeds	125	125	1.92	120	4	25	None
Enriched with nettle leaves	125	125	1.92	120	4	None	25

Note: The amounts of oil (40 g) and water (120 g) were selected to achieve a homogeneous, kneadable dough consistency suitable for rolling to 0.5 cm thickness. These quantities are based on standard cracker dough formulations for a 250 g flour base.

The amount of the ingredients needed to make the crackers was weighed using a tare balance (GM series, GMB5002, Budapest, Hungary). To prepare the control cracker, the dry ingredients (flours, baking soda, and salt) were first placed in the mixing bowl. The oil was then poured into the dry mixture and mixed with a few quick movements. Water was gradually added while kneading until the dough reached the appropriate consistency and became homogeneous. For the nettle-enriched doughs, the previously dried and ground nettle seeds or leaves were added together with the dry ingredients. Samples of the prepared raw dough were taken for further analysis. The dough was rolled out on a floured surface to a thickness of 0.5 cm and then cut into 2 × 2 cm squares. The shaped raw dough pieces were placed on a baking tray lined with baking paper and baked in a preheated oven at 180 °C for 15 minutes.

2.4 Sample preparation for the determination of total antioxidant (TAC) and polyphenol (TPC) content

For the determination of total antioxidant capacity and total polyphenol content, 0.5 g of dried and ground nettle leaves or seeds was weighed into 250 mL Erlenmeyer flasks using an analytical balance (Sartorius, TE214SE, Budakeszi, Hungary). Then, 100 mL of the extractant, consisting of methanol and high-purity water (80:20, v/v), was added.

For the cracker samples, 1 g of raw dough or baked cracker was weighed into 100 mL Erlenmeyer flasks using an analytical balance, and 20 mL of the same methanol: high-purity water mixture (80:20, v/v) was added. The samples were extracted in a shaking thermostat (Bartelt, 3032, Graz, Austria) for 2 hours at 50 °C. After extraction, the mixtures were transferred into centrifuge tubes

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and centrifuged (Hermle, Z 206 A, Wehingen, Germany) at 6000 rpm for 20 minutes. The supernatant was collected for further analysis.

2.5 Determination of total antioxidant content by FRAP assay

The FRAP assay was performed according to the method described by Benzie and Strain (1996). Briefly, 200 µL of supernatant and 3 mL of FRAP reagent were pipetted into a test tube. The mixtures were kept in the dark for 5 min, after which the absorbance was measured at 593 nm against the blank using a Spectroquant Pharo 100 spectrophotometer (Merck, Darmstadt, Germany). Ascorbic acid (40-500 mg/L) was used as the standard, and the results were expressed as milligrams of ascorbic acid equivalents (mg AAE) per gram of dry matter.

2.6 Determination of total polyphenol content by Folin-Ciocalteu assay

Total polyphenol content was determined according to the Folin-Ciocalteu method described by Singleton et al. (1999). Briefly, 200 µL of supernatant was mixed with 1.5 mL of high-purity water. Then, 2.5 mL of 10 % Folin-Ciocalteu reagent was added, followed by 2 mL of 7.5 % Na_2CO_3 solution. The mixtures were kept in the dark for 90 min, after which the absorbance was measured at 725 nm against the blank using a spectrophotometer. Gallic acid (25-1000 mg/L) was used as the standard, and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry matter.

2.7 Consumer acceptance of cracker samples

Consumer acceptance of the cracker samples was evaluated by a panel of 15 untrained panellists (employees and students of the Albert Kázmér Faculty of Agricultural and Food Sciences at Széchenyi István University, Mosonmagyaróvár). The panellists rated the samples on a 5-point hedonic scale (5 = like very much, 1 = dislike very much, 3 = neither like nor dislike). The evaluated sensory attributes were appearance, odour, taste, texture, and overall acceptability.
All three cracker samples (control, nettle seed-enriched, and nettle leaf-enriched) were presented simultaneously to the panellists to allow direct comparison. Evaluations were carried out using a structured questionnaire. The results are presented as mean scores ± standard deviation.
The study was conducted in accordance with ethical guidelines. Informed consent was obtained from all panellists prior to their participation, and appropriate measures were taken to protect their rights and privacy.

2.8 Data analysis

All experimental results for stinging nettle seeds, leaves, and cracker samples were processed using Microsoft Office Excel. The results are expressed as mean values ± standard deviation (n = 3). Calibration curves for the spectrophotometric methods were prepared using second-order polynomial regression (nonlinear least-squares method). The absorbance values of the sample extracts were interpolated from these curves to calculate the concentrations.
One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine significant differences between means. Differences were considered statistically significant at $p < 0.05$.

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3. RESULTS AND DISCUSSION

3.1 Total antioxidant (TAC) and polyphenol (TPC) content of stinging nettle seed and leaf samples

The dry matter content of nettle leaves was 21.2 ± 1.4 % (m/m), while that of nettle seeds was 72.6 ± 2.1 % (m/m). The TAC and TPC values of the nettle leaf and nettle seed samples are presented in *Table 2*.

Table 2: Total antioxidant (TAC) and polyphenol (TPC) content of stinging nettle leaf and seed samples (n = 3), different letters (a and b) denote significant differences (p ≤ 0.05) between different nettle parts

Sample	TAC mg AAE/g	TPC mg GAE/g
Nettle leaf	108.1 ± 7.7[a]	46.6 ± 5.2[a]
Nettle seed	45.7 ± 0.5[b]	23.4 ± 1.2[b]

Based on the results, the total antioxidant capacity of nettle leaves was approximately 2.4 times higher than that of nettle seeds. Albayrak et al. (2011) reported a total antioxidant content of 132.7 ± 2.1 mg AAE/g for nettle using methanol extraction, which is close to the value obtained for nettle leaves in the present study.

In terms of total polyphenol content, nettle leaves also showed markedly higher values, approximately twice that of nettle seeds. Consequently, nettle leaves contain substantially higher amounts of bioactive compounds and may exert more favourable physiological effects than nettle seeds.

Joshi et al. (2015) found that the total phenolic content of various *Urtica dioica* L. extracts was highest in the ethyl acetate fraction (13.06 ± 0.15 mg GAE/g). This value is considerably lower than the results obtained in the present study for both nettle leaves and seeds. Elez Garofulić et al. (2021) reported that the total polyphenol content of nettle leaves ranged from 3.56 ± 0.15 to 23.69 ± 0.30 mg GAE/g depending on the extraction conditions applied. These literature values are in agreement with the phenolic content determined for nettle leaves in this work.

Overall, the results and supporting literature data confirm that Urtica *dioica* L. is a rich source of phenolic compounds and possesses strong antioxidant properties (Kataki et al., 2012).

3.2 Total antioxidant (TAC), and polyphenol(TPC) content of stinging nettle enriched cracker samples

Based on the results presented in *Table 3*, both nettle leaf- and nettle seed-enriched crackers showed higher antioxidant content compared to the control cracker. These findings confirm that *Urtica dioica* L. is suitable for increasing the antioxidant capacity of bakery products.

The baked crackers exhibited significantly higher TAC and TPC values than the corresponding raw doughs. This increase is most likely due to concentration effects caused by moisture loss during baking. The short baking time (15 min at 180 °C) did not appear to cause substantial degradation of the heat-sensitive bioactive compounds.

Interestingly, in both the raw dough and baked cracker samples, the nettle seed-enriched variants consistently showed higher antioxidant and polyphenol contents than the nettle leaf-enriched samples, despite the lower values observed in the pure dried nettle seeds. The highest values were recorded in the baked seed-enriched crackers (TAC: 17.4 ± 1.9 mg AAE/g; TPC: 7.7 ± 0.4 mg GAE/g).

These results are consistent with those of Mitrović et al. (2022), who observed that adding nettle seed flour or nettle seed phenolic extracts to cookies increased the DPPH radical-scavenging capacity and

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reducing power of both free and bound phenolic compounds after heat treatment. Similarly, previous studies have demonstrated that incorporating nettle leaves or extracts into baked goods improves their technological, nutritional, and functional properties (Đurović et al., 2020; Rutakhli et al., 2019). Heat treatment has also been reported to increase the levels of phenolic compounds, such as phenolic acids, tannins, and flavonoids, in various food matrices (Alide et al., 2020; Călinoiu & Vodnar, 2019).

Table 3: The total antioxidant (TAC) and total polyphenol (TPC) content of the nettle-enriched cracker samples, as well as the raw cracker doughs and baked cracker samples (n = 3), different letters (a, b,c, d, e and f) denote significant differences ($p \leq 0.05$)

Cracker sample	TAC mg AAE/g	TPC mg GAE/g
Control raw dough	0.3 ± 0.02^{a}	1.7 ± 0.3^{a}
Raw dough enriched with nettle leaves	2.3 ± 0.2^{b}	3.1 ± 0.4^{b}
Raw dough enriched with nettle seeds	8.2 ± 0.5^{c}	4.6 ± 0.4^{c}
Control cracker	5.4 ± 0.3^{d}	2.5 ± 0.2^{d}
Cracker enriched with nettle leaves	9.4 ± 0.2^{e}	5.0 ± 0.4^{c}
Cracker enriched with nettle seeds	17.4 ± 1.9^{f}	7.7 ± 0.4^{e}

In the polyphenol content analysis, the nettle seed-enriched samples also exhibited higher values compared to the nettle leaf-enriched crackers. The highest polyphenol content was found in the baked crackers enriched with nettle seeds (7.7 ± 0.4 mg GAE/g). Among the nettle-enriched samples, the raw dough containing nettle leaves showed the lowest polyphenol content (3.1 ± 0.4 mg GAE/g). These findings align with previous research demonstrating that increasing the proportion of plant-based ingredients can enhance the polyphenol content of baked products. For example, Drabińska et al. (2018) reported that the addition of broccoli leaves to gluten-free mini sponge cakes resulted in a proportional increase in polyphenol content as the amount of broccoli leaves increased.

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3.3 Consumer acceptance test results

Figure 1 shows the mean ratings of 15 panellists for the five sensory attributes (appearance, odour, taste, texture, and overall acceptability) of the three cracker samples (control, nettle seed-enriched, and nettle leaf-enriched).

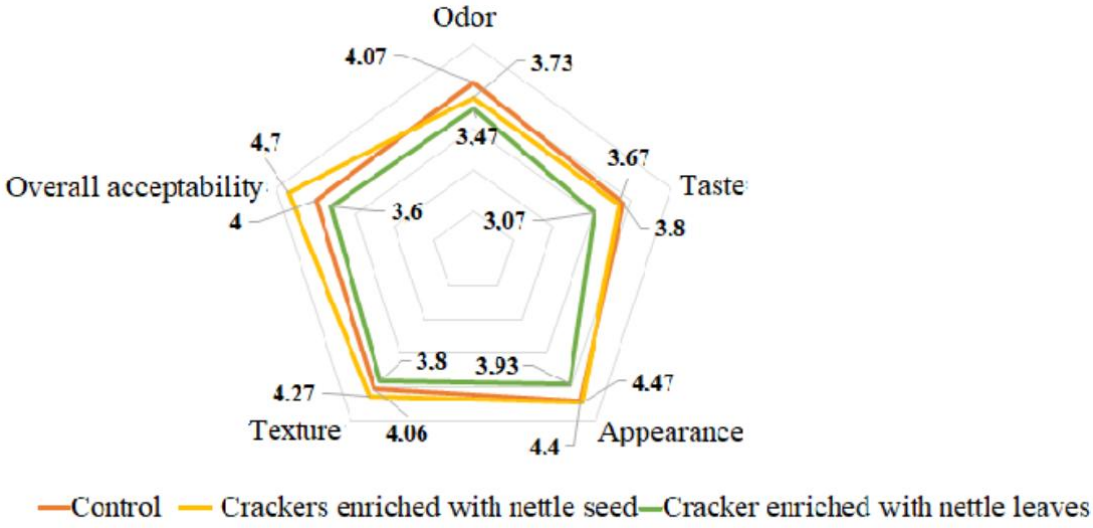

Figure 1. Consumer perception of nettle-enriched crackers

Based on the sensory evaluation, the nettle leaf-enriched cracker received the lowest scores for all attributes. The nettle seed-enriched cracker achieved the highest overall acceptability (4.7). None of the nettle-enriched samples matched the control cracker in odour (control: 4.1). For taste, the control sample was rated highest (3.8), followed closely by the nettle seed-enriched cracker (3.7).
In terms of appearance, the nettle leaf-enriched cracker received the lowest rating, while the difference between the control and nettle seed-enriched crackers was minimal; the latter scored highest (4.5). For texture, the nettle seed-enriched cracker received the highest score (4.3), followed by the control (4.1) and the nettle leaf-enriched cracker (3.8).
Overall, the nettle seed-enriched cracker was the most liked among the two enriched samples. These findings are consistent with those of Chochkov et al. (2025), who reported that control bread samples received higher taste scores than nettle-enriched bread samples. Similarly, Rădulescu et al. (2024) noted that adding nettle powder to bread dough imparts a distinctive taste and texture.

4. CONCLUSION

Higher levels of bioactive components were observed in the nettle-enriched crackers after baking, despite the typically expected baking losses, due to the heat sensitivity of these compounds. The results clearly demonstrate that nettle-enriched crackers have a more beneficial nutritional profile, owing to their higher antioxidant and polyphenol levels.
Nettle seeds proved to be a superior choice compared to nettle leaves for the supplementation of bakery products in terms of both antioxidant and polyphenol content. Overall, the spectrophotometric analyses confirm that both stinging nettle seeds and leaves are suitable ingredients for increasing the antioxidant and polyphenol values of bakery products, thereby potentially enhancing their health-promoting effects. Furthermore, sensory evaluation showed that the nettle seed-enriched sample also received the highest consumer acceptance.

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Nagy csalán (*Urtica dioica* L.) alkalmazhatóságának vizsgálata funkcionális sütőipari termék előállítására

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ÖSSZEFOGLALÁS

Manapság egyre nagyobb az igény az egészség megőrzését elősegítő, kivételes tápértékű és biológiai tulajdonságokkal rendelkező élelmiszerek iránt. A gyógynövények antioxidánsokban és polifenolokban gazdagok, valamint kedvező érzékszervi tulajdonságokkal rendelkeznek. Ezért a tanulmány célja egy csalánnal (*Urtica dioica* L.) dúsított, új funkcionális sütőipari termék kifejlesztése volt. A funkcionális élelmiszer alapja kréker volt, amelyet csalánlevéllel és csalánmaggal egészítettek ki. A célkitűzések között szerepelt annak bizonyítása, hogy a csalánlevél és a csalánmag kiváló bioaktív összetevők forrása, valamint hogy ezek az összetevők az élelmiszer-előállítás során is megmaradnak a termékekben. Emellett a csalánnal dúsított krékerek fogyasztói elfogadottságát érzékszervi bírálattal is vizsgálták.

Az eredmények szerint a szárított csalánlevél mindkét vizsgált paraméterben jelentősen magasabb értékeket mutatott, mint a csalánmag (TAC: 108,1 ± 7,7 mg AAE/g; TPC: 46,6 ± 5,2 mg GAE/g). A krékerminták összehasonlításakor a csalánmaggal dúsított sült krékerek rendelkeztek a legmagasabb polifenol-tartalommal (7,7 ± 0,4 mg GAE/g) és antioxidáns-tartalommal (17,4 ± 1,9 mg AAE/g), valamint ezek bizonyultak a fogyasztók körében a legkedveltebbeknek. Érzékszervi és táplálkozási szempontból a csalánmag a legjobb választás a krékerek dúsítására.

Kulcsszavak: nagy csalán, funkcionális élelmiszer, antioxidánsok, spektrofotometria, fogyasztói elfogadottság

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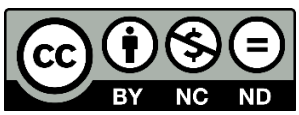

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