








## Original article

# Fortification of wheat white bread: assessing the suitability of *Beta vulgaris* through technological, nutritional, and sensory evaluation

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**Summary** As societies undergo shifts in dietary patterns, there is often an increase in nutrition-related issues. This is particularly evident in the case of wheat bakery products, which have evolved in an unsustainable way. The fortification of wheat flour with vegetables has emerged as a strategy to mitigate the imbalanced composition of white bread. The objective of this study is to address existing knowledge gaps regarding nutritional quality and technological characteristics of food-to-food fortified bakery products. A blend of *Beta vulgaris* powder (up to 5% of the total weight) was incorporated into commercial wheat white flour, and the resulting composition and technological parameters were analysed throughout the bread-making process, using standard methods (e.g., AAAC, ISO). The sensory assessment of the tested fortified baking products formulations was conducted. The 178 volunteer consumers noted the differences conveyed by *B. vulgaris*, but scored the taste and colour as acceptable (6 out of 9). Formulations containing moderate amounts of chard (e.g., 2%) were most appreciated. This study demonstrates that *B. vulgaris* can be employed to enrich white bread, particularly in minerals, such as calcium, magnesium, and iron, in a readily actionable manner that is well accepted by consumers. Furthermore, the use of flour blends contributes to mitigate the impact of fluctuations in wheat availability, thereby enhancing food security.

**Keywords** Bakery products, bioactive phytochemicals, food processing aspects, fortification, nutrition, sensory analysis, white bread.

## Introduction

Wheat (principally *Triticum aestivum* L. and *T. durum*) has historically been a staple grain in the Mediterranean and the Middle East. However, it has evolved into a significant traded commodity for food and feed manufacturing on a global scale. According to the FAO (2022), global wheat markets are currently experiencing high degrees of uncertainty due to consecutive reduced harvests and/or trade blockades, with the majority of the largest world producers affected. The role of centralised production systems in ensuring food security is not straightforward, particularly in the case of wheat (Raj *et al.*, 2022). However, global wheat consumption is projected to grow (FAO, 2022;

Our World in Data, 2024). Wheat stocks are expanding notably in Asia and decreasing in the Maghreb, where wheat is historically, culturally, and nutritionally important (FAO, 2022; UNESCO, 2023).

From a nutritional perspective, the Anses-Ciquel Food Composition Table (2020) indicates that wholemeal flour (type 150) contains approximately 11% water, 65% carbohydrates (of which 57% is starch and less than 2% is sugars), 12% protein, 10% fibres, 1.5% fat, and 1.3% ash (relevant quantities of Ca, Mg, K, and P), and relevant quantities of the vitamins E, B1, B2, B3, B5, B6, B9 and some vit C, in addition to microelements (such as Mn and Fe). It is therefore evident how the increased availability of wheat contributed to the rapid growth of human populations during the Neolithic period and to the emergence of major civilisations in the Mediterranean region, where

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wheat domestication first occurred (Delgado *et al.*, 2022). It is well documented that bread and water are capable of sustaining human survival for quite extended periods. However, bread, which was originally made from whole wheat flour, yeast (mainly *Saccharomyces cerevisiae*), water and salt, subsequently evolved into a complex bakery product.

The refining of wheat flour, which occurs during wet milling, grinding, and bleaching, results in the removal of the bran and germ, as well as polyphenols, fibres, vitamins, and other components. This process has the effect of reducing the antioxidant activity and nutritional value of the flour, which in turn affects the antioxidant activity and nutritional value of the bread (Yu *et al.*, 2013). Moreover, the relative proportion of the remaining components increases when certain components are removed. For instance, the proportion of soluble starch and free sugars in refined flours is higher than in whole or less processed flours, which consequently results in a higher glycaemic index. Such imbalances in composition have been demonstrated to result in negative health outcomes (Iorgachova *et al.*, 2019; Zare *et al.*, 2023). Conversely, numerous authors have discussed the effects of the food matrix on a range of characteristics, including the bioaccessibility of nutrients and sensory properties (Fardet, 2017; Holland *et al.*, 2020; Ahmed *et al.*, 2021; Delgado *et al.*, 2021; Fardet & Rock, 2022). These studies have highlighted the potential detrimental effects of excessive processing on human health and the environment (Kaim & Goluch, 2023; Zare *et al.*, 2023). Some researchers argue that ultra-processing and alterations to food matrices may be essential for upcycling secondary raw materials and reducing food loss (Melini *et al.*, 2020; Capozzi *et al.*, 2021; Capozzi, 2022). In any case, as noted by Aguilera (2019), the term 'food matrix' has been used in the literature in a vague manner, as a synonym for the actual whole food, a microstructure, or a phase. This author posits that the term 'food matrix' is best understood as a 'physical and spatial domain, that contains, interacts with, and/or provides a specific functionality to a constituent (e.g., a nutrient) or element of the food (e.g., starch granules, microorganisms)'. It is described as scale-sensitive, in that different interactions may occur simultaneously at various magnitude levels in the same food. For example, in bread, at the near-visible level, it is possible to describe the textural properties of the porous crumb, which result from the protein–starch reactions that form walls surrounding the air cells. At a smaller scale, starch granules can be regarded as gel inclusions in the continuous gluten matrix. At an even smaller scale, starch granules can be regarded as the matrix in which alpha-amylase is acting and causing gelatinisation and releasing glucose units (Aguilera, 2019). During bread making, the matrix evolves. The

dough is a viscoelastic matrix in which gluten forms a protein network holding the starch particles, and baked bread is a porous material (Aguilera, 2019). It is therefore notable noteworthy that the texture and aroma of bread are largely dependent on the composition and properties of the network (predominantly protein–starch) enclosing the dispersed gas phase. It has recently been documented that fibres, pectins, and polyphenols are involved in the slowing down of the rate of starch digestibility. For example, fibres absorb water, during mastication, resulting in structural alterations to the bread matrix and consequently influencing the accessibility of amylases to the starch granule (Kaim & Goluch, 2023).

The preference of consumers for soft bakery products such as white bread, the convenient and easy preservation and transportation of refrigerated dough, and the fast-raising process were significant drivers for the substitution of traditional bread-making. The coarsely milled wheat flour, which was slightly dark and bitter, and the long fermentation times (which were required by the yeast to break down starches and fibres into CO<sub>2</sub> gas bubbles) were abandoned, despite the superior nutritional quality of such bread. Consumer preferences have arguably contributed to a double burden on public health and the environment, as the bakery industry has evolved to meet mainstream demand. Consequently, standardised processes and bakery products have been developed with the objective of optimising profits through economies of scale. However, the removal of valuable nutrients and health-promoting compounds occurs at the expense of additional energy and water, while producing wastewater requiring treatment (thereby adding costs and environmental damage). The aggregation of such losses and mismanagement of resources when added up to waste at the retail and consumer levels reveals the unsustainability of current practices (Ghaziani *et al.*, 2022). There is a growing awareness of necessity to alter the manner in which food is produced and consumed, particularly in the context of bakery products. This is due to the estimated hidden costs on health and the environment of the current food systems, which are estimated to exceed \$10 trillion annually (FAO, 2023).

The upcycling of wasted bread has been proposed as a potential solution to mitigate food loss issues (Brancoli *et al.*, 2020; Ben Rejeb *et al.*, 2022; Kumar *et al.*, 2023). Other strategies proposed by the bakery industry to minimise the impact of processing on the nutritional value and consumer acceptability include: adjusting formulations to avoid high glycaemic index (Iorgachova *et al.*, 2019), enhancing processing steps to improve the nutritional value of wheat flour, thus avoiding the need for additives (Barros *et al.*, 2023), exploring gluten-free products (Schmid *et al.*, 2023),

and biotechnology applications, such as certain enzymes for breadmaking (Bala & Singh, 2017). An additional avenue for exploration is the development of novel fortified breads and bakery products.

The most common method of fortification is the addition of well-identified and quantified micronutrients to given foods. However, there are alternative approaches, such as food-to-food fortification, whereby a complex nutrient-dense source is used. In any case, the objective is to address the nutritional deficiencies of the population, including by replacing losses during food processing (Kruger *et al.*, 2020). In some countries, governmental regulations dictate the fortification of refined flour with B vitamins and Fe (Yu *et al.*, 2013). Conversely, fortification with by-products, such as olive pomace (Cecchi *et al.*, 2019) or powders and extracts from other plants, such as carob, is gaining traction, as evidenced by in our previous works (Issaoui *et al.*, 2021a, 2021b).

In order to gain a comprehensive understanding of food systems, it is essential to consider a multitude of perspectives, given the intricate nature of value chains and the involvement of numerous stakeholders. Furthermore, the vital act of eating holds significant social, cultural, and archaeological significance, as explored by scholars such as Hastorf (2016), an archaeologist. For the purposes of this study, it is important to recognise that these dimensions are intertwined with emotions and biological characteristics, which influence individual food preferences (Laureati *et al.*, 2024). For various reasons, including marketing, globalised habits, and convenience, consumer preferences have been shifting towards a nutrition transition stage of obesity and nutritional deficiencies (Popkin *et al.*, 2012). This has led to a preference for cheaper and more appealing foods, in terms of taste and convenience (Drewnowski & Monsivais, 2020). Consequently, educating the public on the importance of making healthy choices appears to be a rational option; however, it can be more challenging and less effective than desired. This important and time-consuming task is carried out by numerous national and international organisations, which also prescribe strategies for fortifying staple foods (FDA, 2015; WHO, 2023) with the aim of achieving long-term results.

Upon examination of the culinary traditions of Tunisia, it becomes evident that wheat plays a pivotal role, as does chard (*Beta vulgaris* L.), which is incorporated in sauces that frequently accompany pieces of bread, thereby creating distinctive and popular flavour combinations. *Beta vulgaris* is an edible leafy plant of the *Chenopodiaceae* family with thick stalk (white or reddish-purple) and large green leaves. It is commonly consumed raw (e.g., in salads) or cooked in a manner similar to spinach. In their systematic review of the

nutritional features of Swiss chard, Gamba *et al.* (2021) present evidence of antioxidant and immunomodulating properties that may contribute to the prevention and alleviation of diabetes, cardiovascular diseases, and other positive health outcomes. With respect to nutritional composition, according to Anses-Ciqual (2020), the raw leaves of *B. vulgaris* reported to be rich in dietary fibres (1.8%), minerals as Ca (25 mg/100 g), Mg (17 mg/100 g), Fe (0.85 mg/100 g), Mn (0.16 mg/100 g), vitamins as tocopherols (0.12 mg/100 mg), pantothenic acid (0.2 mg/100 mg), or folate (0.0247 mg/100 g), while only providing 16.4 Kcal/100 g (expressed in wet weight of edible portion). This species was selected for testing as a natural enriching ingredient in the formulation of white bread.

The main objective of the current study is to provide knowledge that will enable an assessment of the potential of *B. vulgaris* to reinforce the nutritional value of white bread. It is anticipated that this will contribute to the achievement of SDG2 and SDG12, by enabling the strategy of delivering nutritious and affordable food to all, while respecting consumer preferences and the constraints of small and medium-sized enterprises (SMEs). Furthermore, since wild and cultivated plants are commonly consumed in Tunisia, their nutritional and technological features were comparatively assessed, with a focus on addressing environmental challenges.

To this end, a portion of white wheat flour was replaced by different proportions of Swiss chard (*Beta vulgaris* L.) powder, to evaluate the impact of this substitution on the nutritional quality of white bread and on consumer preferences.

## Methods

### Plant material, dough, and white bread preparation

To prepare the enriched white bread for this study, we used commercially available refined white soft wheat flour (commercial baker's flour), and chard powder obtained from *Beta vulgaris* cultivated plants or gathered from the wild, in the Sadikia region of the governorate of Sidi Bouzid, Tunisia. The freshly collected plants (leaves and most of the stem) were transported to the lab and immediately sorted, washed, drained, and dried in an oven for 48 h at a temperature of 40 °C. The dehydrated whole plants were then grounded with a blender to a powder sieved to limit the granulometry to 200 µm and stored in opaque glass jars at room temperature. The concentration range of *B. vulgaris* (wild and cultivated) powder to blend with refined wheat flour was chosen based on the experience from previous works (Issaoui *et al.*, 2021a, 2021b). Thus, 1%, 2%, 3%, 4%, and 5% of wild (coded S1–S5) or cultivated (coded C1–C5) chard were blended with wheat white

flour (WF). 100% WF was used as control. Operations from the preparation of flour blends to breadmaking were performed on the bakery pilot scale at the faculty of Science and Technology of Sidi Bouzid, with the same type of equipment found in commercial large-scale bakeries. The number of samples along the process was large enough to allow for the necessary replicates and controls to perform the below-described analysis and the sensory assessments by naïf consumers (hedonic studies). In terms of mass proportions and steps (recipe), the breadmaking process can be described as follows: Baguette-form bakery product was made by mixing 100 g of each of the different flour blends with commercial baking powder (containing  $K_2HCO_3$  as the raising agent), 0.1 g of NaCl (refined kitchen salt), and  $55 \pm 5$  mL of spring water were stepwise incorporated, while dough was stirred by hand, for 4–7 min to obtain the necessary viscoelastic consistency to shape 50 g baguettes. After a resting period of 45 min at 40 °C (allowing some release of  $CO_2$  into the dough, from the dissociation of  $K_2HCO_3$ ), the baguettes were spread with a few water drops, laminated, and cooked in perforated trays, in an electric-ventilated oven for 16 min at 254 °C. Duplicate or triplicate samples were removed for analysis along the process.

#### Flour blends' mineral composition

Determination of the ash rate was performed after incineration of the test sample at 900 °C according to ISO 2171:2007. For nutrient extraction, dried samples were incubated in 1 N  $H_2SO_4$  (20 mL) at 80 °C for 1 h and then left overnight at room temperature.  $K^+$  concentration was determined by flame photometer (BWB), while  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Zn^{2+}$  and  $Fe^{2+}$  were detected and quantified by atomic absorption spectrophotometry (Perkin Elmer, Analyst 300). The results were expressed in  $mg\ g^{-1}$  DW (Zorrig *et al.*, 2019).

#### Rheological parameters of dough

Measurements of flour humidity (%) and dough alveographic parameters (P, L, G, W, Ie), were performed in samples prepared from each blend of WF with 1%–5% of BS or BC.

The humidity was determined by the reference method ISO 712: 2009, in a 5 g sample, kept 1 h 30 min at 130 °C, atmospheric pressure. For alveograph measurements, the Alveo PC (Chopin Technology, La Garenne, France) was used, with the method AACC 54–30 (1999) and the following alveographic parameters were recorded: tenacity (P), extensibility (L), baking strength (W), the swelling ability of the dough (G), and elasticity (Ie). The protocol consisted of four steps: (1) the mixtures of flour and salted water were prepared; (2) five calibrated pieces of dough were

prepared from each mixture; (3) the dough was left to rest to allow for the accumulation of  $CO_2$ , and (4) the resulting bubbles were allowed to burst. Dough resists pressure by swelling in the form of bubbles, and the graphic recording of the pressure variations inside the bubble as a function of time (alveograph) ends up with the rupture of the bubble. The analysis of the alveograph curve informs about the rheological properties of the sample under test, notably the firmness and the extensibility of the dough.

#### Assessment of bakery products' texture and related rheological parameters

A Texture Procedure Analysis (TPA) test was conducted using a Texture Analyser, also known as a texturometer (Perkin Elmer TVT 6700 Texture Analyser). The sample was placed into a moulded Nalgene polypropylene tube, which was secured in a fixture beneath the Texture Analyser. This equipment was connected to a computer, which controlled the instruments and analysed the data using software provided by Texture Technologies Corp. Parameters such as hardness (peak force during the first compression cycle), stickiness (ratio of detected height during the second compression to the original compression distance), cohesiveness (ratio of positive areas during the second cycle to those of the first cycle), and adhesiveness (negative force area during the initial compression, representing the work required to remove the compressing plunger from the sample) were determined.

#### Colour assessment of white bread (enriched with different proportions of chard)

The colour parameters of the samples under study, including flour, Swiss chard powder, white bread, etc., were measured using a colorimeter (Chroma Meters Measuring Head CR 400, manufactured by Konica Minolta, Japan). It functions by illuminating the sample with a controlled light source and measuring the reflectance or transmittance of light across different wavelengths, transposed into different colour parameters. The colorimeter utilises the CIELAB colour scale, which is a standardised colour space defined by the International Commission on Illumination (CIE). The parameters include:

- $L^*$  (lightness) that represents the brightness of the colour and ranges from 0 (black) to 100 (white). A higher L value indicates lighter shades, while lower values indicate darker shades;
- $a^*$  (red-green axis); The a-axis represents the spectrum from green (−60) to red (+60). Positive values indicate a shift towards red, while negative values indicate a shift towards green. Therefore, a higher positive  $a^*$

value suggests a more pronounced red hue in the sample;

- $b^*$  (yellow-blue axis): The  $b$ -axis represents the spectrum from blue ( $-60$ ) to yellow ( $+60$ ). Positive values indicate a shift towards yellow, while negative values indicate a shift towards blue. A higher positive  $b^*$  value suggests a more intense yellow colour in the sample.

### Analysis of aroma's volatile compounds (in flours and bakery products)

The aroma profiles of wheat flour, *B. vulgaris* powder, and bakery products made from the different blends were analysed by gas chromatography and mass spectrometry (GC–MS). For sample preparation, 2 g of the corresponding flour blend or of the resulting bakery product to be tested was placed into a 5 mL glass vial and left to equilibrate (to reach ambient conditions) for 30 min. A Supelco Solid Phase Micro-Extraction (SPME) fibre coated with polydimethylsiloxane (PDMS, 100  $\mu\text{m}$ ) was used. The headspace was sampled for 50 min at room temperature using the same fibre and the same conditions for all the samples and blanks, which were executed before each first SPME extraction and randomly repeated during each series. Comparisons of relative peak areas were performed between the same compounds in different samples. For GC–MS analysis, an Agilent 7890B gas chromatograph (Agilent Technologies Inc., Santa Clara, CA) equipped with an Agilent HB-5MS (Agilent Technologies Inc., Santa Clara, CA) capillary column (30 m  $\times$  0.25 mm; coating thickness 0.25  $\mu\text{m}$ ) and an Agilent 5977B single quadrupole mass detector (Agilent Technologies Inc., Santa Clara, CA) were used with splitless injection. Injector and transfer line temperatures were 220 and 240  $^{\circ}\text{C}$ , respectively; oven temperature was programmed from 60  $^{\circ}\text{C}$  to 240  $^{\circ}\text{C}$ , at a rate of 3  $^{\circ}\text{C min}^{-1}$ ; Helium was used as the carrier gas, flowing at 1 mL  $\text{min}^{-1}$ ; Compounds were identified on the basis of their retention times, in relation to those of pure standards, comparing their linear retention indices (L.R.e) relative to the series of  $n$ -hydrocarbons, using the information from the National Institute of Standards and Technology library (NIST 2014 and ADAMS) and homemade library mass spectra built from pure substances and components of known mixtures, and MS literature data.

### Sensory acceptance tests

The volunteers, 178, were recruited in public places (e.g., open-air markets, schools) at different locations in Tunisia to perform the predefined hedonic tests; informed consent was granted, and personal data were not disclosed; inclusion criteria were age from 18 to 65

years old, frequency of basic school, and a pre-assessment of their overall liking of bread and bakery (deduced from purchasing and consumption frequency, as well as familiarity with white bread). Each volunteer filled out a questionnaire with information on age, gender, region of origin, socio-professional category, and consumption frequency of bread. Group diversity was sought regarding gender, age, and education level as well as professional occupation/socio-economic status. Participants were asked to evaluate samples before and after the enrichment process according to their preference and expressing their degree of liking using a 9-point hedonic scale (scores: like extremely: 9; like very much: 8; like moderately: 7; like slightly: 6; neither like nor dislike: 5; dislike slightly: 4; dislike moderately: 3; dislike very much: 2; dislike extremely: 1). Blind and randomised bakery product samples (approx. 15 g) were served at room temperature, in transparent glasses labelled with a three-digit code, ensuring the consumers were deprived of any background information concerning the samples to be tasted. These naïf consumers (non-trained) were asked to participate in three tasting sessions of eleven samples each and to grant their informed consent (after being informed of the purpose of the study, and how collected data was aggregated and anonymised, ensuring the confidentiality of personal information). The participants were provided with breaks between sample tasting and were given palate cleansers such as apple and water to cleanse the palates between the samplings to avoid any carry-on effects. This ensured a comfortable and relaxed tasting experience. Additionally, it was ensured that participants had adequate rest time between samples to prevent palate fatigue and enable accurate sensory evaluation. This allowed participants to evaluate each sample comfortably and without feeling stressed or pressured.

### Statistical analysis

All experimental datasets underwent statistical analysis using the SPSS statistical package (Version 12.00 for Windows, SPSS Inc., Chicago, Illinois, 2003). This included values obtained from chromatographs and other automated analytical devices, readings from rheological analysis, composition data, and manually collected sensory analysis responses. The information collected from consumers' sensory tests was anonymised before statistical analysis. Results are displayed as means  $\pm$  standard deviations (of at least three repetitions). A one-way analysis of variance (ANOVA) was performed to assess the variations between repetitions and the significance of differences, at a 5% level between mean values, was determined by Tukey's test.

## Results and discussion

### Flour composition and properties

#### Concentration in micronutrients of white wheat flour and of *Beta vulgaris* powder

A first indication of the differences in the mineral content between refined wheat flour (WF), cultivated chard (BC) and wild chard (BS) can be deduced from the results on ash content displayed in Table 1, which are respectively 0.015% (WF), 1.076% (BC), and 1.039% (BS).

Swiss chard powder from both wild and cultivated plants (BS and BC) contains significantly higher levels of  $K^+$ ,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Fe^{2+}$ , and  $Zn^{2+}$  than white wheat flour (WF), as can be verified in Table 1. Furthermore, the anthocyanin content, indicative of antioxidant activity, is considerably higher in Swiss chard (BC and BS) in comparison to white wheat flour, as expected. Swiss chard powder was found to be significantly higher than that of wild plants, indicating the potential influence of genetic factors on anthocyanin production. These results are in accordance with those reported by Mzoughi *et al.* (2019) and Gamba *et al.* (2021), who studied the composition in minerals, phytochemicals, and biological activities of *B. vulgaris* from Tunisia. It is important to note that differences in plant composition are highly variable due to a multitude of factors. These include environmental factors such as soil and weather conditions, as well as plant-specific factors such as genetic traits and plant location/organ. Additionally, differences in the analytical methodologies can contribute to variations in results. Nevertheless, these authors observed the high mineral content of wild *B. vulgaris* cultivars. Furthermore, our findings indicate that wild plants exhibit a higher mineral content than cultivated plants (Table 1).

**Table 1** Composition of wheat white flour (WF) and Swiss chard (*Beta vulgaris*) powder obtained from wild (BS) and from cultivated chard plants (BC)

Concentration (expressed in dry weight)	Wheat white flour (WF)	Wild <i>B. vulgaris</i> (BS)	Cultivated <i>B. vulgaris</i> (BC)
Ash (%)	0.015 <sup>c</sup> ± 0.009	1.039 <sup>b</sup> ± 0.008	1.076 <sup>a</sup> ± 0.00
$K^+$ (mg g <sup>-1</sup> )	5.30 <sup>c</sup> ± 0.10	25.66 <sup>a</sup> ± 0.62	21.30 <sup>b</sup> ± 0.24
$Ca^{2+}$ (mg g <sup>-1</sup> )	6.14 <sup>c</sup> ± 0.11	39.41 <sup>a</sup> ± 1.33	28.61 <sup>b</sup> ± 0.23
$Mg^{2+}$ (mg g <sup>-1</sup> )	0.12 <sup>c</sup> ± 0.003	5.81 <sup>a</sup> ± 0.01	4.72 <sup>b</sup> ± 0.04
$Fe^{2+}$ (mg g <sup>-1</sup> )	0.08 <sup>c</sup> ± 0.003	0.26 <sup>b</sup> ± 0.005	0.28 <sup>a</sup> ± 0.005
$Zn^{2+}$ (mg g <sup>-1</sup> )	0.01 <sup>c</sup> ± 0.00	0.049 <sup>a</sup> ± 0.004	0.033 <sup>b</sup> ± 0.001
Anthocyanin (mg g <sup>-1</sup> )	0.16 <sup>b</sup> ± 0.06	4.21 <sup>a</sup> ± 0.00	5.00 <sup>a</sup> ± 0.51

<sup>a,b,c</sup>Values in the same row with different superscript letters represent significant differences.

Our findings also indicated that the wild *B. vulgaris* contain higher levels of  $Ca^{2+}$  ( $39.41 \pm 1.33 \text{ mg g}^{-1}$ ) in comparison to the Ca values reported by Ullah *et al.* (2017) for several wild species, such as *Amaranthus thunbergii* ( $11.63 \text{ mg g}^{-1}$ ), *Allium astrosanguineum* ( $5.472 \text{ mg g}^{-1}$ ), and *Caralluma edulis* ( $5.378 \text{ mg g}^{-1}$ ).

The presence of Fe and Zn may also be relevant, from a nutritional viewpoint, since the daily recommended intake values of these elements are lower than those of Ca and Mg. Thus, if we consider a serving portion to be a small baguette (manufactured with approximately 1:1 flour blend and water), and the median enriching level of 2.5% (of chard powder blended with WF), then, using Table 1 figures, the concentration of Ca, per serving, would be increased by approximately 18.9 mg (BC) to 24.6 mg (BS) by that moderate level of chard addition. The Mg concentration would increase by approximately 3 mg per serving, plus 0.16–0.17 mg/serving Fe and 0.02–0.03 mg of Zn.

The European Food Safety Authority (EFSA, 2015) and the United States Food and Drug Administration (FDA, 2022) have established that an adult should ingest approximately 1000 mg of Ca and 400 mg of Mg daily. The recommended daily intakes (RDI) for Fe and Zinc, as established by the EFSA and the FDA, respectively, are 8–18 and 8–11 mg per day. If we disregard the issue of bioavailability for the sake of simplicity, the aforementioned contributions can be relevant to the recommended daily intakes of minerals. Therefore, *B. vulgaris* can be employed as a beneficial source of minerals in a strategy of fortification of foodstuffs with minerals.

#### Colour parameters of white wheat flour and of *Beta vulgaris* powder

As expected, chard powder (BS and BC) was darker (more than 10% difference in L\*) and much more pigmented (marked differences in a\* and b\* indexes) than WF (Table 2).

The chromatic properties of chard are a consequence of the presence of pigments such as chlorophylls (green), translated in the low a\* value, and anthocyanins (purple-blue), whose presence explains the negative b\* values. A significant concentration of anthocyanins was registered in chard powder (Table 1). Like other phenolic compounds, they are recognised for their health-promoting properties and have recently been acknowledged as phytonutrients (Mozos *et al.*, 2021; Issaoui *et al.*, 2021a, 2021b). According to our results (Tables 1 and 2), no major differences were found between wild (BS) and cultivated chard (BC), with respect to nutritional composition or chromatic properties.

**Table 2** Colour parameters of wheat white flour (WF) and of Swiss chard (*Beta vulgaris*) powder obtained from wild (BS) and from cultivated chard plants (BC)

Colour index (CIELAB colour scale)	Wheat white flour (WF)	Wild <i>B. vulgaris</i> (BS)	Cultivated <i>B. vulgaris</i> (BC)
L*	88.70 <sup>a</sup> ± 0.06	9.70 <sup>b</sup> ± 0.00	10.74 <sup>b</sup> ± 0.94
a*	0.36 <sup>b</sup> ± 0.05	1.58 <sup>a</sup> ± 0.01	1.54 <sup>a</sup> ± 0.03
b*	11.04 <sup>a</sup> ± 0.09	-1.40 <sup>b</sup> ± 0.00	-1.31 <sup>b</sup> ± 0.017

L\*: dark to light (0–100); a\*: green to red (–60 to +60), and b\*: blue to yellow (–60 to +60); <sup>a,b,c</sup>Values in the same row with different superscript letters represent significant differences.

### Aroma compounds

The presence of volatile compounds was investigated in WF, as well as in chard powder (BS and BC) given that they contribute to the sensory attributes of white bread and hence to consumer acceptance. As expected, when dealing with two different plant species, the profile of aroma compounds of wheat flour diverged. Aromatic compounds were identified in both, wheat flour and Swiss chard powder, although at varying concentrations. Other compounds were found to be more characteristic of each type of flour. For example, limonene was present in greater quantities in wheat flour (16.8%) than in Swiss chard (10%). Similarly, hexanal was found to be more abundant in wheat flour (19.5%) than in Swiss chard (6.2%–10.2%). The same trend (WF vs. BS/BC) was observed for  $\alpha$ -thujene (3% vs. 1%) and 2,2,4,6,6-pentamethylheptane (7.1% vs. 5.7%). Conversely, n-heptane,  $\alpha$ -pinene, sabinene, and 3-ethyl-1-hexanol are present in similar proportions in flours from both plant species (wheat and Swiss chard). However,  $\beta$ -pinene is present in significantly higher quantities in Swiss chard than in wheat flour (7.3% vs. 0.4%). The same applies to ethyl acetate (4.4 vs. 1.6), isovaleraldehyde (6.7 vs. 1.3%), and 2-methylbutanal (4.6% vs. 1.4%). In terms of series, non-terpene derivatives (mostly aldehydes and ketones), representing approximately 29–30% of the total compounds detected, appear to be nearly as abundant in WF as in BS/BC.

A number of differences were observed in the profile of the volatile compounds between wild (BS) and cultivated (BC) chard plants. For example, non-terpene aldehydes/ketones found to be higher in BS (29.1%) than in BC (12.9%), while non-terpene hydrocarbons were present in greater quantities in BC (14.1%) than in BS (7.1%). Conversely, non-terpene alcohols/ethers were found to be present in higher concentrations in BC than in BS (13.2% vs. 7.8%), as well as nitrogen/sulphur derivatives (16% vs. 5.1%). In terms of individual aromatic compounds, it was observed that BS exhibited a higher concentration than BC in ethyl

acetate (4.4 vs. 2.1%), isovaleraldehyde (6.7 vs. 1.2%), 2-methylbutanal (4.6 vs. 0.4%), n-heptane (5.7 vs. 2.3), hexanal (10.2 vs. 6.2),  $\alpha$ -pinene (4.0 vs. 2.6%), sabinene (10.3 vs. 8.9%), and  $\beta$ -pinene (7.3 vs. 5.7). In contrast, BC is richer than BS in tetramethylpyrazine (16.0 vs. 1.0%), acetic acid (17.5 vs. 3.9%), and 1,3-butanediol (9.1 vs. 0.5). Conversely, limonene shows consistent concentration levels in both cultivated and wild varieties of Swiss chard.

The aromatic composition of white and chard-fortified bakery products was also studied. A total of twenty-six aromatic compounds were identified in white bread, with 3-hydroxy-2-butanone (16.9%), ethyl acetate (14.2%), limonene (6.6%), 1,8-cineole (6.7%), and phenylethyl alcohol (6.2%) being the most prevalent. It is important to note that acetoin (or 3-hydroxy-2-butanone) is frequently employed as an industrial flavour enhancer, due of its distinctive creamy yogurt/butter-like aroma (Xiao & Lu, 2014; Issaoui *et al.*, 2021a, 2021b). Furthermore, acetoin is utilised as an energy source by certain fermentative bacteria (Xiao & Lu, 2014).

The presence of compounds absent in WF was also identified, including acetic acid (4.6%), isobutyl alcohol (2.9%), 3-hydroxy-2-butanone (16.9%), 2,3-butanediol (0.7%), methylpyrazine (1.4%), furfuryl alcohol (1.1%), 6-methyl-5-hepten-2-one (2.5%), and phenylethyl alcohol (6.2%). Nevertheless, we encountered significant challenges in establishing a clear causal relationship between these compounds and the aroma notes observed in the final bakery products. This is largely due to the complex nature of the sensorial attributes and perceptions involved. Cho & Peterson (2010) have identified around 540 volatile compounds in bread noting that only a part contributes to bread's aroma because not all are easily detectable by human olfactory receptors. From those, Pico *et al.* (2015) mention in their review that more than 300 were correlated with bread's scent and flavour. Some, directly correlate with positive sensory attributes and good consumer acceptance (e.g., 3-methyl-1-butanol), while others are associated with negative sensory attributes (e.g., hexanal and butyric acid). In between, many aroma compounds may have unclear negative or positive effects, depending not only on concentration but also on physico-chemical interactions in the food matrix. Traces of limonene in the crust release pleasant citrus olfactory sensations during bread mastication, while the presence of sabinene in the crumb conveys a woody aroma (Pico *et al.*, 2015).

Nevertheless, the results (as detailed in the aforementioned concentrations and Table 5) demonstrate that the aromatic profile of enriched white bread differs from that of WF's bread. For example, it was observed that the concentration of ethyl acetate was considerably higher in WF's bread than in

fortified bakery products (14.2% WF vs. 2.3% BC/BS, on average), whereas limonene, sabinene, and β-pinene exhibited an inverse trend (higher in BC/BS). It is also worthy of note that certain compounds are formed as a consequence of the baking process, including furanic compounds and pyrazine compounds, as previously discussed in our article (Issaoui *et al.*, 2021a, 2021b). Pyrazine derivatives, which are commonly used as flavouring agents in the food industry, were found to be present in higher concentrations in BC's fortified white bread than in the other types of tested bakery products. Thus, ten pyrazine compounds were identified in BC fortified white bread, eight pyrazine compounds in BS fortified white bread, and only one pyrazine compound in white bread (WF control). At a 5% BC fortification level, 38.6% of pyrazine derivatives were registered, whereas in the case of BS5, this level was 9.1%, and in white bread (WF) only 1.4% of pyrazine derivatives were detected. Similarly, furanic compounds were detected at higher concentrations in white bread enriched with 5% BC. Furan is a typical by-product of the Maillard reaction, and it is likely that the concentrations of this compound varied according to the specific blends that were tested.

**Properties of dough and white bread enriched with *B. vulgaris***

*Rheological properties of dough*

As can be seen in Table 3, the incorporation of chard (BS or BC) may slightly reduce the dough moisture, similarly to what was previously observed in other works with different plant-extract powders (Issaoui *et al.*, 2021a, 2021b). The tenacity of the dough, P, is an indicator of the resistance to deformation. The lowest P value was recorded for BC2 (67.33 ± 2.92 mm) and the highest for BC5 (120.33 ± 0.66 mm), significantly above the control. The addition of high levels of chard may thus negatively affect the technological properties of the dough, namely by increasing its resistance to deformation. However, when comparing the values for the rheological parameters of the doughs prepared with BS blends (with chard powder from plants gathered in the wild) and with BC blends (with chard powder obtained from cultivated plants), no statistically significant differences were found. Therefore, from a technological point of view, it seems indifferent to use wild or cultivated chard in enriching wheat white flour.

Extensibility, L, is an assessment of the dough elongation (mm) until rupture. Measurements ranged from 41 to 67 mm with an intermediate value of L for the control WF (around 65 mm) and were inconclusive. Also inconclusive were the measurements of the swelling ability of the dough, G, as well as the baking strength, W. The ANOVA test showed no statistically

**Table 3** Humidity of the flour (H), and rheological properties of the dough obtained from wheat white flour (WF) and from WF enriched with different % of powder from wild (BS) and cultivated (BC) *B. vulgaris*

	WF enriched with 1%-5% wild <i>B. vulgaris</i>					WF enriched with 1%-5% cultivated <i>B. vulgaris</i>					
	Control WF	BS1	BS2	BS3	BS4	BS5	BC1	BC2	BC3	BC4	BC5
H (%)	14.00 <sup>a</sup> ± 0.00	14.00 <sup>a</sup> ± 0.00	13.00 <sup>a</sup> ± 0.00	13.00 <sup>a</sup> ± 0.00	13.00 <sup>a</sup> ± 0.00	13.00 <sup>a</sup> ± 0.00	14.00 <sup>a</sup> ± 0.00	13.50 <sup>a</sup> ± 0.00	13.00 <sup>a</sup> ± 0.00	13.00 <sup>a</sup> ± 0.00	13.00 <sup>a</sup> ± 0.00
P (mm H <sub>2</sub> O)	79.33 <sup>de</sup> ± 3.17	91.00 <sup>bcd</sup> ± 1.52	90.00 <sup>bcd</sup> ± 1.52	97.33 <sup>bcd</sup> ± 6.43	96.66 <sup>bcd</sup> ± 3.84	104.66 <sup>ab</sup> ± 1.45	84.00 <sup>cde</sup> ± 3.51	67.33 <sup>e</sup> ± 2.92	79.16 <sup>de</sup> ± 1.87	102.16 <sup>abc</sup> ± 6.20	120.33 <sup>a</sup> ± 0.66
L (mm)	65.33 <sup>a</sup> ± 1.20	67.00 <sup>a</sup> ± 8.08	53.00 <sup>a</sup> ± 1.73	53.00 <sup>a</sup> ± 7.00	47.66 <sup>a</sup> ± 14.05	57.33 <sup>a</sup> ± 1.45	66.00 <sup>a</sup> ± 2.00	41.16 <sup>a</sup> ± 2.77	48.00 <sup>a</sup> ± 0.50	46.66 <sup>a</sup> ± 2.58	65.00 <sup>a</sup> ± 15.52
G (cm <sup>3</sup> )	18.63 <sup>a</sup> ± 0.23	18.30 <sup>a</sup> ± 1.23	15.73 <sup>a</sup> ± 0.64	16.13 <sup>a</sup> ± 1.04	15.00 <sup>a</sup> ± 2.30	16.96 <sup>a</sup> ± 0.49	18.03 <sup>a</sup> ± 0.27	14.20 <sup>a</sup> ± 0.48	15.38 <sup>a</sup> ± 0.08	15.16 <sup>a</sup> ± 0.43	14.86 <sup>a</sup> ± 0.79
W (10 <sup>-4</sup> J)	199.00 <sup>abc</sup> ± 8.02	227.00 <sup>a</sup> ± 8.96	152.33 <sup>de</sup> ± 7.35	177.66 <sup>cd</sup> ± 8.19	237.66 <sup>a</sup> ± 7.83	224.00 <sup>ab</sup> ± 2.08	201.33 <sup>bc</sup> ± 2.84	123.50 <sup>a</sup> ± 12.00	159.33 <sup>abc</sup> ± 7.09	176.33 <sup>cd</sup> ± 7.09	184.00 <sup>bcd</sup> ± 13.05
P/L	1.17 <sup>c</sup> ± 0.04	1.40 <sup>bc</sup> ± 0.20	1.71 <sup>abc</sup> ± 0.19	1.92 <sup>abc</sup> ± 0.37	1.80 <sup>abc</sup> ± 0.20	1.82 <sup>abc</sup> ± 0.03	1.50 <sup>bc</sup> ± 0.05	1.65 <sup>abc</sup> ± 0.05	1.73 <sup>abc</sup> ± 0.12	2.23 <sup>ab</sup> ± 0.27	2.53 <sup>a</sup> ± 0.12
le	56.43 <sup>a</sup> ± 0.51	57.50 <sup>a</sup> ± 4.01	40.76 <sup>ab</sup> ± 8.12	48.83 <sup>a</sup> ± 3.44	49.33 <sup>a</sup> ± 4.66	53.50 <sup>a</sup> ± 0.89	52.96 <sup>a</sup> ± 1.18	55.10 <sup>a</sup> ± 0.91	43.56 <sup>a</sup> ± 1.93	39.26 <sup>ab</sup> ± 0.20	23.93 <sup>a</sup> ± 6.28

H (%): humidity of the flour (w/w); Alveographic parameters: tenacity (P), extensibility (L), baking strength (W), elasticity (le). Bubble swelling index (G) represents the biaxial extensibility of the dough. WF: wheat white flour (refined, commercial type); BS1 to BS5: WF + 1 to 5% of wild *B. vulgaris* powder; BC1 to BC5: WF + 1 to 5% of cultivated *B. vulgaris* powder. Values in the same row with different superscript letters represent statistically significant differences between treatments for the same sample, at *P* < 0.05 by Tukey's test (*n* = 3).

significant differences. However, our measurements of the elasticity values of doughs made from the tested blends seem to point to a decrease in  $I_e$  in the presence of chard powder.  $I_e$  values seem to be concentration-sensitive with a minimum of 23.93 and the control value (WF) around 56. Our results from previous studies point in the same direction: enriching WF with plant-extract powders can lose the elasticity of the dough (Issaoui *et al.*, 2021a, 2021b). Several authors (Wang *et al.*, 2002; Borchani *et al.*, 2011; Fakhfakh *et al.*, 2017) reported similar observations regarding the enrichment of WF with plant materials as diverse as aromatic herbs (*Malvaceae*), mallow, or pea. According to those authors, such changes can be due to the proportional increase in dietary fibres in relation to gluten, which may interfere with the formation of the viscoelastic network to retain the released gas phase, during dough fermentation. On the one hand, the capacity of dietary fibres to retain water may interfere with gluten hydration, and on the other hand, the interactions between fibres and gluten may change the structure of the starch-protein network, thus affecting the plastic properties of the dough and the capacity to contain the gas bubbles. In other words, the tested flour blends may have an impact on the bread's matrix, and if so, effects beyond physical properties can be involved, such as changes in the kinetics of aroma release or in the bioavailability of micronutrients, that may be worth exploring in further studies.

#### Textural and chromatic properties of enriched white bread

When the bakery products were made from blends of WF with chard powder, the most noticeable change was in the colour of the crumb, in accordance with features disclosed in Fig. 1. As can be observed in Fig. 1, the greenish colour of the bakery products' samples on the right (c) is easily traced back to the plant (a), but after baking, a clear colour grading is

not so obvious to the naked eye. It is thus difficult to correlate, visually, the observed pigmentation with the concentration of chard in the flour blend. However, as expected, measurements showed proportional decreases in  $a^*$ ,  $b^*$  and  $L^*$  (brightness) that can be explained by the green colour incorporated in the chard powder (BS and BC). It is worth noting that the Maillard reactions (which occur at baking temperature, between the amino groups in protein and the carbonyl group of reducing sugars) may be different in the presence of BS/BC. The typical colour and aromas of wheat bread (WF) may change because of the different proportions of protein and sugars in the Maillard reactions and by the interference of other compounds such as fibres and pigments. A significant decrease in the brightness indicator  $L^*$  ( $P < 0.01$ ) was observed upon blending BS/BC with WF, as can be inferred from Table 2 and Fig. 1. As anticipated, the  $b^*$  indicator of the typical colour of baked white bread exhibited a notable decline for BS and BC-enriched bakery products (data not shown). Our findings are consistent with those reported by other authors, who have similarly observed that the incorporation of plant-based fortifiers alters the appearance of the newly formulated bakery product notably by reducing its luminosity. This has been attributed to the high ash, fibre, and polyphenol content of plant-based fortifiers (Peng *et al.*, 2010; Fakhfakh *et al.*, 2017; Ning *et al.*, 2017; Issaoui *et al.*, 2021a, 2021b).

Only slight changes in the white bread texture were registered, as can be observed in Fig. 1c. In other words, the rheological properties of the bakery products were much less impacted than the colour, by blending 1%–5% chard powder (BS or BC) with bakers' flour (WF), with noticeable effects mainly on adhesion and mastication. No overall significant differences were found in the rheological parameters of BC and BS samples (Table 4).



**Figure 1** (a) *Beta vulgaris*, cultivated plant (Swiss chard), (b) appearance of the dough made from blended wheat flour with chard powder, during alveograph measurements; (c) bread slices made from enriched wheat flour—from left to right, on top: BS1 to BS5 (1%–5% powder from wild chard), at the bottom row: BC1–BC5 (1%–5% powder from cultivated chard). WF control (white bread, from refined wheat flour) is placed at the centre.

As can be observed in Table 4, the mean values for hardness and mastication were lower than the control for all tested blends (from 1% to 5% of chard powder), as well as adhesion. In practical terms, it means that the consumer will only need to apply little effort (e.g., mastication and chewing) when eating the enriched white bread. On the other hand, textural properties, such as cohesiveness and elasticity, were not significantly affected in general, as also deduced by analysing Table 5.

### Sensory assessment of white bread formulations by consumers

A consumer acceptability test was conducted on five formulations of bakery products enriched with cultivated Swiss chard or wild Swiss chard, based on the physicochemical, nutritional, rheological, and textural properties studied. In order to assess consumer acceptability of the aforementioned new bakery products, a hedonic study was conducted. A total of 178 volunteer naive consumers were recruited in accordance with legal and ethical requirements and following the steps detailed in section 2.7. The participants were predominantly young women (70%), aged 19–29 years. There was considerable variation in educational level (from incomplete basic school to Ph.D.) and profession (with a fair distribution of unemployed/inactive, students, and multiple occupations), ensuring a diverse socioeconomic background in the respondents. These non-trained, naive consumers were asked to assess the overall appreciation of the products, which was mostly based on the aspects and smells. They were also asked to score the texture, taste and odour of the products on a 9-point hedonic scale. The responses were analysed (or further details, please see section 2.8 for details) and are displayed in Table 5.

The results of the overall consumer evaluation of white bread and bakery products enriched with wild and cultivated Swiss chard indicate a clear preference for fortified white bread over unenriched white bread. The appreciation scores for fortified white bread are consistently above 5, whereas white bread receives the lowest score at 4.9 which is rated as ‘Dislike slightly’. It is noteworthy that bread enriched with cultivated Swiss chard received the highest scores, reaching up to 6.33 (BC3) and 6.09 (BC1), indicative of a ‘Like slightly’ appreciation. Consequently, the fortification of white flour with cultivated Swiss chard led to a notable increase in the overall appreciation from ‘Dislike slightly’ to ‘Like slightly’. The overall appreciation of white bread enriched with wild Swiss chard falls between the extremes observed for bread enriched with cultivated Swiss chard, with scores ranging from 5.37 to 5.75.

**Table 4** Textural properties of the bakery products made from white wheat flour (WF) and from WF enriched with 1%–5% of wild (BS) and cultivated (BC) *B. vulgaris* powder

Rheological parameters	WF enriched with 1%–5% cultivated <i>B. vulgaris</i>										
	Control (WF)	BS1	BS2	BS3	BS4	BS5	BC1	BC2	BC3	BC4	BC5
Hardness (N)	1.65 ± 0.09 <sup>a</sup>	0.85 ± 0.10 <sup>bcd</sup>	0.74 ± 0.00 <sup>d</sup>	0.99 ± 0.00 <sup>bc</sup>	0.88 ± 0.00 <sup>bcd</sup>	1.07 ± 0.03 <sup>b</sup>	0.91 ± 0.04 <sup>bcd</sup>	0.72 ± 0.08 <sup>cd</sup>	0.93 ± 0.00 <sup>bcd</sup>	1.11 ± 0.08 <sup>b</sup>	0.65 ± 0.02 <sup>d</sup>
Cohesiveness	0.42 ± 0.00 <sup>bcd</sup>	0.31 ± 0.00 <sup>bcd</sup>	0.40 ± 0.01 <sup>bcd</sup>	0.45 ± 0.01 <sup>ab</sup>	0.46 ± 0.00 <sup>a</sup>	0.28 ± 0.00 <sup>de</sup>	0.24 ± 0.00 <sup>e</sup>	0.29 ± 0.00 <sup>de</sup>	0.31 ± 0.04 <sup>bcd</sup>	0.25 ± 0.04 <sup>e</sup>	0.43 ± 0.06 <sup>abc</sup>
Elasticity (mm)	7.05 ± 0.00 <sup>ab</sup>	7.16 ± 0.1 <sup>ab</sup>	7.28 ± 0.54 <sup>ab</sup>	8.56 ± 0.35 <sup>a</sup>	8.54 ± 0.16 <sup>a</sup>	5.73 ± 0.22 <sup>b</sup>	6.27 ± 0.09 <sup>ab</sup>	6.06 ± 0.38 <sup>b</sup>	7.90 ± 0.42 <sup>ab</sup>	5.76 ± 1.18 <sup>b</sup>	8.62 ± 0.37 <sup>a</sup>
Elasticity index	0.70 ± 0.00 <sup>ab</sup>	0.71 ± 0.01 <sup>ab</sup>	0.60 ± 0.038 <sup>b</sup>	0.85 ± 0.03 <sup>ab</sup>	0.85 ± 0.016 <sup>ab</sup>	0.58 ± 0.02 <sup>b</sup>	0.78 ± 0.00 <sup>ab</sup>	0.72 ± 0.05 <sup>ab</sup>	0.87 ± 0.05 <sup>ab</sup>	0.69 ± 0.18 <sup>ab</sup>	0.98 ± 0.03 <sup>a</sup>
Adhesion (N)	0.75 ± 0.04 <sup>a</sup>	0.33 ± 0.03 <sup>bc</sup>	0.25 ± 0.00 <sup>c</sup>	0.51 ± 0.02 <sup>b</sup>	0.44 ± 0.01 <sup>bc</sup>	0.36 ± 0.02 <sup>bc</sup>	0.28 ± 0.01 <sup>c</sup>	0.34 ± 0.02 <sup>bc</sup>	0.36 ± 0.036 <sup>bc</sup>	0.34 ± 0.08 <sup>bc</sup>	0.33 ± 0.04 <sup>bc</sup>
Mastication (Nmm)	5.35 ± 0.29 <sup>a</sup>	2.38 ± 0.24 <sup>cd</sup>	1.55 ± 0.08 <sup>d</sup>	4.41 ± 0.016 <sup>ab</sup>	3.79 ± 0.18 <sup>abc</sup>	2.11 ± 0.23 <sup>cd</sup>	1.80 ± 0.055 <sup>d</sup>	2.49 ± 0.08 <sup>cd</sup>	2.81 ± 0.13 <sup>bcd</sup>	2.29 ± 0.88 <sup>cd</sup>	2.91 ± 0.54 <sup>bcd</sup>

BS1–BS5 refers to blends of wheat white flour (WF) with 1%–5% of *Beta vulgaris* (collected in the wild in Tunisia) dried and ground to powder, while BC1 to BC5 refer to the powder obtained from cultivated plants.

a, b, c, d, e: Values in the same row with different superscript letters represent significant differences between treatments for the same sample at  $P < 0.05$  by Tukey's test ( $n = 3$ ).

**Table 5** Average sensory evaluation scores (1–9 scale) obtained for white bread (WF, control) and for white bread enriched with 1%–5% powder from wild (BS) or cultivated (BC) plants of *B. vulgaris*, by naïf volunteer consumers

	Wheat white flour + wild <i>B. vulgaris</i>					Wheat white flour + cultivated <i>B. vulgaris</i>					
	WF	BS1	BS2	BS3	BS4	BS5	BC1	BC2	BC3	BC4	BC5
Taste	6.32 <sup>a</sup> ± 0.15	5.41 <sup>b</sup> ± 0.18	5.52 <sup>b</sup> ± 0.16	5.72 <sup>b</sup> ± 0.16	5.92 <sup>b</sup> ± 0.16	5.67 <sup>b</sup> ± 0.17	6.33 <sup>a</sup> ± 0.15	6.11 <sup>b</sup> ± 0.15	6.09 <sup>a</sup> ± 0.14	5.6 <sup>b</sup> ± 0.17	5.7 <sup>b</sup> ± 0.16
Colour	6.05 <sup>a</sup> ± 0.16	5.72 <sup>b</sup> ± 0.18	5.72 <sup>b</sup> ± 0.17	5.39 <sup>b</sup> ± 0.17	5.82 <sup>b</sup> ± 0.16	5.56 <sup>b</sup> ± 0.17	6.16 <sup>a</sup> ± 0.15	6.13 <sup>a</sup> ± 0.15	5.95 <sup>ab</sup> ± 0.16	5.66 <sup>b</sup> ± 0.17	5.77 <sup>b</sup> ± 0.16
Odour	6.09 <sup>a</sup> ± 0.15	5.75 <sup>b</sup> ± 0.17	5.67 <sup>b</sup> ± 0.18	5.55 <sup>b</sup> ± 0.17	5.84 <sup>b</sup> ± 0.17	5.68 <sup>b</sup> ± 0.17	5.95 <sup>ab</sup> ± 0.16	6.05 <sup>a</sup> ± 0.15	5.57 <sup>b</sup> ± 0.16	5.75 <sup>b</sup> ± 0.16	5.77 <sup>b</sup> ± 0.16
Texture	5.97 <sup>ab</sup> ± 0.17	5.64 <sup>b</sup> ± 0.17	5.72 <sup>b</sup> ± 0.17	5.51 <sup>b</sup> ± 0.16	5.72 <sup>b</sup> ± 0.17	5.63 <sup>b</sup> ± 0.17	6.19 <sup>a</sup> ± 0.15	6.05 <sup>a</sup> ± 0.15	5.91 <sup>ab</sup> ± 0.16	5.4 <sup>b</sup> ± 0.17	5.64 <sup>b</sup> ± 0.17
Overall appreciation	4.90 <sup>c</sup> ± 0.19	5.37 <sup>b</sup> ± 0.17	5.43 <sup>b</sup> ± 0.17	5.43 <sup>b</sup> ± 0.17	5.75 <sup>b</sup> ± 0.16	5.52 <sup>b</sup> ± 0.17	6.09 <sup>a</sup> ± 0.15	5.94 <sup>ab</sup> ± 0.16	6.33 <sup>a</sup> ± 0.14	5.72 <sup>b</sup> ± 0.17	5.63 <sup>b</sup> ± 0.17

Values in the same row with different superscript letters represent significant differences between treatments for the same sample at  $P < 0.05$  by Tukey's test.

BC1, white wheat flour enriched with 1% of cultivated Swiss Chard; BC2, white flour enriched with 2% of cultivated Swiss Chard; BC3, white flour enriched with 3% of cultivated Swiss Chard; BC4, white flour enriched with 4% of cultivated Swiss Chard; BC5, white flour enriched with 5% of cultivated Swiss Chard; BS1, white wheat flour enriched with 1% of wild Swiss Chard; BS2, white flour enriched with 2% of wild Swiss Chard; BS3, white flour enriched with 3% of wild Swiss Chard; BS4, white flour enriched with 4% of wild Swiss Chard; BS5, white flour enriched with 5% of wild Swiss Chard; WF, white wheat flour.

In terms of taste, consumers demonstrated a preference for recipes with moderate proportions of BC (ranging from 1% to 3%). This trend is evident through the analysis of aggregated mean scores, which indicate that consumers slightly like this new taste, scoring 6.33 for BC1, 6.11 for BC2, and 6.09 for BC3, on a scale of 1–9.

Regarding odour appreciation, we observed a remarkable stability in the scores, which ranged from 6.09 to 5.55. This reflects an appreciation ranging from 'Neither like nor dislike' to 'Like slightly' (Table 5).

It can be observed that the texture of white bread fortified with cultivated Swiss chard is slightly more appreciated than that of the other bakery products' samples. Thus, the highest appreciation score was observed for white bread enriched with 1% and 2% of cultivated Swiss chard, with scores of 6.19 and 6.05 (out of 9), respectively.

The sensory evaluation of the different bread recipes does not demonstrate a clear and distinct consumer preference for BS or BC, despite the greater variability of responses obtained for formulations containing BS. In brief, the taste of the enriched bread formulations was observed to be differ slightly from that of the control, but was deemed acceptable (6 out of 9). The results of the olfactory were comparable. The texture of the enriched bread formulations was well accepted, and consumers expressed a positive overall appreciation of those containing moderate quantities of chard (e.g., 1% and 2%). The rheological properties of white bread remained acceptable when moderate amounts of chard powder were blended into bakers' flour (control). Despite the changes in colour and flavour, white bread enriched with moderate amounts of chard powder was well accepted by Tunisian consumers (Table 5).

### Health, nutritional, and environmental considerations about enriching white bread

This study demonstrates the potential for enhancing the nutritional quality of mainstream bakery products by blending moderate amounts of a native culinary herb, *B. vulgaris*, which is well-known among Tunisian consumers. It can be reasonably anticipated that the incorporation of this native culinary herb into mainstream bakery products will result in a positive impact on public health. This is due to the fact that the daily intake of green leafy vegetables will increase, while micronutrients are conveyed and the caloric apport is decreased (Gamba *et al.*, 2021). According to Anses-Cinqual (2020), 100 g of the fresh, raw vegetable provides 16.4 Kcal. *B. vulgaris* (Swiss chard) is a promising source of phytonutrients, in addition to minerals, corroborating the findings of other authors (Mohammed *et al.*, 2019; Mzoughi *et al.*, 2019; Gamba

*et al.*, 2021). This suggests the potential for health benefits in the prevention of type 2 diabetes (Sener *et al.*, 2002), atherosclerosis (Freeman *et al.*, 2017) and other conditions.

In light of the main nutritional features and consumer preferences, there is no clear rationale for preferring wild chard to cultivated varieties. From an environmental perspective, the extensive collection of wild plants may contribute to the risk of extinction for certain subspecies (Valavanidis & Vlachogianni, 2013; Delgado *et al.*, 2023). It is therefore recommended that moderate amounts of cultivated chard powder (e.g., 2%) be used to enrich white bread with minerals, fibres, and phytonutrients.

According to Kaim & Goluch (2023) bread fortification can be a powerful tool to sustainably improve public health, notably through responsible and ethical marketing of these products. It is crucial to address both, SDG2 (zero hunger) and SDG12 (sustainable production and consumption) simultaneously. With respect to food security (SDG2), it is possible to simultaneously address nutrition security, through fortification, as well as building resilience against wheat availability by replacing part of it in bread making. With respect to SDG12, a paradigm shift is required in the production of wheat-based products. It is not logical to extract macronutrients from the wheat grain and reassemble them in a wasteful manner. From the perspective of consumer behaviour, there is a need to enhance food literacy and encourage informed, healthier choices.

## Conclusion

As anticipated, the most commonly used refined flour was found to be deficient in minerals, vitamins, and fibres. Such potential nutritional deficiencies can be tackled by the addition of chard, which may also convey minerals, phytonutrients (with antioxidant and anti-inflammatory activities) such as anthocyanins and terpenoids.

The nutritional quality of the resulting bakery product can thus be significantly improved by blending 2% chard powder with refined wheat flour, notably with respect to minerals, without significant changes in the dough's rheological properties or in the bread's texture. The addition of chard resulted in differences in aroma compounds, which may be attributed to the presence of bioactive aroma compounds, such as  $\alpha$ -pinene in chard. In essence, with minimal alterations at the manufacturing stage and with consumer acceptance, it is feasible to produce an innovative bread of enhanced nutritional quality, while sustainably valorising a native culinary vegetable that is easily cultivated.

In light of the current consumer preferences regarding bread (notably in Tunisia) and the

prevalence of unhealthy and unsustainable bakery products, a short-term strategy is proposed to address the acknowledged poor nutritional quality and high environmental impact of such globalised ultra-processed foodstuffs. It is proposed that wheat white bread be enriched with micronutrients from *B. vulgaris* powder, thereby showcasing the benefits of *B. vulgaris* and encouraging its sustainable cultivation. In the long term, a balance between public health and nature conservation may be achieved by educating consumers in making healthier and sustainable choices, and by advocating the adoption of good environmental practices by producers, and law enforcement.

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## Author contributions

**Manel Issaoui:** Conceptualization; methodology; investigation; writing – original draft; writing – review and editing; formal analysis; supervision; resources; funding acquisition; validation. **Samia Oueslati:** Investigation; methodology; validation; data curation; writing – review and editing; formal analysis. **Guido Flamini:** Investigation; methodology; validation; resources. **Amélia M. Delgado:** Conceptualization; investigation; writing – original draft; writing – review and editing; formal analysis; validation; data curation. **Anabela Romano:** Investigation; writing – original draft; validation; writing – review and editing; data curation; supervision; funding acquisition.

## Conflict of interest

All authors declare that there is no conflict of commercial interest or correlative interest in relation to the work herein submitted.

## Data availability statement

Data generated or analysed during this study are mostly included in this published article. Restrictions apply to the availability of some data (e.g., sensory acceptance test). However, data are available from the corresponding authors on reasonable request, if not involving privacy, ethical/legal issues.

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