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Sensory and chemical profiles of bread derived from the novel semi- and wholegrain flour enrichment of *Triticum aestivum* L. old genotypes with organic stinging nettle

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Abstract

Background: Bread made from refined *Triticum aestivum* L. flour, a staple food in large parts of the world, is considered a nutrient security risk. Strategies used to enhance the nutritional and functional properties of wheat-derived food products include the use of semi- or wholegrain flour, old genotypes, and enrichment through fortification. Limited studies exist on biofortifying staples with neglected and underutilized species. The innovative approach of the present study was to combine all aforementioned strategies, which involved the enrichment of semi-wholegrain and wholegrain bakery products derived from an organically cultivated mix of five *T. aestivum* soft wheat landraces (Andriolo and Gentil Rosso) and old varieties (Frassineto, Inallettabile, and Verna) with *Urtica dioica* L. (stinging nettle [SN]), also cultivated under sustainable management.

Results: The organoleptic assessment showed that the enrichment of semi-wholegrain and wholegrain traditional Italian bakery products with 3% SN was appreciated for the first time by free tasters representing the "health conscious" sector of the population. Moreover, the functional component content (fiber, polyphenols, flavonoids, and antiradical activity) significantly exceeded that reported previously for refined flour.

Conclusion: Consumer acceptance of SN-enriched wholegrain bakery products shows potential to meet the increased demand for fortified products, with the raw ingredients suited for cultivation under environmentally sustainable management practices.

KEYWORDS
old wheat genotypes, polyphenols, sensory panel, stinging nettle enrichment, *Urtica dioica* L., wholegrain

INTRODUCTION

Ensuring global food security while protecting the environment and biodiversity is the single greatest scientific challenge facing humankind.[1] The term "food security" was originally coined to describe malnutrition, with the emphasis on averting hunger or lack of nutrition in terms of quantity.[2] However, malnutrition, (synonymous with bad nutrition) is increasingly manifested by the intake of foods with insufficient nutrient content or by the over-consumption of energy-dense foods, necessitating a change in the perception of food security to incorporate food quality and environmental factors,[2] as reflected by the Intergovernmental Platform for Climate Change statement: "Finding the right balance between food and nutritional security, protecting the environment, and addressing climate change remain major challenges for sustainable food systems."[3] "Nutrition security," encompassing food security, was more recently proposed as a more fitting phrase,[2] with the world's staple foods coming under increasing scrutiny for nutrition security. Wheat is the second most important staple crop for 35% of the global population.[4] Approximately 90%–95% of wheat production worldwide is comprised of common or bread wheat (*Triticum aestivum* L.),[5] with bread representing the staple food in the Middle East, Central Asia, North Africa, Europe, the United States, Australia, and Southern Africa. Traditionally, refined wheat flour has been the standard raw material for "white wheat bread" production associated with a significant reduction in the nutritional density, fiber content, and bioactive (functional) components.[6,7]

As a strategy, food fortification is reputed to be a sustainable, scientifically proven, and cost-effective global intervention to malnutrition.[8] Enrichment through fortification is defined as the process of the deliberate addition of nutrients or non-nutrient bioactive components to food products.[9] Clinical trials have shown health benefits from consuming bread fortified with vitamins, minerals, fiber, proteins, and polyphenolic compounds.[9] From the Functional Food Ingredients Global Market Report of 2023,[10] there has been a major increase in the demand for fortified foods and beverages, attributable to the surge in health consciousness and an increasing occurrence of diseases.

Staple food fortification with neglected and underutilized species (NUS) represents a robust and economic-friendly strategy for nutritional security.[11] NUS are cultivated, semidomesticated, or wild plant species, largely "neglected" by agricultural researchers, plant breeders, and policymakers, and hence "underutilized" in larger agricultural production systems.[12] Aside from exceptional nutritional properties, bioactive potential, and proven health benefits with immense potential to contribute to nutrition security.[13] NUS are also suited to sustainable agriculture and marginal areas, being resilient, typically adapted to local conditions, ensuring more stable yield, requiring fewer external inputs than conventional crops, and supporting biodiversity protection (local pollinators, pest, and disease control).[11,12,14,15]

To date, overall research on the NUS *Urtica dioica* L. or stinging nettle (SN) biofortification of wheat products is relatively scarce and has been performed exclusively using highly refined commercial flour.[16–21] Only two of the above-mentioned articles included analyses on bioactive polyphenol content and antioxidant activity.[18,20] In Italy, the NUS, *U. dioica* L. or SN has been widely used since ancient times as an ingredient in foods.[20] Although SN is an ingredient of interest, consumer appreciation of SN-fortified bread in Italy has yet to be demonstrated.

Aside from fortification with SN as a strategy to enhance the nutritional and functional potential of bread wheat, the selection of suitable raw wheat material is also important. Improvements in the functional properties of bioactive components in old (released before 1950, or the advent of the "Green Revolution") compared to modern wheat genotypes have been verified from in vitro, as well as in vivo animal and human experimental trials.[22] In addition, the consumption of wholegrain wheats products over that of refined products is widely reported in enhancing the nutritional and functional properties of wheat-derived food products. Within the context of sustainable agriculture, the utilization of landraces (domesticated, traditional, regional ecotypes, locally adapted to their respective natural and cultural agricultural environments) and old genotypes (as genetic resources have been shown to provide greater resilience and yield stability under unpredictable weather).[22–24] Landraces and old genotypes are typically cultivated under sustainable agricultural management strategies (agroecology/organic) to improve the agricultural carbon footprint, soil fertility, and biodiversity with knock-on benefits on pest/pathogen and disease incidence.[25–28]

The innovative aspect of the present investigation is the utilization of a functional bread, prepared from a mix ("Virgo") of five Italian *T. aestivum* soft wheat landraces (Andriolo, Gentil, and Rosso) and old varieties (Frassineto, Inallettabile, and Verna) cultivated under sustainable agriculture as the basis, with enrichment performed using the NUS, *U. dioica* L. (also cultivated under sustainable agriculture). The objective was to investigate the potential to enrich semi-wholegrain and wholegrain flour derived from old varieties and to ascertain organoleptic appreciation by free tasters representing the "health conscious" sector of the population. The working premise is that supplementation with SN could further enhance the chemical profiles to exceed levels previously reported.

MATERIALS AND METHODS

Plant material

The *T. aestivum* flour was obtained from five old genotypes that were cultivated in mixtures. The genotypes were Andriolo, Gentil Rosso, Verna, Frassineto, and Inallettabile. Cultivation was carried out on source farms, certified for organic farming and incorporating biodynamic principles, as part of the Virgo project aimed at introducing agrobiodiversity in the Emilia Romagna region.[29]

Artisan-based, stone-ground flour was used for the enrichment with SN. In the present study, soft wheat Type 1 brown flour was used with a maximum ash content of 0.80% dry matter.[30] Wholegrain soft wheat flour was also used as a basis, with a minimum and maximum ash content of 1.3% and 1.7%, respectively.[30]

Seed material of a commercial variety of *U. dioica* L. (for the harvest of bioactive compounds) was purchased from Fluxias GmbH (Baden-Württemberg, Germany) and cultivated on an organic farm at Lizzano in Belvedere (latitude 44.214722, longitude 10.863889) in Emilia Romagna, Italy. From the experimental trial reported in Marotti et al.,[31] leaf material from Lizzano was selected based on the significantly higher biomass, polyphenol content, and antiradical activity. Leaf material was blended into fine particles (1–2 mm) for addition to the soft wheat Type 1 and wholegrain soft wheat flour, respectively.

Bread making

The bakery products were prepared to reflect typical products consumed in the region of Emilia Romagna, and included Bolognese Focaccia bread ("Crescenta"), farmhouse loaf ("Pagnotta"), and savory crackers ("Streghe Bolognese"), respectively. The six bakery combinations with and without SN were as follows: BCT (Brown flour [Type 1]

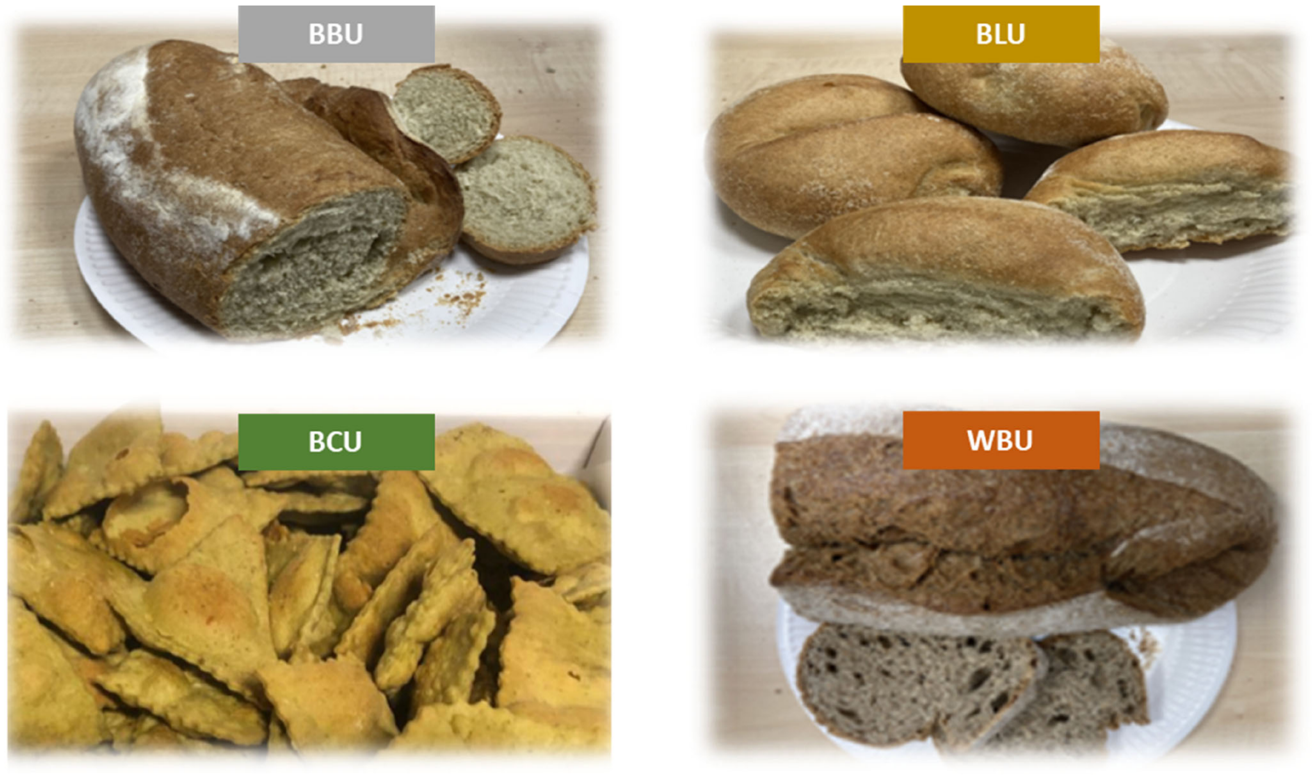

FIGURE 1 A visual representation of the stinging nettle-enriched bakery products. The codes for the bakery products: BBU (Brown flour [Type 1] focaccia Bread with *Urtica*), BLU (Brown flour farmhouse Loaf with *Urtica*), BCU (Brown flour savory Crackers with *Urtica*), and WBU (wholegrain focaccia Bread with *Urtica*).

focaccia Control bread), BBU (Brown flour focaccia Bread with *Urtica*), BLU (Brown flour farmhouse Loaf with *Urtica*), BCU (Brown flour savory Crackers with *Urtica*), WCT (wholegrain focaccia Control bread), and WBU (wholegrain focaccia Bread with *Urtica*). A visual representation of the SN-enriched bakery products is given in Figure 1.

The bread types and crackers were prepared by Associazione Panificatori Bologna (APB). The ratio of ingredients used in the preparation of the Bolognese focaccia and farmhouse loaf was based on that of Maietti et al.,[27] and included flour (1 kg), water (500 mL), Baker's yeast (30 g), extra-virgin olive oil (40 g) and salt (20 g). Sugar (5 g) was also included. Nettle-enriched bread was obtained by adding 3% (30 g per kg) dried nettle leaves (particle size 1–2 mm) to the ingredients during the kneading step. The kneaded breads were left to rise for a period of 3 h in fermentation cells set at 28–30°C. Thereafter, the breads were initially baked at 250°C for a short duration of 5 min, after which the temperature was reduced to 220°C until completion for a total time of 20 min.

The savory crackers were obtained by mixing flour (1 kg), yeast (25 g), salt (24 g), margarine (100 g), and water (500 mL) to form a dough. The nettle-enriched products were, similarly, obtained by adding 3% (30 g for each kg) of dried nettle leaves (particle size 1–2 mm) to the ingredients during the kneading step. The dough was allowed to ferment for 16 h at 4°C. After fermentation, the dough was laminated in order to obtain the same thickness over the entire length, and extra-virgin oil was placed on each cracker. The crackers were baked at 180°C for 12 min.

The bakery products were used for the panel tests designed to evaluate the sensory parameters. Portions of the breads and crackers were dried and then ground to a fine powder for the analysis of the chemical profile.

Sensory analysis

A panel of 13 assessors (7 females and 6 males aged between 27 and 55 years) was selected. These individuals were screened and recruited from the University of Bologna (in food-related disciplines) to represent the "health conscious" sector of the population. The subjects received written information about the test. All the participants provided their informed consent to participate in the study. The study was exempt from an ethical committee review.

A generic sensory descriptive analysis was used to develop the lexicon and methodology for the evaluation of the bakery products.[32] Each panelist completed 12 training sessions (3 h for each session). Overall, the training session included aspects such as concept alignment and agreement, lexicon development (terms and their definitions, references, and use of scale), sample handling, practicing, and product evaluation (quantifying the intensity of attributes). A total of seven attributes (Table 1) were defined and agreed upon by the panel. The attributes were appearance, consistency, smell, taste, flavor, long-lasting taste, and total enjoyment, respectively.

TABLE 1 Sensory parameters of brown flour (Type 1) bread enriched with 10% stinging nettle (SN) compared to non-enriched bread. Comparison of sensory parameters for different brown flour and wholegrain bakery products, each enriched with 3% SN, together with the brown and wholegrain non-enriched flour breads. All results are the mean and standard deviation of four replicate samples.

Code	Appearance	Consistency	Smell	Taste	Taste fragrance	Long-lasting taste	Total enjoyment
Control brown flour bread plus 10% *Urtica*							
BBU	76 ± 2.0^{c}	66 ± 1.7^{c}	67 ± 2.0^{bc}	58 ± 3.0^{d}	85 ± 1.0^{a}	95 ± 3.6^{a}	50 ± 3.6^{f}
Control brown flour bread							
BCT	78 ± 2.0^{c}	67 ± 0.9^{c}	67 ± 2.3^{bc}	60 ± 2.7^{cd}	47 ± 1.0^{c}	47 ± 1.8^{c}	64 ± 1.0^{d}
Brown flour bakery products plus 3% *Urtica*							
BBU	88 ± 2.7^{a}	74 ± 2.0^{ab}	65 ± 2.0^{a}	64 ± 2.0^{bc}	62 ± 2.0^{c}	48 ± 1.7^{c}	74 ± 1.3^{b}
BLU	80 ± 1.0^{bc}	73 ± 1.0^{b}	73 ± 1.0^{b}	67 ± 2.0^{b}	64 ± 1.0^{b}	62 ± 2.0^{b}	69 ± 1.0^{c}
BCU	83 ± 1.0^{b}	56 ± 1.8^{d}	62 ± 1.0^{d}	76 ± 1.0^{a}	66 ± 1.0^{b}	43 ± 2.0^{d}	60 ± 1.0^{e}
Control wholegrain flour bread and with 3% *Urtica*							
WCT	78 ± 2.0^{c}	75 ± 1.0^{ab}	70 ± 2.0^{b}	65 ± 2.0^{bc}	62 ± 1.0^{b}	58 ± 2.0^{b}	73 ± 1.0^{b}
WNU	83 ± 1.0^{b}	77 ± 2.7^{a}	68 ± 2.0^{bc}	61 ± 1.7^{cd}	66 ± 2.0^{b}	61 ± 1.7^{b}	78 ± 2.3^{a}
p value	***	***	***	***	***	***	***

Note: The codes for the bakery products: BCT (Brown flour [Type 1] focaccia Control bread), BBU (Brown flour focaccia Bread with Urtica), BLU (Brown flour farmhouse Loaf with Urtica), BCU (Brown flour savory Crackers with Urtica), WCT (wholegrain focaccia Control bread), and WBU (wholegrain focaccia Bread with Urtica). ***variable is significant at $p < 0.001$ by one-way ANOVA (Analysis of Variance) test. Different letters within each column denote significantly different values at $p \leq 0.05$ from Tukey's post hoc test.

Appearance was graded based on visual appreciation for the product, whereas consistency was assessed according to how much the product crumbled in the mouth. A low degree of consistency was indicative of a product that formed a doughy lump in the mouth, while a high degree of consistency was indicative of a product that crumbled in the mouth. Smell and taste, respectively, referred to the intensity while holding the product to the nose and the intensity during chewing. Flavor was the combination of the smell and taste intensity. The long-lasting taste was defined as flavor persistence, whereas total enjoyment was the overall appreciation of the product. These were evaluated using an unstructured scale of 100 mm anchored at their extremes (0: extremely weak, 100: extremely strong). All bakery products, labeled with a three-digit random number, were presented simultaneously and evaluated in random order among panelists. Water was provided to cleanse the palate between samples during tasting. A 2-min break was allowed between one sample and the next.

The organoleptic properties were evaluated by a panel of 13 tasters, recruited from individuals from the university in food-related disciplines to represent a "health conscious" sector of the population. Initially, the BBU was prepared using 10% SN. The 10% SN was not considered to be suitable from a sensory perspective, and subsequent analyses were performed using 3% SN in the BBU, BLU, BCU, and WBU products. Each panelist was provided with descriptive test analysis sheets in which a mark had to be made on a percentage scale, ranking appearance, smell, taste, consistency (texture), long-lasting taste, and flavor persistence for each product. For the preference or overall enjoyment, each panelist was also asked to rank the products in descending order on the basis of preference (liking) that encompassed all sensory parameters.

Chemical analysis

For the chemical profile, protein, starch, glucose, dietary fiber, polyphenols, flavonoids, and antiradical activity were measured, respectively, and expressed on a dry mass (DM) basis. Four replicates were made for each variable analyzed.

Proteins were extracted according to a modified TCA (trichloroacetic acid)/acetone precipitation method used for proteomic analysis and measured using the Bradford method as described in Niu et al.[33] The total starch (and glucose units) was measured following the instruction protocol for the Total Starch Assay Kit (AA/AMG, Megazyme International, Ireland) developed from McCleary et al.[34] Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were extracted and measured according to the instruction protocol provided with the Megazyme Total Dietary Fiber Assay Procedure kit (Megazyme International, Ireland), as based on the method of Lee et al.[35] The total dietary fiber (TDF) was calculated from summing the respective IDF and SDF contents. Total polyphenol (TP) content, comprising both free and bound constituents, was extracted as described previously.[36] Free and bound polyphenols were individually measured based on the Folin-Ciocalteau spectrophotometric (765 nm) method using gallic acid equivalents (GAE) as a reference standard,[37] and then summed to represent the TP content. Similarly, the free and bound flavonoids (within the polyphenol extracts) were individually measured using a spectrophotometric (510 nm) colorimetric assay with catechin equivalents (CAE) as a reference standard.[38] The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed by measuring the reduction (515 nm) of DPPH•.[39] The antiradical activity contained in both the free and bound fractions was summed and expressed as total DPPH (TDPPH).

Statistical analyses

All analyses were carried out using Statistica 6.0 software (2001, StatSoft, Tulsa, OK, USA). Data were subjected to an analysis of variance (one-way ANOVA). Tukey's honest significant difference was used to determine the differences between means at $p < 0.05$.

The Principal Component Analysis (PCA) was performed as described in our previous article.[31] Using this method, the original space for variable measurements was projected down onto two low-dimensional subspaces. The first was case-related (24 samples reflecting the different bakery products with or without 3% SN) while the other was variable-related. For the sensory analysis PCA, the seven variables were appearance (APP), consistency (CON), flavor (FLA), long-lasting taste (LLT), smell (SME), total enjoyment (TEN), and taste (TST), respectively. For the nutritional and functional component PCA, there were nine variables, which included protein (PRO), starch (STR), glucose (GLU), TP, total flavonoids (TF), total antiradical activity (TDPPH), IDF, SDF, and TDF, respectively. The variable-related subspace was analyzed (factor loading) to understand the correlation between the variables and factors (principal component).

RESULTS AND DISCUSSION

Sensory analysis of stinging nettle-enriched bakery products

It was previously shown that the addition of 10% chopped SN to refined bread significantly increased the level of fiber, calcium, and total phenolic content and antioxidant activity compared to the non-enriched bread.[20] However, consumer appreciation of the 10% SN-fortified bread was not demonstrated in that study.[20] Given that enrichment may influence the sensory properties of foods and that refined flour was not used as a basis in the present study, it was considered important to firstly investigate the sensory parameters to assess from the outset whether any potential barrier to product development could exist for health-conscious individuals. Based on the promising potential of the chemical analyses for 10% SN-fortified bread,[20] light brown flour (Type 1) containing 10% SN was analyzed by a sensory panel. Overall enjoyment was rated at only 50% by the health-conscious tasters, which was not sufficiently promising to merit production (Table 1). The negative aspects were not attributable to appearance, smell, and consistency, but mostly to the unacceptably high values for long-lasting (after) taste and flavor (Table 1). For this reason, the 10% SN was discarded, and an enrichment percentage of 3% was selected.

Motivation for the selection of 3% was based on previous sensory-based results conducted on refined (0.46%–0.60% ash content) bread and pasta. Previously, refined bread enriched with 2% SN was shown to score the highest acceptability, with 4% also showing minimal negative impact on sensory parameters.[17] Then, in a separate study, 5% SN leaf supplementation was unacceptable in taste compared to 2.5%.[18] In pasta, both the lowest starch hydrolysis index and glycemic index were recorded at 3% SN enrichment, with the majority of sensory parameters unaffected.[19] The sensory profiles and appearance of the products prepared from the 3% SN enrichment of organically grown soft wheat semi-wholegrain and wholegrain flour are shown in Table 1.

The WBU was rated the highest for total enjoyment, followed by BBU and BCT (Table 1). No previous research has been published on SN enrichment of brown and wholegrain wheat flour with which to compare the present results. Of note, previous research on refined flour enrichment showed that the refined bakery products were preferred (greater acceptability score) over the SN-enriched products, although SN enrichment scores were not statistically different up to 2.5%–3%.[17–19] Given the significantly lower total enjoyment score for BCT (Table 1) compared to WBU and all SN-enriched breads, the health-conscious tasters were shown to differ from those representing the general population. Nonetheless, the increased demand for fortified foods and beverages reflects the current surge in the health-conscious population,[10] which to the best of our knowledge, are less well represented in fortification studies and warrant investigation. For the health-conscious panel, the appearance was rated highly (78%–88%) for all bakery products (Table 1). The consistency (texture) was ranked significantly higher for the wholegrain products compared to the semi-wholegrain products. Flavor varied minimally for all products enriched with 3% SN.

In order to examine a possible grouping of the bakery products for sensory parameters, a PCA was carried out (Figure 2). The first two components (PC1 and PC2) explained 52.43% and 26.53% of the variance, respectively. Of interest, the lower left quadrant was associated with negative values for consistency, long-lasting taste, flavor, and total enjoyment, distinctive for only WBU (Figure 2). Overall, the crackers enriched with SN (rated the lowest for consistency, long-lasting taste, smell, and total enjoyment; Table 2), clustered at the far end of the lower right quadrant. Also grouped in this quadrant was the brown bread enriched with SN, distinguishable for taste and appearance. Instead, smell was distinctive for both WCT and BLU and were grouped in the upper left quadrant of the PCA (Figure 2). Overall, the addition of 3% chopped SN leaf material was considered acceptable for both the wholegrain and semi-wholegrain bread products. Further investigations of the chemical profile were then undertaken.

Chemical analysis of stinging nettle-enriched bakery products

To test the working premise that supplementation of semi-wholegrain and wholegrain flour with 3% SN flour could exceed the functional component content reported previously, dietary fiber, polyphenols, flavonoids, and antiradical activity, along with protein and starch, were investigated. Lipid content was not included given the presence of olive oil (approximately 4%) in the bread products and butter (approximately 10%) in the savory crackers. Moreover, previous reports

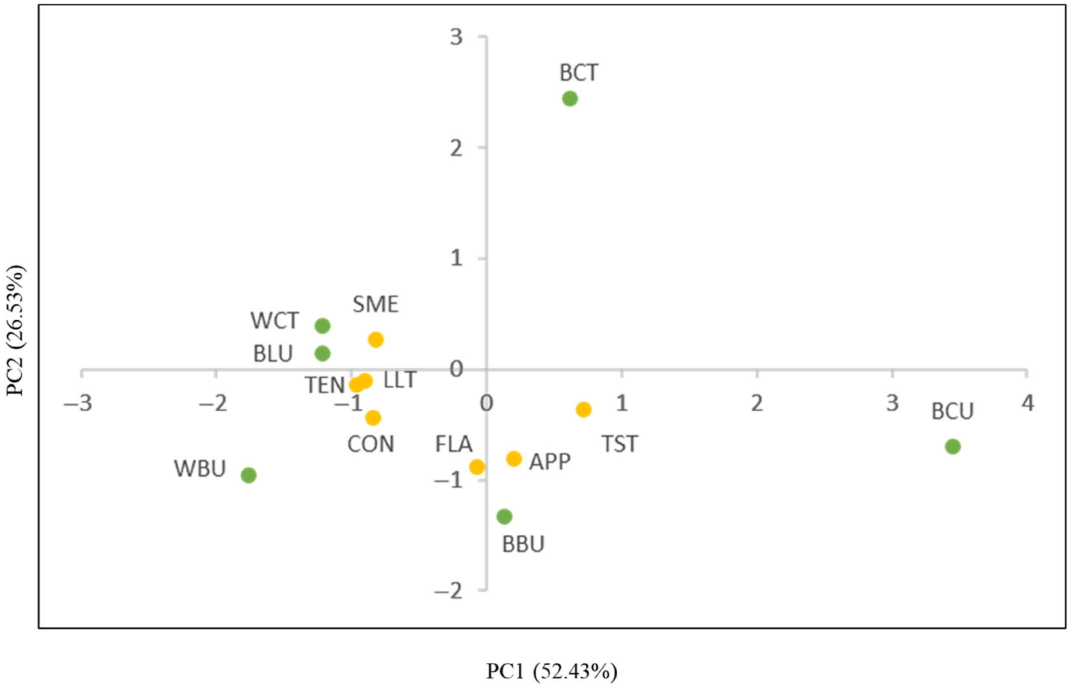

FIGURE 2 Principal Component Analysis (PCA) of the sensory parameters (yellow circles) and bakery products (green circles). The codes for the bakery products: BBU (Brown flour [Type 1] focaccia Bread with *Urtica*), BCT (Brown flour [Type 1] focaccia Control bread), BLU (Brown flour farmhouse Loaf with *Urtica*), BCU (Brown flour savory Crackers with *Urtica*), and WBU (wholegrain focaccia Bread with *Urtica*). The codes for the sensory components are: APP (appearance), CON (consistency or texture), SME (smell), TST (taste), FLA (flavor), LLT (long-lasting taste), and TEN (total enjoyment).

TABLE 2 Chemical constituents of semi-wholegrain and wholegrain bakery products enriched with 3% stinging nettle. All results are the mean and standard deviation of four replicate samples.

Constituents	*p* value	BCT	BBU	BLU	BCU	WCT	WBU
Protein (% DM)	***	14.08 ± 0.10^{d}	15.65 ± 0.04^{ab}	15.65 ± 0.02^{ab}	15.34 ± 0.73^{b}	14.72 ± 0.47^{c}	16.22 ± 0.10^{a}
Starch (% DM)	***	70.14 ± 2.93^{a}	66.03 ± 2.37^{ab}	65.27 ± 3.39^{ab}	68.04 ± 4.43^{a}	61.68 ± 2.93^{b}	52.31 ± 2.01^{c}
Glucose (mg/100 g DM)	***	140.76 ± 5.75^{b}	113.47 ± 13.58^{c}	76.12 ± 5.50^{e}	60.32 ± 5.75^{d}	155.12 ± 10.5^{a}	106.76 ± 5.50^{c}
TDF (% DM)	***	13.91 ± 1.44^{d}	15.87 ± 0.23^{cd}	16.15 ± 0.38^{c}	15.73 ± 2.03^{cd}	22.63 ± 0.88^{b}	24.60 ± 0.31^{a}
IDF (% DM)	***	9.44 ± 0.55^{d}	11.10 ± 0.19^{c}	11.37 ± 0.32^{c}	10.55 ± 1.29^{cd}	16.71 ± 1.17^{b}	18.10 ± 0,17^{a}
SDF (% DM)	***	4.48 ± 1.09 ^{b}	4.77 ± 0.28 ^{b}	4.78 ± 0.25 ^{b}	5.20 ± 0.78 ^{b}	5.93 ± 0.41^{b}	6.53 ± 0.27^{a}
TP (mg GAE/100 g DM)	***	62.37 ± 3.19^{d}	87.46 ± 2.37^{c}	96.61 ± 5.77^{bc}	148.46 ± 24.56^{a}	110.25 ± 6.85^{b}	155.11 ± 7.71^{a}
TF (mg CAE/100 g DM)	***	48.94 ± 1.23^{c}	77.84 ± 6.65^{b}	79.46 ± 6.19^{b}	82.09 ± 8.68^{b}	74.91 ± 2.73^{b}	92.56 ± 7.79^{a}
TDPPH (μmolTE/g DM)	***	12.97 ± 0.33^{b}	26.40 ± 0.49^{a}	24.04 ± 4.22^{a}	31.88 ± 1.98^{a}	34.28 ± 1.04^{a}	37.52 ± 11.56^{a}

Note: The codes for the bakery products: BCT (Brown flour [Type 1] focaccia Control bread), BBU (Brown flour focaccia Bread with *Urtica*), BLU (Brown flour farmhouse Loaf with *Urtica*), BCU (Brown flour savory Crackers with *Urtica*), WCT (wholegrain focaccia Control bread), and WBU (wholegrain focaccia Bread with *Urtica*). Codes for constituents are: catechin equivalents (CAE), gallic acid equivalents (GAE), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total dietary fiber (TDF), total DPPH (TDPPH), total flavonoids (TF), total polyphenols (TP), and Trolox equivalent (TE). ***variable is significant at $p < 0.001$ by one-way ANOVA. Different letters within each row denote significantly different values at $p \leq 0.05$ from Tukey's post hoc test.

showed that lipid content was either unchanged or reduced by the supplementation of wheat with SN.[18–20]

Protein content in the brown semi-wholegrain and wholegrain control flours was within the range of that reported previously for the refined flours on a DM basis.[17,18,20] Addition of 3% SN increased the total protein content in the bakery products prepared from both the semi-wholegrain and wholegrain flours, corroborating previous research reporting increases for equivalent additions of SN.[17,18] Starch content was not significantly different between the BCT and SN-enriched semi-wholegrain products (Table 2). However, the starch content was significantly lower in the WCT compared to the brown flour products and even lower in the WBU, collectively attributable to the higher protein content, ash content, and fiber content, respectively. The glucose content was significantly higher in the

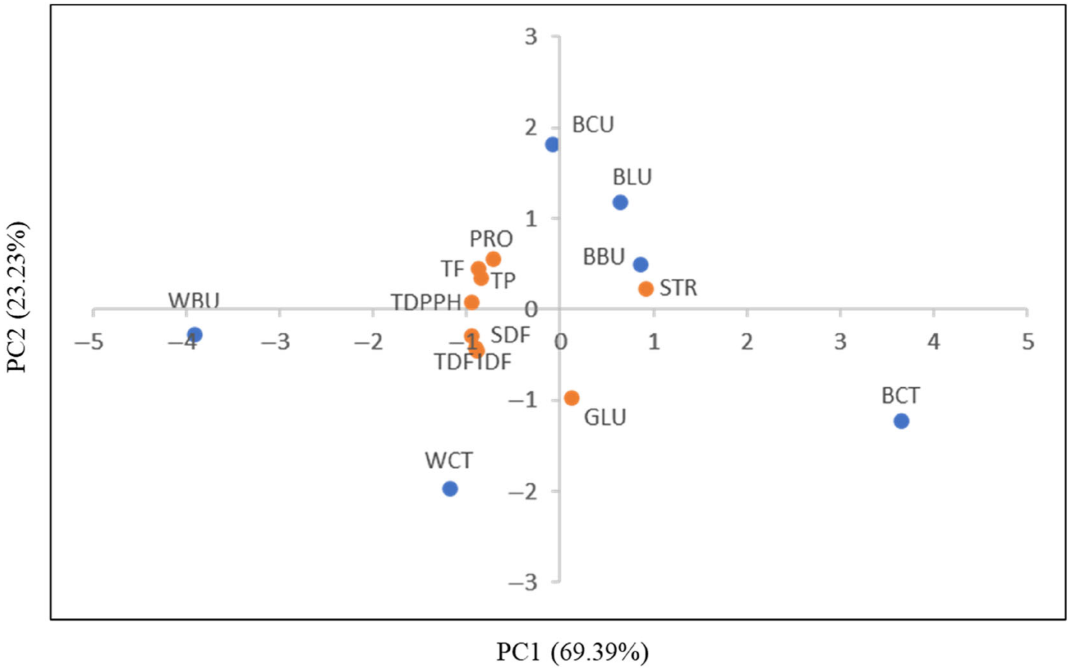

FIGURE 3 Principal Component Analysis (PCA) of the chemical parameters (brown circles) and bakery products (blue circles). The codes for the bakery products: BBU (Brown flour [Type 1] focaccia Bread with *Urtica*), BCT (Brown flour [Type 1] focaccia Control bread), BLU (Brown flour farmhouse Loaf with *Urtica*), BCU (Brown flour savory Crackers with *Urtica*), and WBU (wholegrain focaccia Bread with *Urtica*). The codes for the chemical parameters are: Protein (PRO), starch (STR), glucose (GLU), total polyphenols (TP), total flavonoids (TF), total antiradical activity (TDPPH), insoluble dietary fibers (IDF), soluble dietary fibers (SDF), and total dietary fibers (TDF).

non-enriched controls (BCT and WCT) compared to the bakery products enriched with SN. Interestingly, both the lowest starch hydrolysis and glycaemic indexes were shown in pasta enriched with 3% nettle compared to the control.[19] Hence, the lower levels of glucose, albeit minimal, in the 3% SN-enriched products in the present study may have been indicative of a lower level of starch breakdown, and/or a higher level of resistant starch. This aspect warrants further investigation.

TDF (Table 2) in the BCT and WCT significantly exceeded that of refined flours reported previously, with the addition of SN increasing the TDF by approximately 0.8% to attain values of 1.5%–1.8%.[17,20] The average TDF, IDF, and SDF of WCT was comparable to that reported previously for the wholegrain flours of the individual genotype constituents (14.5%, 16.8%, 15.9%, 17.3%, and 16.3% IDF for Andriolo, Frassineto, Inallettabile, Gentil Rosso, and Verna, respectively, and 5.38%, 5.40%, 5.14%, 5.99%, and 5.52% SDF for Andriolo, Frassineto, Inallettabile, Gentil Rosso, and Verna, respectively), indicating that baking had no effect on fiber content.[25] The use of semi-wholegrain and wholegrain flour as the basis constituted a significant functional advantage in terms of fiber content. Based on the results reported by Marotti et al.,[31] the addition of 3% SN was potentially foreseen to increase TDF, IDF, and SDF in the bakery products by approximately 2.1%, 1.7%, and 0.4%, respectively, which was verified by the present results (Table 2). The only reports of IDF and SDF contents with 3% SN were recorded for refined pasta, which showed that only IDF content increased significantly with SN enrichment.[19] In contrast to the breads of the present study, the SDF content in refined cooked pasta was significantly higher than the IDF content.[19]

The TP content of the control breads (BCT and WCT) exceeded that of refined flour (Table 2), in which 29 and 52 mg GAE/100 g DM were reported previously.[25,27] Addition of SN leaf material significantly increased the TP content of all bakery products made with both semi-wholegrain flour and wholegrain flour (Table 2), corroborating previous work.[18,20] Similarly, flavonoid content and antiradical DPPH activity were also shown to increase in the 3% SN-enriched bakery products (Table 2). Enrichment of with SN, with recognized anti-inflammatory, antimicrobial, antioxidant, cardiovascular, chemopreventative, diuretic, and hepatoprotective, respectively,[39] represents an effective means to improve the functional potential of bread staples.

Interestingly, it was shown that during the fermentation and baking procedures, the polyphenol content increased by approximately 15% in SN-enriched refined bread, presumably due to the breakdown of bound polyphenols during fermentation.[20] The highest polyphenol content in WBU was 3 and 2 times higher than that for refined bread supplemented with 2.5% SN and 10% SN, respectively.[18,20] Previous work showed that enrichment using SN leaf extract rather than chopped leaves was shown to improve the refined bread quality and improved the sensory acceptability scores.[28] Nonetheless, the TP content in WBU exceeded that for the 2.5% chopped SN leaf and 5% SN extract-enriched bread by 2.6 and 1.8 times, respectively.[18] The use of SN extracts (thereby permitting the addition of a greater quantity of SN), rather than chopped leaf material, is also a noteworthy option toward increasing functional properties in semi-wholegrain and wholegrain flours.

The choice of the raw wheat material was also a significant factor in improving the product. Wholegrain products as raw materials provided a higher quantity of functional components than the brown flour products, with both types of flour being superior in quality to refined flour used in all previous investigations. Moreover, all of the genotypes used in Virgo flour mix were shown to possess functional properties that significantly improved cell viability by reducing oxygen radicals, with Verna showing the highest scavenging capacity.[40] Of additional relevance is that landraces and old wheat genotypes, as well as SN, are more suited to sustainable agriculture.[11–15,22–25]

The possible grouping of the bakery products for health-promoting components was then also examined by PCA (Figure 3). The first two components (PC1 and PC2) were shown to explain 69.39% and 23.23% of the variance, respectively. Of relevance, the negative values for protein, TP, TF, TDPPH, IDF, SDF, and TDF were grouped in both the lower and upper left quadrants and were distinctive for WBU, WCT, and BCU (Figure 2). Instead, the positive values for starch and glucose (upper and lower right quadrants) were distinctive of the Type 1 brown flour products. WCT and, more specifically, WBU were more distinctive for IDF, SDF, and TDF and inversely associated with starch, which in turn was distinctive for BLU and BBU (Figure 3). The BCU product was projected on the quadrant border. Although enrichment with 3% SN improved the functional components (polyphenols, antioxidant activity, and fiber) in both wholegrain and Type 1 flour products (Table 2), the wholegrain wheat as a raw material base (regardless of the addition of SN) was distinctive from the brown flour in the PCA (Figure 3).

CONCLUSION

Combining existing strategies to enhance the functional component of wheat bread, a staple food in large parts of the globe, the present study involved the use of a semi-wholegrain and wholegrain flour of a mix of organically cultivated old genotypes enriched with the NUS, SN. Enrichment with 3% SN was shown to enhance the functional component content (fiber, polyphenols, flavonoids, and antiradical activity) of the bakery products to exceed levels previously reported. Although SN has been widely used since ancient times as an ingredient in foods in Italy, appreciation of SN-fortified bread was demonstrated for the first time with the use of health-conscious tasters of the population. It is the surge in the health-conscious sector of the population that constitutes the demand for fortified foods and beverages.[10] The present results show potential consumer acceptance for SN-enriched wholegrain products with significant improvements in functional components.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The data are available on request from the authors.

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