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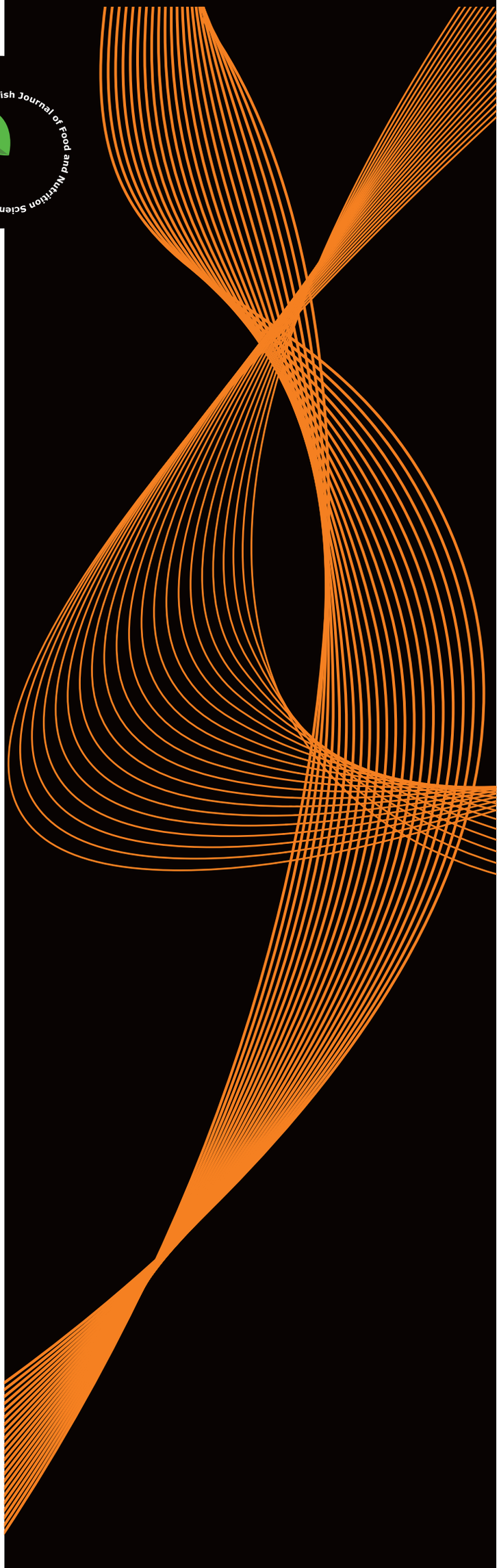

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Contents

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REVIEW ARTICLE

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Edukacji i Nauki

W latach 2022–2024 kwartalnik naukowy ***Polish Journal of Food and Nutrition Sciences*** realizuje projekt nr RCN/SP/0520/2021/1 finansowany ze środków budżetu państwa – Ministerstwo Edukacji i Nauki w ramach programu „Rozwój czasopism naukowych". Dofinansowanie wynosi 120 000 zł. W ramach projektu podejmowane są działania zmierzające do podniesienia poziomu praktyk wydawniczych i edytorskich, zwiększenia wpływu czasopism na rozwój nauki oraz utrzymania się czasopism w międzynarodowym obiegu naukowym.

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Truffle Species Discrimination Based on Their Chemical Composition, Chromaticity Coordinates and Antioxidant Capacity

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Some edible truffle species are more sought after and expensive than others while they have a similar colour and appearance. This therefore leads to high risk of fraud. To prevent these frauds, this study proposes to explore the chemical composition and antioxidant capacity to discriminate the six-truffle species encountered in France. To achieve this, infrared spectrometry analysis, chromaticity measures and atomic absorption analysis were performed on dehydrated truffle powder as well as antioxidant capacity (ABTS and CUPRAC assays) and total phenolic content analyses were performed for truffle aqueous ethanol extracts. Infrared spectrometry analysis provided results allowing to discriminate the six-truffle species using a chitin/chitosan ratio (1,659 cm^{-1}/1,627 cm^{-1}) determined in the range of 0.75 to 0.93 or a β/α-glucan ratio (889 cm^{-1}/850 cm^{-1}) in the range from 1.50 to 1.81. Colour coordinates, including *L**, *a** and *b** values, ranged from 20.56 to 36.35, 1.62 to 4.23 and 2.78 to12.9, respectively, and differed significantly between species. Truffle calcium and magnesium content was 2.62–0.48 mg/g dry weight and 0.91–0.21 mg/g dry weight, which also differentiated truffle species. Total phenolic content and antioxidant capacity analyses allowed to discriminate most of the six-truffle species but not each of the species. Thus, biophysical approaches and, to a lesser extent, the antioxidant activity assays, and total phenolic content are credible means of identifying truffle species found in France.

Keywords: antioxidant activity, atomic absorption, ATR-FTIR, colour parameters, *Tuber* species

ABBREVIATIONS

ABTS, 2-2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ATR-FTIR, Attenuated total reflectance-Fourier transform infrared spectroscopy; CUPRAC, Cupric ion reducing antioxidant capacity; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; ORAC, Oxygen radical absorbance capacity.

INTRODUCTION

Truffles (*Tuber* spp.) are edible Ascomycota, hypogeous ectomycorrhizal funguses which live in symbiosis with the plant. What is classically named "truffle" consists in a developed fruiting body corresponding to hyphae aggregate. Truffles have been used as human food since the Bronze era [Shavit, 2014].

At least 180 truffle species are currently known worldwide [Bonito *et al.*, 2010] although only 70 are undoubtedly established [Ceruti *et al.*, 2003; Jeandroz *et al.*, 2008]. Six truffle species are found and/or commercialised in France: *Tuber aestivum, Tuber brumale, Tuber indicum, Tuber magnatum*, *Tuber melanosporum*, and *Tuber uncinatum*. On the one hand, some truffle species are in great demand due to their special taste and aroma. These are mainly *T. melanosporum*, the most wanted and cultivated species, *T. uncinatum* and to a lesser extent *T. magnatum* (from Italy). On other hand, other species like *T. aestivum, T. brumale* and *T. indicum*, have

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an inferior flavour and are less sought after. However, distinguishing fruitbodies based on their morphology is not possible due to their similar appearance and colour. Therefore, fraud risks may arise by marketing low-value truffle species as high-value species. Thus, molecular methods for the identification of truffle species (DNA marker identification) have been developed since the 1980's, especially for *T. melanosporum* and *T. magnatum* identification [Paolocci *et al.*, 1997; Rubini *et al.*, 1998; Séjalon-Delmas *et al.*, 2000]. However, there are no data available concerning *T. brumale* DNA, and it has never been tried to discriminate up to 6 truffle species. To sum up, there is no approach today, allowing to discriminate the 6 *Tuber* species met in France.

Truffle chemical composition is already known [Yan *et al.*, 2017]. Their cell wall contains chitin, chitosan, glucans, mannans, and proteins [Bowman & Free, 2006] sometimes associated with melanin mainly in the case of black truffles [Gessler *et al.*, 2014]. They contain phenolics including flavonoids [Beara *et al.*, 2014; Shah *et al.*, 2020b] and fatty acids [Sancholle *et al.*, 1988; Yan *et al.*, 2017]. Some information about their mineral composition is also available in the Ciqual French food composition table (**https://ciqual.anses.fr/**). Surprisingly, identification approaches of *Tuber* species based on their composition variation have never been developed.

In addition to their composition, truffles have a number of interesting properties including antioxidant potential [Shah *et al.*, 2020a; Tejedor-Calvo *et al.*, 2021; Wu *et al.*, 2021]. Indeed, antioxidants in food are beneficial for health with an impact at a molecular scale, inhibiting lipid oxidation for instance [Zehiroglu & Ozturk Sarikaya, 2019], and also their broader effects are supposed preventing putatively Alzheimer's disease through, *e.g.*, inhibition of acetylcholinesterase [Wilson *et al.*, 2017]. Tejedor-Calvo *et al.* [2021] demonstrated 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and/or ferric reducing antioxidant power (FRAP) and/or oxygen radical absorbance capacity (ORAC) of truffle species. The antioxidant capacity of truffles is therefore a potential way to discriminate truffle species.

Considering all these data together, it appears interesting to explore variations in truffle chemical composition, chromaticity and antioxidant capacity to discriminate truffle species. This study proposes to evaluate in this respect the six species of truffles found or sold in France including *T. aestivum, T. brumale, T. indicum, T. magnatum*, *T. melanosporum*, and *T. uncinatum*. Firstly, biophysical analysis of the truffles using attenuated total reflectance-Fourier transform infrared spectrometry (ATR-FTIR), chromaticity and atomic absorption spectrometry were carried out. Then, their antioxidant activity was evaluated using hydrogen atom transfer and single-electron transfer approaches. The detection of the authenticity of truffle species can be used in science to detect fraud.

MATERIALS AND METHODS

Biological materials

T. melanosporum was bought from "Truffefrance" (SAS Terroir Gourmand, Millau, France); *T. magnatum* from S.Z. Tartufisnc (Atessa, CH, Italy) and *Tuber aestivum* from La Rabasse De L'enclave (Valreas, France). Listed truffles were purchased in dehydrated slices form. *T. brumale* and *T. uncinatum* were purchased in frozen broken from La Rabasse De L'enclave (Valreas, France). *T. indicum* (Himalayan truffles) was purchased canned from Maison Borde (Saugues, France) and then was frozen. All frozen samples were lyophilised for 3 days. Each sample of dehydrated truffle was powdered using a grinder (IKA A11 classic, Staufen im Breisgau, Germany) for 30 s. These powders were stored in an oven at 40°C (a humidity-free environment) before analysis.

Chemicals

$FeCl_3$, KCl, neocuproine and nitric acid were purchased from Sigma (Saint Louis, MO, USA). 2-2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), absolute ethanol, ammonium acetate, $CaCl_2$, NaCl, $CuCl_2$, $CuSO_4$, gallic acid, $MgSO_4$, potassium persulfate, and Trolox were purchased from Grosseron (Coueron, France).

ATR-FTIR spectrometry analysis

Reflectance spectra were acquired using a Bruker V70 interferometer (Bruker, Billerica, MA, USA) working under a dehydrated airflow in a reflectivity mode, with an ATR accessory containing a gold crystal. The measurements were performed for wave numbers from the middle of the infrared range (between 500 cm^{-1} and 4,500 cm^{-1}). The instrumental resolution was about 1 cm^{-1}, and measurements were averaged on 64 scans. Ten measures were done for each truffle species. ATR-FTIR measure is not a directly quantitative approach; hence, to present different spectra on a comparable scale, they were normalised by their standard deviation. To provide quantitative analyses, ratios between IR bands were established as used in the literature [Renouard *et al.*, 2014; Rytwo *et al.*, 2015].

Chromaticity analysis

Chromaticity analyses were conducted on a dehydrated truffle powder using Konica Minolta CR-400 colorimeter (Tokyo, Japan). Coordinates in the CIELab colour space were recorded, *i.e.*, lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*). Ten measures were performed for each truffle species.

Mineral composition analysis

Dehydrated truffle samples were heated to redness in a muffle furnace at 700°C for 7 h. The ashes obtained were then solubilised in 1 to 5% nitric acid. The main ions found in living organisms: calcium, copper, iron, magnesium, potassium and sodium, were analysed from these solutions using an atomic absorption spectrometer AAnalyst 200 Perkin Elmer (Waltham, MA, USA). Quantification was performed based on the calibration curves (with R^2>0.99) obtained using NaCl, $CaCl_2$, KCl, $FeCl_3$, $CuSO_4$, and $MgSO_4$. Five measurements were performed for each truffle species.

Extraction and quantification of phenolic compounds

Dried truffle powders (30 mg) were mixed with 1 mL of an ethanol/water (40/60, *v/v*) mixture. Samples were sonicated

for 30 min at 40°C and 37 kHz (Elmasonic P30H ultrasonic bath, Singen, Germany); then centrifuged at 525×*g* for 10 min. Lastly, supernatants were collected for phenolic compound content and antioxidant capacity analyses. Five repetitions were conducted *per* species.

Total phenolic compound quantification was carried out using the Folin–Ciocalteu assay [Singleton & Rossi, 1965]. An aliquot of 50 µL of the supernatant (or 40% ethanol as control) was added to 800 µL of distilled water. Then, 50 µL of the Folin–Ciocalteu reagent were added, and the mixture was vortexed and left to stand for 5 min. A volume of 100 µL of 1 M Na_2CO_3 solution was then incorporated, and the mixture was incubated in the dark for 2 h. Finally, the absorbance of the solution was measured at 725 nm with a spectrophotometer microplate (FlexA-200, Allsheng, Hangzhou, China). A gallic acid calibration curve was plotted in the concentration range of 12.5–400 mg/L. Each measurement was performed in triplicate, and the total phenolic content was expressed as mg of gallic acid equivalent (GAE)/g dry weight (DW) of truffle powder.

Antioxidant capacity analysis

Two methods were used to evaluate the antioxidant capacity of truffles. Firstly, the $ABTS^{\bullet+}$ scavenging capacity was determined based on Re *et al.* [1999] method with few adaptations. The $ABTS^{\bullet+}$ was obtained from the reaction between the ABTS solution (7 mM in Milli-Q water) and the 2.45 mM potassium persulfate solution. The mixture was incubated in darkness at 25°C for 16 h. The $ABTS^{\bullet+}$ solution was diluted till its absorbance reached 0.7 at 734 nm. Then, 3 mL of the $ABTS^{\bullet+}$ solution was mixed with 100 µL of the truffle extract at 30°C for 10 min, and its absorbance was measured at 734 nm. All solutions were used no later than 24 h after preparation. The analyses were performed in triplicate, and the $ABTS^{\bullet+}$ scavenging capacity was expressed as µmol Trolox equivalent/g DW using a standard curve for Trolox with the concentration in the range of 31.25–500 µM (R^2=0.9991).

The second method, the cupric ion reducing antioxidant capacity (CUPRAC) of truffles was determined with a protocol adapted from Apak *et al.* [2007]. An aliquot of 10 µL of the truffle extract was added to 190 µL of a 0.01 M copper (II) chloride solution. The copper (II) chloride solution consisted in a mixture of $CuCl_2 \times 2H_2O$, ammonium acetate buffer (pH 7) and 7.5×10^{-3} M neocuproine (2,9-dimethyl-1,10-phenanthroline) diluted in ethanol, with the following proportions: 1:1:1, *v/v/v*). After 30 min rest, the absorbance was measured at 450 nm. The CUPRAC was expressed as µmol Trolox equivalent/g DW. Standard curve plotted for Trolox (31.25–1,000 µM, R^2=0.9993) was used to calculate the results.

Statistical analysis

All data were presented in this study as the mean and standard deviation of ten replicates for ATR-FTIR and chromaticity analysis; five replicates for atomic absorption analysis, and three for CUPRAC, ABTS and Folin-Ciocalteu assays. Comparative statistical analysis of groups was performed using Student's *t*-test. Statistical differences were considered to be significant at $p<0.05$. Graphical and statistical treatments were performed using Microsoft EXCEL 2010 software (Microsoft, Redmond, WA, USA). The principal component analysis (PCA) was performed using XLSTAT 2022.2.1 software (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Chemical characterization of truffles using ATR-FTIR spectroscopy

The ATR-FTIR measurement is not a directly quantitative approach. Considering one sample, ATR-FTIR analysis will provide spectra always presenting the same proportion between the IR bands but from one measure to another – the global spectrum intensity may change. Accordingly, quantitative analysis requires using ratios between IR bands. Then, to present different spectra on a comparable scale, they need to be normalised.

Zhao *et al.* [2006] demonstrated the infrared spectrometry ability to discriminate truffle species on 3 Chinese truffle species. On the other hand, in addition to being a simple, fast and economical biophysical approach, ATR-FTIR is known as a relevant tool in food fraud detection [Valand *et al.*, 2019].

In **Figure 1A**, ATR-FTIR spectra of truffles of different species are presented as normalised by their respective standard deviation. First, the six species presented a global similar profile. Moreover, these profiles were similar to those obtained *via* the FTIR analysis by Zhao *et al.* [2006]. Four common massive absorbance areas were noteworthy in the truffles FTIR spectra (**Figure 1A**). The first area centered at 3,390 cm^{-1} corresponded to the stretching mode of hydroxyl bond (abundant in biological samples) [Grošev *et al.*, 2001; Pappas *et al.*, 2003]. The second area, between 3,000 and 2,800 cm^{-1}, corresponded to fatty acid found in cell membranes with a band at about 2,925 cm^{-1} assigned to out-of-phase CH_2 stretching and another at about 2,855 cm^{-1} assigned to in-phase CH_3 stretching [Lin *et al.*, 2004; Pappas *et al.*, 2003]. The third area, between 1,800 and 1,500 cm^{-1}, showed two main bands centered at about 1,650 and 1,560 cm^{-1} that are respectively associated to amide I and amide II [Payne & Veis, 1988] herein met in chitin and chitosan, which are two important fungal cell wall components. The last area, between 1,250 and 750 cm^{-1} and presenting an important maximum at about 1,042 cm^{-1}, was assigned to C–O stretching in carbohydrate [Grošev *et al.*, 2001; Pappas *et al.*, 2003; Silva *et al.*, 2001].

To discriminate truffle species by ATR-FTIR, it was necessary to look for the discriminating ratio linked to the truffle's composition. Among all the fungal components for which specific IR bands are known (α- and β-glucan, aromatic compounds, chitin, chitosan, fatty acids, melanin and proteins), only two IR band ratios allow to statistically distinguish the six-truffle species: a chitin/chitosan ratio (1,659 cm^{-1}/1,627 cm^{-1}) and a β/α-glucan ratio (889 cm^{-1}/850 cm^{-1}). Dahmane *et al.* [2014] established that 1,659 cm^{-1} and 1,627 cm^{-1} IR bands corresponded respectively to chitin and chitosan from fungi cell wall. For the chitin/chitosan ratio, the ratio values ranged from 0.753 for *T. brumale* to 0.931 for *T. indicum* (**Figure 1B**). Concomitantly, Synytsya & Novak [2014] presented 889 cm^{-1} and 850 cm^{-1} as IR bands of β- and α-glucan respectively. For the β/α-glucan ratio, the ratio values ranged

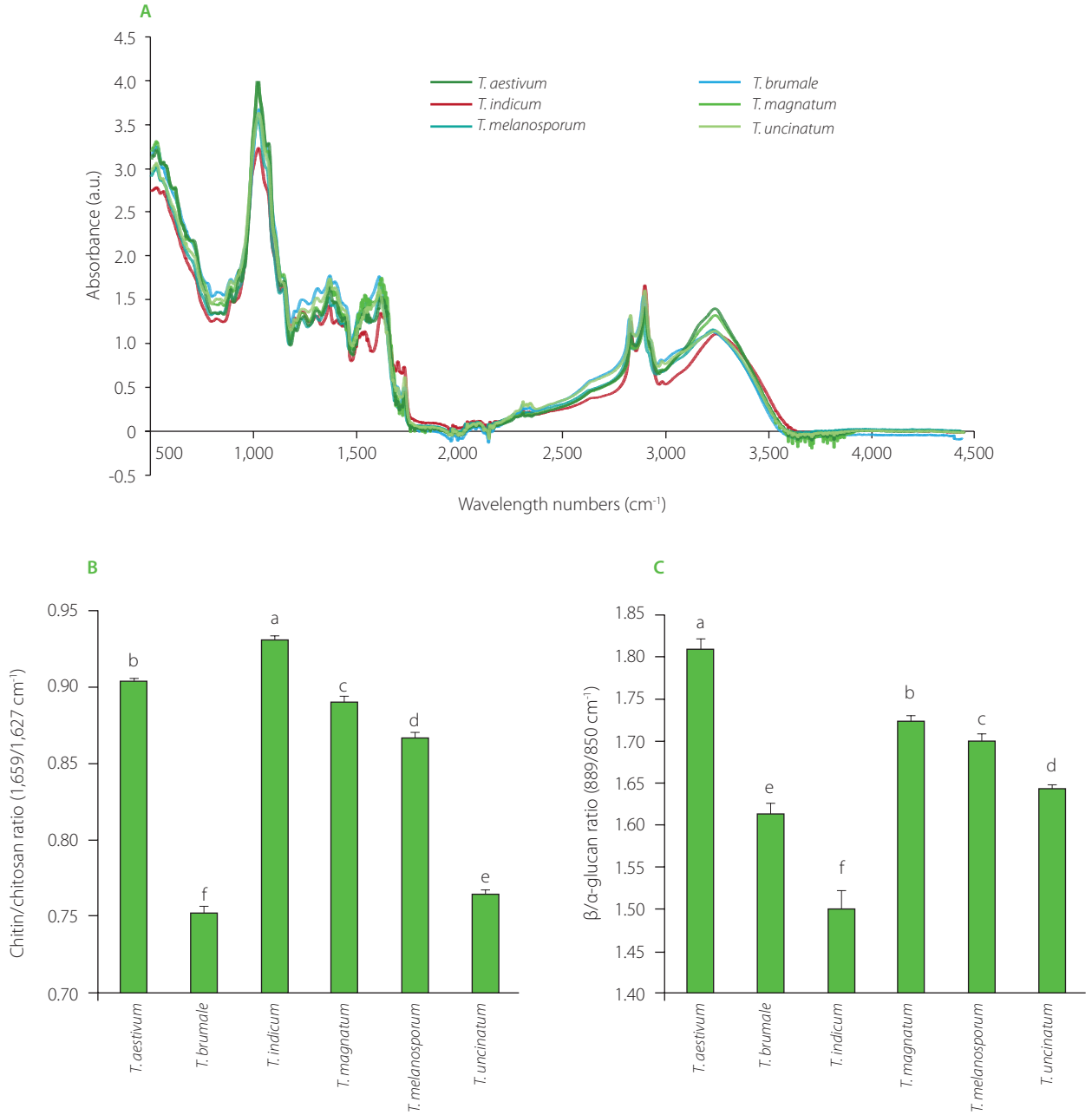

Figure 1. Normalised attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra (**A**), chitin/chitosan ratio (1,659 cm^{-1}/1,627 cm^{-1}) (**B**), and β/α-glucan ratio (889 cm^{-1}/850 cm^{-1}) (**C**) of powders of *Tuber* species. Bars with different letters differ significantly one from another at $p<0.05$.

from 1.50 for *T. indicum* to 1.81 for *T. aestivum* (**Figure 1C**). Thus, both chitin/chitosan ratio and a β/α-glucan ratio allowed to perfectly distinguish the six-truffle species.

Truffles, like all fungi, are characterised by their virulence: their growth time, their resistance to other organisms, their ability to find and interact with their host *etc*. Indeed, truffles are symbiotic fungi associated with a plant, but competition can exist between soil organisms hoping to interact with a plant (for instance *T. brumale* is able to compete with *T. melanosporum*) [Merényi *et al.*, 2016]. The ATR-FTIR results obtained in this study reflect the difference in virulence of each of the six-truffle species through chitin/chitosan ratio because the chitosan (obtained from chitin) is physiologically involved in the fungal virulence and evasion of host immune responses [Mouyna *et al.*, 2020].

Mineral composition of truffles

Atomic absorption analysis was performed to quantify calcium, copper, iron, magnesium, potassium and sodium, that are among the most frequently encountered ions in living organisms. Results indicate (**Table 1**) that only magnesium and calcium contents allowed *T. aestivum, T. brumale, T. indicum, T. magnatum*, *T. melanosporum*, and *T. uncinatum* discrimination.

No detailed data are available on the mineral ion composition of the different truffle species; but, according to the Ciqual French food composition tables (**https://ciqual.anses.fr/**) black truffles (without further species specification) show comparable calcium, copper, iron and sodium contents to our assays, but the magnesium and potassium contents presented therein (2.38 and 44.7 mg/g DW, respectively) were much higher than in our study (0.91 and 19.81 mg/g DW), potentially because the truffle

Table 1. Mineral element content of *Tuber aestivum, Tuber brumale, Tuber indicum, Tuber magnatum, Tuber melanosporum,* and *Tuber uncinatum* (mg/g dry weight) analysed by atomic absorption.

Truffle species	Calcium	Copper	Iron	Magnesium	Potassium	Sodium
T. aestivum	1.29±0.01^{d}	0.05±0.001^{b}	0.04±0.03^{c}	0.91±0.01^{a}	19.81±1.49^{a}	1.32±0.77^{d}
T. brumale	2.62±0.02^{a}	0.07±0.003^{a}	0.11±0.004^{a}	0.73±0.004^{c}	15.79±1.50^{b}	3.38±0.65^{c}
T. indicum	0.69±0.03^{e}	0.02±0.002^{d}	0.03±0.005^{d}	0.21±0.004^{f}	1.39±0.39^{e}	12.37±0.61^{a}
T. magnatum	0.48±0.01^{f}	0.04±0.001^{c}	0.10±0.003^{b}	0.51±0.01^{e}	13.70±1.14^{bc}	0.74±0.37^{e}
T. melanosporum	1.58±0.02^{c}	0.04±0.001^{c}	0.04±0.004^{c}	0.64±0.01^{d}	12.27±1.28^{cd}	2.09±0.96^{d}
T. uncinatum	2.02±0.02^{b}	0.02±0.002^{d}	0.10±0.003^{b}	0.88±0.01^{b}	11.64±1.44^{d}	6.32±0.34^{b}

Results are shown as mean ± standard deviation. Values associated to different letters (*per* column, *i.e., per* mineral element) differ significantly one from another at $p<0.05$ based on Student's t-test.

species studied were different. Additionally, the **https://www.nutritionvalue.org/** website indicates (without specifying the truffle species) similar values (*i.e.,* included in our range of values) to the data obtained in this study (**Table 1**) for all the mineral elements tested.

The ionic analysis confirms virulence variation between truffle species through magnesium and calcium contents since these ions are directly involved in fungal virulence. Lange & Peiter [2020] reviewed fungal virulence modulation to calcium links notably to Ca^{2+} ATPase; and Suo *et al.* [2018] demonstrated magnesium ability to regulate fungal virulence especially using a magnesium transporter named Mgt2, a transporter associated to melanin synthesis.

Chromaticity coordinates of truffle colour

The colour analysis is an interesting approach for food characterisation [Pathare *et al.*, 2013]. Herein, the colour analysis of truffle allowed to discriminate *T. aestivum, T. brumale, T. indicum, T. magnatum*, *T. melanosporum*, and *T. uncinatum* (**Table 2**). The values of *L** ranged from 20.56 to 36.35, the values of *a** ranged from 1.62 to 4.23 and those of *b** ranged from 2.78 to 12.9. The *L**, *a** and *b** values different statistically significant ($p<0.05$) between the six analysed species. The *L** value is the most intuitive to use since the colour of truffles oscillates between black and white. Indeed, the *L** values allowed to clearly discriminate between the six truffle species with a high value for *T. aestivum* and *T. magnatum* considered as white truffle species compared to the 4 other species considered as black truffle species.

Table 2. Colour coordinates of *Tuber aestivum, Tuber brumale, Tuber indicum, Tuber magnatum, Tuber melanosporum,* and *Tuber uncinatum* powders.

Truffle species	*L**	*a**	*b**
T. aestivum	36.35±0.17^{a}	2.63±0.03^{e}	12.90±0.08^{a}
T. brumale	24.50±0.06^{c}	3.92±0.02^{c}	8.68±0.05^{d}
T. indicum	20.56±0.10^{f}	1.62±0.01^{f}	2.78±0.03^{f}
T. melanosporum	22.04±0.32^{e}	2.98±0.04^{d}	7.19±0.14^{e}
T. magnatum	34.21±0.10^{b}	4.23±0.03^{a}	12.69±0.06^{b}
T. uncinatum	23.10±0.06^{d}	4.05±0.02^{b}	10.54±0.06^{c}

Results are shown as mean ± standard deviation. Values associated to different letters (*per* column) differ significantly one from another at $p<0.05$ based on Student's t-test. *L**, lightness; *a**, redness/greenness; *b**, yellowness/blueness.

The chromaticity analysis also shows virulence involvement in truffle species discrimination since the melanin (the truffle black pigment), which we have been able to evaluate using *L** values, enhances the virulence in some fungi [Nosanchuk *et al.*, 2015].

Antioxidant capacity and total phenolic content of truffles

Antioxidants can neutralise reactive oxygen species using different mechanisms, which has led to the development of different methods to assess the antioxidant activity of a molecule. Some analytical methods are based on single-electron transfer reaction, such as CUPRAC; others are based on the hydrogen atom transfer reaction, such as ORAC, and still others can use both modes of action, as ABTS or DPPH assay [Apak *et al.*, 2016].

In this study, the CUPRAC and ABTS assays were used, and results of antioxidant capacity determination of dried truffle powders are shown in **Figure 2 A** & **B**. The CUPRAC was determined for all truffle species, highlighting that they contained antioxidants involved in the single-electron transfer process. ABTS assay results present a significant gain compared to the CUPRAC measures for all *Tuber* species. The only reference data available on truffles antioxidant capacity in the literature were obtained by Tejedor-Calvo *et al.* [2021]. Unlike our study where the results are expressed in relation to dry matter, these authors expressed the results in relation to fresh matter. In addition, the measurement of the antioxidant capacity of truffles was carried out using different techniques but with similar principles. However, the information obtained is similar. Indeed, the values of the tests based on one approach (CUPRAC here for single-electron transfer reaction, ORAC in the publication for hydrogen atom transfer reaction) were lower than those from the tests involving both approaches (ABTS assay here, DPPH assay in the cited publication), which confirms that these two processes were involved in the antioxidant activity of the truffle extracts. In addition, we

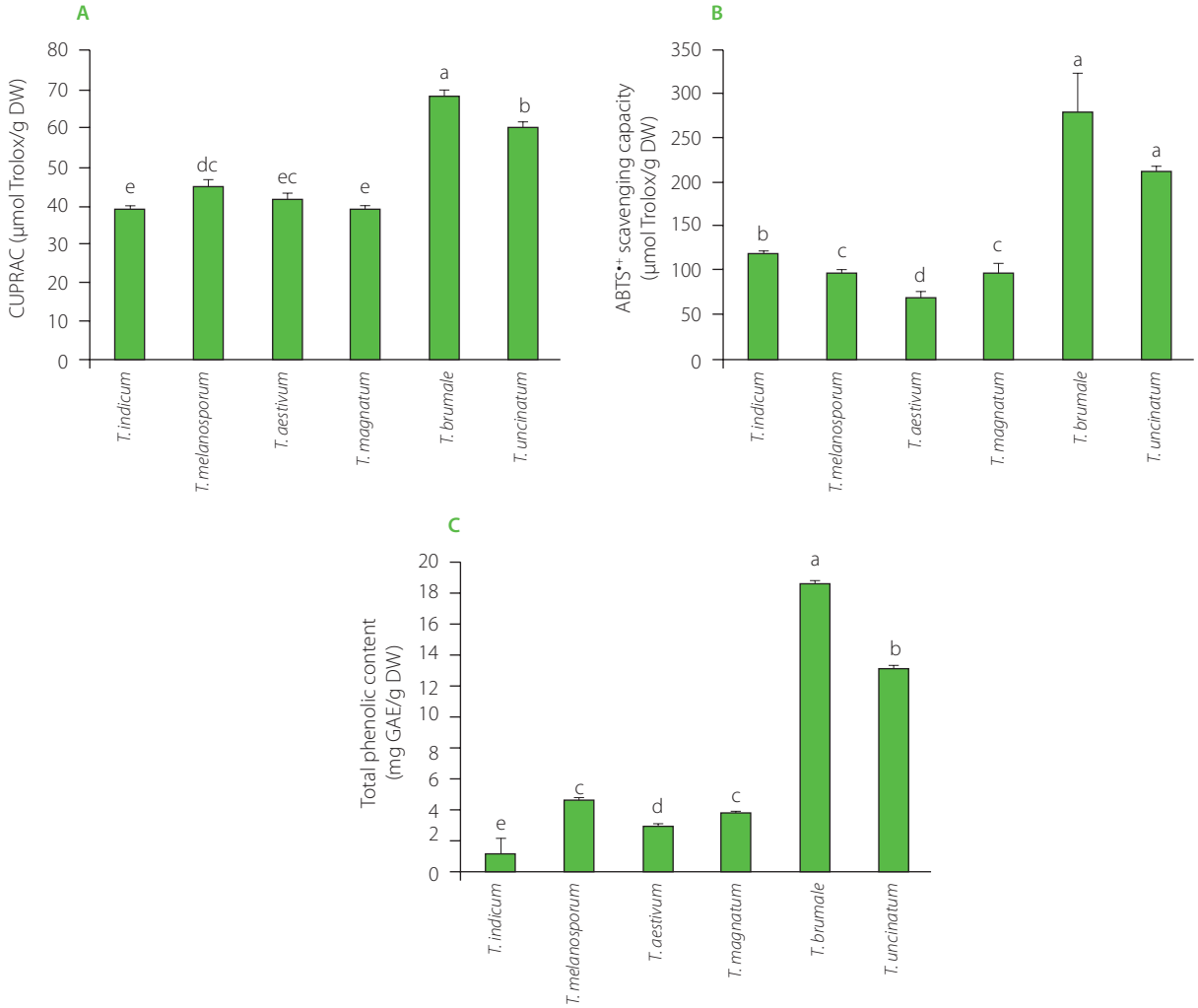

Figure 2. Cupric ion reducing antioxidant capacity (CUPRAC) (**A**), ABTS•+ scavenging capacity (**B**), total phenolic content (**C**) of powders of dried *Tuber* species. Bars with different letters differ significantly one from another at $p<0.05$. DW, dry weight; GAE, gallic acid equivalent.

observed (**Figure 2A**) negligible CUPRAC activity compared to ABTS activity (**Figure 2B**), indicating that antioxidant activity was mainly due to the hydrogen atom transfer reaction. This is confirmed by Tejedor-Calvo *et al.* [2021] who reported ORAC almost as high as DPPH radical scavenging activity for the truffle species studied.

Focusing on truffle species discrimination, ABTS•+ scavenging capacity of truffles allowed to distinguish the different species (**Figure 2B**) except *T. brumale* and *T. uncinatum*. However, CUPRAC allowed to discriminate those two species, *T. brumale* and *T. uncinatum*. Moreover, ABTS•+ scavenging capacity of truffles did not allow to distinguish *T. melanosporum* and *T. magnatum* but it should be noted that it is difficult to confuse these both species since *T. melanosporum* was completely black while *T. magnatum* was white, as indicated by significant differences found in *L** values between these species (**Table 2**).

The molecules directly associated with antioxidant activity are often phenolics [Bešlo *et al.*, 2023]. For this reason, the total phenolic content of truffles was evaluated as a species discrimination approach. It ranged from 1.00 to 18.42 mg GAE/g DW (**Figure 2C**). Tejedor-Calvo *et al.* [2021] reported total phenolic content of different truffle species between 0.22 and 2.9 mg GAE/g FW. The proportion of water in different species of truffles is probably not the same; hence, it is difficult to compare our results with cited literature data expressed on a fresh matter basis [Tejedor-Calvo *et al.*, 2021]. Nevertheless, water representing 75% of the truffle (**https://ciqual.anses.fr/**) and our phenolic compound assays showing amounts 5 to 6 times higher than those reported by Tejedor-Calvo *et al.* [2021] suggest that our extraction was effective. Piatti *et al.* [2024] found that the total phenolic content in *T. magnatum* was 2.41 mg GAE/g DW. This value was lower than that determined in our study for this species (3.69 mg GAE/g DW).

The differences in the total phenolic content between species were comparable to these in ABTS•+ scavenging capacity (**Figure 2**), allowing do discriminate all truffle species except *T. melanosporum* and *T. magnatum*.

Spiteller [2015] explains that mycelial cultures and fruiting bodies (*e.g.,* truffles sold for food consumption) contain fungicidal phenolic compounds constituting a chemical defense. In other words, some truffle phenolic compound inhibits the development of other fungi, avoiding competition and favouring

de facto the truffle development, the truffle virulence. Thus, the truffle phenolic compound content variation is associated to differences in truffle virulence capacities.

Determination of correlation between variables: a principal component analysis

In order to determine the correlation between the discriminate variables studied, a principal component analysis was performed. The principal component 1 (PC1) explained 47.36% of the total variance, while the principal component 2 (PC2) was able to explain 32.36% of the variance, totalizing 79.72% (**Figure 3**). The PCA interpretation showed a strong correlation between the total phenolic content and CUPRAC, which is not surprising as phenolic compounds are significant antioxidants [Bešlo *et al.*, 2023]. Furthermore, there was also a strong negative correlation between the total phenolic content (and CUPRAC) and the ATR-FTIR chitin/chitosan ratio. To explain it, it needs to be first reminded that chitosan is obtained by deacetylation of chitin. Then, knowing that (some) phenolics are capable to act on gene expression using epigenetic mechanisms [Číž *et al.*, 2020] and that acetylation/deacetylation process can be regulated epigenetically [Liu *et al.*, 2023], we can hypothesise that the increased production of phenolic compounds in *Tuber* regulates the conversion of chitin to chitosan through epigenetic mechanisms.

CONCLUSIONS

This study highlights the possibility of using ATR-FTIR, chromaticity and atomic absorption analyses in truffles species discrimination. Indeed, chitin/chitosan and β/α-glucans ratios determined by ATR-FTIR, colour coordinates and lightness as well as magnesium and calcium contents can help discriminate *T. aestivum, T. brumale, T. indicum, T. magnatum*, *T. melanosporum*, and *T. uncinatum*. Also, the ABTS$^{\bullet+}$ scavenging capacity and CUPRAC as well as the total phenolic content allowed to discriminate near than these six truffle species. Only *T. melanosporum* and *T. magnatum* could not be distinguished using antioxidant capacity measures. The potential of biophysical approaches, antioxidant capacity and/or total phenolic content analysis in the discrimination of truffle species has been highlighted in this study demonstrating direct or combined applications in food science such as in fraud detection.

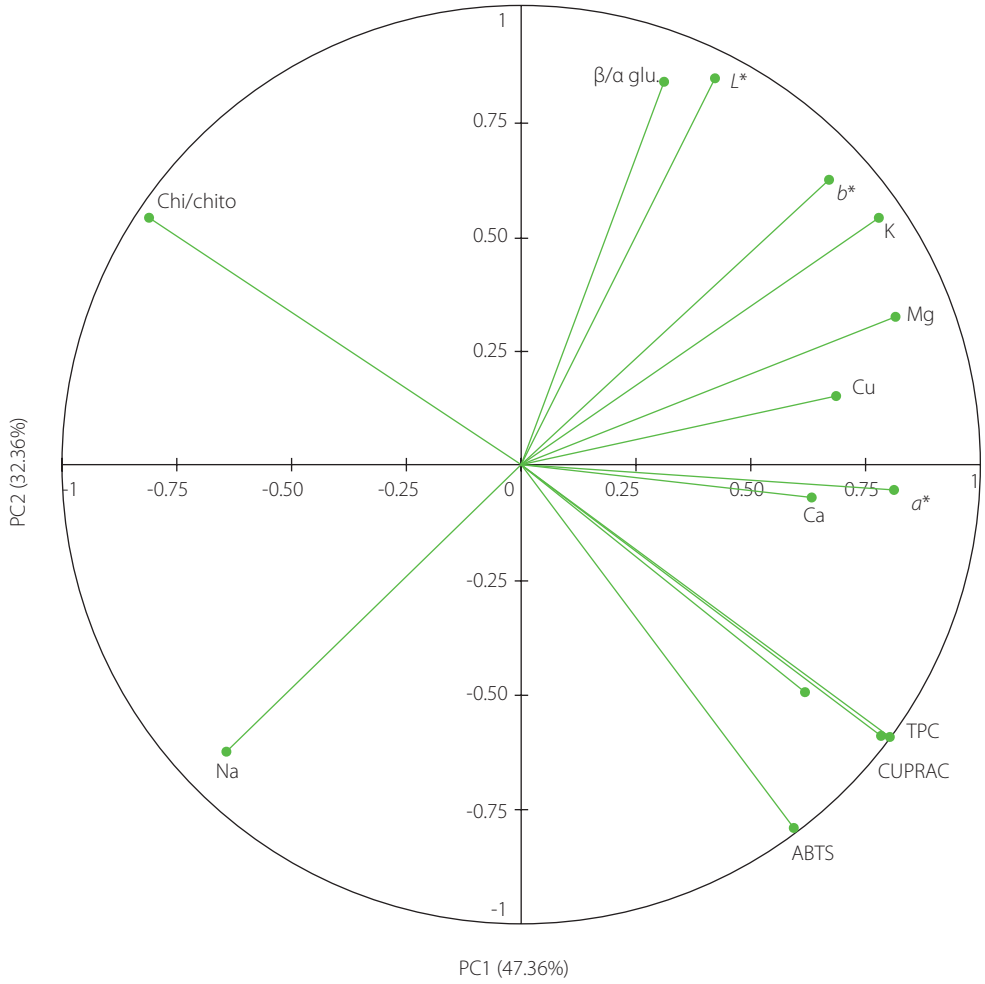

Figure 3. Plot of principal component analysis (PCA) on the set of factors that differentiate truffle species. ABTS, ABTS$^{\bullet+}$ scavenging capacity; β/α gluc., β/α-glucan ratio (889 cm^{-1}/850 cm^{-1}); Ca, calcium; Chi/chito, chitin/chitosan ratio (1,659 cm^{-1}/1,627 cm^{-1}); Cu, copper; CUPRAC, cupric ion reducing antioxidant capacity; Fe, iron; K, potassium; Mg, magnesium; Na, sodium; TPC, total phenolic content.

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CONFLICT OF INTERESTS

Authors declare no conflict of interests.

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REFERENCES

1. Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S.E., Bektaşoğlu, B., Berker, K.I., Özyurt, D. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules, 12*(7), 1496-1547. https://doi.org/10.3390/12071496
2. Apak, R., Özyürek, M., Güçlü, K., Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. 2. Hydrogen Atom Transfer (HAT)-Based, Mixed-Mode (Electron Transfer (ET)/HAT), and lipid peroxidation assays. *Journal of Agricultural and Food Chemistry, 64*(5), 1028-1045. https://doi.org/10.1021/acs.jafc.5b04743
3. Beara, I.N., Lesjak, M.M., Četojević-Simin, D.D., Marjanović, Z.S., Ristić, J.D., Mrkonjić, Z.O., Mimica-Dukić, N.M. (2014). Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of black (*Tuber aestivum* Vittad.) and white (*Tuber magnatum* Pico) truffles. *Food Chemistry, 165*, 460-466. https://doi.org/10.1016/j.foodchem.2014.05.116
4. Bešlo, D., Golubić, N., Rastija, V., Agić, D., Karnaš, M., Šubarić, D., Lučić, B. (2023). Antioxidant activity, metabolism, and bioavailability of polyphenols in the diet of animals. *Antioxidants*, *12*(6), art. no. 1141. https://doi.org/10.3390/antiox12061141
5. Bonito, G.M., Gryganskyl, A.P., Trappe, J.M., Vilgalys, R. (2010). A global meta-analysis of Tuber ITS rDNA sequences: Species diversity, host associations and long-distance dispersal. *Molecular Ecology, 19*(22), 4994–5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x
6. Bowman, S.M., Free, S. (2006). The structure and synthesis of the fungal cell wall. *BioEssays, 28*(8), 799-808. https://doi.org/10.1002/bies.20441
7. Ceruti, A., Fontana, A., Nosenzo, C. (Eds). (2003). *European Species of the Genus Tuber. A Historical Revision*. Monografie di Museo Regionale di Scienze Naturali, Torino, p. 467 (in Italian).
8. Číž, M., Dvořáková, A., Skočková, V., Kubala, L. (2020). The role of dietary phenolic compounds in epigenetic modulation involved in inflammatory processes. *Antioxidants, 9*(8), art. no. 691. https://doi.org/10.3390/antiox9080691
9. Dahmane, E.M., Taourirte, M., Eladlani, N., Rhaz, M. (2014). Extraction and characterization of chitin and chitosan from *Parapenaeus longirostris* from Moroccan local sources. *International Journal of Polymer Analysis and Characterization, 19*(4), 342-351. https://doi.org/10.1080/1023666X.2014.902577
10. Gessler, N.N., Egorova, A.S., Belozerskaya, T. (2014). Melanin pigments of fungi under extreme environmental conditions (Review). *Applied Biochemistry and Microbiology, 50*, 105-113. https://doi.org/10.1134/S0003683814020094
11. Grošev, V., Božac, R., Puppels, G. (2001). Vibrational spectroscopic characterization of wild growing mushroom and toadstools. *Spectrochemica Acta, 57*(14), 2815-2829. https://doi.org/10.1016/S1386-1425(01)00584-4
12. Jeandroz, S., Murat, C., Wang, Y.J., Bonfante, P., Le Tacon, F. (2008). Molecular phylogeny and historical biogeography of the genus *Tuber*, the 'true truffles'. *Journal of Biogeography, 35*(5), 815–829. https://doi.org/10.1111/j.1365-2699.2007.01851.x
13. Lange, M., Peiter, E. (2020). Calcium transport proteins in fungi: The phylogenetic diversity of their relevance for growth, virulence, and stress resistance. *Frontiers in Microbiology, 10*, art. no. 3100. https://doi.org/10.3389/fmicb.2019.03100
14. Lin, M., Al-Holy, M., Al-Qadiri, H., Kang, D., Cavinato, A., Huang, Y., Rasco, B.A. (2004). Discrimination of intact and injured *Listeria monocytogenes* by Fourier Transform Infrared Spectroscopy and Principal Component Analysis. *Journal of Agricultural and Food Chemistry, 52*(19), 5769-5772. https://doi.org/10.1021/jf049354q
15. Liu, Y., Singh, S., Pattanaik, S., Wang, H., Yuan, L. (2023). Light regulation of the biosynthesis of phenolics, terpenoids, and alkaloids in plants. *Communications Biology*, *6*, art. no. 1055. https://doi.org/10.1038/s42003-023-05435-4
16. Merényi, Z., Varga, T., Bratek, Z. (2016). *Tuber brumale*: A controversial *Tuber* species. In A. Zambonelli, M. Iotti, C. Murat (Eds.), *True Truffle (Tuber spp.) in the World. Ecology, Systematics and Biochemistry, Series: Soil Biology*, vol 47, Springer, Cham, pp. 49-68. https://doi.org/10.1007/978-3-319-31436-5_4
17. Mouyna, I., Dellière, S., Beauvais, A., Gravelat, F., Snarr, B., Lehoux, M., Zacharias, C., Sun, Y., de Jesus Carrion, S., Pearlman, E., Sheppard, D.C., Latgé, J.P. (2020). What are the functions of chitin deacetylases in *Aspergillus fumigatus*? *Frontiers in Cellular and Infection Microbiology, 10*, art. no. 28. https://doi.org/10.3389/fcimb.2020.00028
18. Nosanchuk, J.D., Stark, R.E., Casadevall, A. (2015). Fungal melanin: What do we know about structure? *Frontiers in Microbiology, 6*, art. no. 1463. https://doi.org/10.3389/fmicb.2015.01463
19. Paolocci, F., Rubini, A., Granetti, B., Arcioni, S. (1997). Typing *Tuber melanosporum* and Chinese black truffle species by molecular markers. *FEMS Microbiology Letters, 153*(2), 255-260. https://doi.org/10.1111/j.1574-6968.1997.tb12582.x
20. Pappas, C., Tarantilis, P., Harizanis, P., Polissiou, M. (2003). New method for pollen identification by FT-IR spectroscopy. *Applied Spectroscopy*, 57(1), 23-27. https://doi.org/10.1366/000370203321165160
21. Pathare, P.B., Opara, U.L., Al-Said, F.A.L. (2013). Colour measurement and analysis in fresh and processed foods: A review. *Food and Bioprocess Technology, 6*, 36–60. https://doi.org/10.1007/s11947-012-0867-9
22. Payne, K.J., Veis, A. (1988). Fourier transform IR spectroscopy of collagen and gelatin solutions: Deconvolution of the amide I band for conformational studies. *Biopolymers*, *27*(11), 1749–1760. https://doi.org/10.1002/bip.360271105
23. Piatti, D., Marconi, R., Caprioli, G., Zannotti, M., Giovannetti, R., Sagratini, G. (2024). White Acqualagna truffle (*Tuber magnatum* Pico): Evaluation of volatile and non-volatile profiles by GC-MS, sensory analyses and elemental composition by ICP-MS. *Food Chemistry*, *439*, art. no. 138089. https://doi.org/10.1016/j.foodchem.2023.138089
24. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3
25. Renouard, S., Hano, C., Doussot, J., Blondeau, J.P., Lainé, E. (2014). Characterization of ultrasonic impact on coir, flax and hemp fibers. *Materials Letters, 119*, 137-141. https://doi.org/10.1016/j.matlet.2014.05.018
26. Rubini, A., Paolocci, F., Granetti, B., Arcioni, S. (1998). Single step molecular characterization of morphologically similar black truffle species. FEMS *Microbiology Letters, 164*(1), 7-12. https://doi.org/10.1111/j.1574-6968.1998.tb13060.x
27. Rytwo, G., Zakai, R., Wicklein B. (2015). The use of ATR-FTIR spectroscopy for quantification of adsorbed compounds. *Journal of Spectroscopy*, *2015*, art. no. 727595. https://doi.org/10.1155/2015/727595
28. Sancholle, M., Weete, J.D., Kulifaj, M., Montant, C. (1988). Changes in lipid composition during ascocarp development of the truffle, *Tuber melanosporum*. *Mycologia, 80*(6), 900-903. https://doi.org/10.1080/00275514.1988.12025749
29. Séjalon-Delmas, N., Roux, C., Martins, M., Kulifaj, M., Bécard, G., Dargent, R. (2000). Molecular tools for the identification of *Tuber melanosporum* in agroindustry. *Journal of Agricultural and Food Chemistry, 48*(6), 2608-2613. https://doi.org/10.1021/jf9910382
30. Shah, N., Usvalampi, A., Chaudhary, S., Seppänen-Laakso, T., Marathe, S., Bankar, S., Singhal, R., Shamekh, S. (2020a). An investigation on changes in composition and antioxidant potential of mature and immature summer truffle (*Tuber aestivum*). *European Food Research and Technology, 246*, 723-731. https://doi.org/10.1007/s00217-020-03438-7
31. Shah, N.N., Hokkanen, S., Pastinen, O., Eljamil, A., Shamekh, S. (2020b). A study on the fatty acid composition of lipids in truffles selected from Europe and Africa. *3 Biotech, 10*, art. no. 415. https://doi.org/10.1007/s13205-020-02414-y
32. Shavit, E. (2014). The history of desert truffle use. In V. Kagan-Zur, N. Roth-Bejerano, Y. Sitrit, A. Mort (Eds.), *Desert Truffles, Phylogeny, Physiology, Distribution and Domestication, Series: Soil Biology*, Springer, Berlin, pp. 217-241. https://doi.org/10.1007/978-3-642-40096-4_15
33. Silva, C., Lima, C., Pinheiro, A., Goés, J., Figueiró, S., Sombra, A. (2001). On the piezoelectricity of collagen-chitosan films. *Physical Chemistry Chemical Physics, 3*(18), 4154-4157. https://doi.org/10.1039/b100189m
34. Singleton, V., Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144-158. https://doi.org/10.5344/ajev.1965.16.3.144

35. Spiteller, P. (2015). Chemical ecology of fungi. *Natural Product Reports, 32*, 971-993.
https://doi.org/10.1039/C4NP00166D
36. Suo, C., Ma, L., Li, H., Sun, J., Li, C., Lin, M., Sun, T., Du, W., Li, Y., Gao, X., Meng, Y., Sai, S., Ding, C. (2018). Investigation of *Cryptococcus neoformans* magnesium transporters reveals important role of vacuolar magnesium transporter in regulating fungal virulence factors. *MicrobiologyOpen, 7*(3), art. no. e00564.
https://doi.org/10.1002/mbo3.564
37. Synytsya, A., Novak, M. (2014). Structural analysis of glucans. *Annals of Translational Medicine, 2*(2), art. no. 17.
https://atm.amegroups.org/article/view/3397
38. Tejedor-Calvo, E., Amara, K., Reis, F.S., Barros, L., Martins, A., Calhelha, R.C., Venturini, M.E., Blanco, D., Redondo, D., Marco, P., Ferreira, I.C.F.R. (2021). Chemical composition and evaluation of antioxidant, antimicrobial and antiproliferative activities of *Tuber* and *Terfezia* truffles. *Food Research International, 140*, art. no. 110071.
https://doi.org/10.1016/j.foodres.2020.110071
39. Valand, R., Tanna, S., Lawson, G., Bengtström, L. (2019). A review of Fourier Transform Infrared (FTIR) spectroscopy used in food adulteration and authenticity investigations. *Food Additives & Contaminants: Part A, 37*(1), 19-38.
https://doi.org/10.1080/19440049.2019.1675909
40. Wilson, D.W., Nash, P., Buttar, H.S., Griffiths, K., Singh, R., De Meester, F., Horiuchi, R., Takahashi, T. (2017). The role of food antioxidants, benefits of functional foods, and influence of feeding habits on the health of the older person: An overview. *Antioxidants, 6*(4), art. no. 81.
https://doi.org/10.3390/antiox6040081
41. Wu, Z., Meenu, M., Xu, B. (2021). Nutritional value and antioxidant activity of Chinese black truffle (*Tuber indicum*) grown in different geographical regions in China. *LWT – Food Science and Technology, 135*, art. no. 110226.
https://doi.org/10.1016/j.lwt.2020.110226
42. Yan, X., Wang, Y., Sang, X., Fan, L. (2017). Nutritional value, chemical composition and antioxidant activity of three *Tuber* species from China. *AMB Express, 7*, art. no. 136.
https://doi.org/10.1186/s13568-017-0431-0
43. Zehiroglu, C., Ozturk Sarikaya, S.B. (2019). The importance of antioxidants and place in today's scientific and technological studies. *Journal of Food Science and Technology, 56*(11), 4757-4774.
https://doi.org/10.1007/s13197-019-03952-x
44. Zhao, D., Liu, G., Song, D., Liu, J., Zhou, Y., Ou, J., Sun, S. (2006). Fourier transform infrared spectroscopic study of truffles. *ICO20: Biomedical Optics, SPIE Proceedings, 6026,* 117-123.
https://doi.org/10.1117/12.667133

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Impact of Sesame (*Sesamum indicum* L.) Oil Cake on Pasta Physicochemical Properties

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This study was carried out to evaluate the effects of sesame (*Sesamum indicum* L.) oil cake on pasta processing and the functional characteristics of the end product. The pasta produced from wheat flour fortified with 5, 7.5, 10, 12.5, and 15% (*w/w*) sesame oil cake showed a significant increase in total protein (*i.e.*, ranging from 12.3 and 16.0 g/100 g), lipid (*i.e.*, 3-fold) and crude fiber (*i.e.*, 0.58 to 0.91 g/100 g) contents with the increasing addition of sesame oil cake. Consistograph tests showed that the pressure maximum decreased significantly from 267.2 kPa for the wheat flour dough (control) to 157.6 kPa upon the addition of 15% (*w/w*) sesame oil cake to wheat flour. Drop-in pressure after 250 and 450 s was lower for the control sample than for those containing 5, 7.5, 10, 12.5, and 15% (*w/w*) sesame oil cake. The elasticity value for the control dough was 500.1 Pa, and it was reduced to 392.3 Pa with the addition of 15% (*w/w*) sesame oil cake to wheat flour resulting also in a significant reduction in the extensibility. The pasta from the flour blend with 7.5% (*w/w*) sesame oil cake exhibited the highest cooking loss, at 5.40 g/100 g. Sensorial evaluation of the flavor and stickiness of pasta produced from the flour blends containing up to 10% (*w/w*) of sesame oil cake was satisfactory and recommended to open the door for industrial utilization of such valuable and nutritious by-products. Sesame components and gluten network interactions are believed to play a key role in modifying pasta's functional characteristics and require more investigation.

Keywords: pasta, sesame flour, rheological properties, cooking gain, cooking loss

INTRODUCTION

The widespread acceptance of pasta is attributed to its low cost, ease of preparation, sensory attributes, and long shelf life [Kaur *et al.*, 2013; Oliviero & Fogliano, 2016]. "Pasta" usually refers to various shapes and sizes of products made from a basic mixture of wheat (*Triticum durum* or *Triticum aestivum*) endosperm flour and water, cooked as fresh pasta or dried for consumption later on [Bresciani *et al.*, 2022]. The ideal pasta characteristics include its smooth texture, translucence, hardness, brittleness, elasticity, as well as its rich amber color [Sicignano *et al.*, 2015]. The nutrient composition of pasta is similar to that of the semolina used for its production, owing to which it has become recognized as a healthy food with low lipid content, no cholesterol, and a low glycemic index [Animashaun *et al.*, 2017]. Moreover, the consumption of cereal products enriched with different plant materials that are rich in bioactive compounds has increased due to their positive influence on human health [Dziki, 2021]. As a result, the nutritional value of pasta has been increased by adding substances of an organic type or minerals [Wang *et al.*, 2022].

Various ingredients such as tomatoes, spinach, basil, garlic, and flours or protein isolates from other grains and legumes have been incorporated into pasta formulations to enhance both its appeal and nutritional value [Kaur *et al.*, 2013; Oliviero & Fogliano, 2016; Schmidt & de Oliveira, 2023]. Recently, the integration

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of plant-based products into pasta has been extensively tested, *e.g.*, Padalino *et al.* [2014] examined the impact of adding up to 15% pea flour to spaghetti, finding a significant reduction in starch digestibility and optimal cooking time. Other studies have aimed to increase dietary fiber levels in pasta by adding high-fiber materials from sources other than durum wheat [Wang *et al.*, 2022]. Pasta formulated with dietary fiber has shown a lower glycemic index than the non-enriched product and low responses in diabetics [Cui *et al.*, 2024]. The addition of endoxylanases to pasta dough has been reported to reduce required extrusion pressure, decrease cooking time, lower dough hydration levels, and increase soluble fibers [Ingelbrecht *et al.*, 2001]. The incorporation of smoked trout fillet powder enhanced the nutritional value of pasta, though it also increased cooking loss and decreased water absorption capacity and swelling index [Ozgoren & Yapar, 2019]. The combined use of propylene glycol alginate (0.1%), monoglycerides of fatty acids (0.5%), and gelatinization of mixed flour (buckwheat, corn, rice) has produced dried pasta with superior cooking characteristics and texture [De Arcangelis *et al.*, 2020]. Bustos *et al.* [2019] investigated the effects of pasta enrichment with freeze-dried and air-dried blackberry, raspberry, and black and red currant powders on its antioxidant, sensory, and cooking quality. While the enriched pasta showed increased antioxidant activity, the enrichment negatively affected cooked pasta functionality, as evidenced by an increased swelling index. Enrichment with 50% of β-glucans and dietary fiber improved pasta cooking qualities, including stickiness, bulkiness, firmness, and the amount of total organic matter released in cooking water [Marconi *et al.*, 2000]. Incorporation of barley β-glucans into pasta products resulted in a higher fiber content and lower glycemic responses [Yokoyama *et al.*, 1997].

There is an increased demand for incorporating an inexpensive source of proteins that would justify the search for the potential utilization of protein-rich by-products as food [Schmidt & de Oliveira, 2023]. One such source may be sesame oil cake, a by-product of pressing oil from seeds, with a protein content of up to 40–50 g/100 g [Mostashari & Khaneghah, 2024]. Sesame protein provides essential amino acids necessary for human nutrition, supporting muscle growth, repair, and overall bodily functions. Moreover, sesame oil cake is abundant in dietary fiber, primarily cellulose and hemicellulose, which aid digestion and promote gastrointestinal health by regulating bowel movements and supporting beneficial gut bacteria [Wei *et al.*, 2022]. Beyond proteins and fibers, sesame oil cake also contains notable levels of phenolic compounds, such as lignans [Mostashari & Khaneghah, 2024]. These compounds may help reduce oxidative stress, inflammation, and the risk of development of chronic diseases, such as cardiovascular ailments and certain cancers [Wei *et al.*, 2022]. The presence of antioxidants, like tocopherols, further enhances the nutritional profile of sesame oil cake, contributing to its potential role in promoting overall health and well-being [Melo *et al.*, 2021; Mostashari & Khaneghah, 2024]. Sesame oil cake further holds a notable economic value within the agricultural sector due to its dual role as a source of nutrients for livestock feed and as a potential ingredient in various industrial applications. These industrial applications not only contribute to the economic sustainability of sesame oil processing but also support environmental initiatives by promoting waste utilization. Therefore, the objective of this study was to evaluate the effects of using sesame oil cake as an additive ingredient on the pasta dough characteristics, processing ability, and stability of the end product.

MATERIALS AND METHODS

Materials

Semolina flour was obtained from a local mill (The Modern Flour Mills and Macaroni Factories Co., Amman, Jordan). Dried sesame (*Sesamum indicum* L.) oil cake, a by-product of pressing sesame oil, was purchased from Al-Nahda Pressing Mills Co., Amman, Jordan. Sesame oil cake was produced from the crop of 2022, a product of Sudan.

Flour blend preparation

Experimental flour blends were prepared by adding powdered sesame oil cake to durum wheat semolina at levels of 5, 7.5, 10, 12.5, and 15% (*w/w*) and mixing for 5 min using a medium-speed mixer (Pavan, Padova, Italy). The control flour was durum wheat semolina.

Proximate composition analysis

Moisture, ash, and protein contents of wheat flour, sesame oil cake and pasta products were determined using approved methods of the American Association of Cereal Chemists (AACC); methods no. 44-15.02, 08-01, and 46-13, respectively [AACC, 2000]. Crude lipid content was determined according to the AOAC International method 922.06 [AOAC, 2005], and crude fiber content was determined according to the International Organization for Standardization (ISO) standard method no. 5498.1981 [ISO, 1981], while nitrogen-free extract (NFE) was determined by difference. Analyses were carried out in duplicates.

Gluten content and gluten index determination

Gluten content and gluten index of flours were evaluated according to the AACC method 38-12.02 [AACC, 2000]. A 10.0±0.01 g sample was weighed and placed into the Glutomatic wash chamber (Glutomatic® 2200, Perten Instruments, Stockholm, Sweden) equipped with an 88-µm polyester sieve. Then, 4.8 mL of a 2% brine solution was added and mixed to form dough within 20 s. After the mixing period, the dough was washed for 5 min with water. To measure the gluten index, the wet gluten piece was centrifuged for 1 min at 2,415×*g* (centrifuge 2015, Perten Instruments). The fraction that passed through the sieve was scraped off and weighed, while the fraction remaining on the inside of the sieve was collected and added to the balance to obtain the total wet gluten content (g/100 g). For estimating dry gluten content (g/100 g), the total wet gluten piece was dried for 4 min at 150°C using a Glutork 2020 dryer (Perten Instruments). The gluten index (GI) was calculated using formula (1):

$$GI\ (\%) = \frac{\text{Wet gluten remained on sieve (g)}}{\text{Total wet gluten (g)}} \times 100 \qquad (1)$$

Results of five replicates were recorded for statistical analyses and means were calculated.

Falling number test

Flours were analyzed for falling numbers using the AACC method 56-81B [AACC, 2000]. A 7-g sample was combined with 25 mL of distilled water in a glass tube with a stirrer and shaken to form a slurry using Falling Number 1400 apparatus (Perten Instruments). The time (s) it took for the stirrer to fall through the paste was recorded as the falling number value. The measurement for each flour was performed in five repetitions.

Consistograph test

The consistograph test was conducted following the AACC method no. 54-50 [AACC, 2000]. The test was performed using the Chopin Consistograph (Tripette et Renaud, Asnières-sur-Seine, France) at both constant hydration (CH) and adapted hydration (AH) settings. The parameters determined were flour water absorption capacity (WA, %), hydration at 15% water (%), maximum pressure exerted by the dough against the mixer bowl lining (kPa), time needed to reach pressure maximum (s), tolerance time (s), and drop-in pressure at 250 s (D250, kPa) and 450 s (D450, kPa). The test was performed in five replicates.

Alveograph test

The alveograph test was conducted in accordance with the AACC-approved method no. 54-30A [AACC, 2000]. In brief, 250 g of wheat flour or flour blends were weighed, and then water (*i.e.*, 60% of the flour weight) was added. After mixing the flour and water to form dough in a mechanical mixer for 8 min, the dough was allowed to rest for 20 min. After resting, the dough was divided into 150-g portions and was then shaped into smooth balls, and then flattened into a circular disc with a uniform thickness using a rolling pin. The shaped dough discs were then placed into a temperature-controlled chamber at 25°C, for an additional resting period of 30 min. After conditioning, the dough discs were placed onto the alveograph NG-97 (Tripette et Renaud) to run the test according to the manufacturer's instructions. The alveograph test involved inflating the dough discs into bubbles, measuring and recording several parameters including dough strength (P; Pa), extensibility (L, mm), work (W, J), swelling index (G, the square root of the volume of air required to rupture the dough), configuration ratio (P/L), and elasticity index (Ie, %). The results of five tests were recorded for statistical analysis.

Pasta production

Pasta production was carried out in The Modern Flour Mills and Macaroni Factories Co., Amman, Jordan, using a pilot processing line for pasta (Pavan). Flours were mixed in the mixer of the pasta machine, and the water was added through an automatic sprayer to reach a final moisture content of 31±0.5 g/100 g. The mixing time varied from 10 to 12 min. The premix was then subjected to an extruder pressure of 11 MPa under a temperature of 45–50°C, and the dough sheet was formed with a moisture content of 29.5–30 g/100 g. Then it was passed through parallel sheeting rolls to obtain a stable thickness of 1.5 mm. The sheeted dough formed was cut by rotary cutting disks to reach a final length of 16 to 17 cm and a width of 6 to 7 cm. As a result, rectangular-shaped pasta dough was obtained.

The rectangular-shaped pasta dough was arranged on a mesh mounted on wooden frames, and the frame stacks were then pushed into a two-stage pre-drier. In the first stage of pre-drying, the air temperature at the entrance of the pre-drier was 50°C and increased gradually until it reached 56–59°C at the exit side. The relative humidity inside the pre-drier was in the range of 55–60%. The pasta's moisture after the first stage was lowered from 29.5 to 22.0 g/100 g in 50 min drying time. During the second stage, the air temperature was increased to reach 70°C, while the relative humidity reached 75%. The moisture content of the pasta after the second stage of drying decreased from 22.0 to 18 g/100 g in 10 min time.

During the final drying stage, pasta sheets were exposed to three drying stages, which resulted in the production of pasta with a final moisture content below 12.5 g/100 g. After drying, the pasta product was cooled prior to the packaging process. The temperature of the cooling cabinet was around 30°C, the relative humidity was about 50%, and the total resting time was around 360 min. Identical pasta production protocol was replicated four times for each flour blend.

Pasta cooking gain, cooking loss, and cooking time evaluation

To evaluate cooking gain, 300 mL of distilled water were poured into and heated in a stainless-steel container covered with a cap circulated by cold water, and when the water started to boil, a 10-g sample of pasta was dropped in the boiling water to be cooked for different periods (10, 13 and 20 min). Cooking gain was evaluated by weighing pasta before and after cooking and expressed as an increase in cooked pasta weight (g/100 g) [Hummel, 1966].

To determine the amounts of solid substances lost in water after cooking (cooking loss), 30 mL of cooking water was placed in a small dry-weighed beaker and completely evaporated. After complete evaporation, the beaker was cooled in a desiccator and weighed to calculate the percentage of solid substances lost after cooking and expressed as g of matter loss *per* 100 g of dry pasta [Hummel, 1966].

Cooking time was measured by taking a small piece of pasta after being cooked in distilled water and crushed between two glass plates until the center white core could no longer be seen, and that indicated the optimum cooking time [Bergman *et al.*, 1994]. Samples were taken after cooking for 10, 13, and 20 minutes and tested for their doneness states.

Pasta storage and acidity measurement

After processing, pasta samples (100 g) were packed in plastic bags, and stored inside an incubator (Memmert, Model No. INB, E212.0367, Rittersbacher, Schwabach, Germany) set at 25°C and 50% relative humidity. Samples were analyzed for acidity

after zero, three, and six months. Acidity was measured according to the procedure described in Duszkiewicz-Reinhard *et al.* [1988] in which 10 g of ground pasta were weighed into a 250-mL beaker and mixed with 100 mL of 67% (*v/v*) aqueous ethanol. The extracts were titrated after filtration with 0.1 M NaOH, with phenolphthalein as an indicator.

Sensory evaluation

Five sensory experts of pasta quality from the Modern Flour Mills and Macaroni Factories Company in Jordan evaluated the quality of pasta products. Samples for sensory evaluation (pasta immediately after production and after 3 and 6 months of storage) were cooked to optimum cooking time in distilled water, drained, and served warm to sensory panelists. A score sheet was developed for judging the pasta samples according to their color, flavor, stickiness, and overall acceptability by using a hedonic scale test from 1 to 7, with 7 being liked very much and 1 being disliked very much [Duszkiewicz-Reinhard *et al.*, 1988].

Statistical analysis

Data were analyzed using a SAS Institute [2003] software at the University of Jordan / Computer Center. Analysis of variance (ANOVA) of the different flours/doughs/pasta was performed using complete randomized design (CRD) using a *t*-test in which the rheological properties and chemical characteristics were used as a source of variation. The means of replicates from each variable were compared at a significance level of 5% using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Proximate composition of raw materials and end products

The proximate composition of semolina, sesame oil cake, and pasta produced from different formulations of these components is presented in **Table 1**. The ash content of semolina and sesame oil cake was 0.85 and 5.54 g/100 g, respectively. The pasta produced from flour blends containing different levels of sesame oil cake flour showed a significantly ($p<0.05$) higher ash content compared to the control pasta, with a linear increase as the rate of sesame oil cake addition increased. Pasta from the flour blends with 5, 7.5, and 10% (*w/w*) of sesame oil cake met the standard (no. 67) of the Jordanian Institute for Standards and Metrology [1996] for ash content in dry pasta (*i.e.*, less than 1.20%), whereas those with 12.5% and 15% (*w/w*) of sesame oil cake addition had the ash content greater than 1.20%.

Sesame oil cake addition to wheat flour resulted in an insignificant ($p\geq0.05$) effect on pasta moisture content (**Table 1**). Moisture contents of semolina and sesame oil cake were 13.5 and 7.5 g/100 g, respectively. **Table 1** also presents the protein content of durum wheat semolina, sesame oil cake, and pasta samples. Upon the addition of sesame oil cake flour to semolina, the protein content of the pasta products increased significantly ($p<0.05$) from 11.8 g/100 g for the control sample to 16.0 g/100 g for the pasta from the flour blend with 15% (*w/w*) of sesame oil cake, which corresponded to a 36% increase in protein content. A 17% increase in the protein content of pasta samples was accomplished with the addition of 10% (*w/w*) sesame oil cake flour. This was expected since sesame oil cake contained over 4.5 times more protein than semolina (54.2 and 11.9 g/100 g, respectively), which was reflected in the final product composition. A similar trend was obtained by Padalino *et al.* [2014], who reported that the fortification of wheat flour with pea flour considerably increased the protein, ash, and dietary fiber contents of the spaghetti samples when compared to the control sample.

In evaluating the nutritional benefits, one should consider the improvement in protein quantity as well as quality. Although it is no longer believed that plant proteins must be complemented at every meal, it is important to consume a wide variety of plant proteins to provide the body with an adequate supply of the limiting amino acids. Traditional protein combinations from various sources, such as sesame, soybeans, and wheat, can complement requirements for essential amino acids [Animashaun *et al.*, 2017; El-Adawy, 1997].

The lipid contents of semolina and sesame oil cake were 1.19 and 18.31 g/100 g, respectively (**Table 1**). The addition of sesame oil cake to semolina showed a significant ($p<0.05$) increase in the lipid content of pasta samples from 1.13 g/100 g for the control to 3.21 g/100 g for the pasta produced from

Table 1. Proximate composition of semolina wheat flour (S), sesame oil cake (SOC), and pasta from S (control) and blends of S with SOC.

Raw material/product	Moisture (g/100 g)	Protein (g/100 g)	Ash (g/100 g)	Lipid (g/100 g)	Crude fiber (g/100 g)	NFE (g/100 g)
Semolina	13.5±0.4	11.9±0.2	0.85±0.05	1.19±0.07	0.50±0.02	72.1±0.4
Sesame oil cake	7.5±0.1	54.2±0.3	5.54±0.14	18.31±0.44	2.00±0.04	12.4±0.1
Control (100% S)	8.9±0.1^{a}	11.8±0.1^{f}	0.83±0.06^{f}	1.13±0.05^{f}	0.46±0.06^{f}	76.9±0.5^{a}
95% S + 5% SOC	8.9±0.2^{a}	12.3±0.1^{e}	1.04±0.06^{e}	1.36±0.04^{e}	0.58±0.04^{e}	75.8±0.4^{b}
92.5% S + 7.5% SOC	8.9±0.0^{a}	13.1±0.2^{d}	1.15±0.06^{d}	1.70±0.06^{d}	0.65±0.02^{d}	74.5±0.1^{c}
90% S + 10% SOC	8.9±0.2^{a}	13.8±0.1^{c}	1.21±0.05^{c}	2.13±0.06^{c}	0.73±0.02^{c}	73.3±0.4^{d}
87.5% S + 12.5% SOC	8.9±0.1^{a}	14.6±0.4^{b}	1.30±0.04^{b}	2.63±0.04^{b}	0.80±0.04^{b}	71.8±0.1^{e}
85% S + 15% SOC	8.9±0.1^{a}	16.0±0.2^{a}	1.39±0.02^{a}	3.21±0.06^{a}	0.91±0.02^{a}	69.6±0.2^{f}

All results are shown as mean ± standard deviation. Means in the same column (for pasta) with different letters are significantly different ($p<0.05$). NFE, nitrogen-free extract.

the flour blend with 15% (*w/w*) of sesame oil cake. This result was expected since the pasta has been enriched with lipids due to their high content in sesame oil cake, and this enrichment increased the lipid content as well as the nutritional value of the product.

The crude fiber content of semolina was 0.50 g/100 g and that of sesame oil cake was 2.00 g/100 g (**Table 1**). The increasing addition of sesame oil cake to semolina resulted in a gradual, significant increase ($p<0.05$) in the crude fiber content of the pasta samples, from 0.46 g/100 g for the control to 0.91 g/100 g for the pasta produced from the flour blend with 15% (*w/w*) of sesame oil cake. Yaseen *et al.* [2021] reported that the dietary fiber content of sesame seed is about four folds of its crude fiber. Therefore, the dietary fiber contents of the pasta from the flour blends with 10 and 15% (*w/w*) sesame oil cake are expected to be about 2.3 and 7 g/100 g, respectively, which represents an increase of 53% and 80% compared to that of the basic durum pasta products.

The nitrogen-free extract was 12.4 g/100 g for sesame oil cake and 72.0 g/100 g for durum wheat semolina (**Table 1**). As a result, adding sesame oil cake flour with its low nitrogen-free extract to semolina reduced the total nitrogen-free extract of the resulting pasta products from 76.9 g/100 g in the control to 69.6 g/100 g for the pasta samples from the blend with 15% (*w/w*) of sesame oil cake flour.

Quality parameters of wheat flour and flour blends

The wet gluten and dry gluten contents of flours used in pasta production are presented in **Table 2**. The increasing addition of sesame oil cake to semolina resulted in a significant decrease ($p<0.05$) in the wet gluten content of the blends that ranged from 28.8 g/100 g (semolina flour) to 22.7 g/100 g (flour with 15% (*w/w*) of sesame oil cake). These results were directly related to the dilution effect of gluten content due to the fact that sesame oil cake does not contain gluten. This observation agrees with that of El-Adawy [1997], who added different sesame products (*i.e.*, sesame meal and sesame protein isolates) to the pan bread formulation. In fact, pasta made with a high legume flour content (>30%) is often characterized by poor cooking properties, such as high cooking loss, low swelling index, and cooking time, due to the dilution and weakening of the gluten matrix [Romano *et al.*, 2021].

The dry gluten content of semolina was 9.5 g/100 g, and the significantly ($p<0.05$) lower values were found for the flour blends with an increasing proportion of sesame oil cake (**Table 2**). This reduction was parallel to the level of wet gluten content and indicates that the reduction of wet gluten is a true result of gluten drop rather than retardation of water absorption during gluten development.

Semolina had a gluten index of 89.9% (**Table 2**). The addition of 5% (*w/w*) of sesame oil cake to semolina significantly increased ($p<0.05$) the gluten index of the flour. However, this high value decreased significantly ($p<0.05$) for the flour blend containing 7.5% (*w/w*) of sesame oil cake and continued to decrease ($p<0.05$) for the flour with an increasing proportion of sesame oil cake. As a result, the gluten index of only the blend with 15% (*w/w*) of sesame oil cake was lower ($p<0.05$) than that of the control. This behavior may be due to a concentration-dependent interaction between sesame oil cake compounds and the gluten network. Gluten strength is widely recognized as an important factor for superior pasta cooking quality, though the relationship between gluten strength and pasta cooking quality remains complex [Matsuo, 1993]. Moayedi *et al.* [2021] found that the quantity of proteins, including gliadins, albumins, and globulins, as well as complex proteins, significantly influenced the variation in cooked firmness of fresh pasta.

The falling number values for semolina and semolina with 5, 7.5, 10, 12.5, and 15% (*w/w*) of sesame oil cake flour were above 400 s (**Table 2**), indicating that the addition of sesame oil cake to semolina samples at any level tested did not cause any significant difference ($p\geq0.05$) in the falling number values. This result was expected since the sesame oil cake was processed from highly roasted sesame seeds in which all enzymes had been inactivated.

Rheological properties of pasta doughs

Consistograph test parameters

Data in **Table 3** indicates that the maximum pressure decreased significantly ($p<0.05$) from 267.2 kPa for the control sample to 157.6 kPa for dough prepared from the flour blend with 15% (*w/w*) of sesame oil cake. No significant difference ($p\geq0.05$) was observed only between dough from the flours containing 12.5% and 15% (*w/w*) of sesame oil cake. The maximum pressure decrease was attributed to the dilution effect of gluten through

Table 2. Wet gluten and dry gluten contents, gluten index (GI), and falling number (FN) of semolina wheat flour (S) and its blends with sesame oil cake (SOC).

Flour	Wet gluten (g/100 g)	Dry gluten (g/100 g)	GI (%)	FN (s)
Semolina	28.9±0.21^{a}	9.5±0.0^{a}	89.9±0.1^{e}	>400±12.5^{a}
95% S + 5% SOC	25.6±0.1^{b}	8.8±0.0^{b}	95.8±0.2^{a}	>400±8.5^{a}
92.5% S + 7.5% SOC	25.0±0.1^{c}	8.4±0.0^{c}	94.4±0.1^{b}	>400±6.6^{a}
90% S + 10% SOC	23.8±0.2^{d}	8.1±0.0^{d}	93.5±0.2^{c}	>400±6. 5^{a}
87.5% S + 12.5% SOC	23.0±0.1^{e}	7.9±0.0^{e}	91.1±0.1^{d}	>400±6.6^{a}
85% S + 15% SOC	22.7±0.2^{f}	7.8±0.1^{f}	87.8±0.2^{f}	>400±9.8^{a}

All results are shown as mean ± standard deviation. Means in the same column with different letters are significantly different ($p<0.05$).

Table 3. Consistograph test characteristics of doughs made from semolina wheat flour (S) and its blends with sesame oil cake (SOC).

Dough	Constant hydration			Adapted hydration			
	PrMax (kPa)	WA (%)	HYDHA (%)	TPrMax (s)	Tol (s)	D250 (kPa)	D450 (kPa)
Semolina	267.2±0.8^{a}	55.8±2.4^{a}	53.3±2.3^{a}	94.0±1.0^{a}	122±2^{a}	78.7±0.6^{d}	87.5±0.8^{d}
95% S + 5% SOC	219.7±0.6^{b}	52.7±2.2^{b}	51.2±1.4^{b}	92.0±1.1^{a}	120±2^{ab}	82.5±0.6^{c}	104.5±0.5^{c}
92.5% S + 7.5% SOC	169.8±0.9^{c}	51.4±1.2^{b}	49.4±0.5^{c}	90.0±2.2^{a}	114±2^{ab}	88.2±0.6^{b}	109.8±0.9^{b}
90% S + 10% SOC	165.9±0.7^{d}	51.3±0.9^{b}	49.3±1.5^{c}	92.0±1.4^{a}	113±2^{ab}	89.1±0.6^{b}	110.4±0.9^{b}
87.5% S + 12.5% SOC	159.6±0.9^{e}	51.0±1.2^{b}	48.5±2.2^{c}	90.0±2.3^{a}	112±1^{ab}	93.0±0.6^{a}	119.6±0.6^{a}
85% S + 15% SOC	157.6±0.8^{e}	50.9±2.1^{b}	48.4±1.2^{c}	88.0±1.0^{a}	106±2^{b}	88.6±0.6^{b}	115.4±0.5^{a}

All results are shown as mean ± standard deviation. Means in the same column with different letters are significantly different (p<0.05). PrMax, pressure maximum; WA, water absorption; HYDHA, hydration at 15% water; TPrMax, time needed to reach pressure maximum; Tol, tolerance time; D250 and D450, drop-in pressure at 250 and 450 s, respectively.

sesame oil cake, which causes weakening of the dough. Furthermore, it was expected that the increase in lipid content due to the addition of sesame oil cake may have caused dough softening through the lubrication and shortening action of sesame oil. Similar results were reported by El-Adawy [1997], who studied the farinograph characteristics of wheat flour bread supplemented with sesame proteins.

Generally, the addition of sesame oil cake flour resulted in a reduction in the water absorption values (**Table 3**). The water absorption decreased from 55.8% for the control dough to 50.9–52.7% for the samples from the flour blends. This reduction in water absorption was attributed to the high lipid content of the sesame oil cake flour. The higher lipid content made that less moisture was required than in semolina to achieve a similar dough consistency. Despite the different proportions of sesame oil cake in the flour blends the differences in water absorption between doughs were not significant (p≥0.05). This demonstrates that the addition of up to 5% (*w/w*) of sesame oil cake flour resulted in a weakening of the dough gluten matrix, whereas further additions were of minor effect. Padalino *et al.* [2014] concluded that the addition of pea flour considerably increased the cooking loss of spaghetti samples and caused a significant decrease in the swelling index and water absorption when compared to the control. On the other hand, Raungrusmee *et al.* [2022] reported that water absorption was considered an indicator of gluten network extension and gluten matrix maturation during dough formation.

The hydration at 15% water decreased from 53.3% for the control to 48.4% for the dough from the flour blend with 15% (*w/w*) of sesame oil cake, but no significant differences (p≥0.05) were detected between doughs with sesame oil cake flour content above 7.5% (*w/w*) (**Table 3**).

The time needed to reach maximal pressure ranged from 88.0 to 94.0 s and did not differ significantly (p≥0.05) among samples (**Table 3**).

Tolerance is the time during which the dough consistency stands within the pressure maximum and pressure maximum 20% range [Zheng *et al.*, 2000]. Data in **Table 3** indicates that the tolerance time value for the control dough was 122 s and that no significant differences (p>0.05) were found through the increasing addition of sesame oil cake except for the dough from the blend with 15% (*w/w*) of sesame oil case, in which a significant decrease (p<0.05) in the tolerance time was observed when compared to the control. **Table 3** also presents the drop-in pressure from pressure maximum after both 250 and 450 s for the control sample of 78.7 and 87.5 kPa, respectively. A significant (p<0.05) decrease of drop-in pressure was presented with the addition of 5, 7.5, 10, 12.5, and 15% of sesame oil cake. The results agree with a study by El-Adawy [1997], who reported lower stability time with the addition of sesame products to wheat flour.

Sabanis *et al.* [2009] demonstrated that substituting wheat flour with gluten-free flours adversely affected the protein matrix during pasta processing, resulting in an unacceptable product quality. The texture of pasta made from wheat flours is directly related to the protein structure, particularly gluten proteins, due to their unique viscoelastic properties [Larrosa *et al.*, 2016]. Additionally, the protein in sesame oil cake, which mainly consists of globulins [Kinsella & Mohite, 1985], is believed to weakly contribute to the texture of the wheat gluten network. The presence of fiber particles in sesame oil cake flour may also disrupt the protein-starch network stability, leading to lower firmness. Protein-starch interactions significantly impact the formation and strength of the protein gel matrix during dough network formation [Saleh, 2017]. Moreover, sesame seed proteins contain a high percentage of sulfhydryl groups that soften the dough structure [El-Adawy, 1997]. Taking the above into account, the results of the consistograph test in our study were attributed to the weakening effect resulting from higher levels of sesame oil cake addition. The results were in line with Filipović *et al.* [2017] who concluded that the incorporation of sesame in the flour formulation of pasta resulted in a reduction of the speed of starch gelation, which led to a reduction in starch network stability.

■ Alveograph test parameters

The elasticity values (P) determined for the doughs made from wheat flour and flour blends with sesame oil cake ranged from 392.3 to 500.1 Pa (**Table 4**). A significant (p<0.05) difference was only found between the control sample and dough from

Table 4. Alveograph test characteristics of doughs made from semolina wheat flour (S) and its blends with sesame oil cake (SOC).

Dough	P (Pa)	L (mm)	G	W (10^{-4}J)	P/L	Ie (%)
Semolina	500.1±19.6^{a}	81±2^{a}	20.0±1.1^{a}	102.0±1.8^{a}	0.63±0.2^{b}	32.9±1.1^{a}
95% S + 5% SOC	490.3±9.8^{a}	64±1^{b}	17.8±1.5^{ab}	83.0±1.6^{b}	0.78±0.0^{a}	30.0±1.2^{b}
92.5% S + 7.5% SOC	460.9±19.6^{ab}	60±2^{b}	17.3±1.2^{ab}	73.0±1.4^{bc}	0.78±0.1^{a}	27.4±1.7^{bc}
90% S + 10% SOC	441.3±19.6^{ab}	57±2^{b}	16.9±1.1^{b}	72.0±1.4^{c}	0.79±0.1^{a}	26.7±1.1^{c}
87.5% S + 12.5% SOC	421.7±9.8^{ab}	55±1^{b}	16.0±1.5^{b}	70.0±1.8^{c}	0.78±0.0^{a}	26.0±1.2^{c}
85% S + 15% SOC	392.3±19.6^{b}	53±1^{b}	16.1±1.5^{b}	68.6±1.1^{c}	0.75±0.0^{ab}	25.6±1.1^{c}

All results are shown as mean ± standard deviation. Means in the same column with different letters are significantly different (p<0.05). P, elasticity; L, extensibility; G, swelling index; W, work; P/L, configuration ratio; Ie, elasticity index.

the blend with the highest proportion of sesame oil cake. This addition of sesame oil cake resulted in a decrease in the elasticity value. This may reflect an interaction process between the sesame components and the gluten network that also resulted in the dilution effect of gluten. In this regard, Barak *et al.* [2013] reported a positive correlation between P values obtained in the alveograph test and the glutenin content of the dough.

The extensibility (L) of all doughs containing sesame oil cake was significantly lower (p<0.05) when compared to the control dough (**Table 4**). The L values obtained after adding sesame oil cake flour up to 15% (*w/w*) were in the range of 53–64 mm, which is considered acceptable according to the CHOPIN protocol for durum wheat alveograph [Chopin Protocol, 2000].

The work (W) values, which represent the force necessary for the deformation of the dough, showed that the strength of the dough decreased significantly (p<0.05) with the increasing addition of sesame oil cake to the blend (**Table 4**). These results confirm the weakening effect due to the addition of sesame oil cake flour to durum semolina, and agree with a study by El-Adawy [1997], who reported that the addition of sesame cake weakened the bread formulation.

The configuration ratio represents the ratio of elasticity (P) of the dough to its extensibility (L). It was 0.63 for the control dough and increased significantly (p<0.05) in doughs with sesame oil cake (**Table 4**). The configuration ratio summarizes the viscoelastic behavior of the dough, and the results also confirm the weakening effect due to sesame oil cake addition to wheat flour. A configuration ratio between 0.5 and 1.0 was reported to provide an acceptable balance of extensibility [Tribune, 1999].

The effect of sesame oil cake on pasta cooking quality

At the optimum cooking time (10 min), the control sample showed a cooking loss value of 4.5 g/100 g dry pasta, which was significantly (p<0.05) lower than that of pasta from the blend with 7.5% (*w/w*) of sesame oil cake, but not significantly (p≥0.05) different than those of the other samples containing sesame oil cake flour (**Table 5**). However, the highest cooking loss, recorded for the pasta made from 7.5% (*w/w*) sesame oil cake blend (5.40 g/100 g dry pasta), did not exceed 0.9% of the result obtained for the control sample, which indicates that the pasta samples were in their acceptable cooking loss quality range. A cooking loss of 12 g/100 g or less was considered acceptable and indicative of good quality pasta [Fu, 2008]. Almost similar cooking loss trend was obtained for the pasta samples cooked for 13 and 20 min (**Table 5**). Again, the highest cooking loss values were determined for the pasta samples made with 7.5% (*w/w*) of sesame oil cake. The increase in cooking loss values of spaghetti fortified with edible legumes compared to spaghetti made from 100% semolina has also been reported by Filipović *et al.* [2017].

Table 5. Cooking gain and cooking loss of pasta made from semolina wheat flour (control) and blends of semolina (S) with sesame oil cake (SOC) and cooked for different times.

Pasta	Cooking gain (g/100 g)			Cooking loss (g/100 g dry pasta)		
	10 min	13 min	20 min	10 min	13 min	20 min
Control (100% S)	198.0±0.1^{d}	208.3±1.4^{c}	250.0±1.1^{c}	4.50±0.1^{b}	5.0±0.3^{c}	8.50±0.2^{c}
95% S + 5% SOC	214.6±0.2^{a}	221.0±0.4^{a}	263.4±1.4^{b}	4.80±0.6^{b}	5.8±0.1^{ab}	9.50±0.2^{b}
92.5% S + 7.5% SOC	212.7±0.4^{a}	224.3±1.1^{a}	271.2±1.7^{a}	5.40±0.4^{a}	5.9±0.1^{a}	9.60±0.7^{a}
90% S + 10% SOC	202.1±0.3^{b}	215.6±0.9^{b}	262.3±1.1^{b}	4.90±1.1^{b}	5.5±0.2^{bc}	9.00±0.1^{bc}
87.5% S + 12.5% SOC	200.9±0.1^{c}	215.0±0.3^{b}	252.2±0.6^{c}	4.80±0.8^{b}	5.4±0.0^{c}	8.80±0.6^{bc}
85% S + 15% SOC	200.0±0.8^{cd}	211.6±1.1^{c}	251.5±1.0^{c}	4.70±0.1^{b}	5.3±0.3^{c}	8.60±0.2^{bc}

All results are shown as mean ± standard deviation. Means in the same column with different letters are significantly different (p<0.05). Means in the same row (separately for cooking gain and cooking loss) were significantly different (p<0.05), and respective letters indicating significant differences were not shown.

The pasta cooking gain data is presented in **Table 5**. The cooking gain of pasta samples with sesame oil cake levels of 5, 7.5, and 10% (*w/w*) was significantly higher ($p<0.05$) compared to that of the control. The pasta produced from the flour bend with 7.5% (*w/w*) of sesame oil cake showed the greatest cooking gain when cooked for 10, 13, and 20 min. The control sample had a cooking gain of 198.0 g/100 g while the pasta made from semolina and 15% (*w/w*) of sesame oil cake blends had a cooking gain of 200.0 g/100 g at optimal cooking time (10 min). However, increasing the cooking duration for 3 or 10 min over the optimum (13 or 20 min) resulted in significant increases in the cooking gain values ($p<0.05$) for all pasta samples, which was attributed to giving starch granules an extra time to absorb more water. Results in **Table 5** also show a significant decrease ($p<0.05$) in the cooking gain when the sesame oil cake proportion in flour blend was increased from 7.5% to 10% (*w/w*), indicating the optimal level of sesame oil cake affects the starch swelling process and the cooking gain. A significant increase in pasta cooking gain was also reported by Bergman *et al.* [1994] when they supplemented pasta with cowpea meal. In turn, Garcia-Valle *et al.* [2021] reported that the water adsorption capacity was higher in chickpea flour pasta (100.54%) than in durum wheat (59.32%) due to the higher content of dietary fiber in chickpea flour (15.88 g/100 g) as compared to durum wheat semolina (15.88 g/100 g), which might be behind the increased water adsorption capacity of the chickpea flour pasta.

Acidity of pasta during storage

The acidity of pasta produced from wheat flour and fortified with sesame oil cake flour during storage for up to six months is shown in **Table 6**. The volume of 0.1 M NaOH used to neutralize the control pasta at zero time was 1.64 mL. The acidity of pasta made using flour blends with 7.5, 10, 12.5, and 15% (*w/w*) of sesame oil cake recorded a significant increase ($p<0.05$) compared to the control, while the use of the blends with 12.5 and 15% (*w/w*) of sesame oil cake resulted in the highest acidity value of pasta.

During storage, the acidity of each pasta increased significantly ($p<0.05$) (**Table 6**). The significantly ($p<0.05$) higher acidity was also found for the pasta samples made from flours containing sesame oil cake and stored for 3 and 6 months compared to the control sample. This increase can be attributed to the low-molecular-weight volatile fatty acids (secondary products of lipid autoxidation), which are extractable by the ethanol solution. However, the compact dry structure of pasta and the natural

Table 6. Acidity (mL of 0.1 M NaOH) of pasta samples made from semolina wheat flour (control) and blends of semolina (S) with sesame oil cake (SOC) during storage.

Pasta	Storage period (month)		
	0	3	6
Control (100% S)	1.64 ± 0.11^{eC}	2.20 ± 0.01^{eB}	2.38 ± 0.10^{eA}
95% S + 5% SOC	1.71 ± 0.07^{cdeC}	2.66 ± 0.00^{dB}	2.86 ± 0.15^{dA}
92.5% S + 7.5% SOC	1.77 ± 0.11^{cdC}	2.92 ± 0.05^{cB}	3.12 ± 0.13^{cA}
90% S + 10% SOC	1.84 ± 0.08^{bcC}	3.11 ± 0.11^{abcB}	3.25 ± 0.20^{bcA}
87.5% S + 12.5% SOC	1.91 ± 0.10^{abC}	3.21 ± 0.05^{abB}	3.37 ± 0.11^{abA}
85% S + 15% SOC	2.00 ± 0.17^{aC}	3.25 ± 0.06^{aB}	3.51 ± 0.20^{aA}

All results are shown as mean ± standard deviation. Means in the same column with different lowercase letters are significantly different ($p<0.05$). Means in the same row with different uppercase letters are significantly different ($p<0.05$).

Table 7. Results of sensory evaluation using a seven-point hedonic scale of cooked pasta made from semolina wheat flour (control) and blends of semolina (S) with sesame oil cake (SOC).

Storage period (month)	Control (100% S)	95% S + 5% SOC	92.5% S + 7.5% SOC	90% S + 10% SOC	87.5% S + 12.5% SOC	85% S + 15% SOC
Color						
0	6.2 ± 0.3^{a}	4.5 ± 0.1^{b}	4.1 ± 0.1^{d}	4.6 ± 0.3^{b}	4.0 ± 0.5^{d}	3.5 ± 0.3^{e}
3	6.3 ± 0.1^{a}	4.3 ± 0.3^{b}	3.9 ± 0.4^{c}	4.4 ± 0.8^{b}	3.8 ± 0.4^{cd}	3.6 ± 0.2^{d}
6	6.1 ± 0.1^{a}	4.2 ± 0.8^{bc}	4.1 ± 0.0^{bc}	4.4 ± 0.7^{b}	3.9 ± 0.5^{cd}	3.5 ± 0.8^{d}
Flavor						
0	6.3 ± 0.1^{a}	4.4 ± 0.8^{b}	4.1 ± 0.1^{bc}	4.0 ± 0.4^{cd}	3.6 ± 0.4^{d}	3.0 ± 0.1^{e}
3	6.3 ± 0.4^{a}	4.2 ± 0.4^{b}	4.2 ± 0.4^{b}	4.1 ± 0.1^{bc}	3.9 ± 0.3^{c}	3.1 ± 0.2^{d}
6	6.3 ± 0.5^{a}	4.2 ± 0.9^{b}	4.1 ± 0.4^{bc}	3.9 ± 0.7^{cd}	3.8 ± 0.1^{d}	2.8 ± 0.4^{e}
Stickiness						
0	6.3 ± 0.4^{a}	4.3 ± 0.2^{b}	4.3 ± 0.2^{b}	4.2 ± 0.4^{b}	3.8 ± 0.4^{c}	3.1 ± 0.6^{d}
3	6.5 ± 0.1^{a}	4.2 ± 0.2^{b}	4.2 ± 0.1^{b}	4.1 ± 0.1^{bc}	3.9 ± 0.3^{cd}	3.2 ± 0.6^{d}
6	6.4 ± 0.9^{a}	4.3 ± 0.4^{b}	4.2 ± 0.0^{b}	4.2 ± 0.7^{b}	3.7 ± 0.1^{c}	3.1 ± 0.7^{d}
Overall acceptability						
0	6.3 ± 0.2^{a}	4.4 ± 0.8^{b}	4.2 ± 0.5^{bc}	4.2 ± 0.1^{bc}	3.9 ± 0.4^{c}	3.2 ± 0.5^{d}
3	6.3 ± 0.2^{a}	4.2 ± 0.4^{b}	4.1 ± 0.2^{b}	4.2 ± 0.2^{b}	3.9 ± 0.3^{c}	3.3 ± 0.0^{d}
6	6.3 ± 0.4^{a}	4.2 ± 0.2^{b}	4.1 ± 0.8^{b}	4.2 ± 0.2^{b}	3.8 ± 0.1^{c}	3.1 ± 0.1^{d}

All results are shown as mean ± standard deviation. Means for individual characteristics with different letters are significantly different ($p<0.05$)

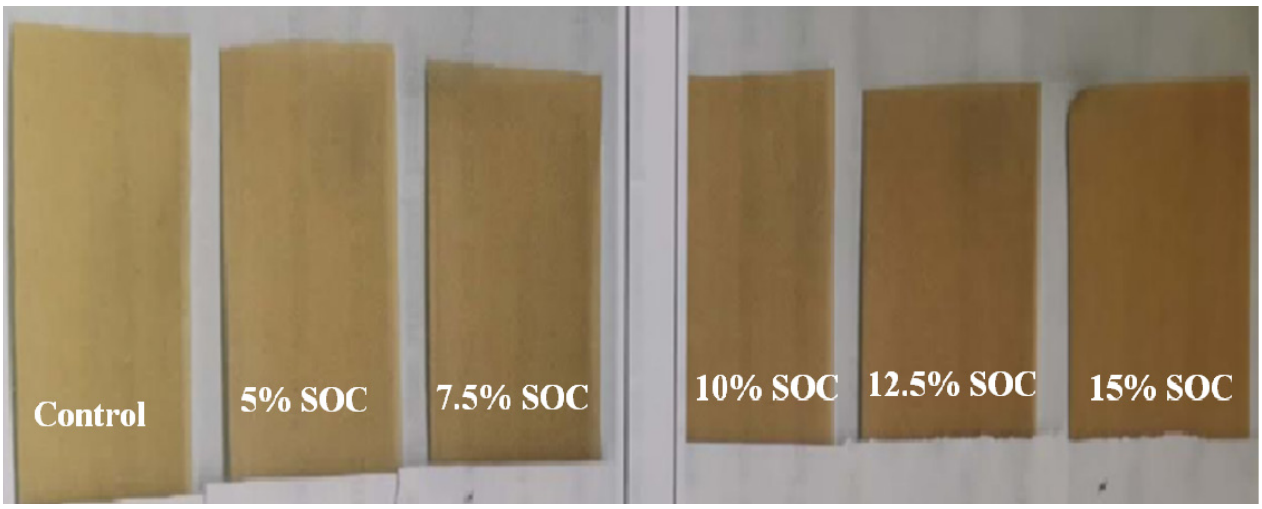

Figure 1. Appearance of dry pasta produced from semolina wheat flour (control) and its blends with 5, 7.5, 10, 12.5, and 15% (*w/w*) of sesame oil cake (SOC).

antioxidants of sesame oil may suggest limited oxidative rancidity. The presence of endogenous antioxidants in sesame oil cake may play a role in the stability of oxidation and contribute to maintaining nutritional quality and extending the shelf life [Melo *et al.*, 2021]. Sesame seed oil is used in food industries as it has a longer shelf life and tremendous antioxidant properties [Yaseen *et al.*, 2021], and sesame oil cake still contains a proportion of oil (32%) with a fatty acid pattern characterized by the abundance of unsaturated fatty acids [Melo *et al.*, 2021]. Kinsella & Mohite [1985] concluded that sesame oil had excellent stability due to the presence of natural antioxidants such as sesamolin, sesamin, and sesamol. Our results agreed with those reported by Duszkiewicz-Reinhard *et al.* [1988], who fortified spaghetti with edible legumes.

Pasta sensory acceptability

Overall, pasta produced from wheat flour with 5, 7.5, and 10% (*w/w*) of sesame oil cake showed higher acceptability to the panelists than the pasta samples made from blends fortified with the higher contents of sesame oil cake, while pasta made from pure semolina recorded the highest acceptability (**Table 7**). Additionally, storage did not affect the panelists' acceptability of pasta samples containing sesame oil cake or those produced only from wheat flour. There was a significant decrease ($p<0.05$) in the color-liking scores of pasta samples produced from flour blends. As the level of sesame oil cake in blended flour increased, the color of the pasta turned darker (**Figure 1**) and became less acceptable to the panelists (**Table 7**). No significant differences ($p\geq0.05$) in the color scores were recorded between all pasta samples at zero time when compared to those stored for three and six months, which indicates color stability throughout the storage period.

The panelists showed a preference for the flavors of pasta produced from the flour blends containing 5, 7.5, and 10% (*w/w*) of sesame oil cake over pasta from the blends containing 12.5 and 15% (*w/w*) of sesame oil cake (**Table 7**). However, the control pasta received the highest flavor score. A similar observation was made by Bergman *et al.* [1994], who supplemented pasta with cowpea meal.

No significant difference ($p\geq0.05$) was observed in the stickiness of cooked pasta from the flour blends containing 5, 7.5, and 10% (*w/w*) of sesame oil cake, while the pasta samples containing 12.5 and 15% (*w/w*) of sesame oil cake were significantly ($p<0.05$) stickier than the control sample (**Table 7**).

CONCLUSIONS

Results suggest the possible utilization of sesame oil cake in bakery products. The addition of sesame oil cake flour to semolina showed significant improvement in the pasta's functional characteristics and nutritional value. The blend flours provided higher protein and lipid contents in the pasta; however, the sesame oil cake addition reduced the percentage of gluten content in the flour, thereby weakening the dough formulation and lowering water absorption capacity, which affected the final product's quality characteristics. Moreover, the increase in cooking loss did not exceed 0.9% in any of the pasta samples containing sesame oil cake, which indicates that the pasta samples were in their acceptable cooking loss quality ranges. Sensorial evaluation of the flavor and stickiness of pasta produced from the flour blends containing up to 10% (*w/w*) of sesame oil cake was satisfactory and recommended to open the door for industrial utilization of such valuable and nutritious by-products. The storage period did not affect the panelists' acceptability of pasta samples containing sesame oil cake.

Sesame compounds and gluten network interactions are believed to have a key role in modifying pasta's functional characteristics. However, further investigation of sesame compounds and the gluten network interactions, including their effects on dough rheological characteristics and studying the protein quality of pasta fortified with sesame oil cake, is required to obtain higher quality pasta products.

ADDITIONAL INFORMATION

The study was approved by the Ethical Committee of the University of Jordan. Sensory evaluation involving human testing

was conducted after oral consent was permitted from each panel. Written informed consent was obtained from all study participants. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTERESTS

The authors declare that they do not have any conflict of interest.

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REFERENCES

1. AACC, American Association of Cereal Chemists. (2000). *Approved Methods of Analysis*, (11th ed.). Cereals and Grains Association, St. Paul, MN, U.S.A.
2. Animashaun, O., Olorode, O., Sofunde, K., Idowu, M. (2017). Quality evaluation of pasta fortified with roasted sesame flour. *Journal of Environmental Science, Toxicology and Food Technology, 11*(7), 29-34. https://doi.org/10.9790/2402-1107012934
3. AOAC, Association of Official Analytical Chemists International. (2005). *Official Methods of Analysis* (17th ed.). Association of Official Analytical Chemistrs, Washington, DC., USA.
4. Barak, S., Mudgil, D., Khatkar, B.S. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. LWT – *Food Science and Technology*, *51*(1), 211-217. https://doi.org/10.1016/j.lwt.2012.09.011
5. Bergman, C., Gualberto, D., Weber, C. (1994). Development of high-temperature dried soft wheat pasta supplemented with cowpea (*Vigna unguiculata* (L.) Walp). Cooking quality, color, and sensory evaluation. *Cereal Chemistry*, *71*(6), 523-727.
6. Bresciani, A., Pagani, M.A., Marti, A. (2022). Pasta-making process: A narrative review on the relation between process variables and pasta quality. *Foods*, *11*(3), art. no. 256. https://doi.org/10.3390/foods11030256
7. Bustos, M.C., Paesani, C., Quiroga, F., Leon, A.E. (2019). Technological and sensorial quality of berry-enriched pasta. *Cereal Chemistry*, *96*(5), 967-976. https://doi.org/10.1002/cche.10201
8. Chopin Protocol 2000. Durum wheat Alveograph/Consistograph Study, pp. 1-8.
9. Cui, C., Wang, Y., Ying, J. Zhou, W., Li. D., Wang, L.J. (2024). Low glycemic index noodle and pasta: Cereal type, ingredient, and processing. *Food Chemistry*, *431*, art. no. 137188. https://doi.org/10.1016/j.foodchem.2023.137188
10. De Arcangelis, E., Cuomo, F., Trivisonno, M.C., Marconi, E., Messia, M.C. (2020). Gelatinization and pasta making conditions for buckwheat gluten-free pasta. *Journal of Cereal Science*, *95*, art. no. 103073. https://doi.org/10.1016/j.jcs.2020.103073
11. Duszkiewicz-Reinhard, W., Khan, K., Dick, J.W., Holm, Y. (1988). Shelf-life stability of spaghetti fortified with legume flours and protein concentrates. *Cereal Chemistry*, *65*(4), 278-281.
12. Dziki, D. (2021). Current trends in enrichment of wheat pasta: Quality, nutritional value and antioxidant properties. *Processes*, *9*(8), art. no. 1280. https://doi.org/10.3390/pr9081280
13. El-Adawy, T. (1997). Effect of sesame seeds protein supplementation on the nutritional, physical, chemical, and sensory properties of wheat flour bread. *Food Chemistry*, *59*(1), 7-14. https://doi.org/10.1016/0308-8146(95)00197-2
14. Filipović, V., Filipović, J., Simonovska, J., Rafajlovska, J. (2017). Effect of sesame flour and eggs on technology and nutritive quality of spelt pasta. *Journal of the Serbian Chemical Society*, *82*(10), 1097-1109. https://doi.org/10.2298/JSC170405060F
15. Fu, B.X. (2008). Asian noodles: History, classification, raw materials, and processing. *Food Research International*, *41*(9), 888–902. https://doi.org/10.1016/j.foodres.2007.11.007
16. Garcia-Valle, D.E., Bello-Pérez, L.A., Agama-Acevedo, E., Alvarez-Ramirez, J. (2021). Effects of mixing, sheeting, and cooking on the starch, protein, and water structures of durum wheat semolina and chickpea flour pasta. *Food Chemistry*, *360*, art. no. 129993. https://doi.org/10.1016/j.foodchem.2021.129993
17. Hummel, C. (1966). *Macaroni Products: Manufacture, Processing and Packing*. Food Trade Press: London, UK.
18. Ingelbrecht, J.A., Moers, K., Abécassis, J., Rouau, X., Delcour, J.A. (2001). Influence of arabinoxylans and endoxylanases on pasta processing and quality. *Cereal Chemistry*, *78*(6), 721-729. https://doi.org/10.1094/CCHEM.2001.78.6.721
19. ISO, International Organization for Standardization, (1981). *Agricultural food products – Determination of crude fibre content - General Method*. https://www.iso.org/standard/11544.html.
20. Jordanian Institute for Standards and Metrology, Hashemite Kingdome of Jordan. Standard No.67. (1996). *Cereal and Cereal Products: Dried Pasta*. Amman, Jordan.
21. Kaur, G., Sharma, S., Nagi, H.P.S., Ranote, P.S. (2013). Enrichment of pasta with different plant proteins. *Journal of Food Science and Technology*, *50*(5), 1000-1005. https://doi.org/10.1007/s13197-011-0404-2
22. Kinsella, J.E., Mohite, R.R. (1985). Chapter 13: The physicochemical characteristics and functional properties of sesame proteins. In A.A. Altschul, H.L. Wilcke (Eds.), *New Protein Foods. Volume 5: Seed Storage Proteins*, Academic Press, London, pp. 435-456. https://doi.org/10.1016/B978-0-12-054805-7.50020-8
23. Larrosa, V., Lorenzo, G., Zaritzky, N., Califano, A. (2016). Improvement of the texture and quality of cooked gluten-free pasta. LWT – *Food Science and Technology*, *70*, 96-103. https://doi.org/10.1016/j.lwt.2016.02.039
24. Marconi, E., Graziano, M., Cubadda, R. (2000). Composition and utilization of barley pearling by-products for making functional pasta rich in dietary fiber and beta-glucan. *Cereal Chemistry*, 77(2), 133-139. https://doi.org/10.1094/CCHEM.2000.77.2.133
25. Matsuo, R.R. (1993). Durum wheat: Production and processing. In E. Bass (Ed.), *Grains and Oilseeds: Handling, Marketing, Processing*, 4th Edition, Canadian International Grains Institute, Winnipeg, Canada, pp. 779-807.
26. Melo, D., Álvarez-Ortí, M., Nunes, M.A., Costa, A.S.G., Machado, S., Alves, R.C., Pardo, J.E., Oliveira, M. (2021). Whole or defatted sesame seeds (*Sesamum indicum* L.)? The effect of cold pressing on oil and cake quality. *Foods*, *10*(9), art. no. 2108. https://doi.org/10.3390/foods10092108
27. Moayedi, S., Ohm, J.-B., Manthey, F.A. (2021). Relationship between cooking quality of fresh pasta made from durum wheat and protein content and molecular weight distribution parameters. *Cereal Chemistry*, *98*(4), 891–902. https://doi.org/10.1002/cche.10431
28. Mostashari, P., Khaneghah, M.A. (2024). Sesame seeds: A nutrient-rich superfood. *Foods, 13*(8), art. no. 1153. https://doi.org/10.3390/foods13081153
29. Oliviero, T., Fogliano, V. (2016). Food design strategies to increase vegetable intake: The case of vegetable enriched pasta. *Trends in Food Science and Technology*, *51*, 58–64. https://doi.org/10.1016/j.tifs.2016.03.008
30. Ozgoren, E., Yapar, A. (2019). Effect of the addition of smoked trout fillet powder to the quality properties of pasta. *Italian Journal of Food Science*, *31*(1), 110-124. **https://doi.org/10.14674/IJFS-1263**
31. Padalino, L., Mastromatteo, M., Lecce, L., Spinelli, S., Contò, F., Del Nobile, M.A. (2014). Chemical composition, sensory and cooking quality evaluation of durum wheat spaghetti enriched with pea flour. *International Journal of Food Science and Technology*, *49*(6), 1544-1556. https://doi.org/10.1111/ijfs.12453
32. Raungrusmee, S., Koirala, S., Anal, A.K. (2022). Effect of physicochemical modification on granule morphology, pasting behavior, and functional properties of riceberry rice (*Oryza Sativa* L.) starch. *Food Chemistry Advances*, *1*, art. no. 100116. https://doi.org/10.1016/j.focha.2022.100116
33. Romano, A., Ferrani, P., Gallo, V., Masi, O. (2021). New ingredients and alternatives to durum wheat semolina for a high quality dried pasta. *Current Opinion in Food Science*, *41*, 249-259. https://doi.org/10.1016/j.cofs.2021.07.005
34. Sabanis, D., Lebesi, D., Tzia, C. (2009). Effect of dietary fibre enrichment on selected properties of gluten-free bread. LWT – *Food Science and Technology*, *42*(8), 1380-1389. https://doi.org/10.1016/j.lwt.2009.03.010
35. Saleh, M.I. (2017). Protein-starch matrix microstructure during rice flour pastes formation. *Journal of Cereal Science*, *74*, 183-186. https://doi.org/10.1016/j.jcs.2017.02.005
36. SAS Institute. (2003). SAS User's Guide in Statistics, 10th International Symposium, Cary, NC., USA: SAS Institutes, Inc.

37. Schmidt, H.O., de Oliveira, V.R. (2023). Overview of the incorporation of legumes into new food options: An approach on versatility, nutritional, technological, and sensory quality. *Foods*, *12*(13), art. no. 2586. https://doi.org/10.3390/foods12132586
38. Sicignano, A., di Monaco, R., Masi, P., Cavella, S. (2015). From raw material to dish: Pasta quality step by step. *Journal of the Science of Food and Agriculture*, *95*(13), 2579-2587. https://doi.org/10.1002/jsfa.7176
39. Tribune. (1999). Chopin SA. Newsletter for Flour Producers and Users. June No.7 Imprimerie Lni SA, France. https://www.scribd.com/document/78846090/Chopin-TRibune-No-05-ENGLISH
40. Wang, J., Brennan, M.A., Serventi, L., Brennan, C.S. (2022). Impact of functional vegetable ingredients on the technical and nutritional quality of pasta. *Critical Reviews in Food Science and Nutrition*, *62*(22), 6069-6080. https://doi.org/10.1080/10408398.2021.1895712
41. Wei, P., Zhao, F., Wang, Z., Wang, Q., Chai, X., Hou, G., Meng, Q. (2022). Sesame (*Sesamum indicum* L.): A comprehensive review of nutritional value, phytochemical composition, health benefits, development of food, and industrial applications. *Nutrients*, *14*(19), art. no. 4079. https://doi.org/10.3390/nu14194079
42. Yaseen, G., Ahmad, M., Zafar, M., Akram, A., Sultana, S., Ahmed, S.N., Kilic, O. (2021). Chapter 15: Sesame (*Sesamum indicum L.*). In Inamudin, R. Boddula, A.M. Asiri (Eds.), *Green Sustainable Process for Chemical and Environmental Engineering and Science*, Elsevier, pp. 253–269. https://doi.org/10.1016/B978-0-12-821886-0.00005-1
43. Yokoyama, W.H., Hudson, C.A., Kunckles, B.E., Chiu, M.M.-Ch., Sayre, R.N., Turnlund, J.R., Schneeman, B.A. (1997). Effect of barley β-glucan in durum wheat pasta on human glycemic response. *Cereal Chemistry, 74*(3), 293-296. https://doi.org/10.1094/CCHEM.1997.74.3.293
44. Zheng, H., Morgenstern, M.P., Campanella, O.H., Larsen, O.G. (2000). Rheological properties of dough during mechanical dough development. *Journal of Cereal Science, 32*, 293–306. https://doi.org/10.1006/jcrs.2000.0339

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Sea Fennel (*Crithmum maritimum* L.) Flowers as an Emerging Source of Bioactive Compounds

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Sea fennel (*Crithmum maritimum* L.) is an edible halophytic species rich in various valuable phytochemicals, and has accordingly, been used in traditional medicine and nutrition since ancient times. The aim of this study was to investigate the chemical composition of sea fennel flowers from three regionally (ecologically) different locations on the Croatian Adriatic coast (Pag, Korčula, Cavtat). The profiles of essential oils (EOs), fatty acids (FAs), tocopherols, and phenolic compounds (total phenolics, flavonoids, and tannins) as well as associated antioxidant capacity were analysed. The flowers collected at the northernmost site had the lowest contents of total phenolic compounds, limonene in the EO and unsaturated FAs, but the highest content of α-tocopherol, one of the best-known plant lipophilic antioxidants. On the other hand, the flowers from Korčula and Cavtat contained high amounts of phenolics, especially chlorogenic acid (7.99 and 13.27 mg/g dry plant matter, respectively), resulting in high antioxidant activity of the samples. Despite these differences in composition, which may be related to the geographical location of the sampling site, sea fennel flowers from all locations can be considered a valuable source of important health-promoting phytochemicals.

Keywords: essential oil, volatile organic compounds, phenolics, fatty acids, tocopherols, antioxidant capacity

INTRODUCTION

Sea fennel (*Crithmum maritimum* L., Apiaceae), also known as rock samphire or crest marine, is a halophytic species commonly found on cliffs and rocks, and somewhat less often on gravel or sandy soils along the Mediterranean, Black Sea, and European Atlantic coasts [Generalić Mekinić *et al.*, 2016; Maleš *et al.*, 2003; Meot-Duros & Magné, 2009]. The flowering period of the sea fennel usually extends from June to September and results in umbels with tiny yellow green flowers [Atia *et al.*, 2011; Franke, 1982; Nartea *et al.*, 2023]. It has been used for years as a source of vitamin C and in folk medicine as a diuretic, digestive and laxative [Franke, 1982; Renna, 2018]. Its essential oils (EOs) have the potential to be used in cosmetics, while the leaves are commonly used in cuisine either as a spice, brined or cooked [Radman *et al.*, 2023; Renna *et al.*, 2017; Renna & Gonnella, 2012]. The plant is also rich in carotenoids, minerals, organic acids, and phenolics with high biological activity [Generalić Mekinić *et al.*, 2024; Maleš *et al.*, 2003; Renna, 2018; Renna *et al.*, 2017; Siracusa *et al.*, 2011], especially substantial antioxidant and anti-inflammatory activity due to the high content of chlorogenic acid and its derivatives [Alemán *et al.*, 2019; Generalić Mekinić *et al.*, 2016, 2024; Meot-Duros & Magné, 2009; Nartea *et al.*, 2023]. Due to its rich chemical composition and health-promoting effects, sea fennel has recently been recognised as a new "cash" crop for saline agriculture.

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There are a large number of studies on the leaves [Generalić Mekinić *et al.*, 2016, 2024; Houta *et al.*, 2011; Kadoglidou *et al.*, 2022; Martins-Noguerol *et al.*, 2022; Nartea *et al.*, 2023] and the aerial parts of the sea fennel [Alves-Silva *et al.*, 2020; Nabet *et al.*, 2017; Özcan *et al.*, 2001, 2006], but only a few studies have investigated the flowers [Generalić Mekinić *et al.*, 2016; Houta *et al.*, 2011; Nartea *et al.*, 2023; Politeo *et al.*, 2023]. Moreover, these studies mainly focus on the EO profile and phenolic content, and contain little or no data on the other nutritionally and biologically important phytochemicals. Therefore, the main objective of this study was to investigate the chemical composition of sea fennel flowers from three different regions on the Croatian Adriatic coast. According to the results of previous reports, Croatian sea fennel could be considered as a specific chemotype; hence, this study aimed to gain an insight into the variability of chemical composition between populations from different geographical locations. The EOs as well as the FAs were analysed by gas chromatography-mass spectrometry (GC-MS), while the individual phenolic compounds and tocopherols were determined by high performance liquid chromatography (HPLC). In addition, the study included the determination of total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) in the sea fennel flowers, and their respective antioxidant capacity. To the best of author's knowledge, this is the first report on the presence and content of valuable antioxidant and nutritive phytochemicals in the flowers of the special chemotype of sea fennel from the Croatian coast.

MATERIALS AND METHODS

Chemicals, reagents and standards

All chemicals, reagents, and solvents used in this study were of analytical grade. HPLC grade acetonitrile and methanol were procured from Sigma–Aldrich GmbH (Steinheim, Germany), as were the phenolic HPLC standards, including chlorogenic acid (3-*O*-caffeoylquinic acid), cryptochlorogenic acid (4-*O*-caffeoylquinic acid), neochlorogenic acid (5-*O*-caffeoylquinic acid), caffeic acid, gallic acid, *p*-hydroxybenzoic acid, sinapic acid, ferulic acid, protocatechuic acid, and rutin. In addition, a series of *n*-hydrocarbons (C8–C40, Supelco), the standard set of fatty acid methyl esters (FAMEs) (Supelco 37 Component FAME Mix) and main reagents for spectrophotometric assays (Folin-Ciocalteu's phenol reagent; vanillin; 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ); 2,2-diphenyl-1-picryl hydrazyl (DPPH); fluorescein; *2,2'-azobis*(2-methylpropionamidine) dihydrochloride (AAPH); and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)) were also procured from Sigma-Aldrich. The tocopherols were purchased from LGC Standards Ltd. (Teddington, Middlesex, *UK*).

Plant material

The flowers of the sea fennel (*C. maritimum* L.) were used as plant material (**Figure 1A**). Sampling took place in early August 2022 along the Croatian Adriatic coast in the Dalmatia region. To cover the area of the Dalmatia region, samples were collected at three different locations: on the island of Pag (44°29'49.74" N, 14°54'58.19" E), the northernmost location, on the island of Korčula (42°57'7.80" N, 17°8'21.84" E) which was the most "pointiest" island in Central Dalmatia, and in the town of Cavtat (42°35'13.29" N, 18°12'41.90" E), the southernmost location (**Figure 1B**). About 500 g of fresh floral material was harvested for the study. The plant material for the analysis of all compounds, except for essential oil volatiles, was frozen and dried by lyophilisation using the FreeZone 2.5 system (Labconco, Kansas City, MO, USA), then homogenised in a hand mill and sieved through a 1 mm diameter screen. The obtained plant powder was used to extract the target compounds. For EO analysis, the plant material was air-dried at room temperature for 15 days and then used for oil extraction without any treatment.

Isolation and analysis of the essential oils

The hydrodistillation of sea fennel flowers was carried out for 3 h in a modified Clevenger apparatus containing a pentane

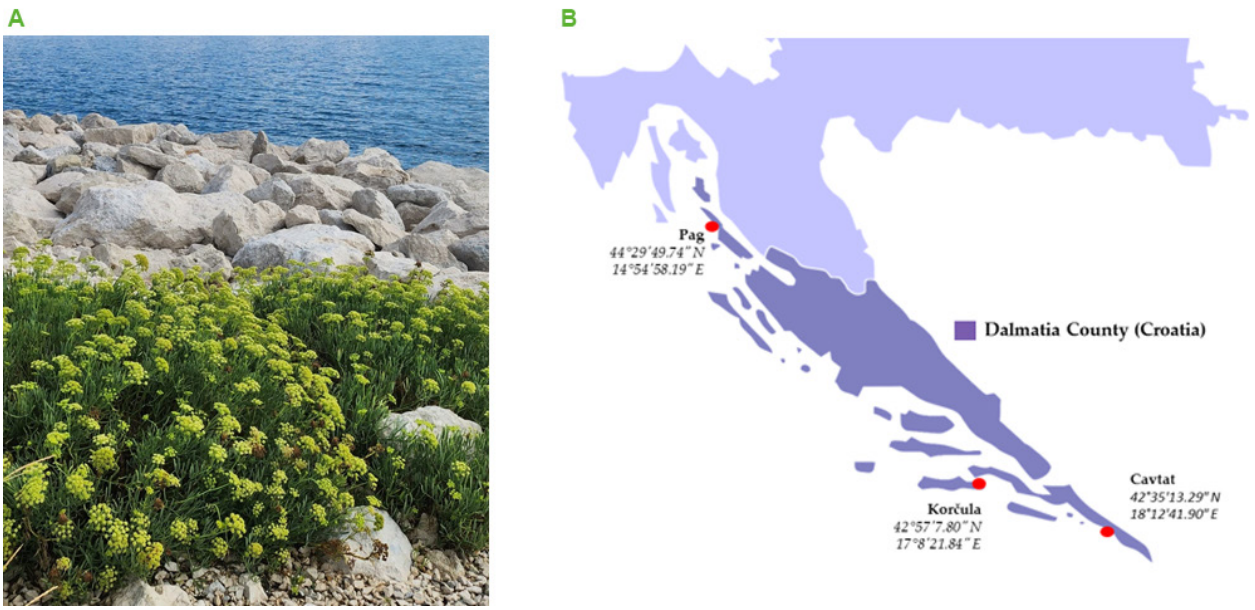

Figure 1. (**A**) Sea fennel (*Crithmum maritimum* L.) in the flowering period. (**B**) Croatian Adriatic coast with marked sea fennel sampling locations.

and diethyl ether mixture (2:1, *v/v*) as an organic solvent trap. The detailed procedure was described by Bektasevic *et al.* [2023]. The isolated EOs were concentrated and stored at 4°C until analysis. The yield of extraction of EOs was calculated on a dry basis of the flowers (%, *w/w*).

The analysis of volatile organic compounds (VOCs) in the EOs was performed using a gas chromatograph (GC) equipped with an automated liquid injector and a tandem mass spectrometer (MS). Specifically, a model 8890 GC with a model 7693A liquid injector and a model 7000D GC/TQ mass spectrometer, all from Agilent Technologies Inc. (Santa Clara, CA, USA), was used for this purpose. VOC separation was performed on a non-polar HP-5ms column (5% phenylmethylpolysiloxane, dimensions: 30 m × 0.25 mm × 0.25 µm, Agilent Technologies Inc.) with helium as carrier gas at a flow rate of 1 mL/min. The injection volume was 1 µL at a split ratio of 1:50, and the inlet temperature was maintained at 250°C. The column temperature was programmed as follows: an initial isothermal phase at 60°C for 3 min, followed by a temperature gradient from 60°C to 246°C at a rate of 3°C/min, with a subsequent isothermal hold for 25 min. The MS detector operated at ionisation energy of 70 eV with an ion source temperature of 200°C. Ion scanning included a full scan mode ranging from 33 to 350 *m/z*.

Individual peaks were identified by comparing their retention indices (RIs) with those of a series of *n*-hydrocarbons (C8–C40) injected under identical conditions. In addition, software matching of mass spectra with commercial databases such as the Wiley 7 MS library (Wiley, NY, USA) and NIST02 (Gaithersburg, MD, USA), and comparison of mass spectra and RIs with literature data, aided in peak identification. The relative content of identified compounds was calculated as the percentage of the peak area. All analyses were conducted in triplicate, and results were expressed as mean ± standard deviation (SD).

Total phenolic, flavonoid, and tannin content determination

Extraction of the homogenised, freeze-dried sea fennel flower samples (0.5 g) was performed in a methanol and water mixture (80:20 *v/v*, 20 mL) by sonication at room temperature for 15 min followed by mixing in an orbital shaker for 3 h at room temperature. The samples were filtered (Chromafil™ Xtra PTFE syringe filter, 0.45 µm, Macherey-Nagel, Düren, Germany) after standing overnight in the dark at 4°C. The absorbance measurements in TPC, TFC, and TTC assays were conducted using a SPECORD 200 Plus, Edition 2010 (Analytik Jena AG, Jena, Germany).

The TPC of sea fennel flower extracts was determined using the Folin–Ciocalteu reagent [Singleton & Rossi, 1965] with results calculated based on a calibration curve for gallic acid and expressed as mg of gallic acid equivalents *per* g of dry plant matter (mg GAE/g d.p.m.).

TFC was determined by the colorimetric method using aluminium chloride [Yang *et al.*, 2004], and the results were calculated based on a calibration curve for rutin, and expressed as mg of rutin equivalents *per* g of dry plant matter (mg RE/g d.p.m.).

TTC assessment was conducted using the vanillin-HCl method [Hagerman & Butler, 1994], with results calculated using a calibration curve for catechin and expressed as mg of catechin equivalents *per* g of dry plant matter (mg CE/g d.p.m.).

Each sample was measured in triplicate, and results were expressed as mean ± SD.

Individual phenolic compounds' analysis

For the analysis of the individual phenolic compounds, the same extracts were used that had previously been prepared to determine the total content of phenolics, flavonoids and tannins. A Shimadzu Nexera HPLC system LC-40 (Shimadzu, Kyoto, Japan), equipped with a UV/VIS detector, was used according to the methodology described by Politeo *et al.* [2023]. Phenolic compounds were separated using a Phenomenex C18 reversed-phase column (250 × 4.6 mm, 5 µm; Torrance, CA, USA). The column temperature (35°C) and flow rate (1.0 mL/min) were constant. The mobile phases consisted of an aqueous solvent (A) containing 0.2% *o*-phosphoric acid in water and an organic solvent (B) comprising methanol and acetonitrile in a 1:1 (*v/v*) ratio. The elution process started isocratically with 4% B for 5 min, followed by a gradient program: 5–16 min (linear gradient up to 15% B), 16–50 min (linear gradient up to 35% B), 50–62 min (linear gradient up to 4% B), and 62–65 min (4% B). Initial conditions were reinstated within 2 min and sustained for 10 min for column equilibration. Peaks were identified by comparing retention times with standards recorded under identical conditions, while quantification at 220 or 320 nm was conducted using calibration curves for external standards. Results were expressed as mg of phenolic compound detected *per* g of dry plant matter (mg/g d.p.m.). Two injections were performed for each sample, and results are expressed as mean ± SD.

Fatty acid analysis

To determine the composition of FAs in sea fennel flower samples, an analysis of FAMEs obtained by methylation was performed. Approximately 1 g of freeze-dried samples was mixed with 4 mL of 2-propanol and heated at 80°C for 15 min for extraction. Subsequently, 6 mL of *n*-hexane and 5 mL of a sodium sulphate solution (6.7%, *w/v*) were added, and the mixture was shaken vigorously. After centrifugation, the upper *n*-hexane layer containing the lipids was transferred to a new tube. The aqueous phase was extracted a second time with 7.5 mL of *n*-hexane and 2-propanol mixture (7:2, *v/v*), and the resulting upper *n*-hexane phase was combined with the previous extract. Subsequently, 3 mL of a methanol-toluene-sulphuric acid solution (88:10:2, *v/v/v*) was added to methylate the FA, and then the mixture was heated at 80°C for 1 h. The FAMEs were then extracted with *n*-heptane.

Analysis of the prepared FAMEs was carried out using a GC system (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with a flame ionisation detector (FID), under the following conditions: a sample injection volume of 1 µL, an injector temperature of 250°C, and a split ratio of 1:50. Helium served as carrier gas with a flow rate of 2 mL/min. Separation was achieved using an RTX 2330 capillary column (30 m × 0.25 mm, 0.2 µm; Restek Corp., Bellefonte, PA, USA), with the detector temperature also

set to 250°C. Samples were injected in duplicate, and a FAME standard set was injected under identical conditions to identify the compounds. The results of two injections (percentage of peak area) were expressed as mean ± SD.

Tocopherol determination

The tocopherols were extracted with *n*-hexane after saponification. First, 0.4 g of freeze-dried sea fennel flower powder was mixed with 1 g of ascorbic acid, 0.1 g of sodium sulphate, 20 mL of ethanol, and 4 mL of 60% potassium hydroxide solution. The mixture was heated in a water bath for 30 min at 85°C, shaking occasionally. After heating, the mixture was cooled, and 12 mL of water was added. A triple extraction was then performed by adding 10 mL of *n*-hexane. The resulting organic phases were combined, washed four times with 10 mL of water, dried at 35°C using a rotary evaporator, and finally dissolved in *n*-hexane [Knecht *et al.*, 2015].

The analysis was performed using an HPLC system (Series 200, Perkin Elmer, Walthamn, MA, USA) equipped with a fluorescence detector (FLD). A volume of 20 µL was injected on an Ultra-silica column (250 × 4.6 mm, 5 µm, Restek Corp.). *n*-Hexane and 2-propanol mixture at a ratio of 96:4 (*v/v*) was used at a flow rate of 0.8 mL/min as the mobile phase to separate the compounds. The temperature of the column was maintained at 25°C. The excitation wavelength of 290 nm and emission wavelength of 330 nm were used for detection. The α-, β-, and γ-tocopherol standards were injected under the same conditions and their retention times were used to identify the tocopherols in the samples. The α-tocopherol calibration curve was used to quantify all tocopherols. The content of individual tocopherols in sea fennel flowers was expressed in mg *per* kg of dry plant matter (mg/kg d.p.m.). All analyses were performed in triplicate, and final results were expressed as mean ± SD.

Antioxidant capacity determination

The antioxidant capacity of sea fennel flowers was determined by three different methods: ferric-reducing antioxidant power (FRAP); 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay; and oxygen radical absorbance capacity (ORAC). The extracts prepared as described in the subsection "Total phenolic, flavonoid, and tannin content determination" were used in assays.

In the FRAP method, the antioxidant activity involved the reduction of iron(III)-tripyridyltriazine (Fe(III)-TPTZ) complexes to iron(II) complexes. A volume of 10 µL of the extract was added to 300 µL of FRAP reagent prepared according to the procedure described by Benzie & Strain [1996]. The absorbance of the reaction mixtures was measured at 593 nm, and the FRAP results were expressed in µmol Trolox equivalents *per* g of dry plant matter of sea fennel flowers (µmol TE/g d.p.m.).

In the DPPH assay, a DPPH radical solution in ethanol was prepared to an initial absorbance of 1.2±0.02. Then, 10 µL of the extract was mixed with 290 µL of the DPPH radical solution, and the mixture was stirred. After a 60-min incubation period, the absorbance was measured at 517 nm [Čagalj *et al.*, 2022; Skroza *et al.*, 2015]. Results were expressed as µmol Trolox equivalents *per* g of dry plant matter of sea fennel flowers (µmol TE/g d.p.m.).

The ORAC assay, which evaluates the ability of antioxidants to inhibit oxidation induced by peroxyl radicals, was performed following a slightly adapted protocol by Prior *et al.* [2003] which was described in detail in our previous paper [Čagalj *et al.*, 2022]. Results were expressed as µmol of Trolox equivalents *per* g of dry plant matter of sea fennel flowers (µmol TE/g d.p.m.).

All measurements were performed in triplicate in 96-well plates using a UV/Vis microplate reader (Synergy HTX Multi-Mode Reader, BioTek Instruments, Inc., Winooski, VT, USA).

Statistical analysis

The results of each determination were subjected to the analysis of variance (one-way ANOVA) with a Fisher's least significant difference post-hoc test to show differences between sea fennel flowers from different locations. Differences were considered significant at $p<0.05$. Analysis was performed using Statgraphics Centurion-Ver.16.1.11 Software (StatPoint Technologies, Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Sea fennel flower essential oils' composition

The EOs from dried sea fennel flowers from three locations (Pag, Korčula and Cavtat) were extracted by hydrodistillation and subjected to GC-MS analysis which resulted in 15 identified volatile organic compounds (VOCs) (**Table 1**). The EO extraction yield ranged from 0.19 to 1.87%, *w/w* (**Table 1**), and results achieved are in agreement with those of our previous study on EO extracted from sea fennel flowers, in which the yield was 1.35% [Politeo *et al.*, 2023]. Other studies reported a high content of EO in sea fennel flowers compared to other parts of the plant (*e.g.*, leaf or stems) [Generalić Mekinić *et al.*, 2016; Pateira *et al.*, 1999].

The detected compounds belong to monoterpenes and monoterpenoids. Limonene was the predominant compound in EOs from flowers from all locations and constituted from 50.82% (Pag) to 96.78% (Cavtat) of VOCs (**Table 1**). The lowest content of limonene was found in EOs from the sea fennel flowers collected in the northernmost location, while the highest content was found in EOs from the flowers collected in the southernmost location. The EOs from the flowers from the island of Pag, as the northernmost location, had the highest content of sabinene (31.43%), while its content in EOs of the flowers from the southern locations was quite low (0.68% in the Korčula sample and 0.32% in the Cavtat sample). Significant amounts of γ-terpinene were found in the EOs from the flowers from the island of Pag (7.08%) and the island of Korčula (8.93%), while it was detected in trace amounts (0.51%) in EOs of the flowers from Cavtat. The results of our previous studies on sea fennel leaves also showed variability in the EO profiles depending on the sites where the plants were collected from [Generalić Mekinić *et al.*, 2024]. However, the major leaf EO compounds were also limonene (range from 24.3 to 93.2%), sabinene (range from 0.8 to 31.7%), terpinene-4-ol (range from 0.5 to 19.2%), and γ-terpinene (range from 0.5 to 8.7%) [Generalić Mekinić *et al.*, 2024]. The Korčula sample had limonene as the major VOC,

Table 1. Volatile organic compound (VOCs) composition of essential oils (EOs) of Croatian sea fennel flowers from three locations (Pag, Korčula and Cavtat), analysed by gas chromatography-mass spectrometry.

Compound	RI	RI (library)	Relative content (%)		
			Pag	Korčula	Cavtat
α-Thujene	931	930	0.12±0.01	-	-
α-Pinene	938	939	3.17±0.93^{a}	1.90±0.33^{b}	1.80±0.72^{b}
Sabinene	977	977	31.43±1.24^{a}	0.68±0.15^{b}	0.32±0.01^{c}
β-Pinene	979	978	0.11±0.01	-	-
β-Myrcene	993	994	0.15±0.01	-	-
α-Terpinene	1,019	1,020	1.12±0.42	-	-
p-Cymene	1,027	1,095	0.67±0.27^{b}	1.34±0.21^{a}	-
Limonene	1,032	1,035	50.82±4.82^{c}	85.92±4.32^{b}	96.78±0.37^{a}
(*Z*)-β-Ocimene	1,035	1,040	-	0.09±0.01	-
(*E*)-β-Ocimene	1,042	1,051	0.17±0.01^{b}	1.12±0.57^{a}	-
γ-Terpinene	1,062	1,064	7.08±1.43^{a}	8.93±3.14^{a}	0.51±0.02^{b}
(*Z*)-Sabinene hydrate*	1,070	1,067	0.19±0.01	-	-
α-Terpinolene	1,090	1,089	0.19±0.01	-	-
Linalool*	1,098	1,104	0.16±0.01	-	-
Terpinen-4-ol*	1,179	1,178	4.58±1.90	-	-
TOTAL			99.96	99.98	99.41
EO yield (%, *w/w*)			1.87	0.99	0.19

*Monoterpenoids. Results (% of total peak area) are expressed as mean ± standard deviation (n=3). Different letters (a–c) in the same row denote statistically significant differences (p<0.05). RI, retention indices.

while the Pag sample had also high contents of sabinene and terpinene-4-ol, which is consistent with the findings reported for sea fennel flowers. Generalić Mekinić *et al.* [2016] analysed the composition of EO from dry sea fennel flowers collected in Split (Croatia) and demonstrated that limonene was the main compound (62.2%), followed by γ-terpinene (13.8%) and sabinene (12.0%). In the later study on Croatian sea fennel EOs from flowers, the contents of sabinene and limonene in samples from Central Dalmatia were almost equal (around 44%), while terpinen-4-ol (3.53%), γ-terpinene (2.79%) and α-pinene (1.76%) prevailed among the other compounds [Politeo *et al.*, 2023]. Different results were reported for the EOs from dried flowers of sea fennel from Sicily, Italy [Pavela *et al.*, 2017]. They contained 46.4% of γ-terpinene, 33.0% of thymol methyl ether, and 11.6% of *p*-cymene as main compounds. The other identified VOCs included α-pinene (accounting for 4.0% of VOCs), sabinene (1.0%), and carvacrol methyl ether (0.8%). γ-Terpinene (43.29%) was the predominant compound in EOs from fresh flowers of sea fennel grown in the garden of the Arid Land Institute (Medenine, southern Tunisia) [Houta *et al.*, 2015]. Other important compounds were methyl thymyl ether (34.39%) and *p*-cymene (14.29%). Dillapiol was detected at 2.39%, while other compounds (α-thujene, α-pinene, sabinene, myrcene, phellandrene, terpinen-4-ol, thymol, α-zingiberene, and *β*-bisabolene) were present in amounts less than 1.0%.

Previous studies on the EOs of sea fennel confirm the presence of different plant chemotypes in the Mediterranean region due to the presence or absence of dillapiole in its EO [Pateira *et al.*, 1999], and this study reconfirms that Croatian sea fennel does not contain it, so it can truly be considered a special chemotype.

Total phenolic, flavonoid, and tannin contents

The TPC, TFC, and TTC of sea fennel flowers from different locations are outlined in **Table 2**. The flowers from Cavtat, the southernmost location, had the highest TPC (26.21 mg GAE/g d.p.m.), while the lowest TPC was detected in the sample from the island of Pag (3.85 mg GAE/g d.p.m.). The TFC of the flowers collected on the island of Korčula was significantly (p<0.05) lower (0.51 mg RE/g d.p.m.) than that of the flowers from other locations (0.94–0.97 mg RE/g d.p.m.). The TTC of sea fennel flowers was in the range of 0.28–0.64 mg CE/g d.p.m. Our previous study [Politeo *et al.*, 2023] reported higher content of all phenolic groups (total phenolics, flavonoids, tannins) in sea fennel flower extract than in the extracts of stems and leaves. However, Generalić Mekinić *et al.* [2016] analysed the TPC in the flowers, leaves, and stems of sea fennel, and found that the highest content of these compounds was found in the leaves (35.1 mg GAE/g), with slightly lower amounts in the flowers (32.6 mg GAE/g). Nartea *et al.* [2023] investigated the TPC of Italian sea fennel flowers and leaves, and the results ranged from 34 to

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) in the Croatian sea fennel flowers from three locations (Pag, Korčula and Cavtat).

Location	TPC (mg GAE/g d.p.m.)	TFC (mg RE/g d.p.m.)	TTC (mg CE/g d.p.m.)
Pag	3.85±0.08^{c}	0.94±0.14^{a}	0.28±0.09^{a}
Korčula	20.43±0.06^{b}	0.51±0.05^{b}	0.40±0.05^{a}
Cavtat	26.21±0.43^{a}	0.97±0.08^{a}	0.65±0.08^{a}

Results are expressed as mean ± standard deviation (n=3). Different letters (a–c) in the same column denote statistically significant differences (p<0.05). GAE, gallic acid equivalent; RE, rutin equivalent; CE, catechin equivalent; d.p.m., dry plant matter.

55 mg GAE/g depending on the harvest location. Interestingly, the highest TPC in their study was detected in the samples from southern Italy (Sicily and Apulia). A similar trend was also observed for TFC. Maleš *et al.* [2003] explored the phenolic content in the aerial parts of Croatian sea fennel at different flowering stages, and found that the samples before and at the beginning of flowering exhibited higher levels of total phenolics, flavonoids, and tannins than those at full flowering and fruiting stages. Houta *et al.* [2011] also compared the phenolic contents of different parts of the sea fennel. The sea fennel flowers were the plant parts with the lowest amounts of phenolics (TPC of 9.43 mg GAE/g; TFC of 3.71 mg CE/g; condensed tannin content of 0.43 mg CE/g).

Phenolic compound profile

The HPLC analysis of the phenolics of sea fennel flowers from different locations resulted in the detection and quantification of ten compounds. Of these, nine were phenolic acids, and one was flavonoid (rutin) (**Table 3**). The contents of most individual phenolics were the highest in the flowers from Cavtat, which was consistent with the TPC (**Table 2**). Chlorogenic acid prevailed in all samples, and a significant (p<0.05) difference was found between the locations where the plant material was collected (**Table 3**). The content of this compound increased in the samples from north to south in a range from 0.30 (Pag) to 13.27 mg/g d.p.m. (Cavtat). Neochlorogenic acid, *p*-hydroxybenzoic acid, cryptochlorogenic acid and rutin were also found in considerable amounts in flowers from different locations. A somehow consistent pattern emerged, demonstrating the lowest content of individual phenolics in the sea fennel flowers collected at the northernmost site and the highest one in the sample from the southernmost location.

Similarly to our research, Nartea *et al.* [2023] found that hydroxycinnamic acids were the main class of phenolics in Italian sea fennel with the highest content of chlorogenic acid, 34 to 53 mg/g, which are significantly higher than the results obtained in our study. Among the caffeoylquinic acids, the authors also identified neochlorogenic acid (1.5–2.3 mg/g), cryptochlorogenic acid (1.17–1.83 mg/g as) and 3,5-di-*O*-caffeoylquinic acid (41.0–57.7 mg/g). In our previous study, Generalić Mekinić *et al.* [2024] investigated the content of individual phenolics in sea fennel leaves from the same locations as in this study and reported the highest content of chlorogenic acid in the Korčula sample (248 mg/g d.p.m.), while the Pag sample had the lowest content of this phenolic compound (32 mg/g d.p.m.). A similar trend was also observed for neochlorogenic and cryptochlorogenic

Table 3. Contents of phenolic compounds in Croatian sea fennel flowers from three locations (Pag, Korčula and Cavtat), analysed by high performance liquid chromatography.

Phenolic compound	t_R (min)	Content (μg/g d.p.m.)		
		Pag	Korčula	Cavtat
Gallic acid	8.55	0.08±0.00^{c}	0.90±0.22^{a}	0.16±0.00^{b}
Protocatechuic acid	14.36	9.66±0.02^{a}	6.12±0.00^{b}	1.28±0.28^{c}
Neochlorogenic acid	16.14	11.20±0.16^{c}	183.08±0.64^{b}	292.06±0.62^{a}
p-Hydroxybenzoic acid	20.44	26.34±0.18^{c}	58.24±0.04^{b}	91.60±1.40^{a}
Chlorogenic acid	23.19	300.02±0.18^{c}	7,986.88±0.12^{b}	13,272.74±0.54^{a}
Cryptochlorogenic acid	24.31	16.02±0.98^{c}	287.88±0.64^{b}	403.56±0.24^{a}
Caffeic acid	25.92	1.60±0.04^{c}	10.12±0.16^{a}	8.62±0.02^{b}
Ferulic acid	47.62	2.88±0.56^{c}	12.84±0.16^{b}	18.72±0.016^{a}
Sinapic acid	48.83	5.36±0.72^{c}	21.62±0.10^{b}	29.42±0.02^{a}
Rutin	51.56	36.06±2.98^{c}	217.68±5.12^{b}	533.40±2.84^{a}

Results are expressed as mean ± standard deviation (n=2). Different letters (a–c) in the same row denote statistically significant differences (p<0.05). d.p.m., dry plant matter; t_R, retention time.

acids, with the highest contents of these phenolic acids found in the Korčula sample.

The content of chlorogenic acid in the Pag sample (**Table 3**) was consistent with the results of our previous study [Generalić Mekinić *et al.*, 2016], in which it was determined at 7.7 mg/g in sea fennel flowers from Split, Croatia, which is geographically located south of the island of Pag and north of the island of Korčula. Meot-Duros & Magné [2009] analysed the phenolic compounds of sea fennel leaves collected from sand hills exposed to the sea spray and from the leaves below the cliffs, which were protected from the sea spray but also rich in organic matter. The phenolic compound content, especially chlorogenic acid, indicated that the plants located in a more stressful environment contained more phenolics. These observations can also be linked to the results of this study, considering the different climatic conditions of the Dalmatian north and the Pag site, which are characterised by notably lower annual temperatures and strong winds. These factors could indeed influence the synthesis and profile of phytochemicals in the plant.

Fatty acid profile

The FA profiles of Croatian sea fennel flowers are shown in **Table 4**. Of the 21 FAs identified, 11 were saturated fatty acids (SFAs), 2 were polyunsaturated fatty acids (PUFAs), namely linolenic acid (C18:3*n*3) and linoleic acid (C18:2*n*6), while 6 were monounsaturated fatty acids (MUFAs), namely pentadecenoic acid (C15:1*n*10), *cis*-7-hexadecenoic acid (C16:1*n*9), palmitoleic acid (C16:1*n*7), heptadecenoic acid (C17:1), oleic acid (C18:1), and eicosenoic acid (C20:1). To the authors' best knowledge, this represents the first report of the FA profile in sea fennel flowers.

Linoleic acid predominated (37.48%) in the Cavtat sample, while its content in the Korčula sample was 42.74%. In contrast, stearic acid (C18:0) predominated in the sample from the island of Pag with 31.29% (**Table 4**). Another four FAs (palmitic, oleic, linolenic and lignoceric acids) were detected in considerable amounts with relatively similar contents in the flowers from different locations. The differences in the dominance of the individual FA in sea fennel flowers collected at the different locations could be due to environmental factors. For example, it has been found that salinity increased the proportion of linoleic acid in sea fennel leaves [Ben Hamed *et al.*, 2005].

The total content of SFAs in the samples ranged from 38.49% (Korčula) to 58.76% (Pag), that of MUFAs from 4.04% (Pag) to 6.34% (Korčula) and that of PUFAs from 37.23% (Pag) to 55.17% (Korčula) (**Figure 2**). SFAs predominated in the sample from the island of Pag, while PUFAs dominated in the samples from the island of Korčula and Cavtat. Although there are no studies on the FA profiles of sea fennel flowers, previous studies on leaves [Ben Hamed *et al.*, 2005; Castillo *et al.*, 2022; Labiad *et al.*, 2021; Maoloni *et al.*, 2021; Ventura *et al.*, 2014] have shown that PUFAs were the major group of FAs, ranging from 33.8% to 86.0% of total FAs. MUFAs were the least represented group of FAs as in the samples of the current study. In turn, Generalić Mekinić *et al.* [2024] reported the highest content of palmitic acid in the Pag leaf sample (22.4%), which had also the highest content of SFAs (56.4%) and the lowest content of PUFAs (37.7%). The share of PUFAs in Korčula and Cavtat leaves was significantly higher, 44.2 and 46.4%, respectively, with the major acids being linoleic acid and linolenic acid.

Table 4. Fatty acid profile of Croatian sea fennel flowers from three different locations along the Adriatic coast (Pag, Korčula and Cavtat).

Fatty acid	Relative content (%)		
	Pag	Korčula	Cavtat
C8:0	0.20±0.01^{a}	0.08±0.00^{b}	0.08±0.00^{b}
C10:0	0.18±0.00^{a}	0.12±0.01^{b}	0.08±0.00^{c}
C11:0	0.38±0.02^{a}	0.19±0.01^{b}	0.13±0.02^{c}
C12:0	0.40±0.00^{b}	0.55±0.01^{a}	0.39±0.01^{c}
C13:0	0.39±0.00^{a}	0.23±0.01^{c}	0.25±0.01^{b}
C14:0	0.69±0.01^{b}	0.83±0.02^{a}	0.25±0.04^{c}
C15:0	0.42±0.01^{a}	0.25±0.01^{b}	0.22±0.01^{c}
C15:1*n*10	0.44±0.00^{a}	0.38±0.00^{c}	0.43±0.00^{b}
C16:0	21.37±0.03^{a}	20.79±0.06^{b}	19.54±0.20^{c}
C16:1*n*9	0.48±0.00^{a}	0.43±0.00^{c}	0.47±0.00^{b}
C16:1*n*7	0.16±0.08^{a}	0.09±0.00^{b}	0.08±0.00^{c}
C17:0	0.27±0.00^{a}	0.20±0.00^{b}	0.19±0.03^{c}
C17:1	0.20±0.08^{a}	0.18±0.02^{b}	0.15±0.01^{c}
C18:0	31.29±0.07^{a}	12.73±0.04^{c}	20.50±0.00^{b}
C18:1	2.41±0.00^{c}	4.77±0.02^{a}	3.53±0.01^{b}
C18:2*n*6	26.46±0.04^{c}	42.74±0.07^{a}	37.48±0.03^{b}
C20:0	0.06±0.01^{c}	0.28±0.01^{b}	0.53±0.01^{a}
C18:3*n*3	10.77±0.02^{c}	12.43±0.01^{b}	12.60±0.04^{a}
C20:1	0.35±0.00^{c}	0.49±0.01^{b}	0.60±0.06^{a}
C22:0	0.07±0.01^{b}	0.04±0.00^{c}	0.43±0.02^{a}
C24:0	3.00±0.00^{a}	2.18±0.01^{b}	2.08±0.18^{c}

Results (% total fatty acids) are expressed as mean ± standard deviation (n=2). Different letters (a–c) in the same row denote statistically significant differences (p<0.05).

To sum up, our study results show the similarity of FA profiles between the samples from the southern regions as well as the specificity of the Pag sample.

Tocopherol profile

Tocopherols can occur in the form of four isomers: α-, β-, γ-, and δ-isomer. They play a protective role in plants [Havaux *et al.*, 2005] but also contribute significantly to the prevention of certain chronic diseases and cancers [Huang *et al.*, 2019; Xiong *et al.*, 2023]. The analysis of sea fennel flowers collected from various locations along the Croatian Adriatic coast identified α-, β- and γ-tocopherol isomers, the profiles of which are shown in **Figure 3**. Furthermore, this is the first report on the tocopherols in the flowers of Croatian sea fennel, which – to the best of our knowledge – have not been investigated before.

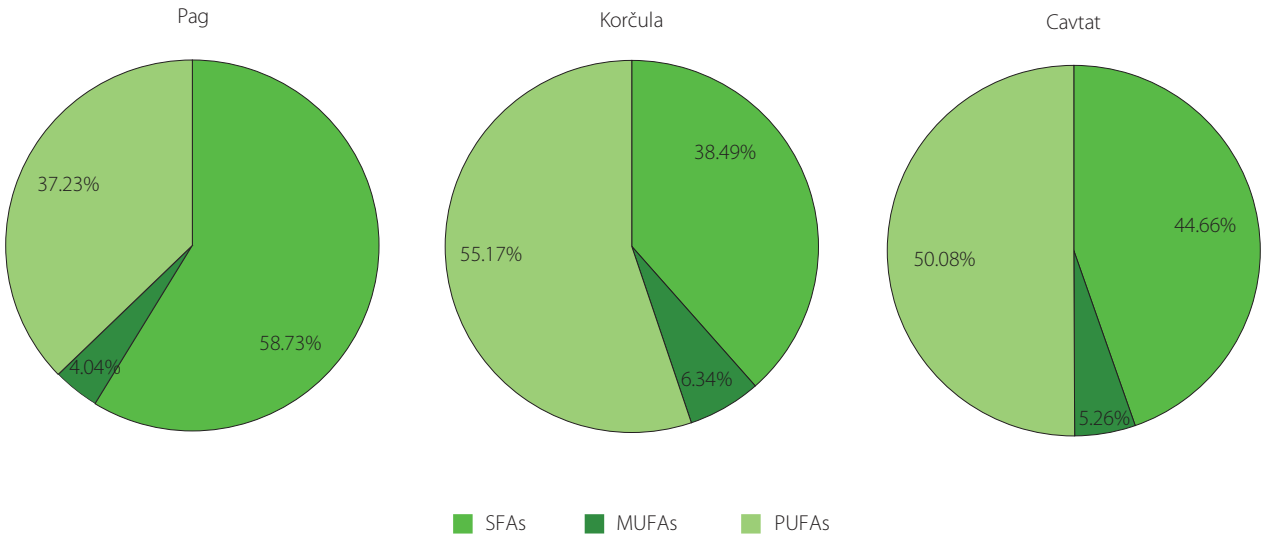

Figure 2. Distribution of main groups of fatty acids of sea fennel flowers collected at different locations. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

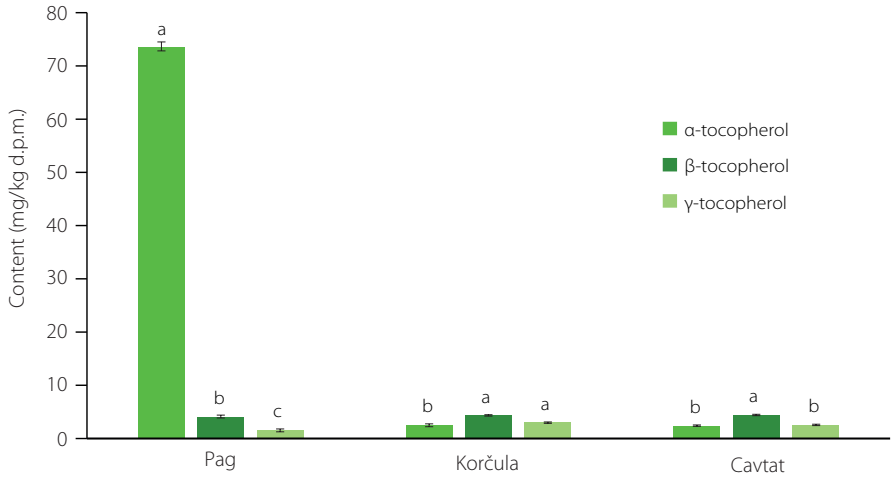

Figure 3. Tocopherol (α-, β- and γ-) profiles of Croatian sea fennel flowers (mg/kg dry plant matter, d.p.m.) from three different locations along the Adriatic coast (Pag, Korčula, Cavtat). Different letters (a–c) above bars, separately for samples from each location, denote statistically significant differences ($p<0.05$).

Similarly to the results described above (contents of EOs, phenolics, PUFAs), the flowers from the island of Pag were richer in tocopherols than the samples from the other locations (**Figure 3**). α-Tocopherol dominated (73.62 mg/kg d.p.m.) among tocopherol isomers in the Pag sample. In the other two samples, the content of *α*-tocopherol was more than 29 times lower, and *β*-tocopherol dominated, but with similar contents as in the flowers from the island of Pag. Our previous study on sea fennel leaves from the same locations and few others along the Adriatic coast showed the preponderance of *α*-tocopherol in the analysed samples (with its content ranging from 9.7 to 53.9 mg/g) [Generalić Mekinić *et al.*, 2024]. This suggests not only the effect of the sampling sites on the profile of sea fennel tocopherols, but also the significant influence of the analysed morphological part of the plant. The content of tocopherols in wild and cultivated sea fennel from Italy was investigated by Nartea *et al.* [2023]. These authors also demonstrated the dominance of *α*-tocopherol (from 6.18 to 33.17 mg/kg d.p.m.), while reported the contents of *β*- and *γ*-tocopherols as the sum of these forms (from 0.93 to 3.33 mg/kg d.p.m.). When we compare these results with those of our study, it appears that the content of tocopherols in the Pag sample was more than 2-fold higher than in the richest Italian samples, while the results of the other two Croatian samples were lower than the value obtained for the Sicilian sample (the southernmost sampling point), which may also be related to the harvest geographical location [Nartea *et al.*, 2023].

Antioxidant capacity

The effect of harvesting location on the antioxidant capacity of the sea fennel flowers was estimated by three antioxidant assays: FRAP, DPPH and ORAC (**Figure 4**). The FRAP was used to evaluate the flowers reducing potential, while the other two methods provided information on their radical scavenging capacity against synthetic DPPH radicals and peroxyl radicals (ORAC assay). The FRAP ranged from 7.37 µmol TE/g (Pag) to 99.74 µmol TE/g (Cavtat), and the ORAC ranged from 125.79 µmol TE/g (Pag) to 595.12 µmol TE/g (Cavtat). The highest ability to

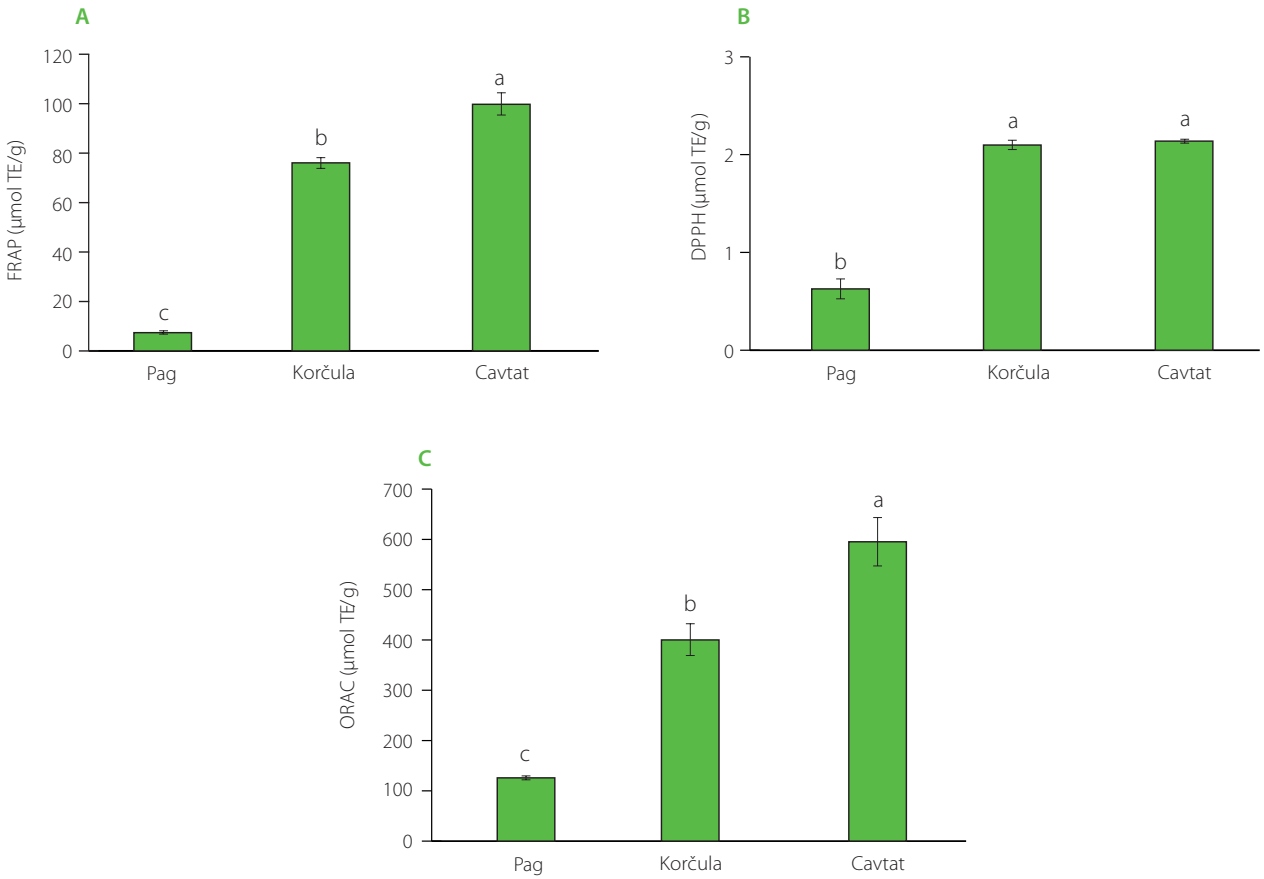

Figure 4. Antioxidant capacity of sea fennel flowers from different locations (Pag, Korčula, Cavtat) determined as (**A**) ferric reducing antioxidant power (FRAP), (**B**) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and (**C**) oxygen radical absorbance capacity (ORAC). Different letters (a–c) above bars denote statistically significant differences ($p<0.05$). TE, Trolox equivalent.

inhibit the DPPH radical was found for the sample from Cavtat but with similar result to the sample from the island of Korčula. The ability to scavenge the DPPH radical ranged from 0.63 µmol TE/g (Pag) to 2.14 µmol TE/g (Cavtat). The DPPH assay conducted for the extract of flowers collected in Split [Generalić Mekinić *et al.*, 2016], which is geographically located between the islands of Pag and Korčula, showed that the ethanol extract of the flowers had a greater antioxidant potential than its EO. Pereira *et al.* [2017] investigated radical scavenging, ferric reducing and metal-chelating activities of infusions and decoctions from stems, leaves and flowers of *C. maritimum* from Portugal, and reported the highest reducing activity obtained for the flower decoction and the activity against DPPH radicals for both flower samples (infusions and decoctions).

A comparison of the antioxidant potential (DPPH assay) of different parts of sea fennel was reported by Houta *et al.* [2011]. The authors found that the extracts from seeds and leaves had higher activity than the extracts from flowers and stems. On the other hand, Nartea *et al.* [2023] investigated the FRAP and free radical scavenging activity of sea fennel flowers and leaves from Italy, and reported the lowest antiradical activity of the flower extract from Sicily, but the highest reducing power of this sample. They also reported a promising antiradical activity of the samples characterized by a low TPC, but showing positive correlations with the content of chlorogenic acids. In addition, it should be taken into account that our previous study [Generalić Mekinić *et al.*, 2018] on the antioxidant activity of sea fennel from different vegetation periods demonstrated variations in the activity of the extracts, and one of the weakest activities was detected for the August sample, which was also the harvest period in this study.

The chemical composition of sea fennel flowers, in this case the phenolic profile, was crucial for their antioxidant capacity. The flowers from Cavtat, which had the highest TPC and contents of chlorogenic, neochlorogenic and cryptochlorogenic acids, but also rutin, provided the highest antioxidant capacity, which could support the suggestion that these compounds were the main antioxidants in sea fennel flowers. As expected, the antioxidant capacity of the samples was correlated with the content of chlorogenic acids, which have been recognized as potent antioxidants [Miao & Xiang, 2020]. Chlorogenic acid is produced in plants during aerobic respiration from caffeic and quinic acid. It is widely distributed in plants, especially in green coffee beans, apples, aubergines, *etc.* [Wang *et al.*, 2022].

CONCLUSIONS

In this study, the chemical composition and antioxidant capacity of the flowers of Croatian sea fennel were investigated. The results showed different chemical profiles depending on

harvesting location. The northernmost sample from Pag had the lowest content of limonene and chlorogenic acids. The latter were mainly responsible for the high antioxidant capacity of the Korčula and Cavtat samples measured by DPPH, FRAP and ORAC assyas. However, the content of tocopherols, in particular of the α-isomer, was extremely high in the flowers from the island of Pag compared to the flowers from the other locations. It can be concluded that the samples from the northern, colder areas had a clearly different chemical profile and, accordingly, the antioxidant capacity, than the samples from the south. In general, however, despite these differences, sea fennel flowers can be considered a valuable source of various phytochemicals with great potential for further use in the food, pharmaceutical and cosmetics industries.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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REFERENCES

1. Alemán, A., Marín, D., Taladrid, D., Montero, P., Gómez-Guillén, M.C. (2019). Encapsulation of antioxidant sea fennel (*Crithmum maritimum*) aqueous and ethanolic extracts in freeze-dried soy phosphatidylcholine liposomes. *Food Research International*, *119*, 665–674. https://doi.org/10.1016/j.foodres.2018.10.044
2. Alves-Silva, J.M., Guerra, I., Gonçalves, M.J., Cavaleiro, C., Cruz, M.T., Figueirinha, A., Salgueiro, L. (2020). Chemical composition of *Crithmum maritimum* L. essential oil and hydrodistillation residual water by GC-MS and HPLC-DAD-MS/MS, and their biological activities. *Industrial Crops and Products*, *149*, art. no. 112329. https://doi.org/10.1016/j.indcrop.2020.112329
3. Atia, A., Barhoumi, Z., Mokded, R., Abdelly, C., Smaoui, A. (2011). Environmental eco-physiology and economical potential of the halophyte *Crithmum maritimum* L. (Apiaceae). *Journal of Medicinal Plants Research*, *5*(16), 3564–3571.
4. Bektasevic, M., Jurin, M., Roje, M., Politeo, O. (2023). Phytochemical profile, antioxidant activity and cholinesterase inhibition potential of essential oil and extracts of *Teucrium montanum* from Bosnia and Herzegovina. *Separations*, *10*(8), art. no. 421. https://doi.org/10.3390/separations10080421
5. Ben Hamed, K., Ben Youssef, N., Ranieri, A., Zarrouk, M., Abdelly, C. (2005). Changes in content and fatty acid profiles of total lipids and sulfolipids in the halophyte *Crithmum maritimum* under salt stress. *Journal of Plant Physiology*, *162*(5), 599–602. https://doi.org/10.1016/j.jplph.2004.11.010
6. Benzie, I.F.F., Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292
7. Čagalj, M., Skroza, D., Razola-Díaz, M.D.C., Verardo, V., Bassi, D., Frleta, R., Mekinić, I.G., Tabanelli, G., Šimat, V. (2022). Variations in the composition, antioxidant and antimicrobial activities of *Cystoseira compressa* during seasonal growth. *Marine Drugs*, *20*(1), art. no. 64. https://doi.org/10.3390/md20010064
8. Castillo, J.M., Mancilla-Leytón, J.M., Martins-Noguerol, R., Moreira, X., Moreno-Pérez, A.J., Muñoz-Vallés, S., Pedroche, J.J., Figueroa, M.E., García-González, A., Salas, J.J., Millán-Linares, M.C., Francisco, M., Cambrollé, J. (2022). Interactive effects between salinity and nutrient deficiency on biomass production and bio-active compounds accumulation in the halophyte *Crithmum maritimum*. *Scientia Horticulturae*, *301*, art. no. 111136. https://doi.org/10.1016/j.scienta.2022.111136
9. Franke, W. (1982). Vitamin C in sea fennel (*Crithmum maritimum*), an edible wild plant. *Economic Botany*, *36*, 163–165. https://doi.org/10.1007/BF02858711
10. Generalić Mekinić, I., Blažević, I., Mudnić, I., Burčul, F., Grga, M., Skroza, D., Jerčić, I., Ljubenkov, I., Boban, M., Miloš, M., Katalinić, V. (2016). Sea fennel (*Crithmum maritimum* L.): phytochemical profile, antioxidative, cholinesterase inhibitory and vasodilatory activity. *Journal of Food Science and Technology*, *53*, 3104–3112. https://doi.org/10.1007/s13197-016-2283-z
11. Generalić Mekinić, I., Politeo, O., Ljubenkov, I., Mastelić, L., Popović, M., Veršić Bratinčević, M., Šimat, V., Radman, S., Skroza, D., Ninčević Runjić, T., Runjić, M., Dumičić, G., Urlić, B. (2024). The alphabet of sea fennel: Comprehensive phytochemical characterisation of Croatian populations of *Crithmum maritimum* L. *Food Chemistry: X*, *22*, art. no. 101386. https://doi.org/10.1016/j.fochx.2024.101386
12. Generalić Mekinić, I., Šimat, V., Ljubenkov, I., Burčul, F., Grga, M., Mihajlovski, M., Lončar, R., Katalinić, V., Skroza, D. (2018). Influence of the vegetation period on sea fennel, *Crithmum maritimum* L. (Apiaceae), phenolic composition, antioxidant and anticholinesterase activities. *Industrial Crops and Products*, *124*, 947–953. https://doi.org/10.1016/j.indcrop.2018.08.080
13. Hagerman, A.E., Butler, L.G. (1994). Assay of condensed tannins or flavonoid oligomers and related flavonoids in plants. *Methods in Enzymology*, *234*, 429–437. https://doi.org/10.1016/0076-6879(94)34113-3
14. Havaux, M., Eymery, F., Porfirova, S., Rey, P., Dörmann, P. (2005). Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. *The Plant Cell*, *17*(12), 3451–3469. https://doi.org/10.1105/tpc.105.037036
15. Houta, O., Akrout, A., Najja, H., Neffati, M., Amri, H. (2015). Chemical composition, antioxidant and antimicrobial activities of essential oil from *Crithmum maritimum* cultivated in Tunisia. *Journal of Essential Oil Bearing Plants*, *18*(6), 1459–1466. https://doi.org/10.1080/0972060X.2013.764209
16. Houta, O., Akrout, A., Neffati, M., Amri, H. (2011). Phenolic contents, antioxidant and antimicrobial potentials of Crithmum maritimum cultivated in Tunisia arid zones: *Journal of Biologically Active Products from Nature*, *1*(2), 138–143. https://doi.org/10.1080/22311866.2011.10719081
17. Huang, J., Weinstein, S.J., Yu, K., Männistö, S., Albanes, D. (2019). Relationship between serum alpha-tocopherol and overall and cause-specific mortality a 30-year prospective cohort analysis. *Circulation Research*, *125*(1), 29–40. https://doi.org/10.1161/CIRCRESAHA.119.314944
18. Kadoglidou, K., Irakli, M., Boutsika, A., Mellidou, I., Maninis, N., Sarrou, E., Georgiadou, V., Tourvas, N., Krigas, N., Moysiadis, T., Grigoriadou, K., Maloupa, E., Xanthopoulou, A., Ganopoulos, I. (2022). Metabolomic fingerprinting and molecular characterization of the rock samphire germplasm collection from the Balkan Botanic Garden of Kroussia, Northern Greece. *Plants*, *11*(4), art. no. 573. https://doi.org/10.3390/plants11040573
19. Knecht, K., Sandfuchs, K., Kulling, S.E., Bunzel, D. (2015). Tocopherol and tocotrienol analysis in raw and cooked vegetables: A validated method with emphasis on sample preparation. *Food Chemistry*, *169*, 20–27. https://doi.org/10.1016/j.foodchem.2014.07.099
20. Labiad, M.H., Giménez, A., Varol, H., Tüzel, Y., Egea-Gilabert, C., Fernández, J.A., Martínez-Ballesta, M.D.C. (2021). Effect of exogenously applied methyl jasmonate on yield and quality of salt-stressed hydroponically grown sea fennel (*Crithmum maritimum* L.). *Agronomy*, *11*(6), art. no. 1083. https://doi.org/10.3390/agronomy11061083

21. Maleš, Ž., Žuntar, I., Nigović, B., Plazibat, M., Vundać, V.B. (2003). Quantitative analysis of the polyphenols of the aerial parts of rock samphire – *Crithmum maritimum* L. *Acta Pharmaceutica*, *53*(2), 139–144.
22. Maoloni, A., Milanović, V., Osimani, A., Cardinali, F., Garofalo, C., Belleggia, L., Foligni, R., Mannozzi, C., Mozzon, M., Cirlini, M., Spaggiari, M., Reale, A., Boscaino, F., Di Renzo, T., Haouet, M.N., Staccini, B., Di Bella, S., Aquilanti, L. (2021). Exploitation of sea fennel (*Crithmum maritimum* L.) for manufacturing of novel high-value fermented preserves. *Food and Bioproducts Processing*, *127*, 174–197. https://doi.org/10.1016/j.fbp.2021.03.001
23. Martins-Noguerol, R., Matías, L., Pérez-Ramos, I.M., Moreira, X., Muñoz-Vallés, S., Mancilla-Leytón, J.M., Francisco, M., García-González, A., DeAndrés-Gil, C., Martínez-Force, E., Millán-Linares, M. del C., Pedroche, J., Figueroa, M.E., Moreno-Pérez, A.J., Cambrollé, J. (2022). Differences in nutrient composition of sea fennel (*Crithmum maritimum*) grown in different habitats and optimally controlled growing conditions. *Journal of Food Composition and Analysis*, *106*, art. no. 104266. https://doi.org/10.1016/j.jfca.2021.104266
24. Meot-Duros, L., Magné, C. (2009). Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiology and Biochemistry*, *47*(1), 37–41. https://doi.org/10.1016/j.plaphy.2008.09.006
25. Miao, M., Xiang, L. (2020). Chapter Three - Pharmacological action and potential targets of chlorogenic acid. In G. Du (Ed.), *Advances in Pharmacology, Volume 87*, Academic Press Inc., pp. 71–88. https://doi.org/10.1016/bs.apha.2019.12.002
26. Nabet, N., Boudries, H., Chougui, N., Loupassaki, S., Souagui, S., Burló, F., Hernández, F., Carbonell-Barrachina, Á.A., Madani, K., Larbat, R. (2017). Biological activities and secondary compound composition from *Crithmum maritimum* aerial parts. *International Journal of Food Properties*, *20*(8), 1843–1855. https://doi.org/10.1080/10942912.2016.1222541
27. Nartea, A., Orhotohwo, O.L., Fanesi, B., Lucci, P., Loizzo, M.R., Tundis, R., Aquilanti, L., Casavecchia, S., Quattrini, G., Pacetti, D. (2023). Sea fennel (*Crithmum maritimum* L.) leaves and flowers: Bioactive compounds, antioxidant activity and hypoglycaemic potential. *Food Bioscience*, *56*, art. no. 103417. https://doi.org/10.1016/j.fbio.2023.103417
28. Özcan, M., Akgül, A., Başçr, K.H.C., Özck, T., Tabanca, N. (2001). Essential oil composition of sea fennel (*Crithmum maritimum*) form Turkey. *Nahrung/Food*, *45*(5), 353–356. https://doi.org/10.1002/1521-3803(20011001)45:5<353::AID-FOOD353>3.0.CO;2-4
29. Özcan, M.M., Pedro, L.G., Figueiredo, A.C., Barroso, J.G. (2006). Constituents of the essential oil of sea fennel (*Crithmum maritimum* L.) growing wild in Turkey. *Journal of Medicinal Food*, *9*(1), 128–130. https://doi.org/10.1089/jmf.2006.9.128
30. Pateira, L., Nogueira, T., Antunes, A., Venâncio, F., Tavares, R., Capelo, J. (1999). Two chemotypes of *Crithmum maritimum* L. from Portugal. *Flavour and Fragrance Journal*, *14*(5), 333–343. https://doi.org/10.1002/(SICI)1099-1026(199909/10)14:5<333::AID-FFJ839>3.0.CO;2-V
31. Pavela, R., Maggi, F., Lupidi, G., Cianfaglione, K., Dauvergne, X., Bruno, M., Benelli, G. (2017). Efficacy of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils against *Culex quinquefasciatus* Say and *Spodoptera littoralis* (Boisd.). *Industrial Crops and Products*, *109*, 603–610. https://doi.org/10.1016/j.indcrop.2017.09.013
32. Pereira, C.M.P., Nunes, C.F.P., Zambotti-Villela, L., Streit, N.M., Dias, D., Pinto, E., Gomes, C.B., Colepicolo, P. (2017). Extraction of sterols in brown macroalgae from Antarctica and their identification by liquid chromatography coupled with tandem mass spectrometry. *Journal of Applied Phycology*, *29*(2), 751–757. https://doi.org/10.1007/s10811-016-0905-5
33. Politeo, O., Popović, M., Veršić Bratinčević, M., Kovačević, K., Urlić, B., Generalić Mekinić, I. (2023). Chemical profiling of sea fennel (*Crithmum maritimum* L., Apiaceae) essential oils and their isolation residual waste-waters. *Plants*, *12*(1), art. no. 214. https://doi.org/10.3390/plants12010214
34. Prior, R.L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, *51*(11), 3273–3279. https://doi.org/10.1021/jf0262256
35. Radman, S., Brzović, P., Radunić, M., Rako, A., Šarolić, M., Ninčević Runjić, T., Urlić, B., Generalić Mekinić, I. (2023). Vinegar-preserved sea fennel: Chemistry, color, texture, aroma, and taste. *Foods*, *12*(20), art. no. 3812. https://doi.org/10.3390/foods12203812
36. Renna, M. (2018). Reviewing the prospects of sea fennel (*Crithmum maritimum* L.) as emerging vegetable crop. *Plants*, *7*(4), art. no. 92. https://doi.org/10.3390/plants7040092
37. Renna, M., Gonnella, M. (2012). The use of the sea fennel as a new spice-colorant in culinary preparations. *International Journal of Gastronomy and Food Science*, *1*(2), 111–115. https://doi.org/10.1016/j.ijgfs.2013.06.004
38. Renna, M., Gonnella, M., Caretto, S., Mita, G., Serio, F. (2017). Sea fennel (*Crithmum maritimum* L.): from underutilized crop to new dried product for food use. *Genetic Resources and Crop Evolution*, *64*(1), 205–216. https://doi.org/10.1007/s10722-016-0472-2
39. Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158. https://doi.org/10.5344/ajev.1965.16.3.144
40. Siracusa, L., Kulisic-Bilusic, T., Politeo, O., Krause, I., Dejanovic, B., Ruberto, G. (2011). Phenolic composition and antioxidant activity of aqueous infusions from *Capparis spinosa* L. and *Crithmum maritimum* L. before and after submission to a two-step *in vitro* digestion model. *Journal of Agricultural and Food Chemistry*, *59*(23), 12453–12459. https://doi.org/10.1021/jf203096q
41. Skroza, D., Generalić-Mekinić, I., Svilović, S., Šimat, V., Katalinić, V. (2015). Investigation of the potential synergistic effect of resveratrol with other phenolic compounds : A case of binary phenolic mixtures. *Journal of Food Composition and Analysis*, *38*, 13–18. https://doi.org/10.1016/j.jfca.2014.06.013
42. Ventura, Y., Myrzabayeva, M., Alikulov, Z., Omarov, R., Khozin-Goldberg, I., Sagi, M. (2014). Effects of salinity on flowering, morphology, biomass accumulation and leaf metabolites in an edible halophyte. *AoB PLANTS*, *6*, art. no. plu053. https://doi.org/10.1093/aobpla/plu053
43. Wang, L., Pan, X., Jiang, L., Chu, Y., Gao, S., Jiang, X., Zhang, Y., Chen, Y., Luo, S., Peng, C. (2022). The biological activity mechanism of chlorogenic acid and its applications in food industry: A review. *Frontiers in Nutrition*, *9*, art. no. 943911. https://doi.org/10.3389/fnut.2022.943911
44. Xiong, Z., Liu, L., Jian, Z., Ma, Y., Li, H., Jin, X., Liao, B., Wang, K. (2023). Vitamin E and multiple health outcomes: An umbrella review of meta-analyses. *Nutrients*, *15*(15), art. no. 3301. https://doi.org/10.3390/nu15153301
45. Yang, J., Meyers, K.J., van der Heide, J., Liu, R.H. (2004). Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *Journal of Agricultural and Food Chemistry*, *52*(22), 6787–6793. https://doi.org/10.1021/jf0307144

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Untargeted Metabolomics Analysis Reveals the Effect of Fixation on the Profile of Volatile Compounds of *Cyclocarya paliurus* Tea

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Fixation is a necessary tea processing step that affects the quality of tea, including its aroma. However, there are no guidelines to follow whether the *Cyclocarya paliurus* leaves need to be fixated to produce tea. The aim of this study was to determine the volatile metabolites in fixated and unfixated *C. paliurus* tea by gas chromatography-mass spectrometry in order to identify the volatile compounds that differentiate them and establish the key aroma compounds. Compared with the unfixated tea, the fixated tea had higher contents of amines, phenols, nitrogen compounds, ether, alcohol, terpenoids, ester and aromatics. A total of 147 metabolites that differentiated both types of teas were identified using principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Among them, 22 and 25 volatile compounds of teas processed without and with fixation, respectively, had relative odor activity values greater than 1. These metabolites had green, fruity, sweet, woody, herbal, floral, fresh, spicy, earthy and waxy odor. This study showed that fixation technology improved the quality of *C. paliurus* tea, which provided the basis for the production of *C. paliurus* tea, and promoted the standardization of the preparation technology of *C. paliurus* tea.

Keywords: fixation technology, HS-SPME-GC-MS, key aroma compounds, OPLS-DA, volatile metabolites

INTRODUCTION

Cyclocarya paliurus is a relicted tree species unique to China that survived the Ice Age and is distributed in mountainous areas at an altitude of 420–2500 meters [Zhang *et al.*, 2021]. Due to the presence of various physiological active substances, such as flavonoids, saponins, terpenoids, polysaccharides, *etc.*, beneficial to the human body, its leaves are often used to brew tea. Its golden color and sweet taste make it known as "sweet tea". *C. paliurus* leaves elicit multiple health benefits, such as preventing and treating diabetes, hypertension, hyperlipidemia, dizziness and swelling and pain, as well as reducing cholesterol level, and modulating the functions of the immune system [Chen *et al.*, 2022b]. The leaves of *C. paliurus* were approved as new food raw material by the National Health and Family Planning Commission of China in 2013. However, the quality of commercially available *C. paliurus* tea is uneven, and the processing technology for professional production of *C. paliurus* tea has not yet been developed. The traditional processing of *C. paliurus* involves mostly direct drying, but in recent years, some producers have adopted the processing method of green tea including first its fixation followed by drying. It has not been reported in literature whether fixation is suitable for processing of *C. paliurus*, and how the profiles of volatile organic compounds (VOCs) and aroma compounds of *C. paliurus* change after fixation. Such knowledge seems important to improve the technology of processing *C. paliurus* leaves for tea.

Fixation is one of the critical steps in fresh leaves processing for tea. By inactivating enzymes at high temperatures, it prevents tea from turning red [Ouyang *et al.*, 2022]. Simultaneously, a series of chemical reactions occur, such as hydrolysis and oxidation

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of proteins and polysaccharides, polyphenol polymerization, pigment degradation, and generation of simple compounds that are beneficial for tea quality [Wang *et al.*, 2021a]. The main task of fixation is to form an acceptable aroma to satisfy consumers [Xu *et al.*, 2020]. Endeavors have been made to increase the formation of "beneficial" chemicals, such as linalool, phenacetaldehyde and benzoic acid, and to avoid the presence of "unfavorable" chemicals, such as L-isoleucine, L-lysine and L-tyrosine from a tea aroma perspective [Wen *et al.*, 2023; Xue *et al.*, 2022].

Non-targeted metabolomics exhibits a high-sensitivity and wide-coverage, and provides a comprehensive view of metabolite changes, which has been widely utilized in tea research for the unbiased analysis and simultaneous identification of a large number of non-volatile and volatile metabolites [Ouyang *et al.*, 2022; Qin *et al.*, 2020]. Its advantage is that unknown metabolites can be discovered, and differences in metabolites associated with treatment states can be identified through multivariate statistical analysis. Its basic principle is to use high-throughput technology to comprehensively analyze metabolites in samples, including mass spectrometry, nuclear magnetic resonance, and other techniques. These techniques allow detecting thousands of metabolites simultaneously, resulting in comprehensive metabolome information. The analysis of metabolome data provides information on metabolite composition, metabolic pathway, and interaction between metabolites in the sample [Liu *et al.*, 2019]. Untargeted metabolomics is a new approach with a wide range of application prospects, and has been successfully applied in investigating geo-tracing of plants [Cao *et al.*, 2019], food processing [Chen *et al.*, 2020], and plant bioactivity [Hasanpour *et al.*, 2020].

Based on this, the purpose of this study was to compare the volatile compound profiles of *C. paliurus* tea prepared with and without fixation by untargeted metabolomics methods, to explore the impact of fixation on the aroma quality of *C. paliurus* tea, and to provide references for the development of processing standards for *C. paliurus* tea.

MATERIAL AND METHODS

Experimental material and chemicals

The *C. paliurus* leaves were randomly picked in Quzhou (Zhejiang, China) in May 2023.

n-Hexane (chromatographic grade) was purchased from Merck (Darmstadt, Germany), and all standards were purchased from BioBioPha Co., Ltd. (KIB, Kunming, China) and Sigma-Aldrich (St. Louis, MO, USA).

C. paliurus leaves processing

To produce tea using fixation, the fresh *C. paliurus* leaves (50 kg) were spread on bamboo woven round plates (Φ2 m; leaf layer thickness on each plate was 2–5 cm) for 12–24 h at 19–22°C with a relative air humidity of 55% until their water content reached about 15–20 g/100 g. Then, fixation was performed using a roller-hot air coupling fixation machine (6CST-80YJ, Shangyang Machinery Co., LTD, Zhejiang, China), with rolling temperatures of 170-180°C and fixation time of 60–120 s. After fixing, the *C. paliurus* leaves were dried at 80°C until the moisture content of the finished tea was less than 5 g/100 g.

Tea without the fixation step was produced by spreading out the collected leaves at room temperature as during the fixated treatment and then drying directly at 8°C until the moisture was less than 5 g/100 g.

Analysis of volatile metabolites in *C. paliurus* teas

Samples were weighed, immediately frozen in liquid nitrogen, and stored at –80°C until used. Before the analysis, the samples were ground to powder in liquid nitrogen. For the analysis, 500 mg of the powders were transferred immediately to a 20 mL head-space vials (Agilent, Palo Alto, CA, USA) containing NaCl saturated solution, to inhibit any enzyme reaction. The vials were sealed with crimp-top caps of tetrafluoroethylene resin (TFE)-silicone headspace septa (Agilent). The solid-phase microextraction (SPME) was carried out by oscillation of each vial at a constant temperature of 60°C for 5 min, then 120 µm VB/CWR/PDMS extraction head (Agilent) was inserted into the sample headspace vial for 15 min of headspace extraction.

The identification and quantification of VOCs in the samples was carried out using an Agilent 7000D triple quadrupole gas chromatography–mass spectrometry (GC/MS) system (Agilent), equipped with a 30 m × 0.25 mm × 0.25 µm (5%-phenyl)-methylpolysiloxane (DB-5MS) capillary column (Agilent). Desorption of VOCs from the fiber coating was performed in the injection port of the chromatograph at 250°C for 5 min in the splitless mode. Injector temperature was maintained at 250°C. The oven temperature was set to 40°C (3.5 min), and then increased to 100°C at 10°C/min, 180°C at 7°C/min, and 280°C at 25°C/min for 5 min. The carrier gas was helium, applied at a flow rate of 1.2 mL/min. The quadrupole mass spectrometer, ion source and transmission line temperatures were set at 150°C, 230°C and 280°C, respectively. The mass spectrum was recorded in an electron shock (EI) ionization mode at 70 eV. The analytes were identified and quantified by mass spectrometry coupled with an ion-monitoring (SIM) mode.

For qualitative analysis, the Agilent MassHunter Unknowns analysis software was used to screen volatile metabolites whose similarity to the NIST11 mass spectrum library standard was >80%. By calculating the linear formula of *n*-alkane (C7–C40), the Kovats retention index of each compound was obtained and compared with the theoretical retention index. For quantitative analysis, the mass concentrations of the volatile metabolites were calculated with reference to the internal standard method using Equations (1) and (2) [Wang *et al.*, 2020]:

$$C_i = \frac{(C_{is} \times A_i)}{A_{is}} \quad (1)$$

$$X_i = \frac{(C_i \times V_i)}{M} \quad (2)$$

where: C_i is the mass concentration of any compound (µg/L), C_{is} is the mass concentration of the internal standard (µg/L) (ethyl caprate, 20 mg/L), A_i is the chromatographic peak area of any compound, A_{is} is the chromatographic peak area of the internal

standard, X_i is the content of compound i in the sample to be measured (μg/g), V_i is the volume of the sample i (L), and M is the amount of the sample to be measured (g).

Calculation of relative odor activity value

Relative odor activity values (rOAVs) of volatile compounds of *C. paliurus* teas were calculated using formula (3) [Xue *et al.*, 2022]:

$$rOAV_i = \frac{C_i}{T_i} \quad (3)$$

where: $rOAV_i$ is the relative odor activity value of compound i, C_i is the content of the compound (μg/L), and T_i is the threshold of the compound (μg/L). Threshold values were taken from the literature [van Gemert, 2011; Yang *et al.*, 2022a; Zhu *et al.*, 2021].

Statistical analysis

SIMCA P13 software (Umetrics, Umea, Sweden) and MEV 9.0 software (**https://mev-tm4-org.caas**) were used to analyze the main components and contributions of volatile metabolites in different tea processing, respectively. Three replicates were taken for each sample.

Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were carried out with the Metware Cloud, a free online platform for data analysis (**https://cloud.metware.cn**). Significantly changed metabolites were selected according to variable importance in the project (VIP) (VIP≥1 and $p<0.05$) obtained by the OPLS-DA model. Duncan's multiple test was applied to identify significant differences ($p<0.05$) among teas. Statistical analysis was performed using SPSS 18 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Types of volatile metabolites in the *C. paliurus* teas

In this study, samples extracted from fixated and unfixated *C. paliurus* tea were analyzed by untargeted metabolomics. A total of 1,222 volatile compounds were detected, including 25 amines (2.05%), 95 alcohols (7.77%), 54 aromatics (4.42%), 29 phenols (2.37%), 10 nitrogen compounds (0.82%), 12 sulfur compounds (0.98%), 4 halogenated hydrocarbons (0.33%), 7 ethers (0.57%), 92 aldehydes (7.53%), 24 acids (1.96%), 309 terpenoids (25.29%), 85 hydrocarbons (6.96%), 99 ketones (8.1%), 172 heterocyclic compounds (14.08%), 199 esters (16.28%), and 6 others (0.49%).

Using these 1,222 volatile metabolites, PCA was carried out on the *C. paliurus* teas prepared with and without the fixation step, and the resulting plot of PCA is shown in **Figure 1A.** The two processing methods were significantly distinguished from each other, and the percentage change of PC1 and PC2 interpretation was 83.18%; thus, these two principal components represented the main characteristics of the sample. Similarly, OPLS-DA, conducted with 1,222 volatile metabolites as independent variables and with fixated and unfixated teas as dependent variables, showed that there were large differences among sample groups, but small differences within groups (**Figure 1B**). The above results indicate that fixation was the key process in the processing of *C. paliurus* leaves, which had a great influence on the VOCs of the tea.

The numbers of volatile metabolites identified in fixated and unfixated teas and classified into different types are shown in **Figure 2**. Of the 1,222 VOCs, 1,196 metabolites were detected in *C. paliurus* tea prepared without the fixation step, while 1,193 volatile compounds were detected in tea obtained after leaf fixation. The main differences in the numbers of VOCs were found for terpenoids, heterocyclic compounds, amines, and phenols. Overall, there was little difference in numbers of compounds of each type between both tea variants, while the total VOC content in the *C. paliurus* tea obtained after fixation was significantly ($p<0.05$) higher than in the unfixated tea (**Figure 3**). Considering the different types of VOCs, contents of amines, phenols, nitrogen compounds, ethers, alcohols, terpenoids, esters, aromatics and others were extremely significantly higher ($p<0.01$) and the content of sulfur compounds was significantly higher ($p<0.05$) in the fixated tea (**Figure 3A, B**). Previous studies have found that the fixation process, due to the transient high temperature, can promote the hydrolysis, isomerization, substitution and redox reactions, and other physical and chemical

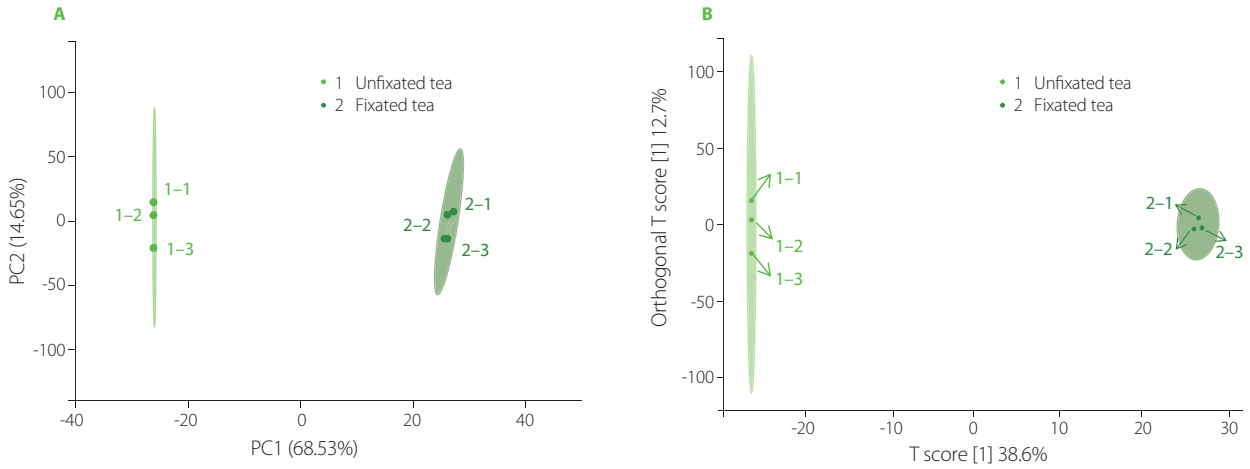

Figure 1. Score plots of principal component analysis (**A**) and orthogonal partial least squares discriminant analysis (**B**) based on volatile metabolites of fixated and unfixated *Cyclocarya paliurus* teas.

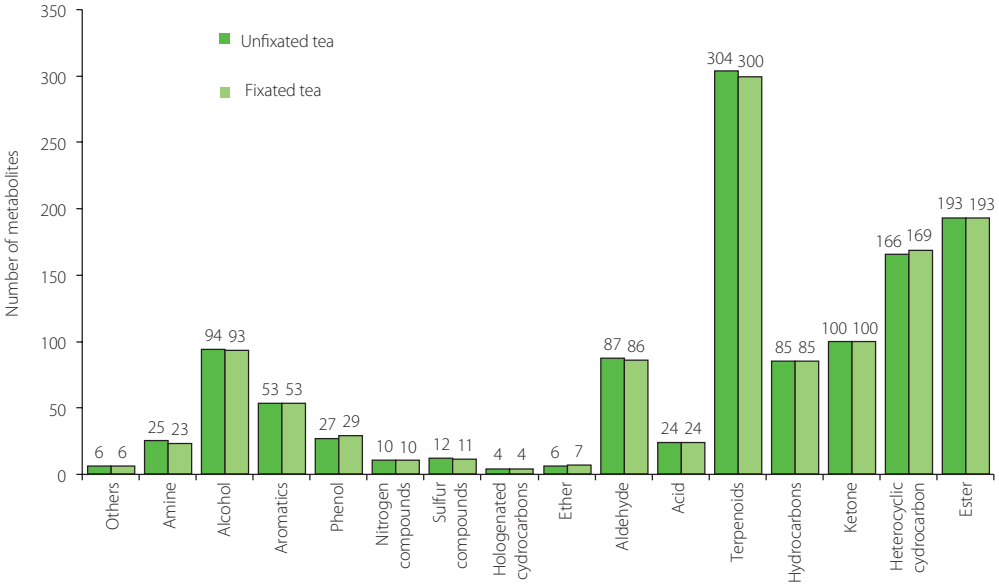

Figure 2. Number of volatile metabolites of different classes identified in *Cyclocarya paliurus* teas produced with and without fixation.

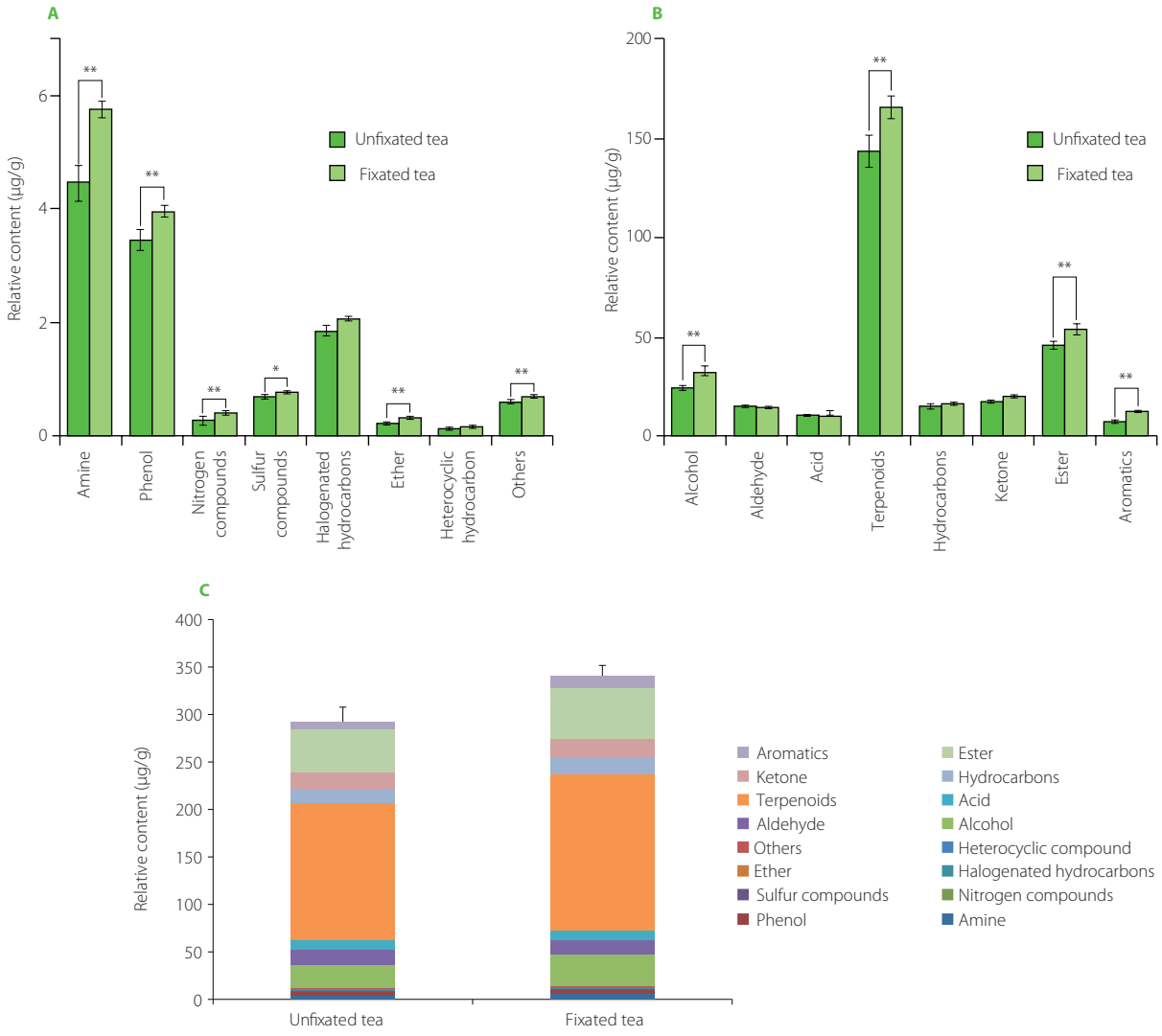

Figure 3. Contents of volatile metabolites of different classes (**A** and **B**) and total content of volatile metabolites (**C**) in *Cyclocarya paliurus* teas produced with and without fixation. Values in pairs (fixated and unfixated teas) marked with * differ significantly at $p<0.05$ and marked with ** differ significantly at $p<0.01$.

thermal changes of the non-volatile compounds of green tea leaves, which, in turn, were the key prerequisite compounds for the VOC formation [Wang *et al.*, 2021a]. The increase in the contents of VOCs of the fixated *C. paliurus* tea found in our study can be explained by a similar transformation.

Individual volatile metabolites in the *C. paliurus* teas

In order to elucidate the effect of fixation on the volatile metabolites in the processing of *C. paliurus* leaves, the differences of main metabolite contents in the key process of fixation were explored. According to the OPLS-DA screening criteria of metabolites: VIP ≥1 and fold change (FC) ≥2 or ≤0.5 [Geng *et al.*, 2023], a total of 147 tea metabolites were screened, of which 68 were up-regulated and 79 down-regulated after fixation (**Figure 4A**). Among them, the top ten up-regulated were succinimide, α-ethylidenebenzeneacetaldehyde, (*Z*)-1-methoxy-4-(1-propenyl)benzene, *trans*-anethole, anethole, 1-(4-ethylphenyl)ethanone, 2,2,3-trimethylcyclobutanone, 4-hexen-3-one, diethyl phthalate, benzenepropanoic acid methyl ester, and the top ten down-regulated were 2-hexenal, (*E*)-2-hexenal, *cis-p*-menth-2--en-7-ol, 2-methyl-5-(1-methylethyl)phenol, thymol, isopropenyl ethyl ketone, 1-cyclopropyl-1-propanone, 2,6-dimethyl-2,6--octadiene, acetic acid cyclohexyl ester, and (*E*)-3-octen-2-one (**Figure 4B**).

As shown in **Table S1** in Supplementary Materials, 147 main metabolites included 13 aldehydes, 10 hydrocarbons, 15 heterocyclic compounds, 18 ketones, 18 esters, 9 aromatics, 18 alcohols, 32 terpenoids, 1 nitrogen compound,1 ether, 2 amines, 2 acids, 3 phenols, 3 sulfurs, and 2 others. In order to observe the differences between metabolite contents of fixated and unfixated teas in a more intuitive way, heat maps of 147 metabolites were obtained and they are show in **Figure S1** in Supplementary Materials.

Terpenoids

Terpenoids, which were the most abundant among the VOCs of teas (**Figure 2** and **3B**) mainly included monoterpenoids, sesquiterpenoids and their derivatives. A total of 32 terpenes showed significant differences between tea variants, including 16 up-regulated and 16 down-regulated terpenes after fixation (**Table S1**, **Figure S1**). The first group included β-guaiene and β-pinene. Previously, these compounds were detected in black tea flavor profile [Yao *et al.*, 2023]. One of the down--regulated terpenoid was α-terpineol, which was considered to be a non-flavor compound in beverages [Pérez-López *et al.*, 2016], and has been identified as a key component of the stale odor of old green tea [Dai *et al.*, 2020]. In our study, the content of L-α--terpineol in the *C. paliurus* tea decreased significantly as a result of fixation (**Figure S1**), indicating that the quality of the *C. paliurus* tea could be improved by this process. In addition, a previous study has shown that terpenoids are important characteristic compounds of *C. paliurus* [Shao *et al.*, 2024], and volatile terpenoids are essential for the aroma quality of tea brews, with typical sweet, floral and woody flavors [Zhu *et al.*, 2017].

Alcohols

There was a significant difference in the total alcohol content between the *C. paliurus* tea processed by fixation and the *C. paliurus* tea prepared without the fixation step (**Figure 3B**), of which 7 compounds were up-regulated and 11 compounds were down-regulated after fixation (**Table S1**, **Figure S1**). 2,3-Butanediol was included in the first group. This compound was also observed in the VOC profile of Huaguo tea [Lu *et al.*, 2023], and Fuzhuan brick tea co-fermented with *Moringa oleifera* leaves [Li *et al.*, 2023b]. (*E*)-2-Hexen-1-ol and (*Z*)-2-hexen-1-ol were identified to be more obvious in Huaguo tea [Lu *et al.*, 2023]. After fixation, the down-regulation of some compounds that have adverse

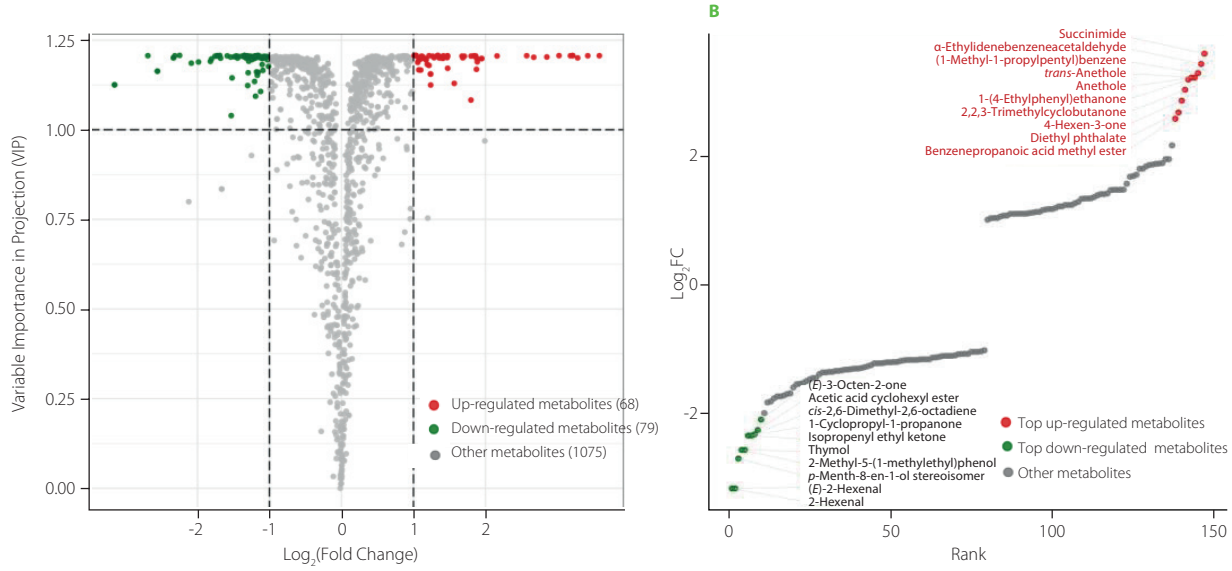

Figure 4. Volcano plot of the volatile metabolites of fixated *vs* unfixated *Cyclocarya paliurus* teas with indicated compounds significantly up-regulated (red points) and down-regulated (green points) after fixation (**A**). Dynamic distribution of metabolites significantly differentiating teas with the top 10 metabolites that were up-regulated (red points) and down-regulated (green points) after fixation (**B**).

reactions to human inhalation was particularly obvious, such as 3-hydroxy-benzenemethanol, *trans*-2-undecen-1-ol, and prenol. Therefore, through the reduction of odor or harmful compounds, fixation offers also an important advantage to improve the quality of *C. paliurus* tea.

Esters

A total of 18 esters were detected in the fixated and unfixated teas, with 9 up-regulated and 9 down-regulated after fixation (**Table S1**, **Figure S1**). Previous studies have shown that high temperature and long-term treatment are conducive to the formation of ester volatiles [Zhou *et al.*, 2023b], and fixation process results in a large amount of dissipation of low boiling point, grassy volatile esters [Ouyang *et al.*, 2022]. These esters, together with ketones, aldehydes, and other metabolites, were responsible for chestnut-like, flowery, and fruity aromas of tea [Wang *et al.*, 2021a]. Among up-regulated esters of *C. paliurus* tea, butyrolactone was determined (**Figure S1**). This compound was also found in the volatile compound profile of Congou black tea [Zhou *et al.*, 2023a], green tea [He *et al.*, 2023] and Dianhong Congou tea [Chen *et al.*, 2022a].

Ketones

Ketones, which mainly contribute to woody and floral aromas [Liu *et al.*, 2023], represent a common class of volatile compounds. In the study, it was found that 15 ketones were significantly down-regulated and only 3 were significantly up-regulated after fixation (**Table S1**, **Figure S1**). Among them, 2-heptanone down-regulated in the fixated tea, is commonly found in the VOCs of fruit and vegetable food products [Fella *et al.*, 2022; Wang *et al.*, 2021b] and is a flavor compound associated with fresh fruit aroma, with special banana aroma. 2-Heptanone can be formed by thermal β-oxidation of fatty acids, then decarboxylation [Valero *et al.*, 2001], and can bind to proteins [Wang & Arntfield, 2015]. This may explain its decreasing content after high temperature treatment in our study. The three up-regulated ketones were 4-hexen-3-one, 2,2,3-trimethylcyclobutanone, and 1-(4-ethylphenyl)ethanone (**Figure S1**). 4-Hexen-3-one is an unsaturated aldehyde that was reported to occur in peels of *Citrus aurantiifolia* fruit [Sandoval-Montemayor *et al.*, 2012], 1-(4-Ethylphenyl)ethanone was found in *Rhus potaninii* Maxim [Zhu *et al.*, 2020], while 2,2,3-trimethylcyclobutanone has not been reported in literature concerning VOCs of plants.

Heterocyclic compounds

Heterocyclic compounds are also important aroma compounds, and occupy an important place in the VOCs of various varieties of tea [Guo *et al.*, 2021]. A total of 15 heterocyclic compounds with significant differences were detected in *C. paliurus* tea variants, of which 8 were up-regulated and 7 were down-regulated after fixation (**Table S1**, **Figure S1**). The up-regulated compound, methylpyrazine, has nutty, roast and sweet aromas [Hu *et al.*, 2021]; hence, its up-regulation endows the tea a unique nutty aroma. It was discovered in chestnut-like aroma green tea [Yang *et al.*, 2022b]. 1*H*-Pyrrole-2-carboxaldehyde is a heat-induced compound (a previous study has shown that its content was higher in tomatoes obtained by heat pump drying [Jeyaprakash *et al.*, 2020]). This may explain up-regulation of this compound after fixation in this study.

5-Ethylhydantoin, succinimide, 2-acetyl-3-ethylpyrazine, 1*H*-pyrrole-2,5-dione, and (3*R*,6*S*)-2,2,6-trimethyl-6-vinyltetrahydro-2*H*-pyran-3-ol were the five significantly up-regulated heterocyclic compounds (**Figure S1**) that have not been reported in the volatility studies of other species, and can be used as characteristic compounds of *C. paliurus* tea obtained through fixation. Among the down-regulated heterocyclic compounds, betahistine, which is used in western medicine for the treatment of cardiovascular disease, was found in *C. paliurus* leaves for the first time. 2-*n*-Heptylfuran is only produced in a thermal reaction at 14°C and is associated with the flavor of cocoa and roasted nuts [Li *et al.*, 2023a]. However, the temperature of the fixation reached 170–180°C; hence, the content of this compound in tea decreased after processing. 1-Methyl-2-pyrrolidinone, 4-(*N*-nitroso-*N*-methylamino)-1-(3-pyridyl)-1-butanone, 2-furanpropanoic acid ethyl ester, dihydro-5-pentyl--2(3*H*)-furanone, and 1,5,6-trimethyl-azacyclohexan-3-one were the five down-regulated heterocyclic compounds (**Figure S1**) that have not been reported in the volatile compound profile of other species.

Aldehydes

Aldehydes with low odor threshold [Culleré *et al.*, 2011] can provide special flavor for *C. paliurus* tea. In addition, they perform a variety of biological functions, *e.g.*, cinnamaldehyde and *trans*-cinnamaldehyde showed antifungal and antibacterial activities [Gu *et al.*, 2024; Mu *et al.*, 2023]. The first of the mentioned aldehydes was up-regulated after fixation in our study (**Table S1**, **Figure S1**). Generally, in the *C. paliurus* teas, 6 aldehydes were significantly up-regulated and 7 others were significantly down-regulated after fixation. Up-regulated 2,4-dimethylbenzaldehyde was also found in a previous study as an odor active compound of the sour bamboo shoot fermentation broth [Long *et al.*, 2023]. 4-Isopropylcyclohexa-1,3-dienecarbaldehyde and 8-isopropyl--1-methyltricyclo[4.4.0.$0^{2,7}$]dec-3-ene-3-carbaldehyde were two significantly up-regulated aldehydes that have not been reported in studies of VOSs of other species, and can be used as the characteristic compounds derived from *C. paliurus* tea.

2-Hexenal and (*E*)-2-hexenal are common volatile aldehydes, which have been recognized as signature characteristic compounds in tea processing [Rong *et al.*, 2023; Yang *et al.*, 2023]. Similarly, 2-undecenal and (*E*)-2-undecenal (the *cis-trans* isomers) are also typical volatile aldehydes sensitive to heating [Zhou *et al.*, 2021], and this is the first time when they had been detected in the VOCs of *C. paliurus* leaves. Studies have shown that (*E,E*)-2,4-nonadienal appeared to be mainly responsible for the stale odor in green tea [Liu *et al.*, 2023], which indicated that the significant down-regulation of (*E,E*)-2,4-nonadienal could increase the flavor quality of *C. paliurus* fixated tea. (*E*)-2-Decenal was the main aroma compound in *Angelica keiskei* tea [Rong *et al.*, 2021], presenting typical flower, fruity, and sweet flavor.

The significant down-regulation of this compound after fixation in our study, may be related to its properties. After heating, it can continue to oxidize to form volatile substances with short carbon chains [Wang *et al.*, 2023]. 5-(Propan-2-yl)bicyclo[3.1.0] hex-2-ene-2-carbaldehyde has not yet been reported in the VOCs of *C. paliurus* leaves.

Hydrocarbons

Weakly polar hydrocarbons are also characteristic flavor compounds in teas [Guo *et al.*, 2021]. The 10 individual hydrocarbons (**Table S1**, **Figure S1**) included down-regulated after fixation olefins (4 compounds) and alkanes (2 compounds), as well up--regulated alkenes (3 compounds) and alkane (1 compound). Among them, 2,6-dimethyl-2,6-octadiene is produced during the thermal decomposition of glycosides [Hattori *et al.*, 2004]. Heating at 130°C/10 min + 110°C/30 min was conducive to its formation in black tea [Yang *et al.*, 2020]. In this study, the fixation temperature was higher (170°C–180°C); hence, its content decreased significantly. Albene has not been found in the VOCs of other species, especially tea, except in the roots of yarrow [Kindlovits *et al.*, 2018]. In addition, other up-regulated olefins and alkanes are rare in the study of volatile compound profile, and can be used as the characteristic compounds of *C. paliurus* tea for further study.

Aromatics

Contents of nine aromatics in *C. paliurus* tea variants showed significant differences, of which six were up-regulated after fixation (**Table S1**, **Figure S1**). Surprisingly, anethole and *trans*-anethole, which exists in fennel seeds [Odeh & Allaf, 2017], were found in *C. paliurus* tea, and they were significantly up-regulated after fixation. Due to their weet, herbaceous, and smooth odor profile, anethole and *trans*-anethole exhibited a comparatively high rOAV, indicating their crucial role in creating distinctive flavors and making them the primary contributor to the overall flavor of Yunan and Fujian tea [Kfoury *et al.*, 2018]. Estragole, a volatile phenylpropanoid contained in a variety of edible herbs [Gross *et al.*, 2009; Yamani *et al.*, 2014], was suspected to be carcinogenic and genotoxic, according to the European Union Committee on Herbal Medicinal Products (https://www.ema.europa.eu/en/committees/committee-herbal-medicinal-products-hmpc). Therefore, further studies are needed on a safe daily intake of *C. paliurus* herbal tea. The 1,2-dihydro--1,1,6-trimethyl-naphthalene was found as a key aroma compound of Pu-erh tea (ripe tea) [Wang *et al.*, 2022]. It was also significantly up-regulated in *C. paliurus* tea after fixation. The other two up-regulated aromatics, *i.e.*, 1,2,3,4-tetrahydronaphthalene and 2-phenylpropenal, have not been reported in any tea volatile compound profile The aromatics down-regulated after fixation were 2-methoxy-4-vinylphenol, (1-methyl-1-propylpentyl) benzene, and 1,1′-(1,3-propanediyl)bis-benzene. Among them, 2-methoxy-4-vinylphenol has the clove-like flavor, which has been reported to be a landmark compound of canned black tea after heating [Kumazawa & Masuda, 2001]. The fixation temperature was significantly higher than that of black tea sterilization; thus, the changes of 2-methoxy-4-vinylphenol caused by the two treatments were inconsistent.

Other compounds

In addition to the volatile compounds mentioned above, the other up-regulated volatile compounds of teas after fixation included carbonochloridodithioic acid methyl ester, ethanediamide, benzoic acid, 2-(1-methylpropyl)phenol, *cis-p*-menth-2--en-7-ol, dipropyl disulfide, (*Z*)-1-methoxy-4-(1-propenyl)benzene, benzyl nitrile, and ethyl 1-methylethyl disulfide (**Table S1**, **Figure S1**). Among them, benzoic acid has a floral and fruity flavor, and its content was also significantly up-regulated after green tea and yellow tea fixation [Wen *et al.*, 2023], which is consistent with our findings. Benzyl nitrile has a pungent odor [Li &Wang, 2020], which has been identified as a key marker for the floral odor of Fenghuang Dancong tea [Qin *et al.*, 2023]. The significantly up-regulated *cis-p*-menth-2-en-7-ol, has been previously found in *Ephedra sinica* Stapf. [Miyazawa *et al.*, 1997].

Four volatiles of *C. paliurus* teas were down-regulated after fixation included butanoic anhydride, *N*-phenylacetamide, (*E*)-2-hexenoic acid, and 2-(1,1-dimethylethyl)phenol. (*E*)-2-Hexenoic acid, with a fruity, herbal odor, has also been found in ferns [Fons *et al.*, 2010].

Aroma compounds of *C. paliurus* teas

Although a variety of volatile components were screened in different processed products of *C. paliurus* tea, not all of the volatile compounds were the material basis for the odor difference between fixated and unfixated *C. paliurus* teas. Therefore, the rOAV method was used to determine the contribution of volatile components to the overall odor of teas, and then to identify the key aroma compounds. In general, rOAV≥1 indicates that the compound has a direct contribution to the sample flavor, and 0.1≤rOAV<1 can be considered to have an important modification effect on the aroma of the sample [Huang *et al.*, 2022; Xue *et al.*, 2022]. As shown in **Table 1**, there were 46 volatile compounds with rOAV value greater than 0.1 in the unfixated *C. paliurus* tea, among which 22 volatile compounds had rOAV greater than 1. In the fixated *C. paliurus* tea, there were 44 volatile compounds with rOAV greater than 0.1, and 25 volatile compounds with rOAV greater than 1.

Many identified ketones, aldehydes, terpenes and other compounds in the tea produced by the two processing technologies had a high content and a relatively low threshold; hence, they contributed a lot to the overall flavor of the tea. The compound that contributed the most was 1-hexen-3-one (ketone), which mainly presents a cooked, vegetable, and metallic aroma. Followed by 3-octen-2-one, also a ketone, mainly presents earthy, spicy, herbal, sweet, mushroom, hay, and blueberry aroma. In addition, 5 terpenoids (2-methylisoborneol, 6,6-dimethyl-bicyclo[3.1.1]hept--2-ene-2-methanol, , β-pinene, α-terpineol and L-α-terpineol), 6 aldehydes (2-hexenal, (*E*)-2-undecenal, (*E*,*E*)-2,4-nonadienal, (E)-2-hexenal, 2-undecenal, (*E*)-2-decenal), 4 aromatics (anethole, 2-methoxy-4-vinylphenol, *trans*-anethole, and 1,2-dihydro-1,1--6-trimethyl-naphthalene), 2 esters (acetic acid cyclohexyl ester

Table 1. Threshold, odor description and relative odor activity value (rOAV) of volatile compounds identified in *Cyclocarya paliurus* tea produced with and without fixation.

Compound	Class	Odor	Threshold (μg/L)	rOAV*	
				Unfixated tea	Fixated tea
1-Hexen-3-one	Ketones	Cooked, vegetable, metallic	2×10^{-5}	25,371±1826	13,291±74
3-Octen-2-one	Ketones	Earthy, spicy, herbal, sweet, mushroom, hay, blueberry	3×10^{-5}	5,419±607	1,810±119
2-Methylisoborneol	Terpenoids	Earthy, musty	0.0005	551±27	273.8±8.3
Anethole	Aromatics	Sweet, exotic, flowery, stewed	0.015	21.3±1.4	212.8±7.8
(*E*,*E*)-2,4-Nonadienal	Aldehydes	Fatty, melon, waxy, green, violet, leafy, cucumber, tropical, fruity, chicken	0.0002	272±12	143.4±4.3
(*E*)-2-Undecenal	Aldehydes	Fresh, fruity, citrus, orange peel	0.0008	296±16	143.1±4.2
Heptanoic acid methyl ester	Esters	Sweet, fruity, green, orris, waxy, floral, berry	0.004	39.0±2.7	88.6±5.7
(*E*)- 6,10-Dimethyl-5,9-undecadien-2-one	Ketones	Fresh, green, fruity, waxy, rose, woody, magnolia, tropical	0.01	162.5±8.1	68.3±2.4
trans-Anethole	Aromatics	Sweet, anisic, licorice, mimosa	0.057	5.62±0.37	56.0±2.1
(*E*)-2-Hexenal	Aldehydes	Green, grassy	0.0031	388±193	47.8±2.1
Acetic acid cyclohexyl ester	Esters	Fruity, sweet, musty, ethereal	0.0016	117.6±9.0	26.6±1.1
β-Pinene	Terpenoids	Dry, woody, resinous, pine, hay, green	0.14	7.32±0.46	16.15±0.50
1,2-Dihydro-1,1,6-trimethyl-naphthalene	Aromatics	Licorice	0.0025	5.19±0.23	12.16±0.40
2-Undecenal	Aldehydes	Fresh, fruity, orange, peel	0.01	23.1±1.3	11.17±0.33
2-Hexenal	Aldehydes	Sweet, almond, fruity, green, leafy, apple, plum, vegetable	0.017	71±35	8.72±0.38
2-Methoxy-4-vinylphenol	Aromatics	Spicy, raisin	0.003	15.62±0.60	8.02±0.34
(*E*)-2-Decenal	Aldehydes	Waxy, fatty, earthy, green, cilantro, mushroom, aldehydic, fried, chicken, fatty, tallow	0.005	14.21±0.68	7.28±0.37
6,6-Dimethyl-bicyclo[3.1.1]hept-2-ene--2-methanol	Terpenoids	Woody, minty	0.007	8.20±0.45	3.56±0.23
Acetic acid 4-methylphenyl ester	Esters	Narcissus, phenol, animalic	0.025	0.766±0.069	2.941±0.090
Diethyl phthalate	Esters	Bitter	0.33	0.384±0.033	2.65±0.25
3-Phenyl-2-propenal	Aldehydes	Sweet, spicy, aldehydic, aromatic, balsamic, cinnamyl, resinous, honey, powdery	0.024	0.58±0.11	2.392±0.099
Dipropyl disulfide	Sulfur compounds	Sulfury, earthy, burnt, green, onion	0.0195	0.495±0.038	1.348±0.051
α-Ethylidenebenzeneacetaldehyde	Aldehydes	Sweet, narcissus, cortex, beany, honey, cocoa, nutty, radish	0.5	0.110±0.007	1.271±0.052
Estragole	Aromatics	Sweet, sassafrass, anisic, spice, green, herbal, fennel	0.035	0.414±0.025	1.188±0.032
Eucalyptol	Terpenoids	Eucalyptus, herbal, camphor, medicinal	0.015	0.365±0.032	1.040±0.058
α-Terpineol	Terpenoids	Pine, iris, teil	0.3	2.28±0.18	0.964±0.032
(*E*)-2-Hexen-1-ol	Alcohols	Fresh, green, leafy, fruity, unripe banana	0.1	0.330±0.015	0.897±0.005
(*Z*)-2-Hexen-1-ol	Alcohols	Green, cortex, leafy, beany, nasturtium, herbal, soapy, aldehydic, narcissus, phenol	0.1	0.330±0.015	0.897±0.005
Dihydro-5-pentyl-2(3*H*)-furanone	Heterocyclic compounds	Coconut, woody	0.0079	2.42±0.27	0.892±0.067
L-α-Terpineol	Terpenoids	Lilac, floral, terpenic	0.33	2.07±0.16	0.876±0.030
Methylpyrazine	Heterocyclic compounds	Nutty, cocoa, roasted, chocolate, peanut, green	0.06	0.306±0.037	0.689±0.020

Table 1 cont. Threshold, odor description and relative odor activity value (rOAV) of volatile compounds identified in *Cyclocarya paliurus* tea produced with and without fixation.

Compound	Class	Odor	Threshold (μg/L)	rOAV*	
				Unfixated tea	Fixated tea
Prenol	Alcohols	Fruity, green, lavender	0.25	1.37±0.10	0.664±0.020
α-Methyl-benzenemethanol	Alcohols	Fresh, sweet, gardenia, hyacinth	0.479	0.238±0.031	0.621±0.023
(1*S*)-6,6-Dimethyl-2-methylene--bicyclo[3.1.1]heptane	Terpenoids	Dry, woody, fresh, pine, hay, green, resinous	4.16	0.246±0.016	0.544±0.017
4-Methyl-1-(1-methylethyl)-bicyclo[3.1.0] hex-3-en-2-one	Terpenoids	Minty, pungent	0.75	0.158±0.009	0.469±0.034
3,7-Dimethyl-1,5,7-octatrien-3-ol	Alcohols	Mouldy	0.11	0.875±0.016	0.421±0.066
Hotrienol	Alcohols	Sweet, tropical, ocimene, fennel, ginger, myrcene	0.11	0.875±0.016	0.421±0.066
4-Methylene-1-(1-methylethyl)--bicyclo[3.1.0]hexane	Terpenoids	Woody, terpene, citrus, pine, spice	0.98	0.144±0.013	0.360±0.010
4-Methyl-1-(1-methylethyl)-bicyclo[3.1.0] hex-2-ene	Hydrocarbons	-	0.98	0.118±0.008	0.260±0.010
Isopentyl hexanoate	Esters	Fruity, banana, apple, pineapple, green	0.32	0.53±0.10	0.251±0.005
(*E*)-3,7,11-Trimethyl-1,6,10-dodecatrien--3-ol	Terpenoids	Floral, green, citrus, woody, waxy	0.25	0.478±0.0145	0.241±0.037
2-(1,1-Dimethylethyl)phenol	Phenols	-	0.05	0.421±0.040	0.202±0.030
(*Z*)-3-Hexen-1-ol benzoate	Esters	Fresh, green, leafy, floral, orchid, balsamic, fatty	0.5	0.046±0.011	0.183±0.009
(*E*)-2-Hexenoic acid	Acids	Powerful, fruity, sweet, warm, herbal	1.9	0.409±0.019	0.175±0.011
3-Hexanone	Ketones	Sweet, fruity, waxy, rummy, grape	0.041	0.396±0.030	0.148±0.041
1,2,3,4-Tetrahydronaphthalene	Aromatics	-	0.05	0.054±0.006	0.135±0.009
Benzyl nitrile	Nitrogen compounds	-	1.2	0.031±0.001	0.105±0.004
Acetic acid 2-ethylhexyl ester	Esters	Earthy, herbal, humus, undergrowth	0.047	0.162±0.017	0.058±0.003
Thymol	Terpenoids	Herbal, thyme, phenol, medicinal, camphor	0.188	0.220±0.070	0.040±0.003

*Values are shown as mean ± standard deviation.

and heptanoic acid methyl ester), 1 heterocyclic compound (dihydro-5-pentyl-2(3*H*)-furanone), 1 alcohol (prenol), and 1 ketone ((*E*)-6,10-dimethyl-5,9-undecadien-2-one) in the unfixated *C. paliurus tea* are its characteristic aroma substances. Green and fruity were the two main aromas, which were presented by a variety of substances. In the fixated *C. paliurus* tea, 8 aldehydes ((*E*,*E*)-2,4-nonadienal, (*E*)-2-undecenal, (*E*)-2-hexenal, 2-undecenal, 2-hexenal, (*E*)-2-decenal, 3-phenyl-2-propenal, and α-Et hylidenebenzeneacetaldehyde), 5 aromatics (anethole, *trans*-anethole, 1,2-dihydro-1,1,6-trimethyl-naphthalene, 2-methoxy--4-vinylphenol, and estragole), 4 terpenoids (2-methylisoborneol, β-pinene, 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol, and eucalyptol), 4 esters (heptanoic acid methyl ester, acetic acid cyclohexyl ester, acetic acid 4-methylphenyl ester, and diethyl phthalate), 1 sulfur compound (dipropyl disulfide), and 1 ketone ((*E*)-6,10-dimethyl-5,9-undecadien-2-one) were its characteristic aroma compounds. Green and sweet were the two main aromas, which were presented by a variety of compounds.

CONCLUSIONS

Herein, the effect of fixation on the volatile compounds of *C. paliurus* tea was investigated and analyzed comprehensively for the first time. A total of 1,222 volatile metabolites were detected and 147 individual volatile metabolites were screened. Fixation had a strong effect on the content of volatile compounds in *C. paliurus* tea. After fixation, the content of amines, phenol, nitrogen, ether, alcohols, terpenoids, ester, aromatics increased significantly. The difference of metabolites before and after fixation indicates that fixation was a key step in *C. paliurus* tea processing. In addition, the study also found a batch of unique volatile compounds of *C. paliurus* tea and key aroma compounds, which present mainly green and sweet odor. The results of this study provided a new theoretical basis for the development of processing technology of *C. paliurus* leaves into tea, which could be used for quality control during the processing of *C. paliurus* leaves. In the follow-up study, we will investigate the potential effects of amino acids, lipids *etc.* added during the fixation step on the flavor characteristics of *C. paliurus* tea.

SUPPLEMENTARY MATERIALS

The following are available online at https://journal.pan.olsztyn.pl/Untargeted-Metabolomics-Analysis-Reveals-the-Effect-of-Fixation-on-the-Profile-of,191754,0,2.html.

Table S1. Profile of volatile metabolites of *Cyclocarya paliurus* teas produced with fixation and without fixation. **Figure S1**. Heatmaps of the significantly up-regulated (red cells) and down-regulated (green cells) volatile metabolites after fixation of *Cyclocarya paliurus* teas; aldehydes (A), hydrocarbons (B), heterocyclic compounds (C), ketones (D), aromatics (E), esters (F), alcohols (G), terpenoids (H) and others including one nitrogen compound, one ether, two amines, two acids, three phenols, and three sulfur compounds (I).

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ADDITIONAL INFORMATION

Data supporting the findings of this study are available upon request from the corresponding author.

CONFLICT OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Cao, T., Liu, J., Zhang, X., Wei, X., Qi, Y., Zhang, B., Liu, H., Xiao, P. (2019). Metabolomics characterization of different geographical origins of *Flos Carthami* using UPLC-QTOF/MS and their correlation with climate factors. *Analytical Methods, 11*(20), 2700-2711. https://doi.org/10.1039/C9AY00368A
2. Chen, J., Yang, Y., Deng, Y., Liu, Z., Shen, S., Zhu, J., Yuan, H., Jiang, Y. (2022a). Characterization of the key differential volatile components in different grades of Dianhong Congou tea infusions by the combination of sensory evaluation, comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry, and odor activity value. *LWT – Food Science and Technology, 165*, art. no. 113755. https://doi.org/10.1016/j.lwt.2022.113755
3. Chen, Q., Shi, J., Mu, B., Chen, Z., Dai, W., Lin, Z. (2020). Metabolomics combined with proteomics provides a novel interpretation of the changes in nonvolatile compounds during white tea processing. *Food Chemistry, 332*, art. no. 127412. https://doi.org/10.1016/j.foodchem.2020.127412
4. Chen, Z., Jian, Y., Wu, Q., Wu, J., Sheng, W., Jiang, S., Shehla, N., Aman, S., Wang, W. (2022b). *Cyclocarya paliurus* (Batalin) Iljinskaja: Botany, ethnopharmacology, phytochemistry and pharmacology. *Journal of Ethnopharmacology, 285*, art. no. 114912. https://doi.org/10.1016/j.jep.2021.114912
5. Culleré, L., Ferreira, V., Cacho, J. (2011). Analysis, occurrence and potential sensory significance of aliphatic aldehydes in white wines. *Food Chemistry, 127*(3), 1397-1403. https://doi.org/10.1016/j.foodchem.2011.01.133
6. Dai, Q., Jin, H., Gao, J., Ning, J., Yang, X., Xia, T. (2020). Investigating volatile compounds' contributions to the stale odour of green tea. *International Journal of Food Science and Technology, 55*(4), 1606-1616. https://doi.org/10.1111/ijfs.14387
7. Fella, P., Kaikiti, K., Stylianou, M., Agapiou, A. (2022). HS-SPME-GC/MS analysis for revealing carob's ripening. *Metabolites, 12*(7), art. no. 656. https://doi.org/10.3390/metabo12070656
8. Fons, F., Froissard, D., Bessiere, J., Buatois, B., Rapior, S. (2010). Biodiversity of volatile organic compounds from five French ferns. *Natural Product Communications, 5*(10), 1655-1658. https://doi.org/10.1177/1934578X1000501028
9. Geng, Z., Wang, J., Zhu, L., Yu, X., Zhang, Q., Li, M., Hu, B., Yang, X. (2023). Metabolomics provide a novel interpretation of the changes in flavonoids during sea buckthorn (*Hippophae rhamnoides* L.) drying. *Food Chemistry, 413*, art. no. 135598. https://doi.org/10.1016/j.foodchem.2023.135598
10. Gross, M., Lewinsohn, E., Tadmor, Y., Bar, E., Dudai, N., Cohen, Y., Friedman, J. (2009). The inheritance of volatile phenylpropenes in bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*, Apiaceae) chemotypes and their distribution within the plant. *Biochemical Systematics and Ecology, 37*(4), 308-316. https://doi.org/10.1016/j.bse.2009.05.007
11. Gu, K., Feng, S., Zhang, X., Peng, Y., Sun, P., Liu, W., Wu, Y., Yu, Y., Liu, X., Liu, X., Deng, G., Zheng, J., Li, B., Zhao, L. (2024). Deciphering the antifungal mechanism and functional components of *Cinnamomum cassia* essential oil against *Candida albicans* through integration of network-based metabolomics and pharmacology, the greedy algorithm, and molecular docking. *Journal of Ethnopharmacology, 319*(Part 2), art. no. 117156. https://doi.org/10.1016/j.jep.2023.117156
12. Guo, X., Ho, C., Schwab, W., Wan, X. (2021). Aroma profiles of green tea made with fresh tea leaves plucked in summer. *Food Chemistry, 363*, art. no. 130328. https://doi.org/10.1016/j.foodchem.2021.130328
13. Hasanpour, M., Iranshahy, M., Iranshahi, M. (2020). The application of metabolomics in investigating anti-diabetic activity of medicinal plants. *Biomedicine & Pharmacotherapy, 128*, art. no. 110263. https://doi.org/10.1016/j.biopha.2020.110263
14. Hattori, S., Kawaharada, C., Tazaki, H., Fujimori, T., Kimura, K., Ohnishi, M., Nabeta, K. (2004). Formation mechanism of 2,6-dimethyl-2,6-octadienes from thermal decomposition of linalyl β-D-glucopyranoside. *Bioscience, Biotechnology & Biochemistry, 68*(12), 2656-2659. https://doi.org/10.1271/bbb.68.2656
15. He, Y., Li, J., Mei, H., Zhuang, J., Zhao, Z., Jeyaraj, A., Wang, Y., Chen, X., Li, X., Liu, Z. (2023). Effects of leaf-spreading on the volatile aroma components of green tea under red light of different intensities. *Food Research International, 168*, art. no. 112759. https://doi.org/10.1016/j.foodres.2023.112759
16. Hu, H., Shi, A., Liu, H., Liu, L., Fauconnier, M.L., Wang, Q. (2021) Study on key aroma compounds and its precursors of peanut oil prepared with normal- and high-oleic peanuts. *Foods, 10*(12), art. no. 3036. https://doi.org/10.3390/foods10123036
17. Huang, W.J., Fang, S.M., Wang, J., Zhuo, C., Luo, Y., Yu, Y., Li, L., Wang, Y., Deng, W., Ning, J. (2022). Sensomics analysis of the effect of the withering method on the aroma components of Keemun black tea. *Food Chemistry, 395*, art. no. 133549. https://doi.org/10.1016/j.foodchem.2022.133549
18. Jeyaprakash, S., Heffernan, J.E., Driscoll, R.H., Frank, D.C. (2020). Impact of drying technologies on tomato flavor composition and sensory quality. *LWT – Food Science and Technology, 120*, art. no. 108888. https://doi.org/10.1016/j.lwt.2019.108888
19. Kfoury, N., Baydakov, E., Gankin, Y., Robbat, A.Jr. (2018). Differentiation of key biomarkers in tea infusions using a target/nontarget gas chromatography/mass spectrometry workflow. *Food Research International, 113*, 414-423. https://doi.org/10.1016/j.foodres.2018.07.028
20. Kindlovits, S., Sárosi, S., Inotai, K., Petrović, G., Stojanović, G., Németh, E. (2018). Phytochemical characteristics of root volatiles and extracts of *Achillea collina* Becker genotypes. *Journal of Essential Oil Research, 30*(5), 330-340. https://doi.org/10.1080/10412905.2018.1470581
21. Kumazawa, K., Masuda, H. (2001). Change in the flavor of black tea drink during heat processing. *Journal of Agricultural and Food Chemistry, 49*(7), 3304-3309. https://doi.org/10.1021/jf001323h
22. Li, J., Ma, Y., Xiao, F., Fu, B., Xu, X. (2023a). Formation and conversion of flavour related compounds of thermal cooked *Rudita pesphilippinarum* soup. *International Journal of Food Science, Technology, 58*(12), 6421-6431. https://doi.org/10.1111/ijfs.16753
23. Li, X., Shi, C., Wang, S., Wang, S., Wang, X., Lü, X. (2023b). Uncovering the effect of *Moringa oleifera* Lam. leaf addition to Fuzhuan Brick Tea on sensory properties, volatile profiles and anti-obesity activity. *Food & Function, 14*(13), art. no. 6274. https://doi.org/10.1039/D3FO90057F
24. Li, Z., Wang, J. (2020). Identification and similarity analysis of aroma substances in main types of Fenghuang Dancong tea. *PLoS One, 15*(12), art. no. 244224. https://doi.org/10.1371/journal.pone.0244224
25. Liu, P., Feng, L., Xu, Y., Zheng, L., Yin, P., Ye, F., Gui, A., Wang, S., Wang, X., Teng, J., Xue, J., Gao, S., Zheng, P. (2023). Characterization of stale odor in green tea formed during storage: Unraveling improvements arising from reprocessing by baking. *LWT – Food Science and Technology, 174*, art. no. 114458. https://doi.org/10.1016/j.lwt.2023.114458
26. Liu, X., Zhou, L., Shi, X., Xu, G. (2019). New advances in analytical methods for mass spectrometry-based large-scale metabolomics study. *TrAC Trends in Analytical Chemistry, 121*, art. no. 115665. https://doi.org/10.1016/j.trac.2019.115665

27. Long, Z., Zhao, S., Xu, X., Du, W., Chen, Q., Hu, S. (2023). Dynamic changes in flavor and microbiota in traditionally fermented bamboo shoots (*Chimonobambusa szechuanensis* (Rendle) Keng f.). *Foods, 12*(16), art. no. 3035. https://doi.org/10.3390/foods12163035
28. Lu, W., Chen, J., Li, X., Qi, Y., Jiang, R. (2023). Flavor components detection and discrimination of isomers in Huaguo tea using headspace-gas chromatography-ion mobility spectrometry and multivariate statistical analysis. *Analytica Chimica Acta, 1243*, art. no. 340842. https://doi.org/10.1016/j.aca.2023.340842
29. Miyazawa, M., Minamino, Y., Kameoka, H. (1997). Volatile components of *Ephedra sinica* Stapf. *Flavour and Fragrance Journal, 12*(1), 15-17. https://doi.org/10.1002/(SICI)1099-1026(199701)12:1<15::AID-FFJ604>3.0.CO;2-5
30. Mu, R., Zhang, H., Zhang, Z., Li, X., Ji, J., Wang, X., Gu, Y., Qin, X. (2023). Trans-cinnamaldehyde loaded chitosan based nanocapsules display antibacterial and antibiofilm effects against cavity-causing *Streptococcus mutans*. *Journal of Oral Microbiology, 15*(1), art. no. 2243067. https://doi.org/10.1080/20002297.2023.2243067
31. Odeh, A., Allaf, A.W. (2017). Determination of polyphenol component fractions and integral antioxidant capacity of Syrian aniseed and fennel seed extracts using GC-MS, HPLC analysis, and photochemiluminescence assay. *Chemical Papers, 71*(9), 1731-1737. https://doi.org/10.1007/s11696-017-0169-9
32. Ouyang, W., Yu, Y., Wang, H., Jiang, Y., Hua, J., Ning, J., Yuan, H. (2022). Analysis of volatile metabolite variations in strip green tea during processing and effect of rubbing degree using untargeted and targeted metabolomics. *Food Research International, 162*(Part B), art. no. 112099. https://doi.org/10.1016/j.foodres.2022.112099
33. Pérez-López, A.J., Saura, D., Lorente, J. Carbonell-Barrachina, Á.A. (2006). Limonene, linalool, α-terpineol, and terpinen-4-ol as quality control parameters in mandarin juice processing. *European Food Research and Technology, 222*, 281-285. https://doi.org/10.1007/s00217-005-0055-5
34. Qin, D., Wang, Q., Jiang, X., Ni, E., Fang, K., Li, H., Wang, Q., Pan, C., Li, B., Wu, H. (2023). Identification of key volatile and odor-active compounds in 10 main fragrance types of Fenghuang Dancong tea using HS-SPME/GC-MS combined with multivariate analysis. *Food Research International, 173*(Part 1), art. no. 113356. https://doi.org/10.1016/j.foodres.2023.113356
35. Qin, D., Wang, Q., Li, H., Jiang, X., Fang, K., Wang, Q., Li, B., Pan, C., Wu, H., (2020). Identification of key metabolites based on non-targeted metabolomics and chemometrics analyses provides insights into bitterness in Kucha [*Camellia kucha* (Chang et Wang) Chang]. *Food Research International, 138*(Part B), art. no. 109789. https://doi.org/10.1016/j.foodres.2020.109789
36. Rong, Y., Gu, X., Li, D., Chen, L., Zhang, Y., Wang, Z. (2021). Characterization of aroma, sensory and taste properties of *Angelica keiskei* tea. *European Food Research and Technology, 247*(7), 1665-1677. https://doi.org/10.1007/s00217-021-03737-7
37. Rong, Y., Xie, J., Yuan, H., Wang, L., Liu, F., Deng, Y., Jiang, Y., Yang, Y. (2023). Characterization of volatile metabolites in Pu-erh teas with different storage years by combining GC-E-Nose, GC-MS, and GC-IMS. *Food Chemistry: X, 18*, art. no. 100693. https://doi.org/10.1016/j.fochx.2023.100693
38. Sandoval-Montemayor, N.E., García, A., Elizondo-Treviño, E., Garza-González, E., Alvarez, L., Del Rayo Camacho-Corona, M. (2012). Chemical composition of hexane extract of *Citrus aurantifolia* and anti-*Mycobacterium tuberculosis* activity of some of its constituents. *Molecules, 17*(9), 11173-11184. https://doi.org/10.3390/molecules170911173
39. Shao, Y., Li, T., Wu, C., Fang, S., Fan, G., Shang, X., Yang, W. (2024). A review on extraction and biological activities of triterpenoid from *Cyclocarya paliurus*. *Food Reviews International, 40*, art. no. 2213307. https://doi.org/10.1080/87559129.2023.2213307
40. Valero, E., Villamiel, M., Miralles, B., Sanz, J., Martínez-Castro, I. (2001). Changes in flavour and volatile components during storage of whole and skimmed UHT milk. *Food Chemistry, 72*(1), 51-58. https://doi.org/10.1016/S0308-8146(00)00203-X
41. van Gemert, L.J. (Ed.), (2011). *Odour Thresholds: Compilations of Odour Threshold Values in Air, Water and Other Media*. 2nd edition, Oliemans Punter, Partners BV, Zeist, The Netherlands.
42. Wang, B., Wang, S., Wang, Y., Zhang, S., Lin, X., Xu, X., Ji, C., Liang, H., Dong, L. (2023). Deep exploration of lipid oxidation into flavor compounds: A density functional theory study on (*E*)-2-decenal thermal oxidative reaction. *Food Chemistry, 428*, art. no. 136725. https://doi.org/10.1016/j.foodchem.2023.136725
43. Wang, H., Hua, J., Jiang, Y., Yang, Y., Wang, J., Yuan, H. (2020). Influence of fixation methods on the chestnut-like aroma of green tea and dynamics of key aroma substances. *Food Research International, 136*, art. no. 109479. https://doi.org/10.1016/j.foodres.2020.109479
44. Wang, H., Hua, J., Yu, Q., Li, J., Wang, J., Deng, Y., Yuan, H., Jiang, Y. (2021a). Widely targeted metabolomic analysis reveals dynamic changes in non-volatile and volatile metabolites during green tea processing. *Food Chemistry, 363*, art. no. 130131. https://doi.org/10.1016/j.foodchem.2021.130131
45. Wang, K., Arntfield, S.D. (2015). Effect of salts and pH on selected ketone flavours binding to salt-extracted pea proteins: The role of non-covalent forces. *Food Research International, 77*(Part 1), 1-9. https://doi.org/10.1016/j.foodres.2015.03.017
46. Wang, Z., Dou, R., Yang, R., Cai, K., Li, C., Li, W. (2021b). Changes in phenols, polysaccharides and volatile profiles of noni (*Morinda citrifolia* L.) juice during fermentation. *Molecules, 26*(9), art. no. 2604. https://doi.org/10.3390/molecules26092604
47. Wang, Z., Su, D., Ren, H., Lv Q., Ren L., Li, Y., Zhou, H. (2022). Effect of different drying methods after fermentation on the aroma of Pu-erh tea (ripe tea). *LWT – Food Science and Technology, 171*, art. no. 114129. https://doi.org/10.1016/j.lwt.2022.114129
48. Wen, S., Sun, L., Zhang, S., Chen, Z., Chen, R., Li, Z., Lai, X., Zhang, Z., Cao, J., Li, Q., Sun, S., Lai, Z., Li, Q. (2023). The formation mechanism of aroma quality of green and yellow teas based on GC-MS/MS metabolomics. *Food Research International, 172*, art. no. 113137. https://doi.org/10.1016/j.foodres.2023.113137
49. Xu, Q., Zhou, Y.J., Wang, J.H., (2020). The impact of fixation method on the biochemical characteristics of hawk tea (*Litsea coreana*). *International Journal of Food Engineering, 16*(8), art. no. 20190290. https://doi.org/10.1515/ijfe-2019-0290
50. Xue, J., Liu, P., Yin, J., Wang, W., Zhang, J., Wang, W., Le, T., Ni, D., Jiang, H. (2022). Dynamic changes in volatile compounds of shaken black tea during its manufacture by GC × GC–TOFMS and multivariate data analysis. *Foods, 11*(9), art. no. 1228. https://doi.org/10.3390/foods11091228
51. Yamani, H., Mantri, N., Morrison, PD., Pang, E. (2014). Analysis of the volatile organic compounds from leaves, flower spikes, and nectar of Australian grown *Agastache rugosa*. *BMC Complementary and Alternative Medicine, 14*, art. no. 495. https://doi.org/10.1186/1472-6882-14-495
52. Yang, G., Zhou, M., Shi, J., Peng, Q., Lin, Z., Lv, H., Simal-Gandara, J. (2023). How anaerobic treatment is controlling the volatile components and key odorants of purple-colored leaf tea. *Journal of Food Composition and Analysis, 122*, art. no. 105451. https://doi.org/10.1016/j.jfca.2023.105451
53. Yang, Y., Hua, J., Deng, Y., Jiang, Y., Qian, M.C., Wang, J., Li, J., Zhang, M., Dong, C., Yuan, H. (2020). Aroma dynamic characteristics during the process of variable-temperature final firing of Congou black tea by electronic nose and comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. *Food Research International, 137*, art. no. 109656. https://doi.org/10.1016/j.foodres.2020.109656
54. Yang, Y., Qian, M.C., Deng, Y., Yuan, H., Jiang, Y. (2022b). Insight into aroma dynamic changes during the whole manufacturing process of chestnut-like aroma green tea by combining GC-E-Nose, GC-IMS, and GC × GC-TOFMS. *Food Chemistry, 387*, art. no. 132813. https://doi.org/10.1016/j.foodchem.2022.132813
55. Yang, Y.J., Ai, L.Z., Mu, Z.Y., Liu, H.D., Yan, X., Ni, L., Zhang, H., Xia, Y.J. (2022a). Flavor compounds with high odor activity values (OAV > 1) dominate the aroma of aged Chinese rice wine (Huangjiu) by molecular association. *Food Chemistry, 383*, art. no. 132370. https://doi.org/10.1016/j.foodchem.2022.132370
56. Yao, H., Su, H., Ma, J., Zheng, J., He, W., Wu, C., Hou, Z., Zhao, R., Zhou, Q. (2023). Widely targeted volatileomics analysis reveals the typical aroma formation of Xinyang black tea during fermentation. *Food Research International, 164*, art. no. 112387. https://doi.org/10.1016/j.foodres.2022.112387
57. Yu, J., Chu, M., Park, H., Park, J., Lee, K. (2021). Analysis of volatile compounds in coffee prepared by various brewing and roasting methods. *Foods, 10*(6), art. no. 1347. https://doi.org/10.3390/foods10061347
58. Zhang, L., Zhang, Z., Fang, S., Liu, Y., Shang, X. (2021). Integrative analysis of metabolome and transcriptome reveals molecular regulatory mechanism of flavonoid biosynthesis in *Cyclocarya paliurus* under salt stress. *Industrial Crops and Products, 170*, art. no. 113823. https://doi.org/10.1016/j.indcrop.2021.113823
59. Zhou, J., He, C., Qin, M., Luo, Q., Jiang, X., Zhu, J., Qiu, L., Yu, Z., Zhang, D., Chen, Y., Ni, D. (2023a). Characterizing and decoding the effects of different fermentation levels on key aroma substances of Congou black tea by sensomics. *Journal of Agricultural and Food Chemistry, 71*(40), 14706-14719. https://doi.org/10.1021/acs.jafc.3c02813
60. Zhou, J., Zhang, X., Liu, W., Zhang, Q., Wu, L., (2023b). Effect of different drying methods on color difference, microorganisms and volatile metabolites profiles of Ganpu Tea. *Food Bioscience, 56*, art. no. 103314. https://doi.org/10.1016/j.fbio.2023.103314

61. Zhou, X., Zhang, Q., Chen, X., Li, X., Han, C. (2021). *In-situ* assessment of olive oil adulteration with soybean oil based on thermogravimetric-gas chromatography/mass spectrometry combined with chemometrics. *Food Control, 130*, art. no. 108251. https://doi.org/10.1016/j.foodcont.2021.108251
62. Zhu, J.C., Niu, Y.W., Xiao, Z.B. (2021). Characterization of the key aroma compounds in Laoshan green teas by application of odour activity value (OAV), gas chromatography-mass spectrometry-olfactometry (GC-MS-O) and comprehensive two-dimensional gas chromatography mass spectrometry (GC×GC-qMS). *Food Chemistry, 339*, art. no. 128136. https://doi.org/10.1016/j.foodchem.2020.128136
63. Zhu, X., Li, L., Hsiang, T., Zha, Y.P., Zhou, Z.X., Chen, R., Wang, X., Wu, Q.L., Li, J.K. (2020). Chemical composition and attractant activity of volatiles from *Rhus potaninii* to the spring aphid *Kaburagia rhusicola*. *Molecules, 25*(15), art. no. 3412. https://doi.org/10.3390/molecules25153412
64. Zhu, Y., Shao, C.Y., Lv, H.P., Zhang, Y., Dai, W.D., Guo, L., Tan, J.F., Peng, Q.H., Lin, Z. (2017). Enantiomeric and quantitative analysis of volatile terpenoids in different teas (*Camellia sinensis*). *Journal of Chromatography A, 1490*, 177-190. https://doi.org/10.1016/j.chroma.2017.02.013

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Physicochemical Properties, Antioxidant Capacity and Sensory Acceptability of Instant Rosehip Teas Prepared by Spray-Drying and Freeze-Drying Methods

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In this study, the physicochemical properties, antioxidant capacity, sensory properties, and phenolic and mineral profiles of instant rosehip teas prepared using spray-drying (SD) and freeze-drying (FD) were compared. The yield of instant tea produced using FD and SD was 23.75 and 21.25 g/100 g, respectively. The total color difference between the FD and SD tea was 50.74, with the SD sample exhibiting higher redness and yellowness. The FD tea was richer in ascorbic acid than SD tea (67.2 and 59.4 mg/100 g dry weight, DW, respectively). The mineral content ranged from 0.20 mg/kg DW (copper) to 2,837 mg/kg DW (potassium) in SD tea, and from 0.31 mg/kg DW (copper) to 3,083 mg/kg DW (potassium) in FD samples. The total phenolic content was 1,315 and 1,495 mg GAE/100 g DW of SD and FD samples, respectively. The antioxidant capacity of instant rosehip teas was determined as total antioxidant capacity, ferric reducing antioxidant power and DPPH radical scavenging activity. In all these assays, FD tea was found to have a higher antioxidant capacity. In the phenolic profile determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique, protocatechuic acid dominated in instant rosehip teas prepared using both methods. The main flavonoids identified were quercetin, quercitrin, hyperoside and luteolin 7-glucoside. Following the sensory analysis, the panelists have generally preferred the FD tea in terms of aroma and flavor, while opting for the SD tea in terms of appearance and color. In conclusion, although freeze-drying allowed obtaining a product with a higher antioxidant capacity and antioxidant content than spray-drying, both drying methods may be considered suitable for the production of instant rosehip teas and ensure obtaining a functional food product.

Keywords: antioxidants, *Rosa canina*, instant beverage, lyophilization, phenolics, soluble powders

INTRODUCTION

Increasing global interest in beverages with high functional properties and nutraceutical compounds is gaining momentum [Corbo *et al.*, 2014]. Among these types of beverages, tea and herbal tea are one of the most important in our diets [Liang *et al.*, 2021]. Teas have been found to contain biologically active compounds that can provide health benefits and reduce the risk of chronic diseases [Lorenzo & Munekata, 2016; Sanlier *et al.*, 2018]. Among these phytochemicals, phenolics are natural antioxidants, and their content is significantly correlated with tea antioxidant capacity [Truong & Jeong, 2021]. However, the antioxidant properties of tea are not only related to the presence of phenolic compounds in raw material used for the infusion, but also to the brewing conditions including

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extraction time and temperature [Sayuti *et al.*, 2021; Xi & Wang, 2013].

Both, the production method and the choice of raw materials significantly influence the antioxidant capacity and phytochemical profile of instant teas [Liu *et al.*, 2021]. Instant teas made from green, black, and dark teas, have been studied by many researchers [Alasalvar *et al.*, 2013; Sinija & Mishra, 2008; Sun *et al.*, 2024]. The criteria for the selection of raw materials for instant tea include availability, processing requirements, and nutritional value. In this context, *Rosa canina*, also known as rosehip, may have important potential for instant tea production. Rosehip is a perennial plant that belongs to the Rosaceae family. The fruit has a round and long elliptical shape and is generally yellow, red, and orange [Guantario *et al.*, 2024; İpek & Balta, 2020]. About a hundred species of rosehips are found in North America, Asia, and Europe. Meanwhile, 27 varieties of rosehips have been grown in Turkey [Ercişli, 2005]. Recent studies have shown that the vitamin C content of rosehip fruit varies in the range of 100–5,300 mg/100 g, depending on the altitude at which plant is grown, its type and species [İpek & Balta, 2020]. Rosehip fruits are also a rich source of phenolic compounds including phenolic acids, flavonoids and tannins, which determine their antioxidant potential and other bioactivities including antiproliferative, antidiabetic and anti-inflammatory effects [Mourabit *et al.*, 2023; Nadpal *et al.*, 2016]. The bioactive compounds of these fruits also include carotenoids [Guantario *et al.*, 2024]. Rosehip is widely used in the food industry, medicine, and cosmetics [Negrean *et al.*, 2024].

Drying is another important factor in instant tea production. Phenolic and aroma profiles, and antioxidant capacity are directly affected by the drying process of instant tea [Kraujalytė *et al.*, 2016; Liu *et al.*, 2021]. The commonly employed methods for producing instant tea powder encompass spray-drying, freeze-drying, and vacuum-drying, which are the primary techniques utilized in this process [Someswararao & Srivastav, 2012]. Spray-drying is a well-established and widely used method for converting liquid and semi-liquid food substances into a powder form. This technique has gained considerable attention in the food industry [Anandharamakrishnan, 2013]. Spray-drying is recognized as an effective and unique method for preserving the quality properties of various products, including color, flavor, and nutrients, owing to its short processing time and controlled operational conditions [George *et al.*, 2023]. When producing instant tea powder from tea infusions, the high temperatures involved in spray-drying can alter the content and composition of the aroma compounds through evaporation, oxidation, and thermal degradation [Jin *et al.*, 2023]. This is a major disadvantage of spray-drying. In contrast, the freeze-drying process involves sublimation of ice under vacuum conditions at temperatures below the freezing point of water [Chakraborty *et al.*, 2006]. These low temperatures preserve the aroma of tea by significantly reducing the thermal degradation and evaporation of the compounds responsible for the aroma of tea [Kraujalytė *et al.*, 2016]. In summary, spray-drying is a conventional and cost-effective method for the production of instant tea, while instant tea produced through freeze-drying exhibits a greater abundance of aromatic compounds than that produced by spray-drying.

Based on the information provided, it may be concluded that the application of different drying techniques during instant tea production has an impact on physicochemical quality, phytochemical profile and antioxidant properties of product. To date, most studies on this issue have focused on black, dark, and green instant teas. However, there are very few studies on the instant fruit teas. Therefore, the aim of this study was to determine the differences between spray-dried and freeze-dried instant rosehip teas in terms of physicochemical properties, mineral content, phenolic profile and antioxidant capacity.

MATERIALS AND METHODS

Chemicals and reagents

Sodium acetate buffer, iron(III) nitrate nonahydrate, 2,4,6-Tris (2-pyridyl)-*s*-triazine (TPTZ), sodium molybdate, Folin-Ciocalteu reagent, aluminum nitrate nonahydrate, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Metaphosphoric acid reagent, high-performance liquid chromatography (HPLC)-grade reagents for HPLC analysis and the standard solutions of minerals (Fe, Zn, Cu, Al, Mn, Ca, Mg, Na, and K) used for mineral analysis were purchased from Merck (Darmstadt, Germany).

Material

Rosehip fruits from *Rosa canina* L., which are widely available in various parts of Turkey, were obtained from a local bazaar in Gümüşhane. The fruits were stored in a refrigerator at 4°C until the drying process commenced.

Preparation of instant tea from rosehip fruits

First, the rosehip fruits were dried in an oven (40°C) for 24 h. The dried rosehip fruits with a moisture content of 46.76±1.25 g/100 g (determined by AOAC International method no. 934.06 [AOAC, 2019]) were utilized for extraction under the following conditions: about 4,000 g of the dried fruits were treated with 10 L of boiling distilled water. The mixture was boiled for 10 min and then mixed for approximately 30 min. While still hot, it was filtered through a cheesecloth and placed into 5-L glass jars. The obtained extract was equally divided, with half reserved for freeze-drying and the other half for spray-drying, and then stored in a refrigerator at 4°C until the drying process. The content of total soluble solids of rosehip tea, determined by AOAC International method no 934.06 [AOAC, 2019], was 4.0±0.25°Bx.

Half of rosehip extract volume was added to broad and shallow cups with a capacity of 400 mL, and vacuum was applied under conditions of –65°C and 0.1 mbar in a freeze-dryer (Scientz-12N laboratory lyophilizer, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). The product was allowed to dry for 96 h. The weight of the resulting freeze-dried instant rosehip tea was 475.0±11.2 g. Second part of the obtained aqueous extract of rosehip was dried in a lab-type spray-dryer (SD-06 spray, Labplant UK, Hunmanby, England) with a liquid flow

velocity of 5 mL/min for 4 h at 200°C, which allowed obtaining 427.0±9.1 g of product. Powders of instant teas were stored until analysis in dark-colored capped bottles at 4°C.

The yield (g/100 g) of the freeze-dried and spray-dried products was calculated according to the formula (1).

$$\text{Yield} = \frac{P}{R} \times 100 \quad (1)$$

where: P is the amount of powder (g) and R is the amount of rosehip (g).

Determination of physicochemical properties and mineral profile

For moisture content determination, 5.0 g of rosehip tea powder were taken, weighed in tared aluminum metal cups and pegged at 70°C until constant weight was achieved. The moisture content was provided as g/100 g of instant tea [Nadeem *et al.*, 2011].

A LabSwift-aw water activity meter (Norasina, Lachen, Switzerland) was used to determine water activity (a_w) of instant rosehip teas. The samples were then placed in the cup of the device. The a_w value was read directly after providing stability to the device.

Color coordinates of instant rosehip teas were determined using a CR-400 Minolta chromameter (Konica Minolta Sensing, Inc. Japan). Approximately 5 g of the instant rosehip sample were placed in the sample container. The values of L^* (darkness/whiteness), a^* (greenness/redness), and b^* (blueness/yellowness) were measured in the CIELab color space. The total color difference (ΔE) between the FD and SD teas was calculated according to the formula (2):

$$\Delta E = \sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2} \quad (2)$$

where: ΔL^* is the difference between lightness values, Δa^* is the difference between greenness/redness values, and Δb^* is the difference between blueness/yellowness values of FD and SD teas.

For determining water solubility of instant rosehip teas, the powder (1.0 g) was weighed, and 100 mL of deionized water were added at ambient temperature. The mixture was then stirred for 5 min at 600 rpm using a magnetic stirrer. This solution was centrifuged at 3,000×*g* (NF1200R centrifuge, Nüve Co., Ankara, Turkey). Twenty milliliters of the supernatant were transferred to Petri dishes and left to dry for 24 h at 70°C using an SH-FDO54 oven (Samheung Energy Co., Ltd, Daejeon, South Korea). After drying, the Petri dishes were weighed, and the differences were stated as *per* 100 g of instant tea [Cam *et al.*, 2020].

A distillation apparatus Vapodest (Gerhardt Vapodest, Gerhardt GmbH & Co. KG, Königswinter, Germany) was used to determine the crude protein content of the instant rosehip teas. Analysis was performed according to the standard Kjeldahl method [AOAC, 2019]. A 6.25 conversion factor was used for protein content calculation. The results were expressed as g/100 g of dry weight (DW) of instant tea.

The ash content was determined using a AOAC International method no. 942.05 [AOAC, 2019]. Instant rosehip powder (2.5 g) was weighed in a porcelain crucible and incinerated at 550°C using a muffle furnace (MF-12, Nuve, Ankara, Turkey). The results were expressed as g/100 g DW of instant tea.

Ash samples obtained during determining the ash content were used for mineral profile analysis. Ashed instant teas were dissolved in 10% nitric acid, and ultrapure water was added until the sample volume reached 50 mL volume [AOAC, 2019]. The microwave plasma-atomic emission spectrometer (MP-AES) (MP-AES system, Agilent Technology, Santa Clara, CA, USA) was used to determine the metal ions in the solutions. Before measuring, first, a stock solution containing 50 mg/L of the mixed intermediate was prepared by combining separate 1,000 mg/L solutions of Fe, Zn, Cu, Al, Mn, Ca, Mg, Na, and K minerals. Following this, calibration curves with concentrations of 0.5, 1, 2, 3, 4, and 5 mg/L were prepared using the intermediate stock mixture. The concentrations of minerals in tea solutions measured in mg/L were converted to mg/kg DW of instant tea.

Determination of total phenolic content

For the total phenolic content (TPC) determination, 3.4 mL of deionized water was added to 300 µL of the instant tea solution (200 µg/mL) [Kasangana *et al.*, 2015]. Then, methanol (0.5 mL) and Folin–Ciocalteu reagent (200 µL) were added to the mixture. The mixture was vortexed and incubated for 10 min under ambient temperature, after which 600 µL of a 10% Na_2CO_3 solution were added. The final mixture was vortexed again and incubated in the dark for 120 min. At the end of the incubation period, the absorbance of the solution was read at 760 nm. The results were presented as mg of gallic acid equivalent (GAE) *per* 100 g DW.

Determination of total flavonoid content

The total flavonoid content (TFC) of rosehip instant tea was quantified in accordance with a method developed by Chang *et al.* [2002] with slight modifications. A portion of 0.5 mL of an instant tea solution (200 µg/mL) was combined with 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL and 1 M $NH_4(CH_3COO)$. The mixture was left to stand at ambient temperature for 40 min. The absorbance was measured against a blank at 415 nm. A series of dilutions of quercetin in methanol was prepared and assayed to prepare a calibration curve. The total flavonoid content in the teas was expressed as mg quercetin equivalent (QE) *per* 100 g DM.

Determination of ascorbic acid content

The content of ascorbic acid of rosehip instant tea was determined using an HPLC system with a UV detector (Thermo Finnigan, San Jose, CA, USA). Approximately 70 mL of metaphosphoric acid reagent was combined with 10 g of tea powder in a homogenizer. The flask containing the homogeneous mixture was wrapped with aluminum foil to protect against sunlight, and the mixture was then filtered through blue band filter paper [Brubacher *et al.*, 1985]. The filtrate was filtered again

with a 0.45-µm syringe filter and transferred to vials for HPLC analysis. The HPLC conditions were as follows: the column was a Supelcosil C18 (5 µm, 250×4.6 mm; Sigma-Aldrich); the mobile phase was methanol – H_2O (5:95, *v/v*) adjusted to pH 3.00 with H_3PO_4; the column temperature was maintained at 20°C; the injection volume was 20 µL; the detection wavelength was set at 254 nm, covering a range from 210 to 360 nm; and the flow rate was 1.0 mL/min [Ibrahim *et al.*, 2015]. For the calibration curve, standard solutions of L-ascorbic acid were prepared at concentrations of 10, 30, 60, 90, and 120 mg/L. Ascorbic acid content of the sample was calculated in accordance with the linear regression line (y =8467.7x +24.6, R^2=0.997) method. The results were expressed in mg/100 g DW.

Determination of antioxidant capacity

Antioxidant capacity of rosehip instant teas was evaluated as total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP) and as DPPH radical scavenging activity. In all assays, instant teas were dissolved in water at a concentration of 200 µg/mL, and a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure absorbance of reaction mixtures.

To determine DPPH radical scavenging activity, 100 µL of a tea solution was combined with 3,000 µL of a 0.1 mM DPPH radical solution. After a 30-min waiting period, the absorbance of the mixture was measured at 517 nm [Brand-Williams *et al.*, 1995]. The results were expressed as mg Trolox equivalent *per* 100 g DW.

FRAP was determined by Benzie & Strain [1996] method. For the FRAP reagent, a 300 mM sodium acetate buffer solution (pH 3.6), a 20 mM aqueous $FeCl_3$ solution, and a 10 mM aqueous TPTZ solution were mixed at a ratio of 10/1/1 (*v/v/v*). The FRAP reagent (3 mL), the instant rosehip tea solution (100 µL), and methanol (900 µL) were mixed in a spectrometer cuvette and kept at ambient temperature for 30 min, and absorbance measurements were then conducted at 593 nm. $FeSO_4$ was used to construct a reference curve (62.5–1,000 mg/L), FRAP was expressed as mmol $FeSO_4$ equivalent *per* 100 g DW.

For the TAC analysis, 2,500 µL of deionized water was added to 500 µL of the sample solution. A molybdate reagent solution (1,000 µL), comprised of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, was added to the mixture. The mixture was vortexed and incubated in a water bath at 95°C for 90 min. Subsequently, it was kept for 20–30 min until it reached ambient temperature. Purified water was used as a blank sample. The absorbance of the obtained reaction mixture was read at 695 nm [Prieto et al, 1999]. The standard calibration curve was obtained utilizing ascorbic acid (AA). The total antioxidant capacity values were determined in terms of mg of ascorbic acid equivalent (AAE) *per* 100 g DW.

Phenolic profile analysis

The instant rosehip teas (1.0 g) were dissolved in 25 mL of distilled water. Solutions were filtered using a 0.2 µm microfiber syringe filter and placed in HPLC vials before injection onto the column. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) device (LCMS-8040, Shimadzu, Kyoto, Japan), which was equipped with an electrospray ionization (ESI) source that operated in both negative and positive ionization modes, was used for phenolic compound determination. Chromatographic separation was achieved using a C18 reversed phase column (250×4.6 mm, particle size of 5 µm; Bio-Rad, Hercules, CA, USA) stabilized at 35°C. The elution gradient consisted of solvents A (5 mM ammonium formate solution in water with 0.1% (*v/V*) formic acid) and B (5 mM ammonium formate solution in methanol with 0.1% (*v/V*) formic acid) [Akdeniz *et al.*, 2020]. The following gradient elution profile was used: 20%–100 B (0–25 min), 100% B (25–35 min), and 20% B (35–45 min). The mobile phase flow rate and injection volume were set at 0.5 mL/min and 20 µL, respectively. The operating conditions for the MS were as follows: drying gas (N_2) flow, 15 L/min; nebulizing gas (N_2) flow, 3 L/min; DL temperature, 250°C; heat block temperature, 400°C; and interface temperature, 350°C. Multiple reaction monitoring (MRM) mode was used for the quantification of phytochemicals. Contents of individual compounds in teas were expressed in µg/g DM.

Sensory analysis

A total of twelve panelists (experienced in tea tasting) from the Food Engineering Department of Gümüşhane University (Turkey), six women and six men, examined the instant rosehip teas and evaluated them in terms of flavor, aroma, appearance, color, and overall acceptability, according to previously determined criteria. They evaluated the samples using a nine-point hedonic scale: 1 – dislike extremely, 2 – dislike very much, 3 – dislike moderately, 4 – dislike slightly, 5 – neither like nor dislike, 6 – like slightly, 7 – like moderately, 8 – like very much, and 9 – like extremely. Samples were prepared for sensory analysis based on ISO standards for brewing [ISO8586:2023; ISO 8589:2007; ISO 6658:2017; ISO4120:2021]. Each tea (0.4 g) was taken, 100 mL of boiled water was added, and the samples were placed in porcelain cups. The panelists were given water to rinse their mouths when they passed from one sample to another [Lim *et al.*, 2009]. The findings were calculated as percentages (%). The results of the sensory analysis were presented as a preference map, which was generated using XLSTAT software on the Lumivero platform (Denver, CO, USA). Specifically, the 'Preference Mapping' module of the software was employed to visualize the liking levels of each product based on various sensory attributes, highlighting the differences and similarities among the products.

Statistical analysis

All analyses of teas were executed with six repetitions. The results of individual determinations were analyzed using *t*-test to show significant differences ($p<0.05$) between FD and SP teas. Moreover, principal component analysis (PCA) was performed to show connections between variables (all determined parameters) and differences among the samples. The XLSTAT software

for Excel on the Lumivero platform (Denver, CO, USA) was used for statistical analyses.

RESULT AND DISCUSSION

Physicochemical properties of the freeze-dried and spray-dried instant rosehip teas

The main physicochemical parameters of instant rosehip tea are shown in **Table 1**. The instant rosehip teas were obtained by freeze-drying and spray-drying with the yield of 23.75 g/100 g for FD samples and 21.25 g/ 100 g for SD samples. The moisture content and water activity of the FD sample were 9.99 g/100 g and 0.45 g/100 g, respectively. In contrast, the moisture content and water activity of the SD sample were significantly (p<0.05) lower and reached 1.85 g/100 g and 0.26 g/100 g, respectively. According to the literature, the optimal moisture content for instant green tea packaging and storage was reported to be <5 g/100 g [Sinija & Mishra, 2008]. FD tea, in our study, did not meet this requirement. The high moisture content could make it difficult to store.

Food processing techniques, such as drying, freezing, curing, and baking, can affect water activity levels. Water activity is a crucial factor in determining the growth of microorganisms and shelf life of food products. Low water activity can result in slow microbiological growth [Tiwari *et al.*, 2014]. The water activity of the instant rosehip teas was relatively low. Comparable values were obtained in a study on green tea extracts; the water activity of microencapsulated green tea extract ranged from 0.36 to 0.28, while the water activity of the free green tea extract was measured at 0.45 [Zokti *et al.*, 2016].

The impact of the drying method on color coordinates of instant rosehip teas was significant (p<0.05). As demonstrated in **Table 1**, there was a contradiction between the spray-dried and freeze-dried teas with respect to L^*, a^*, and b^* values. These values suggest that the SD sample exhibited a redder and yellower color than that of the FD sample. The lightness of the SD sample was higher than that of the FD sample. The total color difference between the two samples was calculated as 50.74±2.65.the impact of spray-drying and freeze-drying methods was also analyzed in research conducted on carrot waste extract [Seregelj *et al.*, 2021]. The findings indicated that the spray-drying method resulted in lighter-colored products in contrast to the freeze-drying method which resulted in products with darker coloration.

Solubility is a crucial quality parameter for solid food systems because it influences the functional properties of powders. In the present study, the solubility of the FD samples was 87.8 g/100 g, while that of the SD samples was 98.6 g/100 g (**Table 1**). A statistically significant (p<0.05) difference was observed in the water solubility of both type of teas. Vardanega *et al.* [2019] investigated powdered tea production from Brazilian ginseng roots using freeze-drying and spray-drying methods. The results showed that the solubility rates were 89% for spray-drying and 90% for freeze-drying. Furthermore, another study compared the effect of different drying methods, including spray-drying and freeze-drying, on the extracts of *Thymus serpyllum* L. (wild thyme) and found that the powders obtained by spray-drying had higher thermal stability and solubility than those obtained by freeze-drying [Jovanović *et al.*, 2021].

A comparison of the crude protein content of instant rosehip teas obtained using the two different drying methods revealed that the FD product had a crude protein content of 2.22 g/100 g DW, while the SD product had a protein content of 1.86 g/100 g DW (**Table 1**), with the difference between the products found to be statistically significant (p<0.05). Notably, the protein content of rosehip fruit, as reported in the literature, was 1.60 g/100 g [Fan *et al.*, 2014].

The ash content of the SD sample was found to be 3.87 g/100 g DW, whereas the FD sample had a significantly (p<0.05) higher ash content of 4.12 g/100 g DW. In a study by Özer [2017], ash content for fresh and dried rosehip fruits was reported to be 1.09% for fresh fruit, 2.58% for freeze-dried fruit, and 2.70% for conventionally-dried fruit.

Table 1. Yield of production and physicochemical parameters of spray-dried (SD) and freeze-dried (FD) instant rosehip teas.

Parameter	SD tea	FD tea
Product yield (g/100 g fruits)	21.25±0.79^b	23.75±0.98^a
Moisture content (g/100 g)	1.85±0.07^b	9.99±0.98^a
Water activity	0.26±0.01^b	0.45±0.01^a
Solubility (g/100 g)	98.6±2.5^a	87.8±1.9^b
Crude protein content (g/100 g DW)	1.86±0.09^b	2.22±0.14^a
Ash content (g/100 g DW)	3.87±0.43^b	4.12±0.66^a
L^*	63.13±0.88^a	20.77±0.32^b
a^*	8.45±0.67^a	0.25±0.04^b
b^*	27.44±0.42^a	0.74±0.09^b

Results are presented as the mean ± standard deviation (n=3). Values with different letters (a and b) in the same row are significantly different (p<0.05). DW, dry weight; L^*; darkness/whiteness, a^*, greenness/redness; b^*, blueness/yellowness.

Mineral profile of the freeze-dried and spray-dried instant rosehip teas

In this study, the mineral contents of the instant rosehip teas were determined using an atomic emission technique, and results of analysis are shown in **Table 2**. As expected, the contents of the macroelements, notably Na, Mg, K, and Ca, were considerably higher than those of the other elements. The Na, Mg, K, and Ca contents of the SD samples were 194; 328; 2,837; and 337 mg/kg DW, respectively. The corresponding values for the FD samples were 261; 339; 3,083; and 340 mg/kg DW, respectively. When the studies in the literature are considered, 11.0 mg/kg for Na, 217.5 mg/kg for Mg, 1,454.5 mg/kg for K, and 844.2 mg/kg for Ca were determined in fresh *Rosa canina* fruit [Kazaz *et al.*, 2009]. Heavy and/or trace metals such as Cd, Co, Ni, Pb, and Cr were not detected (above the detection limit) in instant rosehip teas in our study. The contents of Fe, Zn, Cu, and Mn in the samples were <3.5 mg/kg DW (**Table 2**). No statistically significant (p≥0.05) differences between the instant tea

Table 2. Mineral content (mg/kg dry weight) of instant rosehip teas obtained by spray-drying (SD) and freeze-drying (FD).

Mineral	SD tea	FD tea
Fe	3.15±0.27[b]	3.34±0.68[a]
Zn	0.62±0.12[b]	1.20±0.18[a]
Cu	0.20±0.01[b]	0.31±0.01[a]
Al	16.40±0.64[b]	17.90±2.01[a]
Mn	2.56±0.66[a]	2.90±0.50[a]
Ca	337±73[a]	340±40[a]
Mg	328±22[a]	339±18[a]
Na	194±5.5[b]	261±12[a]
K	2,837±142[b]	3,083±78[a]
Total	3,719	4,049

Results are presented as the mean ± standard deviation (n=3). Limit of quantitation (LOQ)=0.01 mg/kg dry weight. Cd, Co, Ni, Pb, Cr are <LOQ.

obtained by spray-drying and freeze-drying were found only for Ca, Mg, and Mn content. Consumers prefer diets rich in specific nutrients that enhance their overall health. The Na, Mg, K, and Ca, which were found in our study in the highest quantities in rosehip instant teas, play important roles in bodily functions. Sodium is essential for maintaining osmotic pressure, extracellular volume, and neuronal excitability, playing a significant role in nerve impulse transmission and muscle function [Bernal *et al.*, 2023]. Magnesium supports over 300 enzymatic reactions, including those involving ATP for energy transfer, and is vital for muscle and nerve function [Fiorentini *et al.*, 2021]. In turn, potassium is critical for maintaining cellular membrane potential and proper nerve function, particularly through the Na^+/K^+-ATPase enzyme [Abdul Kadir *et al.*, 2018]. Finally, calcium is crucial for bone health, muscle contraction, and nerve transmission, with the majority of the body's calcium stored in bones [Vaskonen, 2003].

Total phenolic content, total flavonoid content, ascorbic acid content and antioxidant capacity of the freeze--dried and spray-dried instant rosehip teas

The data on antioxidant capacity, TPC, TFC and ascorbic acid content of instant rosehip teas are presented in **Table 3**. The TPC of the teas was determined to be 1,495 mg GAE/100 g DW for the FD samples and 1,315 mg GAE/100 g DW for the SD products. The TFC was found to be 146.29 mg QE/100 g DW in the samples obtained through the SD method and significantly (p<0.05) higher (183.67 mg QE/100 g DW) in the samples obtained through the FD method. After reviewing the literature, no studies were found on the TPC and TFC of rosehip-soluble tea. However, according to the literature, the TPC of rosehip fruit powder was 2,482 mg GAE/100 g [Igual *et al.*, 2022]. In turn, TFC of water extracts of fresh and air-dried rosehips was reported to be 1.22 and 1.14 mg QE/g DW, respectively [Nadpal *et al.*, 2016].

Results of ascorbic acid analysis were found as 59.4 mg/100 g DW for the spray-dried tea and as 67.2 mg/100 g DW for the freeze--dried tea, and difference between the samples was statistically significant (p<0.05) (**Table 3**). In the literature concerning the rosehip fruit, Kazaz *et al.* [2009] reported the ascorbic acid content at 411.0 mg/100 g for *Rosa canina* L. and as 332.0 mg/100 g for *Rosa damascena* Mill. In turn, Fan *et al.* [2014] performed a study in which they determined the vitamin C content of rosehip fruit to be 426.0 mg/100 g. The same study found that the ascorbic acid content in instant rosehip teas was lower when the powder was dissolved in hot water compared to the rosehip fruit. The researchers explained this by noting that ascorbic acid decomposes in hot water into various compounds such as dehydroascorbic acid and diketogulonic acid. Heat exposure increases the oxidation of ascorbic acid, leading to the formation of these compounds and resulting in the loss of vitamin activity [Pavlovska *et al.*, 2013].

The FRAP of the FD samples was 154.12 mmol $FeSO_4$/100 g DW and that of the SD samples was 133.69 mmol $FeSO_4$/100 g DW (**Table 3**). DPPH radical scavenging activity of instant rosehip teas was 83.62 mg Trolox/100 g DW in the FD sample and 64.23 mg Trolox/100 g DW in the SD sample. TAC of the samples was determined as 3,804 mg AAE/100 g DW for the freeze-dried rosehip tea and as 3,338 mg AAE/100 g DW for the spray-dried rosehip tea. In a study on the encapsulation of rosehip powder, the total antioxidant capacity (TAC) was found to be 1,793 mg TE/100 g [Igual *et al.*, 2022]. In all antioxidant assays, the antioxidant capacity of the FD instant tea was significantly

Table 3. Total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid content, and antioxidant capacity measured as total antioxidant capacity (TAC), DPPH radical scavenging activity (DPPH), and ferric reducing antioxidant power (FRAP) of freeze-dried (FD) and spray-dried (SD) instant rosehip teas.

Parameter	SD tea	FD tea
TPC (mg GAE/100 g DW)	1,315±6.5[b]	1,495±15[a]
TAC (mg AAE/100 g DW)	3,338±15[b]	3,804±62[a]
DPPH (mg Trolox/100 g DW)	64.23±0.10[b]	83.62±0.14[a]
FRAP (mmol $FeSO_4$/100 g DW)	133.69±0.21[b]	154.12±0.14[a]
TFC (mg QE/100 g DW)	146.29±0.65[b]	183.67±0.35[a]
Ascorbic acid content (mg/100 g DW)	59.4±2.0[b]	67.2±1.1[a]

Results are presented as the mean ± standard deviation (n=3). Values with different letters (a and b) in the same row are significantly different (p<0.05). GAE, gallic acid equivalent; AAE, ascorbic acid equivalent; QE, quercetin equivalent; DW, dry weight.

Table 4. Retention times (RT), precursor ions and fragment ions of phenolic compounds identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, and their contents in rosehip instant teas obtained by spray-drying (SD) and freeze-drying (FD).

No	Analytes	RT (min)	Precursor ion (*m/z*)	Fragment ion (*m/z*)	Ionizaion mode	FD tea (µg/g DW)	SD tea (µg/g DW)
1	Protocatechuic acid	7.04	153.4	109.1	Neg	14.40±0.22^{b}	29.53±0.19^{a}
2	Chlorogenic acid	8.05	353.3	191.2	Neg.	5.50±0.24^{a}	2.81±0.12^{b}
3	Luteolin 7-glucoside	13.24	447.0	285.1	Neg.	6.75±0.43^{a}	2.39±0.10^{b}
4	Hesperidin	13.69	611.1	303.0, 449.3	Poz.	1.01±0.02^{b}	2.60±0.09^{a}
5	Hyperoside	13.70	463.0	300.1	Neg.	N.D.	8.05±0.75
6	Quercitrin	14.99	447.0	301.1	Neg.	N.D.	7.14±0.56
7	Astragalin	15.15	447.0	227.1, 255.0, 284.1	Neg.	2.30±0.11^{b}	3.32±0.23^{a}
8	Quercetin	17.14	301.2	151.1. 179.1	Neg.	0.77±0.07^{b}	7.43±0.43^{a}
9	Luteolin	17.82	285.2	133.1, 151.0	Neg.	0.40±0.01^{a}	0.13±0.01^{b}
10	Apigenin	19.24	269.2	117.0, 151.1	Neg.	0.40±0.01^{a}	0.25±0.02^{b}

Results are presented as mean ± standard deviation. Values with different letters (a and b) in the same row are significantly different ($p<0.05$). N.D., not detected; DW, dry weight.

($p<0.05$) higher than that of the SP instant tea. This finding is consistent with literature data. A study conducted on papaya puree revealed that samples dried through freeze-drying had a higher content of phenolic compounds and antioxidant activity as compared to those dried using spray-drying [Gomes *et al.*, 2018]. The rationale behind this could be that the freeze-drying process operates at low temperatures, thereby minimizing the degradation of sensitive antioxidants and preserving the bioactive components more effectively.

Phenolic profile of the freeze-dried and spray-dried instant rosehip teas

The results of phenolic compounds analyzed by the LC-MS/MS method in rosehip instant teas dried using freeze-drying and spray-drying methods are given in **Table 4**. Protocatechuic acid was detected as the main compound for both drying methods (FD: 14.40 µg/g DW, SD: 29.53 µg/g DW). Following this, luteolin 7-glucoside with the content of 6.75 µg/g DW was found in the FD tea, while in the SD sample it was hyperoside with 8.05 µg/g DW. Furthermore, hyperoside (8.05 µg/g DW) and quercitrin (7.14 µg/g DW) were detected only in the samples dried by the SD method. When the literature was examined, it was reported that different quercetin derivatives including rutin, quercetin 3-glucoside, quercetin glucuronide, and quercetin rhamnoside were detected in rosehip fruits [Guantario *et al.*, 2024; Peña *et al.*, 2023]. In another study examining the stability and bioavailability of phenolic compounds of rosehip extracts during *in vitro* digestion, a total of 34 phenolic compounds were determined using UPLC-MS/MS [Odriozola *et al.*, 2023]. Among these compounds, protocatechuic acid, hyperoside, quercetin, quercitrin, and luteolin 7-*O*-glucoside were reported at 4.9 µg/g, 73.6 µg/g, 64.6 µg/g, 53.7 µg/g, and 7.8 µg/g, respectively. When comparing these literature data for extracts with those of this study, the results are consistent in terms of the detected compounds, but differ in terms of quantity, which can be explained primarily by the different extraction methods.

The drying method affected the contents of individual phenolics of instant rosehip teas. The contents of most individual phenolics were significantly ($p<0.05$) higher in SD tea than in FD tea. The exceptions were chlorogenic acid, luteolin 7-glucoside, luteolin and apigenin, for which higher values were determined in the FD sample. The results of this study are consistent with a study which determined the content of phenolic compounds in papaya pulps dried by FD and SD methods [Gomes *et al.*, 2018]. Oxidative reactions during the drying process affect phenolic losses. While the freeze-drying method can lead to the release of enzymes such as polyphenol oxidase and peroxidase due to low oxygen exposure, the spray-drying method prevents the loss of phenolic compounds by inactivating these enzymes due to high-temperature applications, and more bound phenolic acids are released by the breakdown of cellular components [Gomes *et al.*, 2018]. Therefore, optimizing the drying method and parameters significantly affects product quality in terms of the preservation of bioactive components and antioxidant activity.

Sensory analysis

A food product has to have pleasing appearance, be convenient, and should satisfy consumers' demands to enter the market and hold on. Sensory analysis can provide information about these subjects. In the total preference mapping scores, the flavor, aroma, and overall acceptability of the FD tea were found to be higher than those of the SD samples. In contrast, the color and appearance of the SD tea were found to be higher (**Figure 1**).

A numerical hedonic scale is useful in evaluation of new products. If a new product reaches a score of 9, 8, or 7, it can be accepted as a suitable product for the market [Pimentel *et al.*, 2016]. In our study, teas obtained with the FD method received 8.67±0.49 points and SD products received 7.00±1.13 points for overall acceptability. Preference map showed distinct differences

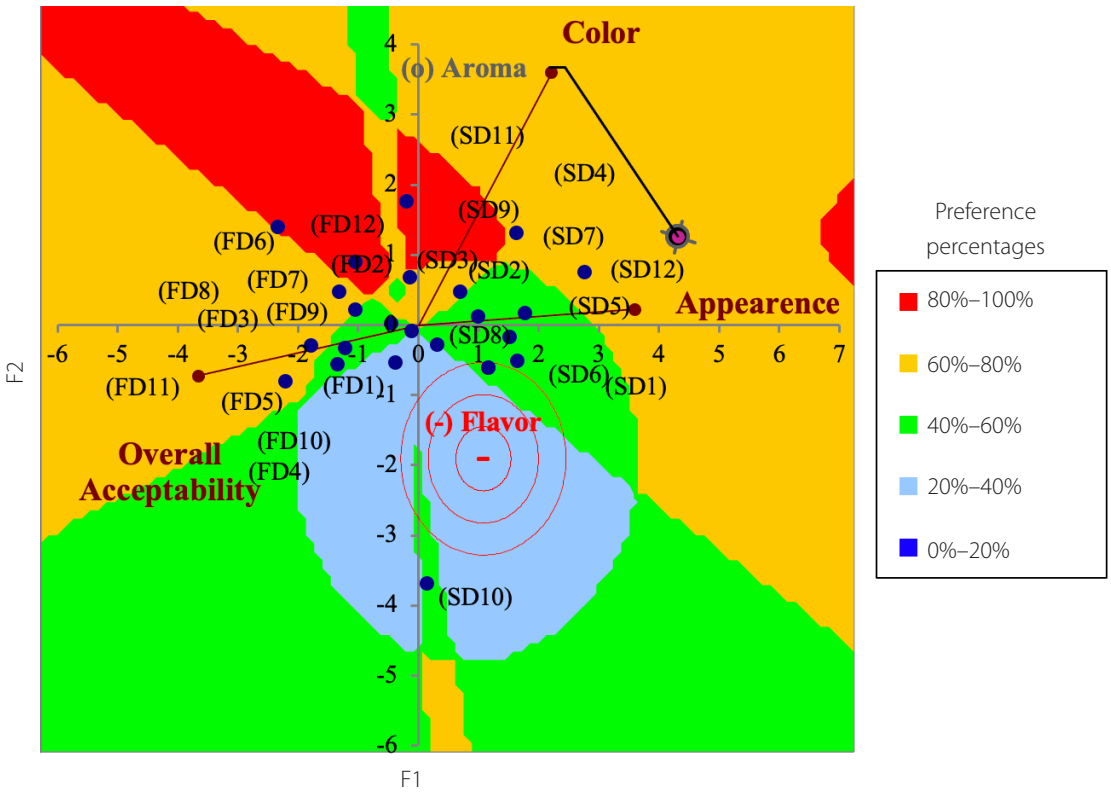

Figure 1. Preference map for sensory acceptability of instant rosehip teas obtained with spray-drying (SD) and freeze-drying (FD).

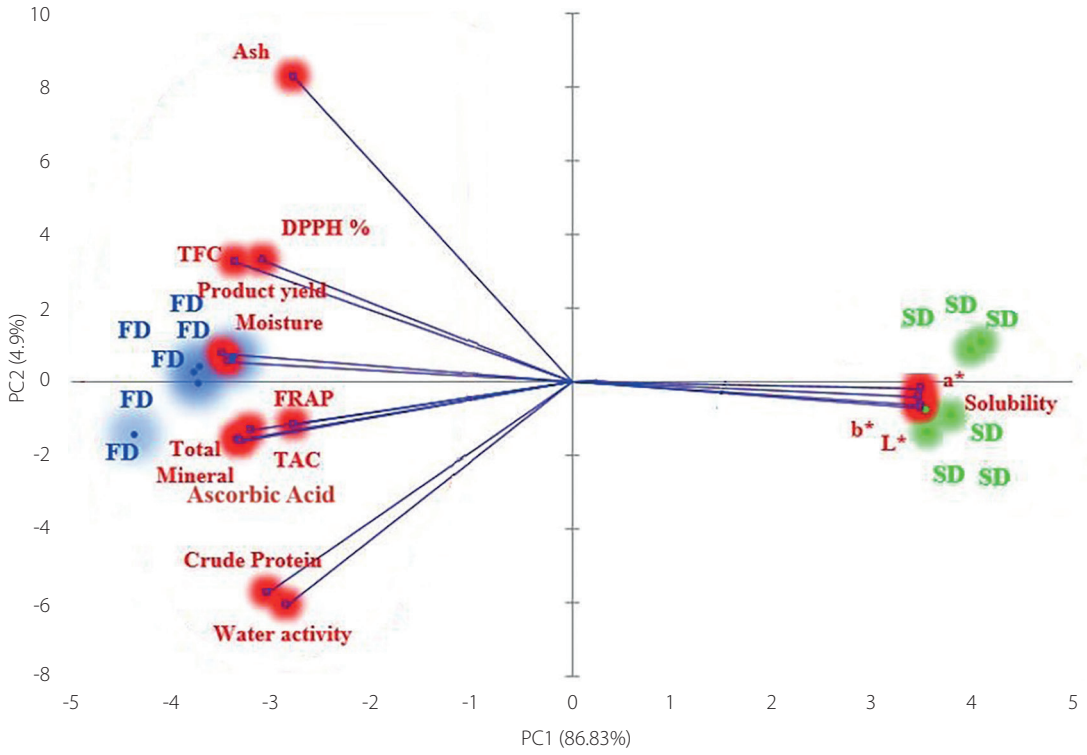

Figure 2. Plot of principal component analysis (PCA) with distribution of variables including production yield, physicochemical parameters, total mineral content, total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid content, antioxidant capacity (total antioxidant capacity, TAC; ferric reducing antioxidant power, FRAP; DPPH radical scavenging activity) and color coordinates (*L**, *a** and *b**) of instant rosehip teas obtained by spray-drying (SD) and freeze-drying (FD).

in the sensory properties of the two instant rosehip tea types produced using different drying methods (**Figure 1**). The scores were arranged into four areas. The separation between the samples indicated differences in certain investigated sensory parameters. Panelists, in general, preferred the FD tea in terms of aroma and flavor, with a preference rate of 68%, while they preferred the SD tea in terms of appearance and color, with a preference rate of 68% and 64%, respectively.

Multivariate analysis

PCA of the instant tea samples was carried out on the values of 14 quality parameters to identify similarities and differences among them. As shown in **Figure 2**, the PCA indicated 91.73% of the initial data variability in the total variance.

The PCA study revealed that drying methods affected the physicochemical properties, color, and antioxidant capacity of instant rosehip tea. The PCA loading plot also showed that physicochemical, antioxidant, and color properties contributed the most to the scattering patterns of the different drying methods. A clear separation was observed between the spray-dried and freeze-dried teas. Solubility and color coordinates (L^*, a^*, b^*) (maximum in SD tea) contributed the most positively along PC1, whereas ascorbic acid content, FRAP, total mineral, TAC, crude protein content, and water activity (maximum in FD tea) contributed the most negatively along PC1. Product yield, moisture and ash contents, TPC, and DPPH radical scavenging activity (maximum in FD tea) contributed the most negatively along PC2.

CONCLUSIONS

The findings of this study indicate that the method of drying has an impact on the physical and chemical properties, as well as the sensory acceptability, of instant rosehip tea. Both, freeze-drying and spray-drying are effective methods for preserving the bioactive components and physicochemical properties of rosehip instant tea. However, the spray-drying method was found to be less effective in product yield and preserving the ascorbic acid and crude protein contents compared to the freeze-drying method. In addition, the freeze-dried tea showed higher antioxidant capacity. Furthermore, the study found that the negative effect of temperature applied in the spray-drying method on phenolic compounds was limited. The preservation of certain phenolic compounds, such as hyperoside and quercitrin, was aided by spray-drying. The sensory analysis revealed that both freeze-dried and spray-dried instant rosehip teas were well-received, with freeze-dried teas being preferred for their aroma and flavor, while spray-dried teas were preferred for their color and appearance. In conclusion, the results of this study indicate that both freeze-drying and spray-drying are viable options for preserving the main nutrients of rosehip fruit and producing instant rosehip tea for the end consumer.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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REFERENCES

1. Abdul Kadir, L., Stacey, M., Barrett-Jolley, R. (2018). Emerging roles of the membrane potential: Action beyond the action potential. *Frontiers in Physiology*, *9*, art. no. 1661. https://doi.org/10.3389/fphys.2018.01661
2. Akdeniz, M., Yilmaz, M.A., Ertas, A., Yener, I., Firat, M., Aydin, F., Kolak, U. (2020). Method validation of 15 phytochemicals *in Hypericum lysimachioides* var. *spathulatum* by LC–MS/MS, and fatty acid, essential oil, and aroma profiles with biological activities. *Journal of Food Measurement and Characterization*, *14*, 3194-3205. https://doi.org/10.1007/s11694-020-00562-6
3. Alasalvar, C., Pelvan, E., Özdemir, K.S., Kocadagili, T., Mogol, B.A., Pasli, A.A., Ozkan, N., Gökmen, V. (2013). Compositional, nutritional, and functional characteristics of instant teas produced from low- and high-quality black teas. *Journal of Agricultural and Food Chemistry, 61*(31), 7529-7536. https://doi.org/10.1021/jf4015137
4. Anandharamakrishnan, C. (Ed.) (2013). Computational fluid dynamics applications in spray-drying of food products. In: *Computational Fluid Dynamics Applications in Food Processing*. SpringerBriefs in Food, Health, and Nutrition. Springer, New York, NY, pp. 11-15. https://doi.org/10.1007/978-1-4614-7990-1_2
5. AOAC, (2019). *Official Methods of Analysis*. 21st Edition. Gaithersburg, MD: AOAC International.
6. Benzie, I.F., Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292
7. Bernal, A., Zafra, M.A., Simón, M.J., Mahía, J. (2023). Sodium homeostasis, a balance necessary for life. *Nutrients*, *15*(2), art. no. 395. https://doi.org/10.3390/nu15020395
8. Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
9. Brubacher, G., Müller-Mulot, W., Southgate, D.A.T. (1985). Vitamin C (ascorbic and dehydroascorbic acids) in foodstuffs: HPLC method. In: G. Brubacher, W. Müller-Mulot, D.A.T. Southgate (Eds.), *Methods for the Determination of Vitamins in Food*. Elsevier Applied Science Publishers, Springer, Dordrecht, pp. 66-75. https://doi.org/10.1007/978-94-009-4944-7_5
10. Cam, M., Basyigit, B., Alasalvar, H., Yilmaztekin, M., Ahhmed, A., Sagdic, O., Konca, Y., Telci, I. (2020). Bioactive properties of powdered peppermint and spearmint extracts: Inhibition of key enzymes linked to hypertension and type 2 diabetes. *Food Bioscience*, *35*, art. no. 100577. https://doi.org/10.1016/j.fbio.2020.100577
11. Chakraborty, R., Saha, A.K., Bhattacharya, P. (2006). Modeling and simulation of parametric sensitivity in primary freeze-drying of foodstuffs. *Separation and Purification Technology*, *49*(3), 258-263. https://doi.org/10.1016/j.seppur.2005.10.008
12. Chang, C.C., Yang, M.H., Wen, H. M., Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*(3), 178-182. https://doi.org/10.38212/2224-6614.2748
13. Corbo, R.M., Bevilacqua, A., Petruzzi, L., Casanova, P.F., Sinigaglia, M. (2014). Functional beverages: The emerging side of functional foods commercial trends, research, and health implications. *Comprehensive Reviews in Food Science and Food Safety, 13*(6), 1192-1206. https://doi.org/10.1111/1541-4337.12109

14. Ercişli, S. (2005). Rose (*Rosa* spp.) germplasm resources of Turkey. *Genetic Resources and Crop Evolution, 52,* 787-795. https://doi.org/10.1007/s10722-003-3467-8
15. Fan, C., Pacier, C., Martirosyan, D.M. (2014). Rosehip (*Rosa canina* L): A functional food perspective. *Functional Foods in Health and Disease*, *4*(12), 493-509. https://doi.org/10.31989/ffhd.v4i12.159
16. Fiorentini, D., Cappadone, C., Farruggia, G., Prata, C. (2021). Magnesium: Biochemistry, nutrition, detection, and social impact of diseases linked to its deficiency. *Nutrients*, *13*(4), art. no. 1136. https://doi.org/10.3390/nu13041136
17. George, S., Thomas, A., Kumar, M.V.P., Kamdod, A.S., Rajput, A., Joshi, J.T., Abdullah, S. (2023). Impact of processing parameters on the quality attributes of spray-dried powders: a review. *European Food Research and Technology*, *249*(3), 241–257. https://doi.org/10.1007/s00217-022-04170-0
18. Gomes, W.F., França, F.R.M., Denadai, M., Andrade, J.K.S., da Silva Oliveira, E.M., de Brito, E.S., Rodrigues, S., Narain, N. (2018). Effect of freeze- and spray-drying on physico-chemical characteristics, phenolic compounds and antioxidant activity of papaya pulp. *Journal of Food Science and Technology*, *55*, 2095-2102. https://doi.org/10.1007/s13197-018-3124-z
19. Guantario, B., Nardo, N., Fascella, G., Ranaldi, G., Zinno, P., Finamore, A., Pastore, G., Mammano, M.M., Baiamonte, I., Roselli, M. (2024). Comparative study of bioactive compounds and biological activities of five rose hip species grown in Sicily. *Plant, 13*(1), art. no. 53. https://doi.org/10.3390/plants13010053
20. Ibrahim, F., El-Enany, N., El-Shaheny, R.N., Mikhail, I.E. (2015). Development and validation of a new HPLC method for the simultaneous determination of paracetamol, ascorbic acid, and pseudoephedrine HCl in their co-formulated tablets. Application to *in vitro* dissolution testing. *Analytical Sciences, 31*, 943–947. https://doi.org/10.2116/analsci.31.943
21. Igual, M., García-Herrera, P., Cámara, R.M., Martínez-Monzó, J., García-Segovia, P., Cámara, M. (2022). Bioactive compounds in rosehip (*Rosa canina*) powder with encapsulating agents. *Molecules*, *27*(15), art. no. 4737. https://doi.org/10.3390/molecules27154737
22. İpek, P., Balta, F. (2020). Fruit properties of Rose hip (Rosa spp.) genotypes selected from Akkuş, Ordu Province. *Yüzüncü Yıl University Journal of Agricultural Sciences*, *30*(2), 338-344. https://doi.org/10.29133/yyutbd.680453
23. ISO 4120:2021 (2021). Sensory analysis – Methodology – Triangular test: This standard outlines the procedure for the triangle test, a method used to determine whether there is a sensory difference between two products. https://www.iso.org/standard/76666.html (accessed: 20 March 2024).
24. ISO 6658:2017 (2017). Sensory analysis – Methodology – General guidance: This provides general guidance on the methodologies used in sensory analysis, covering aspects like test design, sample preparation, and data analysis. https://www.iso.org/standard/65519.html (accessed: 20 March 2024).
25. ISO 8586:2023 (2023). Sensory analysis – General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. https://www.iso.org/obp/ui/en/#iso:std:iso:8586:ed-2:v1:en (accessed: 20 March 2024).
26. ISO 8589:2007 (2007). Sensory analysis – General guidance for the design of test rooms: This standard specifies the design and setup requirements for sensory testing environments to minimize bias and ensure accurate results. https://www.iso.org/standard/60215.html (accessed: 20 March 2024).
27. Jin, J.C., Liang, S., Qi, S.X., Tang, P., Chen, J.X., Chen, Q.S., Chen, Y.F., Yin, J.F., Xu, Y.Q. (2023). Widely targeted metabolomics reveals the effect of different raw materials and drying methods on the quality of instant tea. *Frontiers in Nutrition*, *10*, art. no. 1236216. https://doi.org/10.3389/fnut.2023.1236216
28. Jovanović, A.A., Lević, S.M., Pavlović, V.B., Marković, S.B., Pjanović, R.V., Đorđević, V.B., Nedović, V., Bugarski, B.M. (2021). Freeze *vs.* spray-drying for dry wild thyme (*Thymus serpyllum* L.) extract formulations: The impact of gelatin as a coating material. *Molecules*, *26*(13), art. no. 3933. https://doi.org/10.3390/molecules26133933
29. Kasangana, P.B., Haddad, P.S., Stevanovic, T. (2015). Study of polyphenol content and antioxidant capacity of *Myrianthus arboreus* (Cecropiaceae) root bark extracts. *Antioxidants*, *4*(2), 410-426. https://doi.org/10.3390/antiox4020410
30. Kazaz, S., Baydar, H., Erbaş, S. (2009). Variations in chemical compositions of *Rosa damascena* Mill. and *Rosa canina* L. fruits. *Czech Journal of Food Sciences*, *27*(3), 178-184, https://doi.org/10.17221/5/2009-CJFS
31. Kraujalytė, V., Pelvan, E., Alasalvar, C. (2016). Volatile compounds and sensory characteristics of various instant teas produced from black tea. *Food Chemistry*, *194*, 864-872. https://doi.org/10.1016/j.foodchem.2015.08.051
32. Liang, S., Granato, D., Zou, C., Gao, Y., Zhu, Y., Zhang, L., Yin, J.-F., Zhou, W., Xu, Y.-Q. (2021). Processing technologies for manufacturing tea beverages: From traditional to advanced hybrid processes. *Trends in Food Science & Technology*, *118*(Part A), 431–446. https://doi.org/10.1016/j.tifs.2021.10.016
33. Lim, J., Wood, A., Green, B.G. (2009). Derivation and evaluation of a labeled hedonic scale. *Chemical Senses*, *34*(9), 739-751. https://doi.org/10.1093/chemse/bjp054
34. Liu, H.Y, Liu, Y., Mai, Y.H., Guo, H., He, X.Q., Xia, Y., Li, H., Zhuang, Q.G, Gan, R.Y. (2021). Phenolic content, main flavonoids, and antioxidant capacity of instant sweet tea (*Lithocarpus litseifolius* [Hance] Chun) prepared with different raw materials and drying methods. *Foods*, *10*(8), art. no. 1930. https://doi.org/10.3390/foods10081930
35. Lorenzo, J.M., Munekata, P.E.S. (2016). Phenolic compounds of green tea: Health benefits and technological application in food. *Asian Pacific Journal of Tropical Biomedicine, 6*(8), 709-719. https://doi.org/10.1016/j.apjtb.2016.06.010
36. Mourabit, Y., El Hajjaji, S., Taha, D., Badaoui, B., El Yadini, M., Rusu, M.E., Lee, L.-H., Bouyahya, A., Bourais, I. (2023). HPLC-DAD-ESI/MS phytochemical investigation, antioxidant, and antidiabetic activities of Moroccan *Rosa canina* L. extracts. *Biocatalysis and Agricultural Biotechnology*, *52*, art. no. 102817. https://doi.org/10.1016/j.bcab.2023.102817
37. Nadeem, H.Ş., Torun, M., Özdemir, F. (2011). Spray-drying of the mountain tea (*Sideritis stricta*) water extract by using different hydrocolloid carriers. *LWT – Food Science and Technology*, *44*(7),1626-1635. https://doi.org/10.1016/j.lwt.2011.02.009
38. Nadpal, J.D., Lesjak, M.M., Šibul, F.S., Anačkov, G.T., Četojević-Simin, D.D., Mimica-Dukić, M.N., Beara, I.N. (2016). Comparative study of biological activities and phytochemical composition of two rose hips and their preserves: *Rosa canina* L. and *Rosa arvensis* Huds. *Food Chemistry*, *192*, 907-914. https://doi.org/10.1016/j.foodchem.2015.07.089
39. Negrean, O.R., Farcas, A.C., Nemes, S.A., Cic, D.E., Socaci, S.A. (2024). Recent advances and insights into the bioactive properties and applications of *Rosa canina* L. and its by-products. *Heliyon*, *10*(9), art. no. e30816. https://doi.org/10.1016/j.heliyon.2024.e30816
40. Odriozola-Serrano, I., Nogueira, D.P., Esparza, I., Vaz, A.A., Jiménez-Moreno, N., Martín-Belloso, O., Ancín-Azpilicueta, C. (2023). Stability and bioaccessibility of phenolic compounds in rosehip extracts during *in vitro* digestion. *Antioxidants, 12*(5), art. no. 1035. https://doi.org/10.3390/antiox12051035
41. Özer, C.O. (2017). The use of Rose hip fruit (*Rosa canina* L.) in fermented meat model system. *Gıda, 42*(4), 372-381. https://doi.org/10.15237/gida.GD17021
42. Pavlovska, G., Tanevska, S. (2013). Influence of temperature and humidity on the degradation process of ascorbic acid in vitamin C chewable tablets. *Journal of Thermal Analysis and Calorimetry*, *111*(4), 1971-1977. https://doi.org/10.1007/s10973-011-2151-z
43. Peña, F., Valencia, S., Tereucán, G., Nahuelcura, J., Jiménez-Aspee, F., Cornejo, P., Ruiz, A. (2023). Bioactive compounds and antioxidant activity in the fruit of rosehip (*Rosa canina* L. and *Rosa rubiginosa* L.). *Molecules, 28*, art. no. 3544. https://doi.org/10.3390/molecules28083544
44. Pimentel, T.C., Gomes da Cruz, A., Deliza, R. (2016). Sensory evaluation: Sensory rating and scoring methods. In B. Caballero, P.M. Finglas, F. Toldrá (Eds). *Encyclopedia of Food and Health*. Academic Press, pp. 744-749. https://doi.org/10.1016/B978-0-12-384947-2.00617-6
45. Prieto, P., Pineda, M., Aguilar, M., (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, *269*(2), 337–34. https://doi.org/10.1006/abio.1999.4019
46. Sanlier, N., Gokcen, B.B., Altuğ, M. (2018). Tea consumption and disease correlations. *Trends in Food Science & Technology, 78*, 95-106. https://doi.org/10.1016/j.tifs.2018.05.026
47. Sayuti, N., Kamarudin, A., Saad, N., Razak, N., Mohd Esa, N. (2021). Optimized green extraction conditions of matcha green tea (*Camellia sinensis*) using central composite design for maximal polyphenol and antioxidant contents. *BioResources*, *16*(2), 3255-3271. https://doi.org/10.15376/biores.16.2.3255-3271
48. Seregelj, V., Cetković, G., Canadanović-Brunet, J., Tumbas Saponjac, V., Vulić, J., Lević, S., Nedović, V., Brandolini, A., Hidalgo, A. (2021). Encapsulation of carrot waste extract by freeze and spray-drying techniques: An optimization study. *LWT – Food Science and Technology*, *138*, art. no. 110696. https://doi.org/10.1016/j.lwt.2020.110696
49. Sinija, V., Mishra, H. (2008). Moisture sorption isotherms and heat of sorption of instant (soluble) green tea powder and green tea granules. *Journal of Food Engineering, 86*(4), 494-500. https://doi.org/10.1016/j.jfoodeng.2007.10.026

50. Someswararao, C., Srivastav, P.P. (2012). A novel technology for production of instant tea powder from the existing black tea manufacturing process. *Innovative Food Science & Emerging Technologies, 16*, 143-147. https://doi.org/10.1016/j.ifset.2012.05.005
51. Sun, H., Fan, R., Fang, R., Shen S., Wang, Y., Fu, J., Hou, R., Sun, R., Bao, S., Chen, Q., Yue, P., Gao, X. (2024). Dynamics changes in metabolites and pancreatic lipase inhibitory ability of instant dark tea during liquid-state fermentation by *Aspergillus niger*. *Food Chemistry*, *448*, art. no. 139136. https://doi.org/10.1016/j.foodchem.2024.139136
52. Tiwari, U., Walsh, D., Rivas, L., Jordan, K., Duffy, G. (2014). Modelling the interaction of storage temperature, pH, and water activity on the growth behaviour of *Listeria monocytogenes* in raw and pasteurised semi-soft rind washed milk cheese during storage following ripening. *Food Control*, *42*, 248-256. https://doi.org/10.1016/j.foodcont.2014.02.005
53. Truong, V.L., Jeong, W.S. (2021). Cellular defensive mechanisms of tea polyphenols: structure-activity relationship. *International Journal of Molecular Sciences*, *22*(17), art. no. 9109. https://doi.org/10.3390/ijms22179109
54. Vardanega, R., Muzio, A.F.V., Silva, E.K., Prata, A.S., Meireles, M.A.A. (2019). Obtaining functional powder tea from Brazilian ginseng roots: Effects of freeze and spray-drying processes on chemical and nutritional quality, morphological and redispersion properties. *Food Research International, 116*, 932-941. https://doi.org/10.1016/j.foodres.2018.09.030
55. Vaskonen, T. (2003). Dietary minerals and modification of cardiovascular risk factors. *Journal of Nutritional Biochemistry*, *14*(9), 492-506. https://doi.org/10.1016/S0955-2863(03)00074-3
56. Xi, J., Wang, B. (2013). Optimization of ultrahigh-pressure extraction of polyphenolic antioxidants from green tea by response surface methodology. *Food and Bioprocess Technology*, *6*, 2538-2546. https://doi.org/10.1007/s11947-012-0891-9
57. Zokti, J.A., Baharin, B.S., Mohammed, A.S., Abas, F. (2016). Green tea leaves extract: Microencapsulation, physicochemical and storage stability study. *Molecules, 21*(8), art. no. 940. https://doi.org/10.3390/molecules21080940

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Spontaneous Fermentation of Beetroot – Effect of Fermentation Time and Temperature and Slice Thickness on Leaven Quality

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The aim of this study was to evaluate the influence of selected processing conditions of beetroot spontaneous fermentation, including slice thickness (2, 4, and 6 mm), temperature (15, 20, and 25°C) and fermentation time (1–15 days), on the physicochemical properties of the resulting leaven (total soluble solid content, turbidity, pH, titratable acidity, color parameters, and content of total carbohydrates, total phenolics, total betacyanins and total betaxanthins). All tested conditions had a significant impact on the properties of beet leaven. Among them, fermentation time and temperature were mostly decisive. Slice thickness was important only for short-time fermentation. The preferable pH below 4.1 was achieved between the 4th and 6th day of fermentation, depending on process temperature. Titratable acidity steadily increased and pH decreased throughout the fermentation process, with more pronounced changes observed at the highest temperature. The highest values of total soluble solids (7.25%) and turbidity (1,100 NTU) were noted on the 13th day of fermentation of the thinnest slices at lower temperatures (15°C and 20°C, respectively). The color of the leaven darkened with increasing fermentation time, but the changes in the *b** parameter were the most notable. Changes in the content of bioactive compounds were dynamic during beetroot spontaneous fermentation, but higher temperatures promoted increased total phenolic content and total betaxanthin content in the leaven. The optimal fermentation conditions in terms of all tested leaven properties were determined at 6 mm, 20°C and 7 days for slice thickness, temperature and processing time, respectively.

Keywords: *Beta vulgaris* L., bioactive compounds, color, lactic acid fermentation, physicochemical properties

INTRODUCTION

Beetroots are a rich source of main nutrients (especially protein and carbohydrates) with a relatively low energy value (43 kcal/100 g of fresh product). A 100-g portion of fresh beetroot contains about 86.3 g water, 0.2 g fat, 1.0 g protein, 8.4 g carbohydrates, 2.6 g fiber, and 1.5 g ash [Abdo *et al.*, 2020]. In addition, it contains large amounts of biologically active compounds and micronutrients, such as betalains, carotenoids, phenolics, and B-group vitamins (B_1, B_2, B_3, B_6, and B_9), as well as inorganic nitrates [Chhikara *et al.*, 2019]. Phenolics, carotenoids, and vitamins in beetroots determine their antioxidant, anti-inflammatory, anticancer, antidepressant, and liver-protecting effects [Slavov *et al.*, 2013], as well as antidiabetic, anti-obesity, antimicrobial, antihypertensive and cognitive improvement properties [Hadipour *et al.*, 2020]. Natural pigments found in beetroots, such as betanins, are used to enhance the red color of various food products, *e.g.*, ice cream, jams, jellies, tomato pastes, sauces, drinks, sweets, and desserts [Punia Bangar *et al.*, 2023]. To take advantage of the health-promoting properties of beetroot, they can be eaten raw, fermented, cooked, baked, or dried as an addition to

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various types of dishes and salads, but also in the form of juice or beetroot leaven [Szekely & Mate, 2022].

Recently, fermented products have become increasingly popular. This is related to new nutritional trends and growing consumer awareness of food processing [Janiszewska-Turak *et al.*, 2022]. Beetroot leaven (also called beetroot kvass) is, next to fermented beets, the main product of the beetroot lactic fermentation process and is an element of Polish cultural heritage [Staninska-Pięta *et al.*, 2023]. According to traditional Polish recipes, beet pickling (beetroot fermentation) involves placing fresh beets (and spices, *e.g.*, garlic, pepper, allspice or bay leaves) in glass (clay, stoneware) jars and pouring brine over them. The mixture is then stored in a dark, warm place for about 7–14 days. After this time, both the fermented beets and beetroot leaven (solution) are consumed. Fermentation is considered a simple and valuable biotechnological process to maintain and/or enhance the safety, nutritional, sensory, and shelf-life properties of vegetables and fruits [Di Cagno *et al.*, 2013]. Lactic acid bacteria convert carbohydrates contained in vegetables and fruits mainly into lactic acid, which lowers the pH of fermented products to about 4.0, thus ensuring the microbiological stability of the product by inhibiting the development of unfavorable and pathogenic bacteria [Montet *et al.*, 2014]. Lactic acid bacteria are often considered probiotics beneficial to human health. They improve the microbiological homeostasis in the human intestines, inhibit the growth of pathogens such as *Escherichia coli*, *Salmonella*, and *Staphylococcus* [Zommiti *et al.*, 2020], stimulate the immune system [Latif *et al.*, 2023], or break down some enterotoxins in food [Petrova *et al.*, 2022].

Beetroot leaven is an example of a fermented food that is associated with high nutritive and taste values as well as salubrious effects [Walkowiak-Tomczak & Zielińska, 2006]. It can be a source of valuable probiotic bacteria, and beetroot juice subjected to lactic acid fermentation has been characterized by even stronger antioxidant properties than fresh juice [Jakubczyk *et al.*, 2024]. Despite the high health-promoting value of beetroot leaven consumption, there are few studies determining the impact of individual process parameters on the physicochemical properties of the leaven. An important aspect of the natural fermentation of vegetables is maintaining the appropriate process conditions. For this purpose, the addition of salt is used, the temperature is controlled, and the time of the process needs to be investigated [Janiszewska-Turak *et al.*, 2022]. So far, for example, the effect of boiling on the betalain profiles and antioxidant capacities of red beetroot products has been studied [Sawicki & Wiczkowski, 2018]. In this work, boiling was used as a pre-treatment before the fermentation process, while the fermentation itself was carried out in constant conditions at 23°C, 2–3 mm slices of beetroot and for a maximum period of 14 days. In the cited study, boiling and fermentation reduced the content of betalains by 51–61% and 61–88%, respectively. In another work, the effect of spontaneous fermentation of beetroot against three commercially available starters (Sacco System by Sacco S.r.l., Cadorago, Italy; *L. mesenteroides*; Sacco System, Italy; Kefir; "Kefir d'acqua" Bionova S.r.l., Villanova sull'Arda, Italy) was compared in relation to volatilomes (fermentation was conducted at 22°C for 19 days) [Casciano *et al.*, 2022]. The cited authors stated that spontaneous fermentation was mostly described by molecules with positive aromatic notes, such as 2-undecanone and 2-nonanone. Fermented beetroot juice or beet leaven were used as a raw material in the spray drying process to produce functional powders [Janiszewska-Turak *et al.*, 2022]. These powders can be used as an additive to food production or as an independent dietary supplement [Janiszewska, 2014].

So far, no standardized production recipes have been developed that would allow for controlled spontaneous fermentation of beetroot slices to obtain leaven of the desired quality. Knowledge about optimizing the lactic acid fermentation process of beetroot in terms of the physicochemical properties of the leaven may also be useful in the subsequent use of fermentation products. Therefore, the aim of this study was to determine the optimal thickness of beetroot slices, temperature and time of fermentation for the physicochemical properties of beet leaven, such as titratable acidity, pH, turbidity, total soluble solid content, color parameters, and contents of total carbohydrates, total phenolics, total betacyanins and total betaxanthins.

MATERIAL AND METHODS

Material

Fresh, red beetroots were obtained from a local supermarket (Olsztyn, Poland). The initial moisture content of fresh beetroots was estimated at 6.29±0.60 kg H_2O/kg dry matter (DM) by drying the material in a vacuum dryer (DZ ZBC II, Chemland, Stargard Szczeciński, Poland) at 70°C and 13.3 kPa for 24 h. The ripe vegetables were free from diseases and comparable in freshness and size. Before the fermentation process, they were cleaned with tap water and cut with a vegetable cutter (V-type, HENDI, Robakowo, Poland) into 2-, 4-, and 6-mm slices.

Chemicals and reagents

Analytical-grade reagents such as concentrated sulfuric acid and Folin–Ciocalteau reagent were purchased from Sigma-Aldrich (Saint Louis, MO, USA), and others including phenol, phosphate buffer, sodium carbonate, sodium chloride, and sodium hydroxide from POCH (Gliwice, Poland). Analytical standards such as lactic acid, gallic acid, betanin, vulgaxanthin-I, and glucose were purchased from Sigma-Aldrich, and tocopherols from Calbiochem (Nottingham, United Kingdom). Deionized water was obtained from an HLP 5 deionizer (Hydrolab, Gdańsk, Poland).

Lactic acid fermentation

Each type of beetroot slice (650 g) and spices such as peppercorns (0.5 g), allspice (1 g), bay leaves (1 g), and fresh garlic (15 g) were placed in glass jars of 1.6 L volume. Then, the contents of the jars were poured with a 1.8% sodium chloride water solution. The material-to-solution ratio was 1:1.2 (*w/v*). The jars were then tightly capped and placed in a dark place for immediate fermentation. The fermentation continued at 15, 20, and 25°C in a laboratory incubator (Q-Cell00/40, Pol-Lab, Wilkowice, Poland) for a maximum of 15 days. During the fermentation,

the contents of the jars were mixed once a day, and the leaven samples (100 mL) were taken, then filtered, and frozen at –18°C (GT 4932 Comfort, Liebherr, Bischofshofen, Austria) for the assessment of their quality. The physicochemical properties of the leaven were determined daily for the first seven days of fermentation and then every two days until the 15th day of fermentation. None of the collected samples had any extrinsic tastes or odors and did not show any other irregularities in the organoleptic assessment. Fermentation experiments (*per* one variant, *i.e.,* for a specific thickness of beetroot slices, temperature and fermentation time) were conducted in separate jars and performed in duplicate (in two parallel jars).

Determination of total soluble solid content and turbidity

The content of total soluble solids (TSS) of leaven was measured at 20°C using the refractometer (type G, Carl Zeiss Jena, Germany), and expressed in Brix % (1 g of dissolved solids in 100 g of solution). The turbidity (TR) was determined using a turbidimeter (TB 211 IR, Lovibond Tintometer, Sarasota, FL, USA), and expressed in nephelometric turbidity units (NTU). Both measurements were performed in triplicate.

Determination of pH and titratable acidity

The pH of leaven was measured using a pH meter (CP-451, Elmetron, Zabrze, Poland). In turn, titratable acidity (TA) was determined according to Abdo *et al.* [2020] by titration with a 0.2 M sodium hydroxide solution to pH 8.2. Results of titratable acidity determination were calculated based on an equation for a lactic acid curve and expressed as g lactic acid equivalent (LA) *per* 100 mL of leaven. Both measurements were performed at least in duplicate.

Determination of total carbohydrate content

Total carbohydrate content (TC) was determined according to the phenol-sulfuric acid method described by Zhang *et al.* [2020]. Firstly, 500 µL of the leavens were mixed in the probe with 200 µL of a 5% aqueous solution of redistilled phenol. Next, 1,250 µL of concentrated sulfuric acid were added. The contents of the test tubes were thoroughly mixed, and then the tubes were placed in a boiling water bath for 20 min. Afterwards, they were cooled in an ice bath for 2 min and left for 15 min at room temperature. Absorbance was measured at 490 nm and recalculated to the standard curve prepared for glucose in the range of 1–100 µg/mL. The total carbohydrate content was expressed in g/100 mL. The analyses were performed at least in duplicate.

Determination of color parameters

Color coordinates of leavens were measured using a spectrophotometer (3Color 9000Neo, TRI-COLOR, Narama, Poland) in a reflection mode under standard D65 illumination, a 10° observer, and an 18 mm diaphragm. The color was expressed in the CIE$L^*a^*b^*$ space, where L^*, a^*, and b^* represented lightness, (+)redness/(–)greenness, and (+)yellowness/(–)blueness, respectively. The total color difference (ΔE) was calculated according to the equations (1-4) [Zielinska & Markowski, 2012]. The color results were averaged from six measurements.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$$\Delta L^* = L^*_{\text{reference}} - L^*_{\text{sample}} \quad (2)$$

$$\Delta a^* = a^*_{\text{reference}} - a^*_{\text{sample}} \quad (3)$$

$$\Delta b^* = b^*_{\text{reference}} - b^*_{\text{sample}} \quad (4)$$

where the reference values of color coordinates were measured for pure brine before fermentation (day 0), and the sample values were measured for beetroot leavens fermented at specified process parameters.

Determination of total phenolic content

Total phenolic content (TPC) of leavens was determined according to the assay with Folin-Ciocalteu (FC) reagent described in the literature [Yasaminshirazi *et al.*, 2020], with some modifications. Briefly, 125 µL of the leaven, 125 µL of the FC reagent (diluted at 1:2 with water, *v/v*), 180 µL of a saturated solution of sodium carbonate, and 1,500 µL of deionized water were mixed and left to react for 1 h at room temperature. Absorbance was measured at 760 nm using a FLUOstar Omega microplate reader (BMG LabTech, Ortenberg, Germany). TPC was expressed in mg of gallic acid equivalent (GAE) *per* 100 mL of beetroot leaven. The analyses were performed at least in duplicate.

Determination of contents of total betacyanins and total betaxanthins

The content of total betacyanins (expressed as betanin) and total betaxanthins (expressed as vulgaxanthin-I) in leavens was determined spectrophotometrically according to the procedure described by von Elbe [2001]. Leaven samples were diluted with 0.05 M phosphate buffer (pH 6.5) to obtain an absorbance between 0.4 and 0.5 at 538 nm. The light absorption of the samples was recorded at 476, 538, and 600 nm, and the corrected light absorption of betanin and vulgaxanthin-I was calculated according to the equations (5–7) [von Elbe, 2001]:

$$x = 1.095 \times (a - c) \quad (5)$$

$$z = a - x \quad (6)$$

$$y = b - z - \frac{x}{3.1} \quad (7)$$

where: x is absorption of betanin minus colored impurities, y is absorption of vulgaxanthin-I corrected for betanin and impurities, z is absorption of impurities, a is absorption of sample at 538 nm, b is absorption of sample at 476 nm, and c is absorption of sample at 600 nm.

Total betacyanin content (TBC) and total betaxanthin content (TBX) were expressed as mg of betanin equivalent (BE) *per*

100 mL and mg of vulgaxanthin-I equivalent (VG-I) *per* 100 mL, respectively. All measurements were performed in triplicate.

Statistical analysis

A three-way analysis of variance (ANOVA) by applying a Shapiro-Wilk test was used to assess the effect of the process parameters on the properties of beetroot leaven. Moreover, the correlation coefficient (*r*) was determined (Pearson test) to analyze the correlations between different parameters of beetroot leaven. All calculations were done at a significance level of α=0.05 using STATISTICA 13.0 software (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS AND DISCUSSION

Content of total soluble solids and turbidity of beetroot leaven

The changes in TSS content in beetroot leaven during fermentation under different conditions are shown in **Figure 1**. Additionally, regression equations describing these changes are presented in **Table S1** in the Supplementary Materials. The TSS content of the brine (fermentation time, 0 day) was 0.18%, and this value increased to 7.25% for the beetroot leaven obtained from the fermentation of 2-mm slices at 15°C for 13 days (**Figure 1**). Generally, at the beginning of the process, TSS content increased over the fermentation time for all temperature and slice thickness

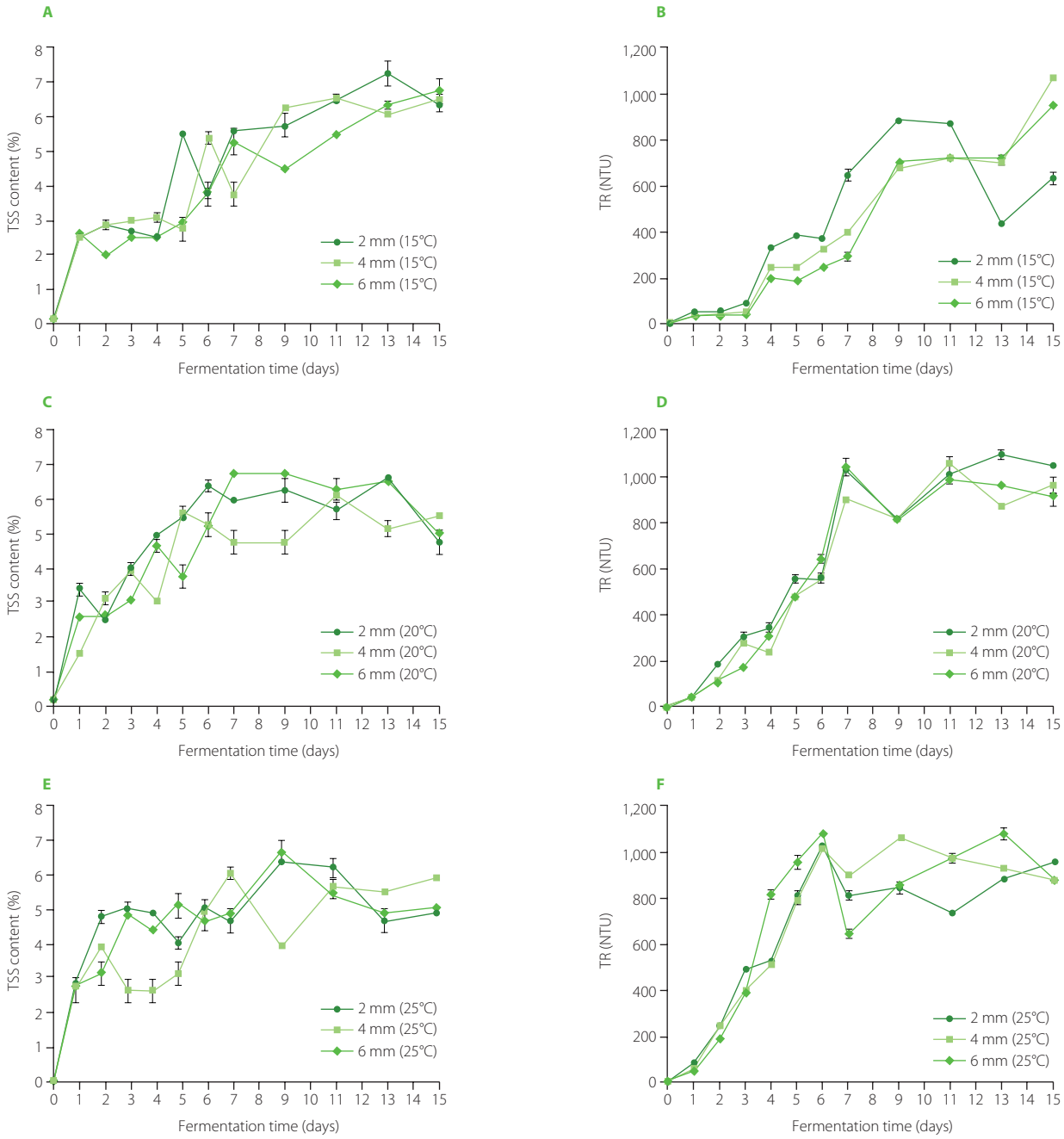

Figure 1. Changes in total soluble solid (TSS) content and turbidity (TR) of beetroot leaven during spontaneous fermentation of beetroot slices of different thicknesses at temperatures of 15°C (**A** and **B**, respectively), 20°C (**C** and **D**, respectively), and 25°C (**E** and **F**, respectively).

values. The higher the process temperature was, the higher was TSS content after a shorter fermentation time. In terms of slice thickness, the lowest values were noted for 4-mm slices. However, all the highest values of TSS content were noted on or after the 7th day of fermentation. After reaching the maximum, TSS content was observed to decrease. An exception was the leaven from 6-mm beetroot slices fermented at 15°C, for which a linear relationship was found (**Table S1**). Increasing TSS content during fermentation can be due to the diffusion of fermentable sugars and other solids from beetroot tissue to the surrounding solution (leaven), as well as NaCl and lactic acid from the solution to the material [Rodriguez-Gomez *et al.*, 2012]. Moreover, at the same time, sugars are converted to lactic acid. A sudden decrease in TSS after reaching the maximum value may indicate a significant decrease in the rate of diffusion of the solids from the beet tissue and a significant increase in the rate of decomposition of sugars into lactic acid [Janiszewska-Turak *et al.*, 2022].

Turbidity of beetroot leavens ranged from 31 NTU for the product obtained from the fermentation of 6-mm slices at 15°C for 1 day to 1,100 NTU for the leaven from 2-mm slices fermented at 20°C for 13 days (**Figure 1**). A similar tendency for turbidity changes along with fermentation time can be observed as in the case of TSS, *i.e.*, an increase to the maximum point followed by a decline (**Figure 1**, **Table S1**). The linear equations were only found for fermentation of 4-mm and 6-mm slices at 15°C. For all tested slice thicknesses, turbidity reached

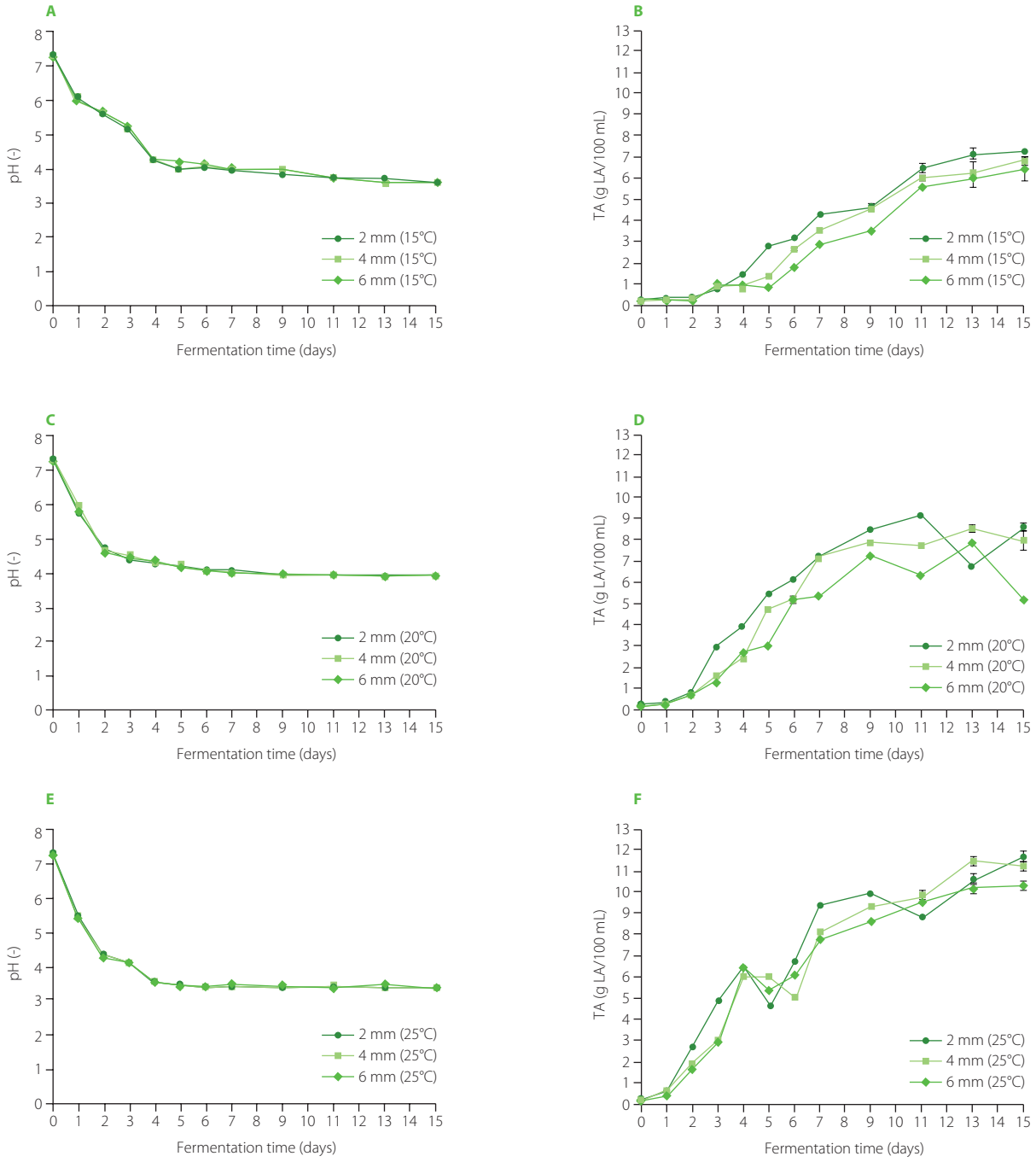

Figure 2. Changes in pH and titratable acidity (TA) of beetroot leaven during spontaneous fermentation of beetroot slices of different thicknesses at temperatures of 15°C (**A** and **B**, respectively), 20°C (**C** and **D**, respectively), and 25°C (**E** and **F**, respectively). LA, lactic acid equivalent.

its highest value the fastest at a temperature of 25°C. Higher temperatures significantly accelerate the rate of lactic acid fermentation and mass exchange during the process. In most cases, the highest turbidity was noted on the same day or two days before the highest content of TSS. It is due to the fact that turbidity is strongly related to the solids content in beetroot leaven and the rate of solid diffusion during the fermentation. Solid substances diffusing from the material into the solution increase its turbidity [Molner *et al.*, 2023].

pH, titratable acidity and total carbohydrate content in beetroot leaven

During the lactic acid fermentation of beetroot, the pH decreased from 7.23 for the brine (fermentation time, 0 day) to 3.36 for the leavens from the fermentation of 2-mm slices for 9 days and 6-mm slices for 15 days at 25°C (**Figure 2**). The lowest pH values were reached by the leaven at the end of the process (13 < fermentation time < 15 day). In general, fermentation allowed for a reduction of pH and the production of a leaven with a pleasant sour taste and a characteristic aroma. However, acidity below pH 3.6 may make the product undesirable in terms of sensory characteristics [Karovicova & Kohajdova, 2003]. From this point of view, more advantageous is achieving pH approx. 4.1, which ensures product stability and, at the same time, its desired sensory properties [Buckenhueskes, 2015]. In our study, depending on the temperature of the process, this pH value was noted between the 4th and 6th day of fermentation (**Figure 2**). The results are similar to those presented in the literature, where beetroot leaven (natural, without any additions) reached a pH of 4.0 on the 4th day of fermentation [Walkowiak-Tomczak & Zielińska, 2006]. The most intensive changes in pH were observed at the beginning of the process. After two days of fermentation, the pH of the leaven dropped even by 41%, whereas for the next 13 days of fermentation, it changed by 21%. The rapid drop in pH at the beginning of fermentation is important for the quality of the final product, because it minimizes the growth of spoilage bacteria [Viander *et al.*, 2003]. Moreover, in slowly acidified environments, lactic acid bacteria can be inhibited by butyric acid bacteria [Lund *et al.*, 2020].

Titratable acidity (TA) increased during fermentation from 0.25 g LA/100 mL for the brine to even 11.62 g LA/100 mL for the leavens from the fermentation of 4-mm beetroot slices for 13 days at 25°C. The rate and nature of changes depended both on process temperature and slice thickness (**Figure 2**; **Table S1**).

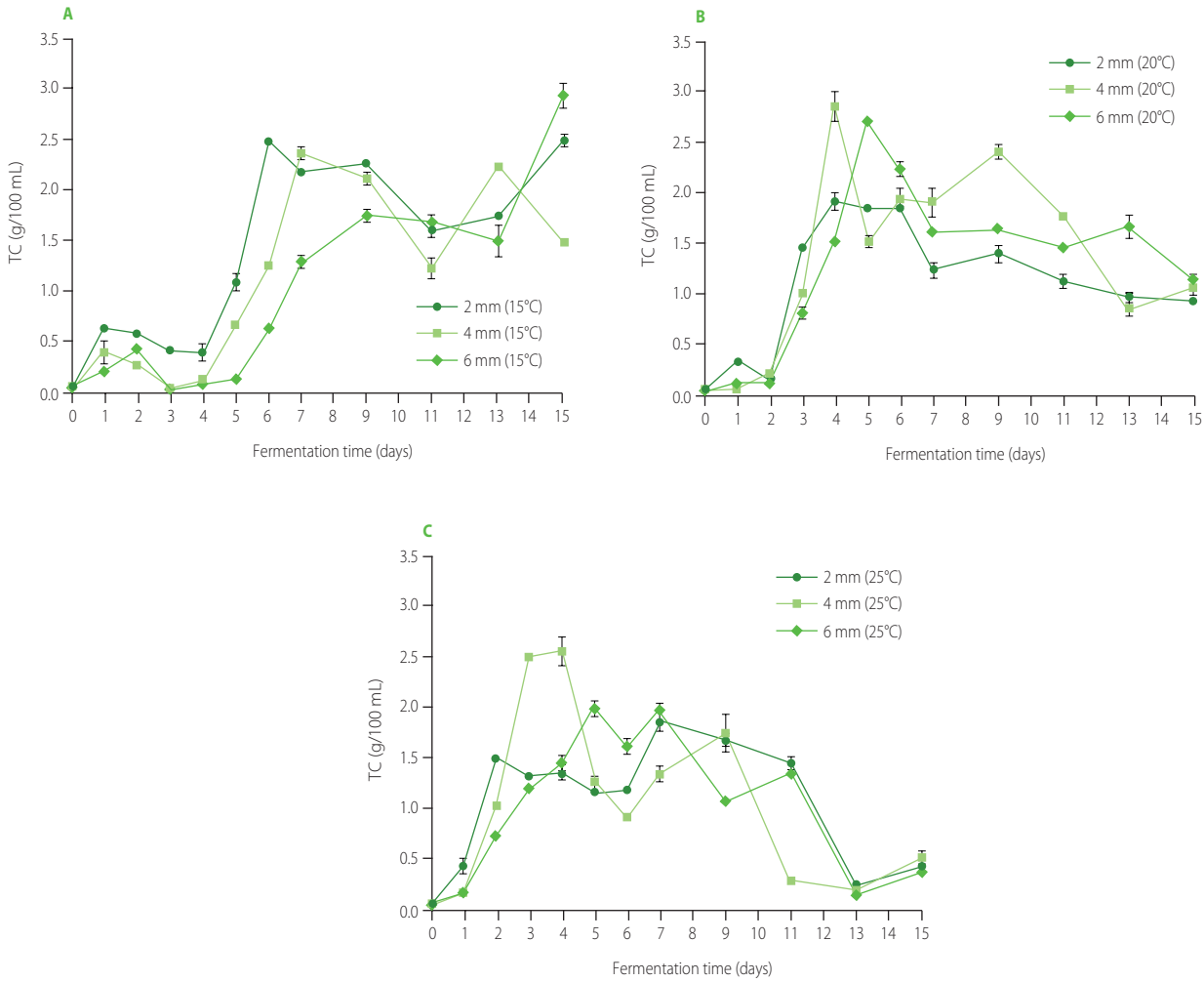

Figure 3. Changes in total carbohydrate content (TC) of beetroot leaven during spontaneous fermentation of beetroot slices of different thicknesses at temperatures of 15°C (**A**), 20°C (**B**), and 25°C (**C**).

The highest TA values were determined at the end of fermentation, *i.e.*, on the 11th day and after. Generally, for all slice thicknesses, it was observed that the higher the process temperature, the higher the TA on specific fermentation days. For example, for 2-mm slices and 7th day of fermentation, the TA values were 4.27, 7.20, and 9.52 g LA/100 mL at temperatures of 15, 20, and 25°C, respectively. It was previously found that the lactic acid fermentation of vegetables and fruits consists of several stages, the first of which is the most important. At this stage, facultative anaerobic microorganisms synthesize carbon dioxide and create conditions for the development of other anaerobic microorganisms, which play a dominant role in subsequent phases [Sun *et al.*, 2021]. Most likely, conducting the process at a higher temperature will result in the acceleration of the metabolism of microorganisms and the faster development of anaerobic conditions. This leads to faster fermentation in further stages, lower pH, a higher concentration of acids (like lactic and formic acids), and therefore higher TA values [Staninska-Pięta *et al.*, 2023].

Total carbohydrate content (TC) of leavens ranged from 0.03 g/100 mL (slice thickness of 6 mm, temperature of 15°C, fermentation time of 3 days) to 2.94 g/100 mL (slice thickness of 6 mm, temperature of 15°C, fermentation time of 15 days) (**Figure 3**). During fermentation, numerous TC fluctuations were observed, which might have been due to the simultaneous occurrence of two phenomena, *i.e.*, the migration of solid substances (including sugars) from the tissue into the solution and the microbial conversion of sugars into organic acids (mainly lactic acid). Additionally, the brine used to pour the beetroot slices at the beginning of the process (day 0) did not contain sugars; hence, the carbohydrate content in the leaven depended only on the above-mentioned phenomena. Changes in TC are much more difficult to interpret in this case than, *e.g.*, during the lactic acid fermentation of vegetable juices. During this type of fermentation, the material contains large amounts of sugars already before fermentation; thus, their content mainly decreases during the process due to their conversion by lactic acid bacteria to lactic acid [Manea & Buruleanu, 2010]. However, a certain influence of temperature on the kinetics of TC changes in leavens during beetroot fermentation was observed (**Figure 3**; **Table S1**). In the case of the samples subjected to fermentation at 15°C, the highest TC were achieved on 7 < fermentation time < 15 day, while at 20 and 25°C it was on 4 < fermentation time < 7 day. During fermentation, sugars are partially extracted into the brine, and temperature is one of the main factors affecting that mechanism. Generally, higher temperatures induce higher water extraction efficiency for sugars [Lopez *et al.*, 2009]. In this case, it can be noted that with increasing temperatures of fermentation, the rate of extracted sugars was higher, and the highest values obtained for TC were observed in increasingly earlier stages of fermentation (**Figure 3**).

Color of beetroot leaven

Figure 4 shows changes in leaven color parameters during the fermentation process. In all tested samples, the greatest color changes (ΔE) occurred in the first few days of fermentation. This was most likely closely related to the greater intensity of pigment release into the brine at the beginning of fermentation. Moreover, the greatest drop in pH was observed during the first 2 days, which may also have a significant impact on the color of the leavens. As the fermentation temperature increased, the highest ΔE values appeared earlier and were recorded on the 4th, 2nd, and 1st day of fermentation for temperatures of 15, 20, and 25°C, respectively. Moreover, with an increase in the fermentation temperature, the maximum L^*, a^*, b^*, and ΔE values of leavens decreased (**Figure 4**). For example, leaven redness (a^*) ranged from 34.92 to 60.67, from 32.03 to 57.60, and from 28.57 to 47.93 for fermentation temperatures of 15, 20, and 25°C, respectively. It indicates faster decomposition of red colorants at higher fermentation temperature, which is consistent with the literature data [Staninska-Pięta *et al.*, 2023].

Content of total phenolics, total betacyanins and total betaxanthins in beetroot leaven

During fermentation of beetroot, total phenolic content in the leaven increased from 1.12 mg GAE/100 mL (slice thickness of 6 mm, temperature of 25°C, fermentation time of 1 day) to even 103.93 mg GAE/100 mL (slice thickness of 4 mm, temperature of 25°C, fermentation time of 4 day) (**Figure 5**). Generally, in all tested samples, the lowest TPC was recorded on the 1st day of fermentation, and the greatest increase in its value was observed at the very beginning of the process, *i.e.*, between days 1 and 2 (even by 97%). Then, there was a further increase in TPC (with numerous fluctuations) at a rate dependent on the process temperature. Phenolic compounds of beetroots, which include flavonoids and phenolic acids [Płatosz *et al.*, 2020], are important secondary metabolites of plant and have highly varied structures and properties. Increased TPC in beetroot leaven during fermentation may be related to microorganisms' ability to break down the food matrix, releasing associated phytochemicals. These microorganisms produce enzymes like cellulase, β-glucanase and lichenase capable to cleave the glycosidic bounds and release phenolic acids from plant cell walls [Matthews *et al.*, 2006]. Breaking these bonds contributes to the release of conjugated acids, which is expressed in a higher TPC [Płatosz *et al.*, 2020].

Betalains are classified based on structural characteristics into betacyanins (betanin and isobetanin) with red-violet coloration, which account for 75–80% and betaxanthins (vulgaxanthin-I, II) with yellow-orange coloration, which constitute 20–25% of total betalains present in beetroot [Guine *et al.*, 2018]. In the present study, changes in total betacyanin content and total betaxanthin content of the leaven during fermentation are presented in **Figure 6**. TBC and TBX ranged from 0.27 mg BE/100 mL (slice thickness of 6 mm, fermentation at 15°C for 1 day) to 27.96 mg BE/100 mL (slice thickness of 2 mm, fermentation at 20°C for 11 days) and from 0.39 mg VG-I/100 mL (slice thickness of 6 mm, fermentation at 20°C for 1 day) to 12.15 mg VG-I/100 mL (slice thickness of 2 mm, fermentation at 25°C during 5 days), respectively. These results agree with literature data for red (from 0.9 to 32.3 mg/100 g) and yellow (from 1.5 to 15.1 mg/100 g) pigment contents in natural leaven [Walkowiak-Tomczak & Zielińska,

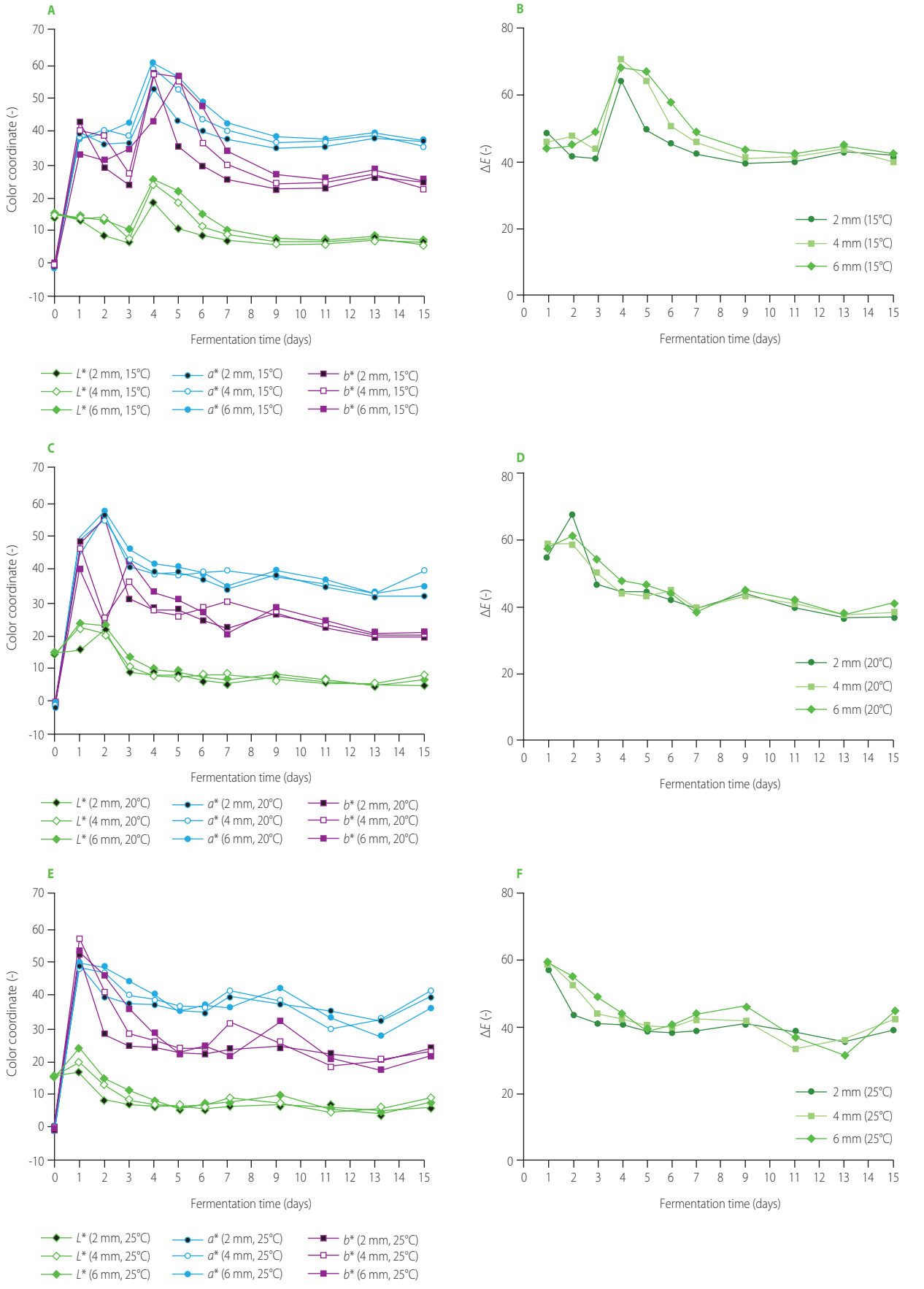

Figure 4. Changes in color coordinates and total color difference (Δ*E*) of beetroot leaven during spontaneous fermentation of beetroot slices of different thicknesses at temperatures of 15°C (**A** and **B**, respectively), 20°C (**C** and **D**, respectively), and 25°C (**E** and **F**, respectively). *L**, lightness; *a**, (+)redness/(–)greenness; *b**, (+)yellowness/(–)blueness.

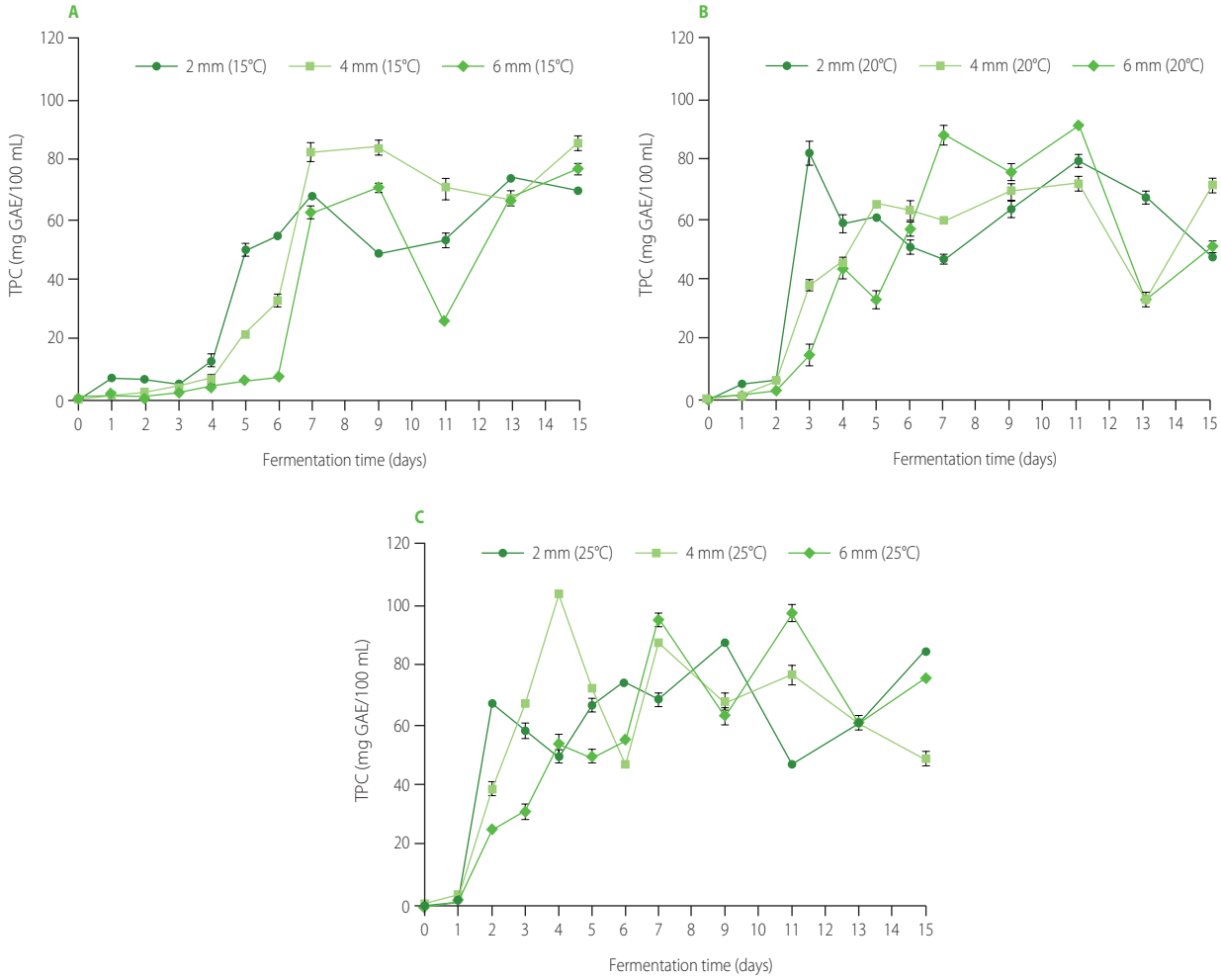

Figure 5. Changes in total phenolic content (TPC) of beetroot leaven during spontaneous fermentation of beetroot slices of different thicknesses at temperatures of 15°C (**A**), 20°C (**B**), and 25°C (**C**). GAE, gallic acid equivalent.

2006]. Changes in TBC and TBX during fermentation showed a similar trend, *i.e.*, the contents of the analyzed betalains increased to their maximum value, and then their fluctuations with a tendency to decrease were observed (**Figure 6**; **Table S1**). For example, the TBC of the leaven from the fermentation of 6-mm slices at 20°C increased during the first seven days from 0.31 to 25.06 mg BE/100 mL, and then decreased in the following days of processing to 16.20 mg BE/100 mL on the 15th day of fermentation. Decreasing or increasing contents of both betacyanins and betaxanthins in the leaven during the fermentation of beetroot resulted from the intensity of two processes occurring, *i.e.*, the release of pigments from the matrix and their degradation. At the beginning of the process, when the leaching of pigments from the food matrix was dominant, an increase was observed in betacyanin content. Next, when these compounds reached their maximum concentrations, the most likely mechanism of their degradation started to be a leading one. The sudden increases in TBC and TBX in the periods with a decreasing tendency may result from a temporary higher leaching efficiency of the compounds due to more intensive maceration of the inner layers of beetroot slices [Sawicki & Wiczkowski, 2018].

Effect of process parameters on beetroot leaven properties

Effect of process parameters and interactions between them on beetroot leaves properties was analyzed by three-way ANOVA. Respective results are presented in **Table 1**. All independent variables had a significant effect on all beetroot leaven properties (dependent variables). Considering the significance of the influence of parameters alone (without their interactions), the order is as follows: fermentation time (FT) > temperature of fermentation (T) > slice thickness (ST), and the % of explained variance in these cases ranged from 33.87% (total carbohydrate content) to 83.83% (total betacyanin content), from 1.37% (total carbohydrate content) to 27.79% (total betaxanthin content), and from 0.01% (pH) to 4.03% (L^*), respectively. Overall, the fermentation time itself turned out to be the most important process parameter, accounting for 75% of all studied properties. In the other 25% (for a^*, total carbohydrate content, and ΔE), it was the second most significant variable, just after the combination of fermentation time and temperature (FT×T).

In the case of the impact of the interactions of process parameters, their order in terms of significance was as follows:

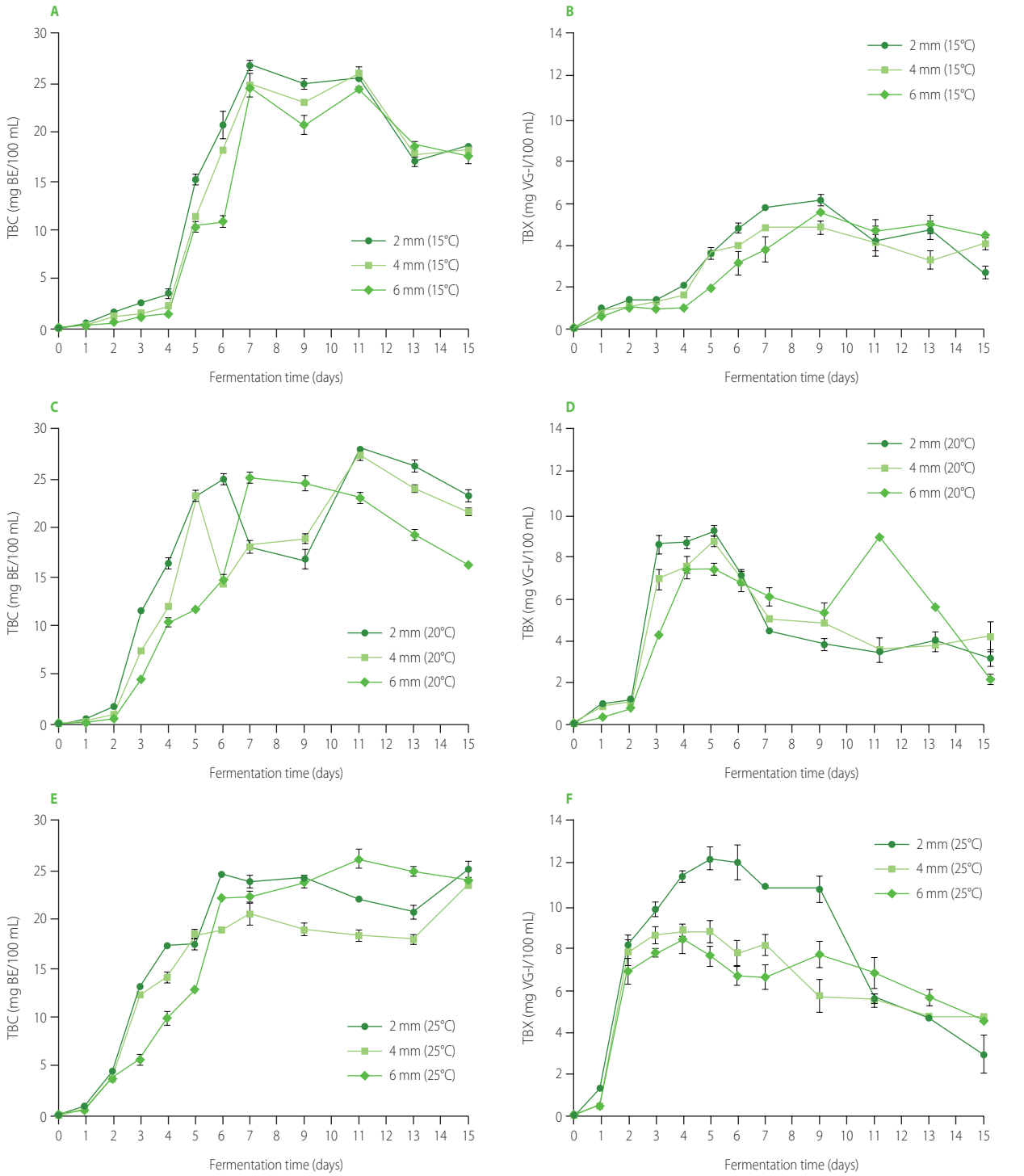

Figure 6. Changes in contents of total betacyanins (TBC) and total betaxanthins (TBX) of beetroot leaven during spontaneous fermentation of beetroot slices of different thicknesses at temperatures of 15°C (**A** and **B**, respectively), 20°C (**C** and **D**, respectively), and 25°C (**E** and **F**, respectively). BE, betanin equivalent; VG-I, vulgaxanthin-I equivalent.

FT×T > ST×FT×T > ST×FT > ST×T, where the % of explained variance ranged from 5.12% (titratable acidity) to 44.49% (a^*), from 0.16% (pH) to 13.42% (total soluble solid content), from 0.07% (turbidity) to 6.21 % (total carbohydrate content), and from 0.04% (pH) to 2.34% (total carbohydrate content), respectively (**Table 1**).

Correlations between beetroot leaven properties

Lactic acid fermentation is a complex process, and the tested properties of the leaven were influenced not only by the parameters of the process itself, but also by their interactions. For a better understanding of the interrelationships between all the analyzed properties of beet leaven, Pearson correlation coefficients were determined and presented in **Table 2**. All correlations were significant ($p<0.05$), and the correlation coefficients were in the range of $0.31 \leq |r| \leq 0.97$.

The positive correlations between the parameters of beet leaven with the highest coefficients ($r \geq 0.80$) were recorded for a^* *vs.* ΔE ($r=0.97$), L^* *vs.* ΔE ($r=0.94$), L^* *vs.* a^* ($r=0.93$), b^* *vs.* ΔE

Table 1. Results (% of the explained variance) of three-way analysis of variance showing the effects of slice thickness (ST), fermentation time (FT) and temperature (T) as well as their interactions on physicochemical parameters of beetroot leavens.

Parameter	ST	FT	T	ST×FT	ST×T	FT×T	ST×FT×T	Other
TSS (%)	2.07*	65.71*	1.42*	4.32*	1.16*	10.92*	13.42*	0.98^{ns}
TR (NTU)	0.12*	73.57*	11.60*	0.70*	0.46*	9.22*	4.21*	0.12^{ns}
pH (–)	0.01*	83.08*	10.30*	0.07*	0.04*	6.32*	0.16*	0.02^{ns}
TA (g LA/100 mL)	1.29*	72.39*	18.94*	0.73*	0.14*	5.12*	1.29*	0.10^{ns}
TC (g/100 mL)	0.38*	33.87*	1.37*	6.21*	2.34*	42.73*	12.83*	0.27^{ns}
TPC (mg GAE/100 mL)	1.41*	56.00*	9.49*	5.82*	0.33*	14.98*	11.80*	0.17^{ns}
TBC (mg BE/100 mL)	1.42*	83.83*	2.34*	2.43*	0.29*	6.15*	3.27*	0.27^{ns}
TBX (mg VG-I/100 mL)	1.43*	35.20*	27.79*	5.58*	1.51*	22.57*	4.35*	1.57^{ns}
L^* (–)	4.03*	51.63*	5.46*	1.76*	0.44*	33.01*	3.62*	0.05^{ns}
a^* (–)	3.86*	38.04*	7.58*	1.72*	0.41*	44.49*	3.76*	0.14^{ns}
b^* (–)	1.18*	44.74*	6.86*	4.54*	1.56*	29.00*	12.04*	0.08^{ns}
ΔE (–)	3.00*	40.67*	6.99*	1.99*	0.58*	41.71*	4.94*	0.12^{ns}

*Significant at $p<0.05$; ns, not significant at $p\geq0.05$; TSS, total soluble solid content; TR, turbidity; TA, titratable acidity; TC, total carbohydrate content; TPC, total phenolic content; TBC, total betacyanin content; TBX, total betaxanthin content; L^*, lightness; a^*, (+)redness/(–)greenness; b^*, (+)yellowness/(–)blueness; ΔE, total color difference; LA, lactic acid equivalent; GAE, gallic acid equivalent; BE, betanin equivalent; VG-I, vulgaxanthin-I equivalent.

Table 2. Coefficients of Pearson correlations of physicochemical parameters of beetroot leaven obtained during fermentation at different conditions.

	TSS	TR	TC	pH	TA	TPC	TBX	TBC	L^*	a^*	b^*
TR	0.76*										
TC	0.48*	0.38*									
pH	–0.69*	–0.80*	–0.48*								
TA	0.73*	0.87*	0.31*	–0.80*							
TPC	0.71*	0.74*	0.64*	–0.77*	0.75*						
TBX	0.41*	0.44*	0.57*	–0.61*	0.43*	0.65*					
TBC	0.80*	0.83*	0.53*	–0.80*	0.81*	0.81*	0.50*				
L^*	–0.68*	–0.71*	–0.56*	0.61*	–0.72*	–0.73*	–0.58*	–0.73*			
a^*	–0.57*	–0.66*	–0.39*	0.42*	–0.67*	–0.61*	–0.43*	–0.63*	0.93*		
b^*	–0.62*	–0.71*	–0.43*	0.58*	–0.71*	–0.67*	–0.45*	–0.67*	0.84*	0.81*	
ΔE	–0.62*	–0.71*	–0.43*	0.48*	–0.71*	–0.66*	–0.47*	–0.67*	0.94*	0.97*	0.89*

*Significant correlation at $p<0.05$. TSS, total soluble solid content; TR, turbidity; TA, titratable acidity; TC, total carbohydrate content; TPC, total phenolic content; TBC, total betacyanin content; TBX, total betaxanthin content; L^*, lightness; a^*, (+)redness/(–)greenness; b^*, (+)yellowness/(–)blueness; ΔE, total color difference.

(r=0.89), TR *vs*. TA (r=0.87), L^* *vs*. b^* (r=0.84), TR *vs*. TBC (r=0.83), TA *vs*. TBC (r=0.81), TPC *vs*. TBC (r=0.81), a^* *vs*. b^* (r=0.81), and TSS *vs*. TBC (r=0.80). Apart from the expected high correlations between color parameters ($0.89\leq r\leq0.97$) of the beetroot leaven, the relationship between TR *vs*. TA also showed a high positive correlation (r=0.87). This can be due to the fact that lactic acid produced by lactic acid bacteria leads to acidification of the environment and increases the TA of beetroot leaven. At the same time, the growing lactic acid bacteria population resulted in an increase in leaven turbidity [Reina *et al.*, 2005]. Most probably, a strong positive correlation between TR *vs*. TBC and TSS *vs*. TBC (**Table 2**) was related to the leaching of betalains into the surrounding solution during the fermentation process. These extracted compounds enrich the total extract present in the leaven while, at the same time, increasing its turbidity [de Jesus Junqueira *et al.*, 2018]. In addition, betanin is more stable in an acidic environment. During fermentation, when the TA of beetroot leaven increased, betanin became more stable; hence, TBC content increased. A similar effect of a positive correlation between titratable acidity and content of betanins in beetroot leaven was previously noted by Sadowska-Bartosz & Bartosz [2021].

In the case of the strongest negative correlations ($-r \geq 0.80$), they were observed between pH *vs*. TR ($r=-0.80$), pH *vs*. TA ($r=-0.80$), and pH *vs*. TBC ($r=-0.80$) (**Table 2**). Due to the fact that both pH and TA indicate the acidification of the environment (with opposite phrases), there was such a strong negative correlation between these two properties of the leaven. Additionally, for the same reason, high negative correlations between pH *vs*. TR and TBC should be interpreted in the same way as high positive correlations between TA *vs*. TR and TBC.

As can be seen in **Table 2**, two individual parameters of the beetroot leaven strongly correlating with all other parameters were TPC ($0.64 \leq |r| \leq 0.81$) and L^* ($0.61 \leq |r| \leq 0.94$). On the other hand, the beetroot leaven parameters characterized by the largest number of weak correlations ($0.3 < |r| < 0.5$) were TC (8 out of 11) and TBX (7 out of 11).

CONCLUSIONS

In this work, beetroot slices with a thickness of 2, 4, and 6 mm were subjected to spontaneous lactic acid fermentation at 15, 20, and 25°C for a maximum of 15 days to determine the most desirable conditions in terms of physicochemical properties and color of the resulting leaven. Although all variables had a significant impact on the properties of beet leaven, the fermentation time and temperature were of key importance. Study results showed that a preferable pH below 4.1 was achieved in leavens between 4 and 6 days of fermentation. However, titratable acidity reached the highest values only in the final phase of fermentation. In contrast, total carbohydrate content was generally the highest between 4 and 7 days of fermentation. To obtain a leaven with higher total soluble solids and turbidity, beetroots need to be fermented for at least 7 days. In order to increase the content of bioactive compounds in the leaven, the most advantageous process parameters were thinner beetroot slices (4 mm for total phenolic content and 2 mm for total betacyanin content) and higher fermentation temperature (20°C–25°C). The highest contents of total phenolics, total betaxanthins and total betacyanins were determined in the leaven on the 4th, 5th and 11th day of fermentation, respectively. On the other hand, the highest values of color parameters (L^*, a^*, b^*, and ΔE) were obtained when thicker beetroot slices (4-mm and 6-mm) were fermented at lower temperature (15°C) for 4 days. The presented research allows for the selection of parameters of the beetroot fermentation process focusing on the specific properties of the leaven (their maximum or minimum values). However, in terms of all tested properties, the optimal process parameters were determined at: slice thickness of 6 mm, fermentation temperature of 20°C and fermentation time of 7 days.

SUPPLEMENTARY MATERIALS

The following are available online at **https://journal.pan.olsztyn.pl/Spontaneous-Fermentation-of-Beetroot-Effect-of-Fermentation-Time-and-Temperature,192122,0,2.html.**
Table S1. Regression equations for curves shown in Figures 1–6.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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REFERENCES

1. Abdo, E., El-Sohaimy, S., Shaltout, O., Abdalla, A., Zeitoun, A. (2020). Nutritional evaluation of beetroots (*Beta vulgaris* L.) and its potential application in a functional beverage. *Plants*, *9*(12), art. no. 1752. https://doi.org/10.3390/plants9121752
2. Buckenhueskes, H.J. (2015). Chapter 22 – Quality improvement and fermentation control in vegetables. In W. Holzapfel (Ed.), *Advances in Fermented Foods and Beverages*. Woodhead Publishing, Sawston, England, pp. 515-539. https://doi.org/10.1016/B978-1-78242-015-6.00022-0
3. Casciano, F., Mayr, H., Nissen, L., Putti, A., Zoli, F., Gianotti, A., Conterno, L. (2022). Red beetroot fermentation with different microbial consortia to develop foods with improved aromatic features. *Foods*, *11*(19), art no. 3055. https://doi.org/10.3390/foods11193055
4. Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., Panghal, A. (2019). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*, *272*, 192-200. https://doi.org/10.1016/j.foodchem.2018.08.022
5. de Jesus Junqueira, J.R., Correa, J.L.G., de Mendonca, K.S., de Mello Junior, R.E., de Souza, A.U. (2018). Pulsed vacuum osmotic dehydration of beetroot, carrot and eggplant slices: Effect of vacuum pressure on the quality parameters. *Food and Bioprocess Technology*, *11*(10), 1863-1875. https://doi.org/10.1007/s11947-018-2147-9
6. Di Cagno, R., Coda, R., De Angelis, M., Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiology*, *33*(1), 1-10. https://doi.org/10.1016/j.fm.2012.09.003
7. Guine, R.P.F., Goncalves, F., Lerat, C., Idrissi, T.E., Rodrigi, E., Correia, P.M.R., Goncalves, J.C. (2018). Extraction of phenolic compounds with antioxidant activity from beetroot (*Beta Vulgaris* L.). *Current Nutrition and Food Science*, *14*(4), 350-357. https://doi.org/10.2174/1573401313666170609102336
8. Hadipour, E., Teleghani, A., Tayarani-Najaran, N., Tayarani-Najaran, Z. (2020). Biological effects of red beetroot and betalains: A review. *Phytotherapy Research*, *34*(8), 1847-1867. https://doi.org/10.1002/ptr.6653
9. Jakubczyk, K., Melkis, K., Janda-Milczarek, K., Skonieczna-Żydecka, K. (2024). Phenolic compounds and antioxidant properties of fermented beetroot juices enriched with different additives. *Foods*, *13*(1), art. no. 102. https://doi.org/10.3390/foods13010102
10. Janiszewska, E. (2014). Microencapsulated beetroot juice as a potential source of betalain. *Powder Technology*, *264*, 190-196. https://doi.org/10.1016/j.powtec.2014.05.032
11. Janiszewska-Turak, E., Walczak, M., Rybak, K., Pobiega, K., Gniewosz, M., Woźniak, Ł., Witrowa-Rajchert, D. (2022). Influence of fermentation beetroot juice process on the physico-chemical properties of spray dried powder. *Molecules*, *27*(3), art. no. 1008. https://doi.org/10.3390/molecules27031008
12. Karovicova, J., Kohajdova, Z. (2003). Lactic acid fermented vegetable juices. *Horticultural Science (Prague)*, *30*(4), 152-158. https://doi.org/10.17221/3878-HORTSCI
13. Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M.W., Rehman, A., Riaz, T., Aadil, R.M., Khan, I.M., Ozogul, F., Rocha, J.M., Esatbeyoglu, T., Korma,

S.A. (2023). Probiotics: mechanism of action, health benefits and their application in food industries. *Frontiers in Microbiology*, *17*(14), art. no. 1216674. https://doi.org/10.3389/fmicb.2023.1216674
14. Lopez, N., Puertolas, E., Condon, S., Raso, J., Alvarez, I. (2009). Enhancement of the solid-liquid extraction of sucrose from sugar beet (*Beta vulgaris*) by pulsed electric fields. *LWT – Food Science and Technology*, *42*(10), 1674-1680. https://doi.org/10.1016/j.lwt.2009.05.015
15. Lund, P.A., De Biase, D., Liran, O., Scheler, O., Mira, N.P., Cetecioglu, Z., Fernandez, E.N., Bover-Cid, S., Hall, R., Sauer, M., O'Byrne, C. (2020). Understanding how microorganisms respond to acid pH is central to their control and successful exploitation. *Frontiers in Microbiology*, *11*(24), art. no. 556140. https://doi.org/10.3389/fmicb.2020.556140
16. Manea, I., Buruleanu, L. (2010). Study of the effects shown by the action of various microorganisms on the lactic fermentation of juices. *Annals: Food Science and Technology*, *11*(1), 60-63.
17. Matthews, A., Grbin, P.R., Jiranek, V. (2006). A survey of lactic acid bacteria of interest to oenology. *Australian Journal of Grape and Wine Research*, *12*(3), 235-244. https://doi.org/10.1111/j.1755-0238.2006.tb00063.x
18. Molner, J.V., Soria, J.M., Perez-Gonzalez, R., Soria-Perpinya, X. (2023). Measurement of turbidity and total suspended matter in the Albufera of Valencia lagoon (Spain) using Sentinel-2 images. *Journal of Marine Science and Engineering*, *11*(10), art. no. 1894. https://doi.org/10.3390/jmse11101894
19. Montet, D., Ray, R.C., Zakhia-Rozis, N. (2014). Lactic acid fermentation of vegetables and fruits. In R.C. Ray, D. Montet. (Eds.), *Microorganisms and Fermentation of Traditional Foods*. 1st edition, CRC Press, Boca Raton, United States, pp. 108-140. https://doi.org/10.1201/b17307
20. Petrova, P., Arsov, A., Tsvetanova, F., Parvanova-Mancheva, T., Vasileva, E., Tsigoriyna, L., Petrov, K. (2022). The complex role of lactic acid bacteria in food detoxification. *Nutrients, 14*(10), art. no. 2038. https://doi.org/10.3390/nu14102038
21. Płatosz, N., Sawicki, T., Wiczkowski, W. (2020). Profile of phenolic acids and flavonoids of red beet and its fermentation products. Does long-term consumption of fermented beetroot juice affect phenolics profile in human blood plasma and urine? *Polish Journal of Food and Nutrition Sciences*, *70*(1), 55-65. https://doi.org/10.31883/pjfns/116613
22. Punia Bangar, S., Singh, A., Chaudhary, V., Sharma, N., Lorenzo, J.M. (2023). Beetroot as a novel ingredient for its versatile food applications. *Critical Reviews in Food Science and Nutrition*, *63*(26), 8403-8427. https://doi.org/10.1080/10408398.2022.2055529
23. Reina, L.D., Breidt, F., Fleming, H.P., Kathariou, S. (2005). Isolation and selection of lactic acid bacteria as biocontrol agents for nonacidified, refrigerated pickles. *Journal of Food Science*, *70*(1), M7-M11. https://doi.org/10.1111/j.1365-2621.2005.tb09050.x
24. Rodriguez-Gomez, F., Bautista-Gallego, J., Romero-Gil, V., Arroyo-Lopez, F.N., Garrido-Fernandez, A., Garcia-Garcia, P. (2012). Effects of salt mixtures on Spanish green table olive fermentation performance. *LWT – Food Science and Technology*, *46*(1), 56-63. https://doi.org/10.1016/j.lwt.2011.11.002
25. Sadowska-Bartosz, I., Bartosz, G. (2021). Biological properties and applications of betalains. *Molecules*, *26*(9), art. no. 2520. https://doi.org/10.3390/molecules26092520
26. Sawicki, T., Wiczkowski, W. (2018). The effects of boiling and fermentation on betalain profiles and antioxidant capacities of red beetroot products. *Food Chemistry*, *259*, 292-303. https://doi.org/10.1016/j.foodchem.2018.03.143
27. Slavov, A., Karagyozov, V., Denev, P., Kratchanova, M., Kratchanov, C. (2013). Antioxidant activity of red beet juices obtained after microwave and thermal pretreatments. *Czech Journal of Food Sciences*, *31*(2), 139-147. https://doi.org/10.17221/61/2012-CJFS
28. Staninska-Pięta, J., Czarny, J., Wolko, Ł., Cyplik, P., Drożdżyńska, A., Przybylak, M., Ratajczak, K., Piotrowska-Cyplik, A. (2023). Temperature, salinity and garlic additive shape the microbial community during traditional beetroot fermentation process. *Foods, 12*(16), art. no. 3079. https://doi.org/10.3390/foods12163079
29. Sun, L., Bai, C., Xu, H., Na, N., Jiang, Y., Yin, G., Liu, S., Xue, Y. (2021). Succession of bacterial community during the initial aerobic, intense fermentation, and stable phases of whole-plant corn silages treated with lactic acid bacteria suspensions prepared from other silages. *Frontiers in Microbiology*, *12*, art. no. 655095. https://doi.org/10.3389/fmicb.2021.655095
30. Szekely, D., Mate, M. (2022). Red beetroot (*Beta vulgaris* L.). In P. Kaushik (Ed.), *Advances in Root Vegetables Research*. IntechOpen, Budapest, Hungary, pp. 1-22. https://www.intechopen.com/chapters/83567
31. Viander, B., Maki, M., Palva, A. (2003). Impact of low salt concentration, salt quality on natural large-scale sauerkraut fermentation. *Food Microbiology*, *20*(4), 391-395. https://doi.org/10.1016/S0740-0020(02)00150-8
32. von Elbe, J.H.B. (2001). Betalains. *Current Protocols in Food Analytical Chemistry*, F3.1.1-F3.1.7. https://doi.org/10.1002/0471142913.faf0301s00
33. Walkowiak-Tomczak, D., Zielińska, A. (2006). Effect of fermentation conditions on red-beet leaven quality. *Polish Journal of Food and Nutrition Sciences*, *56*(4), 437-444.
34. Yasaminshirazi, K., Hartung, J., Fleck, M., Graeff-Hoenninger, S. (2020). Bioactive compounds and total sugar contents of different open-pollinated beetroot genotypes grown organically. *Molecules*, *25*(21), art. no. 4884. https://doi.org/10.3390/molecules25214884
35. Zhang, W.H., Wu, J., Weng, L., Zhang, H., Zhang, J., Wu, A. (2020). An improved phenol-sulfuric acid method for the determination of carbohydrates in the presence of persulfate. *Carbohydrate Polymers*, *227*, art. no. 115332. https://doi.org/10.1016/j.carbpol.2019.115332
36. Zielinska, M., Markowski, M. (2012). Color characteristics of carrots: Effect of drying and rehydration. *International Journal of Food Properties*, *15*(2), 450-466. https://doi.org/10.1080/10942912.2010.489209
37. Zommiti, M., Feuilloley, M.G.J., Connil, N. (2020). Update of probiotics in human world: A nonstop source of benefactions till the end of time. *Microorganisms*, *8*(12), art. no. 1907. https://doi.org/10.3390/microorganisms8121907

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Cellulase Treatment of Acerola Seeds and Its Effect on Physicochemical Properties and Antioxidant Potential of Dietary Fiber-Rich Cookies

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Acerola seeds are a by-product of the food industry, which is rich in dietary fiber and antioxidants. This study evaluated the effects of cellulase treatment conditions, including the initial moisture content, enzyme dose, and incubation time, on the insoluble, soluble, and total dietary fiber content of acerola seed powder (ASP). The blends of wheat flour with untreated and cellulase-treated ASP (0, 10, 15, 20, 25, and 30%, *w/w*) were then used to produce cookies. The suitable conditions for the enzymatic treatment were the initial moisture content of 6 g/g dry weight (DW), an enzyme dose of 10 U/g DW and incubation time of 90 min. The cookies produced from flour blends with ASP had higher dietary fiber, ascorbic acid and total phenolic contents, and antioxidant capacity, compared to the control cookies without ASP. Phytate content in the cookies obtained with the lowest level of fortification (10%, *w/w*) was similar to that in the control cookies. The use of cellulase-treated ASP resulted in a lower ratio of insoluble to soluble dietary fiber in the cookies compared to when the untreated ASP was used. In addition, the cookies with cellulase-treated ASP had lower hardness and higher fracturability values than those fortified with the untreated ASP. The overall acceptability of the cookies produced with ASP was higher or comparable to the control cookies. For the first time, the low-cost ASP was used to improve the nutritional quality of cookies. The treated ASP is a novel promising dietary fiber- and antioxidant-rich ingredient for cookie preparation.

Keywords: bakery product, cellulolysis, dimensional characteristics, *Malpighia emarginata;* proximate composition, textural analysis

ABBREVIATIONS

ASP, acerola seed powder; D, diameter; DPPH•, 2,2-diphenyl--1-picrylhydrazyl radical; DW, dry weight; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; IDF, insoluble dietary fiber; OHC, oil holding capacity; SDF, soluble dietary fiber; T, thickness; TDF, total dietary fiber; TE, Trolox equivalent; WHC, water holding capacity.

INTRODUCTION

Acerola (*Malpighia emarginata*) also known as Barbados cherry or West Indian cherry belongs to Malpighiaceae family, which has recently attracted the attention of consumers thanks to its high ascorbic acid content in fruits [Poletto *et al.,* 2021]. The global acerola extract market is anticipated to reach 17.5 billion dollars by 2026 due to the high demand for natural bioactive-rich fruits

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and derivatives [Belwal *et al.*, 2018]. The processing of acerola fruit generates considerable amounts of by-products which account for 30% of seeds and peels, and 10% of sludge [Poletto *et al.*, 2021]. Developing new methods that add value to these by-products to minimize the cost of waste management and promote their valorization is therefore essential.

Acerola seeds contain high levels of insoluble dietary fiber (IDF) (75.66 g/100 g dry weight, DW), soluble dietary fiber (SDF) (4.76 g/100 g DW), phenolic compounds (4.73 g/100 g DW) and even ascorbic acid (457.32 mg/100 g DW) [Marques *et al.*, 2013]. Consequently, acerola seeds can be considered as a supplement, rich in both dietary fiber and phenolic compounds, to develop new food products with various health benefits. Dietary fiber has significant implications for gastrointestinal disorders [Gill *et al.*, 2021] and elicits other various benefits to human health, such as supporting the treatment of eating disorders, diabetes and obesity [Basu *et al.*, 2021]. Moreover, products rich in phenolic compounds and dietary fiber exert preventive effects against cardiovascular diseases, obesity, type 2 diabetes and cancers [Khan *et al.*, 2022]. It was reported that acerola seed flour was added to the recipe of cereal bars to enhance their dietary fiber and phenolic contents [Marques *et al.*, 2015].

SDF and IDF serve different physicochemical and physiological functions. IDF improves the movement of material through the gastrointestinal tract facilitating laxation, while SDF supports the growth of gut microbiota and plays an essential role in attenuating the glycemic reaction and plasma cholesterol level [Gill *et al.*, 2021]. The IDF to SDF ratio of a food product provides important information on its nutritional and physiological effects on the consumers. According to the American Dietetic Association, the IDF to SDF ratio should be 3:1 to ensure the maximum health benefits of dietary fiber [Borderías *et al.*, 2005]. Nevertheless, the IDF to SDF ratio of the cereal bars enriched with acerola seed flour was very high and varied from 8:1 to 17:1 [Marques *et al.*, 2015].

Many techniques, such as mechanical degradation, chemical and biochemical treatment, and microbiological fermentation of food by-products, were applied to reduce the IDF to SDF ratio by partial conversion of IDF to SDF [Gan *et al.*, 2021]. Among these methods, enzymatic treatment has attracted considerable attention due to a reduced loss in bioactive compounds and environmental friendliness [Ta *et al.*, 2023]. Deoiled rice bran processed via cellulase treatment improved the quality of dietary fiber attributed to the increase in SDF and a decrease in the IDF to SDF ratio [Cao *et al.*, 2022]. Moreover, cookies incorporated with the cellulase-treated deoiled rice bran, wheat bran, or spent tea leaves were found to have an improved soluble dietary fiber content, a decreased hardness and an increased overall acceptability [Cao *et al.*, 2022; Nguyen *et al.*, 2021; Nguyen *et al.*, 2022].

To the best of our knowledge, the cellulolytic treatment of acerola seeds has not been reported in the literature. In this study, ASP was treated by cellulase at different conditions to investigate the effects of this treatment on its fiber profile and evaluate the quality of cookies fortified with the cellulase-treated ASP.

MATERIALS AND METHODS

Materials and reagents

Acerola (*Malpighia emarginata*) fruits originated from a local farm in Tien giang province (Vietnam). At the laboratory, the fruits were selected, washed with potable water, blanched at 90°C for 2 min and manually pulped to collect acerola seeds. The seeds were subsequently dried at 50°C for 7–8 h to the moisture content of about 13 g/100 g. The dried seeds were ground, and the obtained acerola seed powder (ASP) was preserved in plastic bags at room temperature for further experimentation. Wheat flour originated from Binh dong Flour Co. (Ho Chi Minh City, Vietnam).

Celluclast® 1.5L with endo-1,4-β-glucanase activity (optimal temperature and pH – 50°C and 4.5–6.0, respectively; catalytic activity – 600 endoglucanase units (EGU)/mL) was purchased from Novozymes A/S (Bagsværd, Denmark). Chemicals of the analytical grade were bought from Aldrich-Sigma (Saint Louis, MO, USA).

Enzymatic treatment of acerola seed powder

The enzymatic treatment of ASP with Celluclast® 1.5L was carried out in 500-mL Erlenmeyer flasks. About 50 g of ASP were placed in each flask. The cellulase preparation was diluted in different volumes of tri-sodium citrate buffer (pH 5.0) and subsequently added into the flasks to achieve the required moisture content of ASP and endoglucanase activity. In the first series, the initial moisture contents of ASP were adjusted to 2, 4, 6, 8, and 10 g water/g DW of seeds, while the cellulase dose and incubation time were fixed at 10 U/g DW of seeds and 60 min, respectively. In the second series, the initial enzyme doses were 0, 2.5, 5, 7.5, 10, and 12.5 U/g DW of seeds, while the treatment was conducted at the initial moisture content of 6 g water/g DW of seeds for 1 h. In the third series, the treatment time was 0, 30, 60, 90, and 120 min, while the initial moisture content and cellulase dose were 6 g water/g DW of seeds and 10 U/g DW of seeds, respectively. The reactions were performed at 50°C in an incubator (Memmert, Schwabach, Germany). The heat treatment by using a water bath at 95°C for 10 min was used to inactivate the enzyme. The obtained products were dried at 60°C in a convection oven until the final moisture content of 10 g/100 g was reached. Dried cellulase-treated ASP was sieved through a 40-mesh screen and stored at 4°C. The chemical composition, antioxidant activity, water holding capacity (WHC) and oil holding capacity (OHC) of the untreated ASP and cellulase-treated ASP were determined under following conditions: initial moisture content of 6 g/g DW, enzyme dose of 10 U/g DW, and treatment time of 90 min. Both ASP types were also used in cookie recipe.

Cookie production at a laboratory scale

The cookie formula used in this study included 200 g flour blend or wheat flour, 70 g isomalt, 70 g raw whole egg, 70 g shortening, 2.4 g sodium bicarbonate, 1.4 g lecithin, 1.0 g table salt, 0.18 g acesulfame potassium, 0.6 g vanilla extract, and 13.0 g water. Flour blends were prepared from wheat flour partially replaced (10, 15, 20, 25 and 30%, *w/w*) by the untreated or cellulase-treated ASP. Cookies were coded as C (control cookie produced without

ASP); AS10, AS15, AS20, AS25 and AS30 (cookies prepared using flour blends with 10, 15, 20, 25 and 30% (*w/w*) untreated ASP, respectively); CAS10, CAS15, CAS20, CAS25 and CAS30 (cookies from flour blends with 10, 15, 20, 25 and 30% (*w/w*) cellulase--treated ASP, respectively).

The cookie production was conducted as previously described [Cao *et al.*, 2022]. The cream was performed using a mixer (Model M8, Vietnam Unie Co., Ltd, Vietnam). Whole eggs were whipped at 200 rpm for 4 min. Isomalt and the solution of table salt and acesulfame potassium were incorporated into the whipped egg. The mixture was whipped at 200 rpm for 4 min and creamed with lecithin and butter (200 rpm, 3 min). Vanilla extract and sodium bicarbonate were added to the cream (200 rpm, 3 min). The dough was formed by mixing the final cream with wheat flour or wheat flour with ASP (100 rpm, 2 min). Cookies with a thickness of 4 mm and a diameter of 35 mm were formed by a rolling pin and circular mold, and then baked in a reel oven (VH509S, Sanaky Co., Ho Chi Minh City, Vietnam) at 175°C for 10 min and flowed at 150°C for 9 min. The baked cookies were cooled to room temperature for 30 min and sealed in transparent low-density polyethylene (LDPE) zip bags (Saigon Indochina Co., Ho Chi Minh City, Vietnam). Afterwards, they were kept at room temperature for a minimum of 24 h prior to further analysis.

Determination of chemical composition

The moisture content of wheat flour, ASP and cookies was measured by drying to constant weight at 105°C using a moisture analyzer (A&D Co., Tokyo, Japan). The protein content of wheat flour, ASP and cookies was analyzed using the AOAC International 979.09 method (Kjeldahl method) [AOAC, 2000]. Lipid content was determined by Soxhlet extraction with diethyl ether solvent according to the AOAC International 920.39 method [AOAC, 2000]. Ash content was quantified by incineration at 600°C in a muffle furnace (Hope Valley, Lenton Co., UK) using AOAC International 942.05 method [AOAC, 2000]. Total carbohydrate content was determined by the phenol–sulfuric acid method [Nielsen, 2010]. Starch was quantified using the starch digestion method previously described by Landhäusser *et al.* [2018]. Starch was broken down using α-amylase (A4551, Sigma Aldrich, Saint Louis, MO, USA) at 85°C for 2 h, and the solids were separated by a centrifuge at 13,000×*g* for 1 min; the supernatant was hydrolyzed into glucose using amyloglucosidase (Sigma Aldrich, A7095) at 55°C for 2 h, and the resulting glucose in the hydrolysate was quantified using an assay with 3,5-dinitrosalicylic acid (DNS) [Miller, 1959]. Total dietary fiber (TDF), IDF and SDF contents were analyzed following the AOAC International 991.43 method [AOAC, 2010]. Briefly, starch and protein were solubilized and hydrolyzed using α-amylase, protease, and amyloglucosidase. For the measurement of IDF, the digested sample was filtered, and the IDF was determined after subtracting ash and protein weight in the residue. To measure SDF, ethanol 95% (*v/v*) was added to the filtrate at 60°C with a ratio of 1:4 (*v/v*) to precipitate soluble fibers. The precipitate was collected by filtration. The SDF was determined after correcting its ash and protein in the precipitate. The sum of IDF and SDF contents represents TDF content. The results of the above mentioned analyses were expressed as g/100 g DW of wheat flour, ASP or cookies.

The phytate content of wheat flour, ASP and cookies was determined by spectrophotometric method formerly outlined by Sureshkumar *et al.* [2015]. About 1 g of the sample was extracted with 20 mL of 0.5 M HNO_3. The filtrate was adjusted to an appropriate volume with distilled water. Then, 1.4 mL of this solution was boiled with 1 mL of a ferric ammonium sulfate solution (21.6 mg/100 mL distilled water) for 20 min. The mixture was cooled and mixed with 5 mL of isopentanol, followed by adding and shaking with 0.1 mL of ammonia solution. The alcoholic layer was separated by centrifugation (500×*g*, 10 min) to determine the color intensity at 465 nm. Alcohol was used as the blank, and sodium phytate was used as the standard. Ascorbic acid content was measured by the spectrophotometric method previously reported by Bajaj & Kaur [1981]. Results of both analyses were expressed as mg/100 g DW of wheat flour, ASP or cookies.

Determination of total phenolic content and antioxidant capacity

About 1 g of ASP, wheat flour or cookies was extracted with 10 mL of an aqueous acetone solution (60%, *v/v*) at room temperature for 30 min. The mixture was then centrifuged at 1,600×*g* for 20 min (3K30, Sigma Zentrifugen Ltd., Osterodeam Harz, Germany). The volume of the supernatant was adjusted to 100 mL with a solvent, and the extract was stored at –4°C in the dark. It was used within 2 days for the determination of total phenolic content and antioxidant capacity.

The total phenolic content was determined by a spectrophotometric method developed by Singleton & Rossi [1965] using Folin–Ciocalteu reagent and the result was expressed as mg gallic acid equivalent (GAE)/g DW.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured using a previously published method [Brand-Williams *et al.*, 1995]. The diluted extract (0.1 mL) was mixed with 3.9 mL of 60 µM methanol DPPH solution. The absorbance at 515 nm was measured at 0 and after 30 min of incubation against methanol as a blank. Iron (III) reducing antioxidant power (FRAP) assay was carried out using the method of Benzie & Strain [1999] with some modifications. The reagent solution was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tris(2-pyridyl)-*s*-triazine solution in 40 mM HCl (2.5 mL), and 20 mM $FeCl_3 \times 6H_2O$ (2.5 mL). The diluted extract (0.2 mL) was mixed with 3.8 mL of the FRAP reagent solution and incubated in the dark at 37°C for 5 min. The absorbance was measured at 593 nm against a reagent blank. The results were expressed as µmol Trolox equivalent (TE)/g DW.

Determination of physical properties

WHC and OHC of untreated, cellulase-treated ASP and wheat flour were measured following AACC approved methods of analysis (56-30.01 method) [AACC, 1983] and the method described by Nguyen *et al.* [2022], respectively. Briefly, to determine WHC, 3 g of the sample were mixed with 30 mL of distilled water. After 24 h at room temperature, the mixture was centrifuged (1,000×*g*

for 20 min at 20°C). The supernatant was removed to determine the weight of the hydrated material. WHC was expressed as g H_2O/g DW. Similarly, OHC was determined in the same procedure in which distilled water was replaced by soybean oil. OHC was expressed as g oil/g DW.

The thickness and diameter of cookies were measured by the method described by Park *et al.* [2015] using a micrometer. Thickness (T) was calculated by the average thickness of the stack of six cookies. Diameter (D) was determined by the average width of six cookies put edge to edge. The spread factor (D/T) was the quotient of diameter and thickness.

Hardness and fracturability of cookies were explored by a three-point break test with a test speed of 1.0 mm/s, distance between two beams of 20 mm, trigger force auto of 50 g, with a 5 kg loading cell and 5 readings taken with TA-XT plus texture analyzer (Stable Micro Systems, Haslemere, UK).

Color of wheat flour and ASP, and surface color of cookies was measured by Konica Minolta CR400 Chromameter (Osaka, Japan) in CIELAB space. The color parameters included lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*). The total color difference (ΔE) was calculated according to the formula (1):

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

where: L_0^*, a_0^*, b_0^* are the color values of the control cookie; L^*, a^*, b^* are the color values of the cookie prepared using flour blends.

Determination of overall acceptability

The test of overall acceptability of cookies was carried out with 60 participants (both men and women from 18 to 50 years old) recruited among students and staff of the Ho Chi Minh City University of Technology (Ho Chi Minh City, Vietnam). Each cookie samples were coded by a random three-digit number and served randomly. The overall acceptability of cookies was rated on a nine-point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like) [Nguyen *et al.*, 2021].

Statistical analysis

Each type of cookies was prepared at least three times and all experimental results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was applied to determine the differences between means using Minitab 16 software (Minitab Co., State College, PA, US). Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Effects of cellulolytic reaction conditions on fiber contents of acerola seed powder

The significant alteration was found in dietary fiber profile of the ASP treated with cellulose under different conditions including initial moisture content, cellulose preparation dose, and reaction time (**Figure 1**). Increasing initial moisture content from 2 to 6 g water/g DW of seeds remarkably increased the SDF content from 3.9 to 7.4 g/100 g DW, but the SDF content was reduced when the initial moisture content further increased (**Figure 1A–C**). Meanwhile, the IDF content gradually decreased from 57.2 to 51.1 g/100 g DW. Hence, the lowest IDF to SDF ratio was found at 6 g water/g DW of acerola seeds. Regarding the effects of enzyme dose on ASP dietary fiber content, the SDF content was significantly improved by 185% as the enzyme dose increased from 0 to 10 U/g DW of seeds, then slightly dropped to 6.2 g/100 g DW at 12.5 U/g DW (**Figure 1D–F**). As expected, the lowest IDF to SDF ratio was achieved at an enzyme dose of 10 U/g DW. In addition, the SDF content was elevated by 204% while the IDF content was decreased by 15% after 90 min of cellulolysis (**Figure 1G–I**). The prolonged incubation time to 120 min led to a drop in both SDF and IDF contents. As a result, the IDF to SDF ratio reached its minimum value of 6.5 at 90 min of enzymatic reaction, however this value did not differ significantly ($p\geq0.05$) from the ratios determined at incubation times of 60 and 120 min.

The changes in the ASP dietary fiber profile with respect to the initial water content were in agreement with our previous studies, in which wheat bran was treated with a cellulase preparation at different conditions and the increased moisture content from 0.25 to 0.75 g water/g DM enhancing the content of SDF and decreasing IDF to SDF ratio [Nguyen *et al.*, 2021]. Moisture plays an important role in hydrolytic yield since it probably affects cellulase diffusion and absorption on the cellulose surface which is a prerequisite step in cellulolysis mechanism [Wang *et al.*, 2011]. In the enzymatic treatment of deoiled rice bran, as the cellulase dose increased from 0 to 2 U/g DM, the IDF content decreased by 5% while the SDF content increased by 26% [Cao *et al.*, 2022]. The higher cellulase doses up to 20 U/g resulted in a noticeable transformation of IDF to SDF in the enzymatic treatment of spent green tea leaves [Nguyen *et al.*, 2022]. Generally, doses of cellulase causing a significant effect on the dietary fiber profile in the current study did not agree with those in the previous studies, because of the different origins of cellulosic materials. However, the SDF content of ASP was significantly reduced in all three set experiments at the high initial moisture content (8–10 g water/g DW), excess cellulase dose (12.5 U/g DW), and elongating incubation time (120 min). This phenomenon could be attributed to the intense hydrolysis of dietary fiber into low molecular-weight carbohydrates such as short-chain oligosaccharides and sugars [Bera *et al.*, 2015]. It should be noted that the carbohydrates with the degrees of polymerization 3–9 scarcely precipitate in 78% ethanol solution contributing to the loss in SDF content [Dai & Chau, 2017].

Physicochemical properties and antioxidant capacity of untreated and cellulase-treated acerola seed powder

The cellulolytic reaction conditions at the initial moisture content of 6 g/g DW with an enzyme dose of 10 U/g DW for 90 min were applied for the preparation of the cellulase-treated ASP, whose the chemical composition, antioxidant capacity and physical properties are shown in **Table 1**. The results achieved for the untreated ASP and wheat flour were also presented (**Table 1**). The cellulase-treated ASP had a 207% higher SDF content

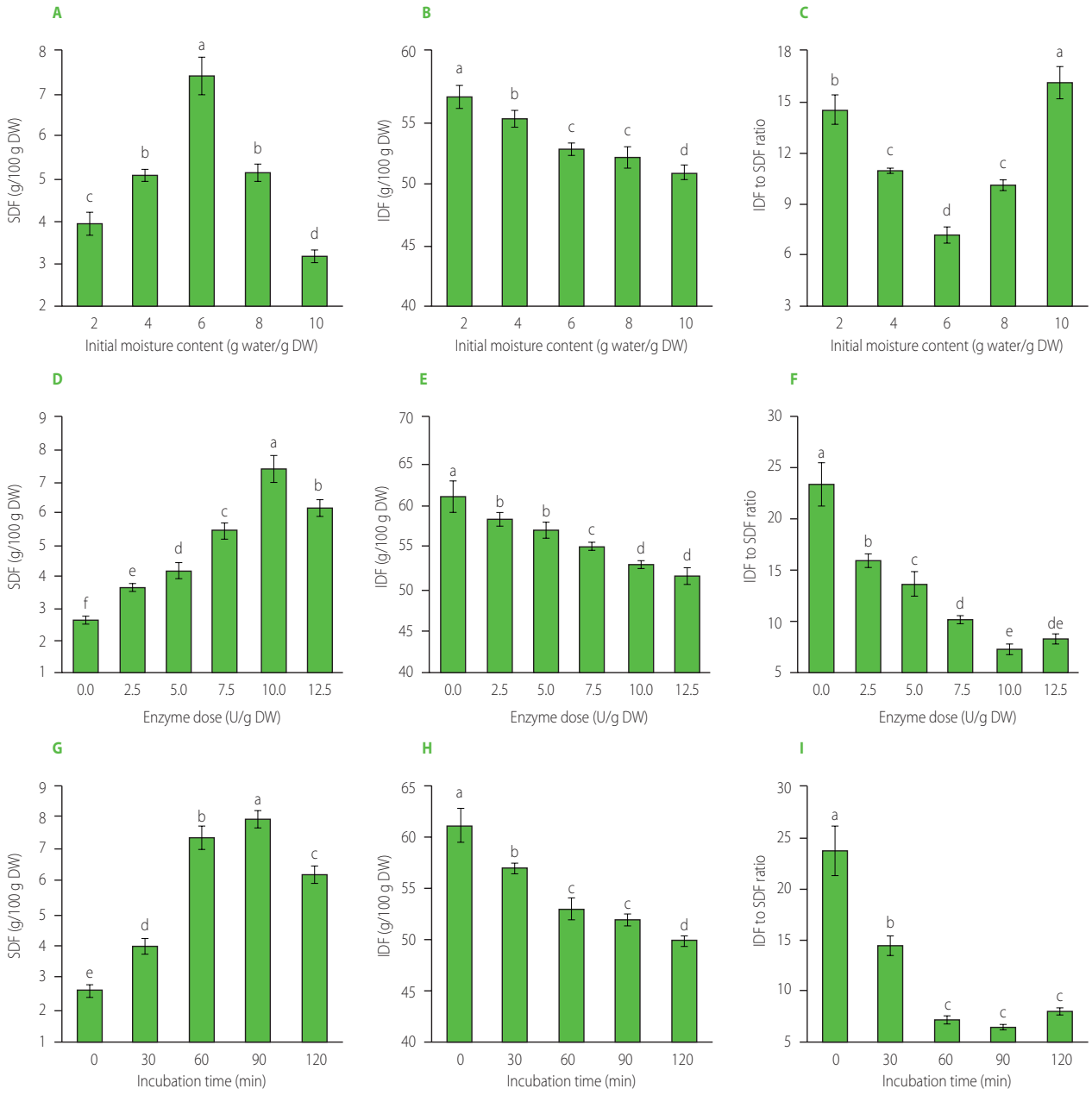

Figure 1. Content of insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and ratio of IDF to SDF of acerola seed powder treated with cellulase at different initial moisture contents and a constant cellulase dose of 10 U/g dry weight (DW) for 60 min (**A–C,** respectively), different enzyme doses and a constant initial moisture content of 6 g water/g DW for 60 min (**D–F**, respectively), and different reaction times and an initial moisture content of 6 g water/g DW and an enzyme dose of 10 U/g DW (**G–I**, respectively). Different letters (a-e) above bars indicate significant differences ($p<0.05$).

and 73% lower IDF to SDF ratio than the untreated counterpart, whereas the protein, lipid, and starch contents changed insignificantly ($p\geq0.05$) after the treatment. Both cellulase-treated and untreated ASP contained a significantly ($p<0.05$) higher amount of IDF, SDF, TDF, and ash, and lower amount of starch and protein than wheat flour. Moreover, phytate content was higher in both types of ASP (87.2–89.8 mg/100 g DW) than in wheat flour. The dietary fiber profile of the untreated ASP was comparable to that reported by Marques *et al*. [2013] who determined the SDF and IDF contents of 4.8 and 75.7%, respectively. Besides, the higher contents of ash in the powder of acerola fruit residue, consisting of seeds and peels (2.07%) and phytates in seed flour (230 mg/100 g DW) respectively, were previously reported [Marques *et al.*, 2013; Sancho *et al.,* 2015]. The differences could be due to the dissimilarities in acerola cultivars, the growing and processing conditions. Furthermore, the higher ash content of the treated ASP compared to that of the untreated one may be explained by using trisodium citrate buffer in the enzymatic process.

The contents of ascorbic acid and total phenolics of the untreated ASP were 476 mg/100 g DW and 25.6 mg GAE/g DW, respectively (**Table 1**). Similar ascorbic acid content was found previously in acerola seed flour (457.32 mg/100 g DW) [Marques *et al.*, 2013] and lower one in the powder from seeds and peels

Table 1. Proximate composition, antioxidant contents, antioxidant capacity, and physical properties of untreated acerola seed powder (ASP), cellulase-treated ASP and wheat flour.

Parameter	Untreated ASP	Cellulase-treated ASP	Wheat flour
Protein (g/100 g DW)	7.6±0.1^{b}	7.8±0.2^{b}	10.7±0.6^{a}
Lipid (g/100 g DW)	2.6±0.0^{a}	2.7±0.1^{a}	2.4±0.4^{a}
Ash (g/100 g DW)	1.3±0.1^{b}	5.5±0.2^{a}	0.4±0.0^{c}
Starch (g/100 g DW)	5.6±0.4^{b}	5.5±1.2^{b}	82.3±1.8^{a}
Carbohydrate (g/100 g DW)	88.4±0.1^{a}	84.0±0.4^{b}	84.3±1.1^{b}
SDF (g/100 g DW)	2.6±0.2^{b}	8.0±0.1^{a}	1.6±0.2^{c}
IDF (g/100 g DW)	61.0±1.8^{a}	51.5±1.7^{b}	1.9±0.4^{c}
TDF (g/100 g DW)	63.6±1.7^{a}	59.5±1.7^{b}	3.5±0.3^{c}
IDF to SDF ratio	23.7±2.1^{a}	6.4±0.2^{b}	1.2±0.3^{c}
Phytate (mg/100 g DW)	87.2±4.3^{a}	89.8±3.1^{a}	45.0±1.0^{b}
Ascorbic acid (mg/100 g DW)	476±21^{a}	247±12^{b}	ND
Total phenolic content (mg GAE/g DW)	25.6±0.6^{a}	23.9±0.5^{b}	1.4±0.1^{c}
DPPH$^{\bullet}$ scavenging activity (μmol TE/g DW)	319.1±4.5^{a}	233.3±4.9^{b}	0.2±0.0^{c}
Ferric reducing antioxidant power (μmol TE/g DW)	334.7±1.1^{a}	152.5±8.0^{b}	1.3±0.2^{c}
Water holding capacity (g water/g DW)	3.88±0.20^{a}	2.88±0.06^{b}	0.91±0.05^{c}
Oil holding capacity (g oil/g DW)	2.98±0.14^{a}	2.72±0.10^{b}	0.94±0.02^{c}
L^*	61.4±0.4^{b}	60.3±0.5^{c}	94.3±0.1^{a}
a^*	9.2±0.1^{b}	9.3±0.0^{a}	0.4±0.0^{c}
b^*	21.6±0.2^{a}	21.6±0.3^{a}	6.9±0.0^{b}
ΔE	37.1±0.4^{b}	38.1±0.4^{a}	0.0±0.0^{c}

Results are shown as mean ± standard deviation. Different letters in the same rows indicate significant differences ($p<0.05$). IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber; DW, dry weight; DPPH$^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; GAE, gallic acid equivalent; TE, Trolox equivalent; L^*, lightness; a^*, greenness (–) to redness (+); b^*, blueness (–) to yellowness (+); ΔE, total color difference; ND, not detected.

(170.73 mg/100 g) [Sancho *et al.,* 2015]. The total phenolic content of ASP was higher than that in the range of 173.30–1155.2 mg GAE/100 g DW reported in different studies of the acerola fruit residue (seeds and peels) powder [Carvalho Gualberto *et al.*, 2021; Sancho *et al.,* 2015; Silva *et al.*, 2021]. For the cellulase-treated ASP, the ascorbic acid content, total phenolic contents and the antioxidant capacity determined by DPPH and FRAP assays were reduced by 48, 7, 27 and 54%, respectively, as compared to those of the untreated counterpart (**Table 1**). A decrease in the total phenolic content and antioxidant capacity during the cellulase treatment and drying of dietary-fiber rich material was also reported in previous studies [Garau *et al.*, 2007; Nguyen *et al.*, 2022]. According to Garau *et al.* [2007], soluble dietary fiber and antioxidants may be modified or degraded during the extended air-drying period and/or high temperature.

It was noticed that the WHC and OHC of the untreated and cellulase-treated ASP were much higher than those of wheat flour (**Table 1**). A possible reason is related to the enormous amount of TDF in both ASP samples. The cellulolytic treatment caused a decrease in both WHC and OHC of ASP by 26 and 9%, respectively due to the lower IDF content. In comparison to SDF, IDF has a porous matrix structure formed by polysaccharide chains that can hold large amounts of water through hydrogen bonds [Zhu *et al.*, 2010].

The results in **Table 1** also reveal that redness (a^* values) and yellowness (b^* values) of both untreated and cellulase-treated ASP were higher, and that both powders were darker than wheat flour. The possible reason is that acerola seeds may contain color compounds.

■ Effects of partial replacement of wheat flour with acerola seed powder on the cookie quality

The chemical composition, antioxidant contents and antioxidant capacity of cookies produced from blends of wheat flour with untreated and cellulase-treated ASP are shown in **Table 2**. The use of untreated or cellulase-treated ASP in the recipe resulted in a slight enhancement of moisture, lipid, ash, and phytate contents in the cookies, but also in a considerable reduction of protein and starch contents. It can be explained by the significantly different composition between ASP and wheat flour

Table 2. Proximate composition, antioxidant contents and antioxidant capacity of cookies from wheat flour (control) and from blends of wheat flour with different proportion (10, 15, 20, 25 and 30%, *w/w*) of untreated acerola seed powder (AS10, AS15, AS20, AS25 and AS30, respectively) and cellulase-treated acerola seed powder (CAS10, CAS15, CAS20, and CAS30, respectively).

Parameter	Control	AS10	AS15	AS20	AS25	AS30	CAS10	CAS15	CAS20	CAS25	CAS30
Moisture (g/100 g)	4.3±0.2^{b}	4.3±0.2^{bA}	4.4±0.1^{abA}	4.4±0.1^{abA}	4.4±0.1^{abA}	4.5±0.1^{aA}	4.3±0.1^{bA}	4.3±0.1^{abA}	4.4±0.1^{abA}	4.4±0.1^{abA}	4.5±0.1^{aA}
Protein (g/100 g DW)	8.0±0.1^{a}	7.4±0.2^{bA}	7.2±0.0^{cA}	6.8±0.3^{dA}	6.6±0.0^{deA}	6.5±0.1^{eA}	7.4±0.2^{cA}	7.2±0.1^{cA}	6.9±0.1^{dA}	6.6±0.1^{eA}	6.5±0.1^{eA}
Lipid (g/100 g DW)	24.3±0.2^{b}	24.8±0.2^{bA}	24.9±0.5^{bA}	25.9±0.3^{aA}	26.0±1.0^{aA}	26.2±0.6^{aA}	24.8±0.4^{bA}	24.9±0.2^{bA}	26.0±0.4^{aA}	26.1±0.4^{aA}	26.3±0.4^{aA}
Ash (g/100 g DW)	1.1±0.1^{f}	1.1±0.0^{fA}	1.1±0.1^{efA}	1.2±0.1^{efA}	1.2±0.0^{eA}	1.2±0.0^{eA}	1.5±0.1^{dB}	1.7±0.1^{cB}	1.9±0.0^{bB}	2.0±0.0^{aB}	2.1±0.1^{aB}
Starch (g/100 g DW)	42.3±2^{a}	38.8±2.3^{bA}	37.3±1.9^{bcA}	34.6±0.9^{cA}	30.5±0.2^{dA}	26.6±2.7^{eA}	38.2±0.5^{bA}	36.8±0.5^{bA}	34.0±0.6^{cA}	29.8±0.6^{dA}	26.0±1.0^{eA}
SDF (g/100 g DW)	0.7±0.1^{f}	0.8±0.0^{bcB}	0.8±0.0^{bcB}	0.8±0.0^{bB}	0.8±0.0^{bB}	0.9±0.0^{aB}	1.0±0.0^{eA}	1.2±0.0^{dA}	1.3±0.1^{cA}	1.5±0.1^{bA}	1.7±0.1^{aA}
IDF (g/100 g DW)	0.9±0.1^{f}	3.2±0^{eA}	4.6±0.2^{dA}	5.9±0.1^{cA}	7.5±0.3^{bA}	8.3±0.2^{aA}	2.8±0.1^{eB}	4.1±0.0^{dB}	5.1±0.1^{cB}	6.4±0.2^{bB}	7.3±0.3^{aB}
TDF (g/100 g DW)	1.6±0.0^{f}	4.0±0.1^{eA}	5.4±0.2^{dA}	6.7±0.1^{cA}	8.3±0.3^{bA}	9.2±0.1^{aA}	3.8±0.1^{eA}	5.3±0.0^{dA}	6.4±0.1^{cA}	7.8±0.2^{bA}	9.0±0.3^{aA}
IDF to SDF ratio	1.2±0.1^{e}	4.3±0.2^{dA}	5.9±0.4^{cA}	7.4±0.2^{bA}	9.2±0.7^{aA}	9.1±0.5^{aA}	2.8±0.1^{dB}	3.5±0.2^{cB}	4.0±0.3^{abB}	4.4±0.3^{aB}	4.4±0.2^{aB}
Phytate (mg/100 g DW)	20.3±0.4^{e}	20.6±0.3^{eA}	23.4±0.8^{dA}	23.7±0.6^{cA}	25.3±0.9^{bA}	27.8±0.9^{aA}	21.3±0.8^{eA}	23.6±0.6^{dA}	24.1±1.0^{cA}	25.4±0.8^{bA}	27.1±0.5^{aA}
Ascorbic acid (mg/100 g DW)	30.2±4.2^{e}	61.3±2.4^{dA}	81.5±4.8^{cA}	86.2±2.1^{bcA}	89.0±2.0^{bA}	97.1±4.4^{aA}	39.3±1.8^{dB}	42.7±1.9^{cB}	44.6±1.4^{bB}	47.3±1.8^{abB}	51.3±2.9^{aB}
Total phenolic content (mg GAE/g DW)	0.7±0.01^{f}	2.5±0.1^{eA}	2.7±0.1^{dA}	3.0±0.1^{cA}	3.2±0.1^{bA}	3.4±0.1^{aA}	2.0±0.1^{eB}	2.1±0.1^{dB}	2.5±0.1^{cB}	2.7±0.1^{bB}	3.1±0.1^{aB}
DPPH$^{\bullet}$ scavenging activity (µmol TE/g DW)	0.3±0.1^{e}	7.0±0.8^{dA}	8.2±0.7^{cA}	9.8±22^{bA}	11.2±0.3^{aA}	11.5±0.5^{aA}	5.6±0.4^{dB}	6.1±0.2^{dB}	7.6±0.3^{cB}	8.3±0.6^{bB}	9.2±0.5^{aB}
Ferric reducing antioxidant power (µmol TE/g DW)	4.7±0.7^{f}	18.8±1.0^{eA}	22.4±0.8^{dA}	28.0±0.6^{cA}	38.2±0.7^{bA}	47.1±0.8^{aA}	14.1±1.4^{eB}	16.8±0.7^{dB}	20.7±0.6^{cB}	23.9±1.0^{bB}	29.2±1.0^{aB}

Results are shown as mean ± standard deviation. In the same row, different lower-case letters (a–f) indicate significant differences between all cookie types, while upper-case letters (A and B) show significant differences between cookies produced with cellulase-treated and untreated acerola seed powder separately for each fortification level ($p<0.05$). IDF, insoluble dietary fiber; SDF, soluble dietary fiber; TDF, total dietary fiber; DW, dry weight; DPPH$^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; GAE, gallic acid equivalent; TE, Trolox equivalent.

(**Table 1**). The significantly higher moisture contents determined in the fortified cookies with 30% (*w/w*) replacement (AS30 and CAS30) compared to the control were due to the high WHC of dietary fiber in the supplement. At the same replacement levels, the differences between the cookies with cellulase-treated and untreated ASP were insignificant ($p \geq 0.05$) in terms of moisture, protein, lipid, and starch contents.

The use of ASP in the cookie recipe greatly improved its dietary fiber contents, specifically, at the 30% (*w/w*) replacement of wheat flour with ASP (**Table 2**). The TDF, IDF and SDF contents of the cookies produced from the flour blend with this level of replacement with the untreated ASP increased by 475, 822 and 29%, while those of the cookies with the treated counterpart were enhanced by 463, 711 and 143%, respectively, as compared to those of the control sample. The cookies produced from flour blends with 20, 25, and 30% (*w/w*) ASP were "high in fiber" food because their IDF contents were higher than 6 g/100 g [European Parliament & Council, 2006]. In the case of the developed cookies with the untreated ASP, an impressive increase in SDF content was not achieved compared to the control cookies (**Table 2**), because of the minor differences in SDF content between the untreated ASP (2.6 g/100 g DW) and wheat flour (1.6 g/100 g DW) (**Table 1**). However, the use of the cellulase-treated ASP led to 37–130% higher SDF content of these cookies compared to that of the control ones (**Table 2**). Interestingly, the enzymatic treatment successfully improved the ratio of IDF to SDF which was comparatively close to the recommendation for a well-balanced proportion to enhance the health benefit of both fiber fractions [Jha *et al.*, 2017].

Ascorbic acid content, total phenolic content, and antioxidant capacity of the cookies increased significantly as the proportion of both untreated and cellulase-treated ASP was increased in the flour blends used to cookie production (**Table 2**). However, the cookies fortified with the cellulase-treated ASP had significantly ($p<0.05$) lower ascorbic acid content, total phenolic content, and antioxidant capacity than the cookies produced with an equal amount of the untreated ASP. A close correlation was observed in this study between the antioxidant capacity of cookies and their total phenolic content. The cookies produced from the flour blends with the 30% (*w/w*) of cellulase-treated and untreated ASP had a total phenolic content of 3.4 mg GAE/g DW and 3.1 mg GAE/g DW, respectively, whereas that determined in the control cookies was barely 0.7 mg GAE/g DW. The obtained results suggested that the use of ASP in cookies could elicit important health benefits.

The phytate content of the ASP-fortified cookies increased with the increasing proportion of ASP in the flour blend used in the production of cookies (**Table 2**). Similar values ($p \geq 0.05$) were found compared to the control cookies only for the AS10 and CAS 10 samples. The phytate contents did not differ significantly ($p \geq 0.05$) between the cookies with the untreated and cellulase-treated ASP (**Table 2**). It could be attributed to the significantly higher levels of phytates in the cellulase-treated and untreated ASP compared to that of wheat flour (**Table 1**). Phytates are an antinutrient that forms a chelate with minerals such as iron, calcium, copper, and zinc and prevents their bioavailability [Atuna *et al.*, 2022].

The use of ASP significantly contributed to physical characteristics of the cookies. Their thickness and diameter decreased as the ratio of ASP in the flour blend increased, while the reverse result was observed for the spread ratio (**Table 3**). The diameters of the AS30 and CAS30 cookies decreased by 5.5 and 1.5%,

Table 3. Physical properties of cookies from wheat flour (control) and from blends of wheat flour with different proportion (10, 15, 20, 25 and 30%, *w/w*) of untreated acerola seed powder (AS10, AS15, AS20, AS25 and AS30, respectively) and cellulase-treated acerola seed powder (CAS10, CAS15, CAS20, and CAS30, respectively).

Cookie	Fracture strength		Dimension		
	Hardness (N)	Fracturability (mm)	Diameter (D, mm)	Thickness (T, mm)	Spread ratio (D/T)
Control	8.19 ± 0.18^{e}	7.98 ± 0.13^{a}	47.25 ± 0.25^{a}	9.09 ± 0.05^{a}	5.20 ± 0.02^{e}
AS10	8.25 ± 0.34^{eA}	7.19 ± 0.30^{abB}	45.83 ± 0.42^{bB}	8.87 ± 0.03^{bA}	5.17 ± 0.06^{eB}
AS15	9.98 ± 0.31^{dA}	6.80 ± 0.30^{abcB}	45.89 ± 0.37^{bB}	8.59 ± 0.03^{cA}	5.34 ± 0.03^{dB}
AS20	13.26 ± 0.22^{cA}	6.62 ± 0.30^{cdeB}	45.22 ± 0.25^{cB}	7.95 ± 0.05^{dA}	5.69 ± 0.06^{cB}
AS25	15.83 ± 1.12^{bA}	5.71 ± 0.30^{deB}	44.82 ± 0.31^{cB}	7.91 ± 0.02^{dA}	5.66 ± 0.04^{cB}
AS30	19.90 ± 1.40^{aA}	5.58 ± 0.20^{eB}	44.67 ± 0.13^{cB}	7.51 ± 0.07^{cA}	5.95 ± 0.04^{bB}
CAS10	8.22 ± 0.17^{eA}	7.40 ± 0.30^{bA}	47.23 ± 0.20^{aA}	8.26 ± 0.03^{bB}	5.72 ± 0.05^{dA}
CAS15	9.03 ± 0.23^{dB}	7.40 ± 0.10^{bA}	47.10 ± 0.25^{aA}	8.06 ± 0.02^{cB}	5.85 ± 0.03^{cA}
CAS20	9.62 ± 0.26^{cB}	7.20 ± 0.10^{bA}	46.92 ± 0.09^{bA}	7.75 ± 0.02^{dB}	6.05 ± 0.03^{bA}
CAS25	10.57 ± 0.71^{bB}	7.20 ± 0.30^{bA}	46.64 ± 0.21^{cA}	7.73 ± 0.05^{dB}	6.04 ± 0.05^{bA}
CAS30	15.62 ± 0.38^{aB}	7.10 ± 0.00^{bA}	46.52 ± 0.49^{cA}	7.61 ± 0.06^{cB}	6.12 ± 0.04^{aA}

Results are shown as mean ± standard deviation. In the same column, different lower-case letters (a–f) indicate significant differences between all cookie types, while upper-case letters (A and B) show significant differences between cookies produced with cellulase-treated and untreated acerola seed powder separately for each fortification level ($p<0.05$).

respectively, and their thickness was reduced by 17 and 16%, respectively, but their spread ratios increased by 16 and 18%, respectively, compared to those of the control cookies. The decrease in cookie thickness was also reported in our previous study [Nguyen *et al*., 2021] for cookies with cellulase-treatment wheat bran. The reason could be the reduction of gluten content in the dough and the disruption of the gluten network, which is responsible for the vertical expansion of the cookie [Handa *et al.*, 2012]. However, the contrary findings were found by Arun *et al*. [2015], who reported an increase in thickness of cookies with an increasing level of Nendran peel flour. It can be suggested that the addition of fiber reduces the amount of water available to dissolve soluble components in the cookie dough, increasing dough viscosity and reducing the spread of the product.

The cookies produced with the untreated ASP had a smaller ($p<0.05$) diameter and a higher ($p<0.05$) thickness compared to those of the cookies with the cellulase-treated ASP at the same replacement levels (**Table 3**). This could be related to the reduced IDF content since IDF has been proven to be unfavorable for the development of the gluten network [Foschia *et al.*, 2015].

The use of ASP in the recipe led to an increase in the hardness and a decrease in the fracturability of the cookies (**Table 3**). However, the cookies produced from the flour blends

Table 4. The color parameters and overall acceptability of cookies from wheat flour (control) and from blends of wheat flour with different proportion (10, 15, 20, 25 and 30%, *w/w*) of untreated acerola seed powder (AS10, AS15, AS20, AS25 and AS30, respectively) and cellulase-treated acerola seed powder (CAS10, CAS15, CAS20, and CAS30, respectively).

Cookie	*L**	*a**	*b**	*ΔE*	Overall acceptability
Control	58.21±0.22^{a}	12.50±0.12^{a}	28.56±0.11^{a}	0.00±0.00^{f}	5.33±1.61^{cd}
AS10	48.56±0.18^{bA}	9.10±0.09^{bA}	16.10±0.41^{bA}	16.10±0.33^{eA}	6.45±1.37^{aA}
AS15	48.44±0.02^{bA}	9.14±0.04^{bA}	15.67±0.12^{cA}	16.58±0.17^{dA}	6.52±1.43^{aA}
AS20	47.52±0.14^{cA}	8.78±0.12^{cA}	14.56±0.10^{dA}	18.01±0.23^{cA}	6.01±1.37^{abA}
AS25	46.56±0.02^{dA}	8.67±0.11^{bcA}	13.67±0.07^{eA}	19.22±0.15^{bA}	5.73±1.82^{bcB}
AS30	46.33±0.01^{dA}	8.53±0.07^{cA}	13.33±0.22^{fA}	19.76±0.25^{aA}	5.12±1.41^{dB}
CAS10	48.26±0.12^{bA}	9.22±0.10^{bA}	16.67±0.15^{bA}	15.81±0.24^{eA}	6.78±1.12^{aA}
CAS15	47.78±0.14^{eB}	9.13±0.09^{bA}	15.89±0.11^{cB}	16.72±0.10^{dA}	6.70±1.31b^{A}
CAS20	47.05±0.11^{dB}	8.90±0.11^{cA}	15.05±0.23^{dB}	17.83±0.11^{cA}	6.56±1.22^{bB}
CAS25	46.23±0.12^{eB}	8.74±0.03^{bcA}	14.90±0.11^{cB}	18.45±0.18^{bB}	6.53±1.53^{bA}
CAS30	46.01±0.02^{eB}	8.62±0.10^{cA}	14.62±0.19^{dB}	19.03±0.12^{aB}	5.62±1.48^{cA}

Results are shown as mean ± standard deviation. In the same column, different lower-case letters (a–f) indicate significant differences between all cookie types, while upper-case letters (A and B) show significant differences between cookies produced with cellulase-treated and untreated acerola seed powder separately for each fortification level ($p<0.05$); *L**, lightness; *a**, greenness (-) to redness (+); *b**, blueness (-) to yellowness (+); *ΔE*, total color difference."

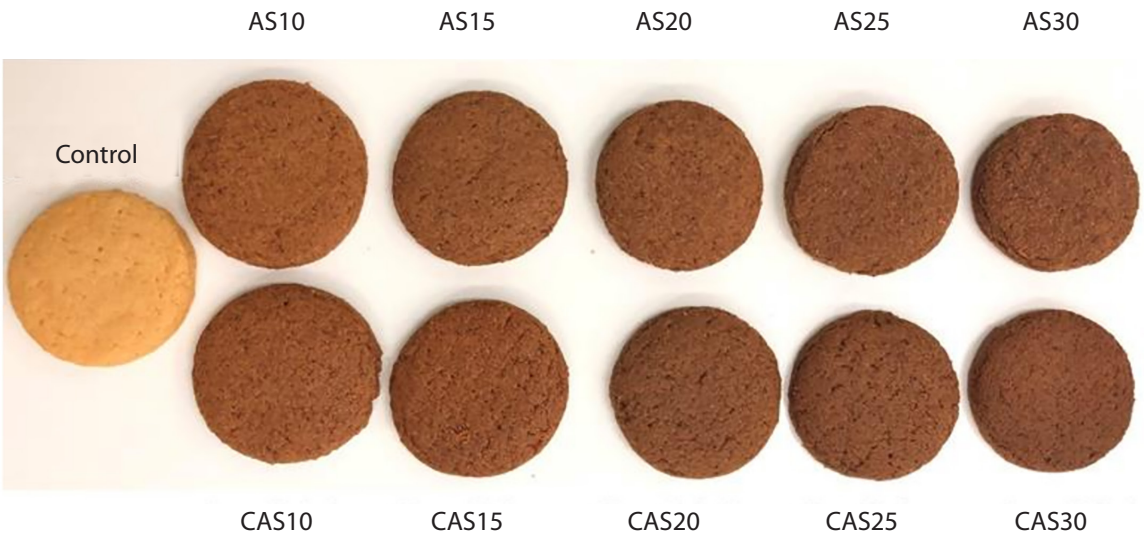

Figure 2. Appearance of cookies from wheat flour (control) and cookies from blends of wheat flour with different proportions (10, 15, 20, 25 and 30%, *w/w*) of untreated acerola seed powder (AS10, AS15, AS20, AS25 and AS30, respectively) and cellulase-treated acerola seed powder (CAS10, CAS15, CAS20, and CAS30, respectively).

with the cellulase-treated ASP (all proportions), interestingly, had lower (p<0.05) hardness and higher (p<0.05) fracturability than the samples with the untreated one. The CAS30 cookies showed 31% lower hardness and 11% higher fracturability than the AS30 samples. Previous studies reported that with increasing brewer's spent grain addition to cookie recipe, the hardness of the final product increased [Heredia-Sandoval *et al.*, 2020], while flaxseed flour-fortified cookies had decreasing hardness [Kaur *et al.*, 2019]. The variations in the results may be caused by the different water absorption capacity of by-products. The increases in hardness could be due to the contribution of the high protein and IDF content, while the attenuation of hardness may be correlated with the competition between sugar and flour protein for water, which leads to the reduction of gluten formation [Morales-Polanco *et al.*, 2017]. Future studies on interactions between wheat protein and dietary fiber of ASP are therefore essential to clarify the insight mechanisms that determine the physical properties of the final product.

The cookie color parameters and cookies appearance are shown in **Table 4** and **Figure 2**, respectively. The cookie color became darker (lower L^* values), more green (lower a values) and less yellow (lower b^* values) with increasing levels of the untreated and cellulase-treated ASP. At the same proportion of both types of ASP in the flour blend, there were no differences in ΔE between the cookies with the cellulase-treated and untreated ASP except for the cookies incorporated with 25 and 30% (*w/w*); cookies with the cellulase-treated ASP had lower ΔE values than their counterparts with the untreated ASP. In general, the incorporation of higher-fiber ingredients usually causes a darker color of bakery products [Bolek, 2020]. Moreover, the natural pigment of ASP could contribute to the darkening of fortified cookies [Marques *et al.*, 2015]. The Maillard reaction occurring between reducing sugars and amino acids, and caramelization of sugars might also be responsible for cookie color darkening [Žilić *et al.*, 2021].

The overall acceptability of the cookies fortified with different levels of both ASP types was significantly (p<0.05) higher than of the control cookies excluding the AS20, AS30 and CAS30 samples (**Table 3**). The previous study revealed that cereal bars with the addition of acerola seed flour were more preferred than the bars without the additive, because of the higher scores received for texture, flavor, overall appearance, and purchase intention [Marques *et al.*, 2015]. However, the overall acceptability declined at high addition ratios (**Table 4**). The overall acceptability of the cookies was reduced as the fortified levels were 25% (*w/w*) or higher for the AS samples and 30% (*w/w*) for the CAS samples. The reduction in the sensory score of the cookies was due to the increased hardness, as the soft and crumbly texture is the most desirable characteristics of cookies [Lara *et al.*, 2011].

CONCLUSIONS

The cellulase treatment was demonstrated to lower the IDF to SDF ratio of ASP owing to the conversion of IDF to SDF. The replacement of wheat flour with the cellulase-treated ASP in the recipe improved the IDF to SDF ratio of the cookies, making it close to the recommended value that efficiently provides health benefits. The cookies produced from the blend of wheat flour with the cellulase-treated ASP had a higher dietary fiber content and antioxidant capacity compared to the control cookies produced from wheat flour only. Moreover, the use of the cellulase-treated ASP at appropriate levels had a negligible effect on the geometric features of the fortified cookies, preserving their round shape and diameter. As compared with the cookies incorporated with the untreated ASP, the cookies prepared from the flour blend with the cellulase-treated ASP had lower hardness, higher fracturability, and generally higher overall acceptance. In the future, health effects of ASP-incorporated products, such as reduction in dietary postprandial glycemic response, should be investigated to confirm the potential of this by-product.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ADDITIONAL INFORMATION

Data are available upon request from the corresponding author.

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REFERENCES

1. AACC (1983). *Approved Methods of Analysis* (8th edition). American Association of Cereal Chemists. Cereals and Grains Association, St. Paul, MN, U.S.A.
2. AOAC (2000). *Official Methods of Analysis* (17th edition). The Association of Official Analytical Chemists International. Gaithersburg, MD, USA.
3. AOAC (2010). *Official Methods of Analysis of AOAC* (18th ed., revision 3). The Association of Official Analytical Chemists International. Gaithersburg, MD, USA.
4. Arun, K.B., Persia, F., Aswathy, P.S., Chandran, J., Sajeev, M.S., Jayamurthy, P., Nisha, P. (2015). Plantain peel – A potential source of antioxidant dietary fibre for developing functional cookies. *Journal of Food Science and Technology*, *52*(10), 6355–6364. https://doi.org/10.1007/s13197-015-1727-1
5. Atuna, R.A., Ametei, P.N., Bawa, A.-A., Amagloh, F.K. (2022). Traditional processing methods reduced phytate in cereal flour, improved nutritional, functional and rheological properties. *Scientific African*, *15*, art. no. e01063. https://doi.org/10.1016/j.sciaf.2021.e01063
6. Bajaj, K.L., Kaur, G. (1981). Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. *Analyst*, *106*(1258), 117–120. https://doi.org/10.1039/an9810600117
7. Basu, A., Feng, D., Planinic, P., Ebersole, J.L., Lyons, T.J., Alexander, J.M. (2021). Dietary blueberry and soluble fiber supplementation reduces risk of gestational diabetes in women with obesity in a randomized controlled trial. *The Journal of Nutrition*, *151*(5), 1128–1138. https://doi.org/10.1093/jn/nxaa435
8. Belwal, T., Devkota, H.P., Hassan, H.A., Ahluwalia, S., Ramadan, M.F., Mocan, A., Atanasov, A.G. (2018). Phytopharmacology of Acerola (Malpighia spp.) and its potential as functional food. *Trends in Food Science & Technology*, *74*, 99–106. https://doi.org/10.1016/j.tifs.2018.01.014
9. Benzie, I.F., Strain, J.J. (1999). [2] Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified

version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology, 299*, 15–27. https://doi.org/10.1016/S0076-6879(99)99005-5
10. Bera, A., Ghosh, A., Mukhopadhyay, A., Chattopadhyay, D., Chakrabarti, K. (2015). Improvement of degummed ramie fiber properties upon treatment with cellulase secreting immobilized *A. larrymoorei* A1. *Bioprocess and Biosystems Engineering*, *38*(2), 341–351. https://doi.org/10.1007/s00449-014-1274-6
11. Bolek, S. (2020). Olive stone powder: A potential source of fiber and antioxidant and its effect on the rheological characteristics of biscuit dough and quality. *Innovative Food Science & Emerging Technologies*, *64*, art. no. 102423. https://doi.org/10.1016/j.ifset.2020.102423
12. Borderías, A.J., Sánchez-Alonso, I., Pérez-Mateos, M. (2005). New applications of fibres in foods: Addition to fishery products. *Trends in Food Science & Technology*, *16*(10), 458–465. https://doi.org/10.1016/j.tifs.2005.03.011
13. Brand-Williams, W., Cuvelier, M.-E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
14. Cao, T.C., Nguyen, T.P., Nguyen, S.N., Tran, T.T.T., Ton, N.M.N., Le, V.V.M. (2022). Cellulase-treated deoiled rice bran: Effects of treatment conditions on dietary fiber content and utilization for formulation of cookies. *Journal of Food Measurement and Characterization*, *16*(1), 840–848. https://doi.org/10.1007/s11694-021-01209-w
15. Carvalho Gualberto, N., Santos De Oliveira, C., Pedreira Nogueira, J., Silva De Jesus, M., Caroline Santos Araujo, H., Rajan, M., Terezinha Santos Leite Neta, M., Narain, N. (2021). Bioactive compounds and antioxidant activities in the agro-industrial residues of acerola (*Malpighia emarginata* L.), guava (*Psidium guajava* L.), genipap (*Genipa americana* L.) and umbu (*Spondias tuberosa* L.) fruits assisted by ultrasonic or shaker extraction. *Food Research International*, *147*, art. no. 110538. https://doi.org/10.1016/j.foodres.2021.110538
16. Dai, F.-J., Chau, C.-F. (2017). Classification and regulatory perspectives of dietary fiber. *Journal of Food and Drug Analysis*, *25*(1), 37–42. https://doi.org/10.1016/j.jfda.2016.09.006
17. European Parliament & Council. (2006). *Regulation (EC) no 1924/2006 of the European Parliament and of the council on nutrition and health claims made on foods.* Official Journal of the European Union.
18. Foschia, M., Peressini, D., Sensidoni, A., Brennan, M.A., Brennan, C.S. (2015). How combinations of dietary fibres can affect physicochemical characteristics of pasta. *LWT – Food Science and Technology*, *61*(1), 41–46. https://doi.org/10.1016/j.lwt.2014.11.010
19. Gan, J., Xie, L., Peng, G., Xie, J., Chen, Y., Yu, Q. (2021). Systematic review on modification methods of dietary fiber. *Food Hydrocolloids*, *119*, art. no. 106872. https://doi.org/10.1016/j.foodhyd.2021.106872
20. Garau, M.C., Simal, S., Rosselló, C., Femenia, A. (2007). Effect of air-drying temperature on physico-chemical properties of dietary fibre and antioxidant capacity of orange (*Citrus aurantium* v. Canoneta) by-products. *Food Chemistry*, *104*(3), 1014–1024. https://doi.org/10.1016/j.foodchem.2007.01.009
21. Gill, S.K., Rossi, M., Bajka, B., Whelan, K. (2021). Dietary fibre in gastrointestinal health and disease. *Nature Reviews Gastroenterology & Hepatology*, *18*(2), 101–116. https://doi.org/10.1038/s41575-020-00375-4
22. Handa, C., Goomer, S., Siddhu, A. (2012). Physicochemical properties and sensory evaluation of fructoligosaccharide enriched cookies. *Journal of Food Science and Technology*, *49*, 192–199. https://doi.org/10.1007/s13197-011-0277-4
23. Heredia-Sandoval, N.G., Granados-Nevárez, M.D.C., Calderón De La Barca, A.M., Vásquez-Lara, F., Malunga, L.N., Apea-Bah, F.B., Beta, T., Islas-Rubio, A.R. (2020). Phenolic acids, antioxidant capacity, and estimated glycemic index of cookies added with brewer's spent grain. *Plant Foods for Human Nutrition*, *75*(1), 41–47. https://doi.org/10.1007/s11130-019-00783-1
24. Jha, S.K., Singh, H.R., Prakash, P. (2017). Chapter 1: Dietary fiber and human health: An introduction. In R.A. Samaan (Ed.), *Dietary Fiber for the Prevention of Cardiovascular Disease.* Elsevier, pp. 1-22. https://doi.org/10.1016/B978-0-12-805130-6.00001-X
25. Kaur, P., Sharma, P., Kumar, V., Panghal, A., Kaur, J., Gat, Y. (2019). Effect of addition of flaxseed flour on phytochemical, physicochemical, nutritional, and textural properties of cookies. *Journal of the Saudi Society of Agricultural Sciences*, *18*(4), 372–377. https://doi.org/10.1016/j.jssas.2017.12.004
26. Khan, J., Khan, M., Ma, Y., Meng, Y., Mushtaq, A., Shen, Q., Xue, Y. (2022). Overview of the composition of whole grains' phenolic acids and dietary fibre and their effect on chronic non-communicable diseases. *International Journal of Environmental Research and Public Health*, *19*(5), art. no. 3042. https://doi.org/10.3390/ijerph19053042
27. Landhäusser, S.M., Chow, P.S., Dickman, L.T., Furze, M.E., Kuhlman, I., Schmid, S., Wiesenbauer, J., Wild, B., Gleixner, G., Hartmann, H., Hoch, G., McDowell, N.G., Richardson, A.D., Richter, A., Adams, H.D. (2018). Standardized protocols and procedures can precisely and accurately quantify non-structural carbohydrates. *Tree Physiology*, *38*(12), 1764–1778. https://doi.org/10.1093/treephys/tpy118
28. Lara, E., Cortés, P., Briones, V., Perez, M. (2011). Structural and physical modifications of corn biscuits during baking process. *LWT – Food Science and Technology*, *44*(3), 622–630. https://doi.org/10.1016/j.lwt.2010.10.007
29. Marques, T.R., Corrêa, A.D., De Carvalho Alves, A.P., Simão, A.A., Pinheiro, A.C.M., De Oliveira Ramos, V. (2015). Cereal bars enriched with antioxidant substances and rich in fiber, prepared with flours of acerola residues. *Journal of Food Science and Technology*, *52*(8), 5084–5092. https://doi.org/10.1007/s13197-014-1585-2
30. Marques, T.R., Corrêa, A.D., Lino, J.B.D.R., Abreu, C.M.P.D., Simão, A.A. (2013). Chemical constituents and technological functional properties of acerola (*Malpighia emarginata* DC.) waste flour. *Food Science and Technology*, *33*(3), 526–531. https://doi.org/10.1590/S0101-20612013005000085
31. Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030
32. Morales-Polanco, E., Campos-Vega, R., Gaytán-Martínez, M., Enriquez, L.G., Loarca-Piña, G. (2017). Functional and textural properties of a dehulled oat (*Avena sativa* L.) and pea (*Pisum sativum*) protein isolate cracker. *LWT - Food Science and Technology*, *86*, 418–423. https://doi.org/10.1016/j.lwt.2017.08.015
33. Nguyen, N.D.T., Phan, T.T.H., Tran, T.T.T., Ton, N.M.N., Vo, D.L.T., Le, V.V.M. (2022). Enzymatic treatment of spent green tea leaves and their use in high-fiber cookie production. *Food Technology and Biotechnology*, *60*(3), 396–405. https://doi.org/10.17113/ftb.60.03.22.7474
34. Nguyen, S.N., Vien, M.D., Le, T.T.T., Tran, T.T.T., Ton, N.M.N., Le, V.V.M. (2021). Effects of enzymatic treatment conditions on dietary fibre content of wheat bran and use of cellulase-treated bran in cookie. *International Journal of Food Science & Technology*, *56*(8), 4017–4025. https://doi.org/10.1111/ijfs.15022
35. Nielsen, S.S. (2010). Phenol-sulfuric acid method for total carbohydrates. In S.S. Nielsen (Ed.), *Food Analysis Laboratory Manual,* Springer, US, pp. 47–53. https://doi.org/10.1007/978-1-4419-1463-7_6
36. Park, J., Choi, I., Kim, Y. (2015). Cookies formulated from fresh okara using starch, soy flour and hydroxypropyl methylcellulose have high quality and nutritional value. *LWT – Food Science and Technology*, *63*(1), 660–666. https://doi.org/10.1016/j.lwt.2015.03.110
37. Poletto, P., Álvarez-Rivera, G., López, G.-D., Borges, O.M.A., Mendiola, J.A., Ibáñez, E., Cifuentes, A. (2021). Recovery of ascorbic acid, phenolic compounds and carotenoids from acerola by-products: An opportunity for their valorization. *LWT – Food Science and Technology*, *146*, art. no. 111654. https://doi.org/10.1016/j.lwt.2021.111654
38. Sancho, S.D.O., Da Silva, A.R.A., Dantas, A.N.D.S., Magalhães, T.A., Lopes, G.S., Rodrigues, S., Da Costa, J.M.C., Fernandes, F.A.N., Silva, M.G.D.V. (2015). Characterization of the industrial residues of seven fruits and prospection of their potential application as food supplements. *Journal of Chemistry*, *2015*, art. no. 264284. https://doi.org/10.1155/2015/264284
39. Silva, N.C.D., Barros-Alexandrino, T.T.D., Assis, O.B.G., Martelli-Tosi, M. (2021). Extraction of phenolic compounds from acerola by-products using chitosan solution, encapsulation and application in extending the shelf-life of guava. *Food Chemistry*, *354*, art. no. 129553. https://doi.org/10.1016/j.foodchem.2021.129553
40. Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158. https://doi.org/10.5344/ajev.1965.16.3.144
41. Sureshkumar, S., Narmatha, S., Priya, R.M., Ramalingam, J., Bharathi, M., Kumaravadivel, N., Tamilkumar, P., Subashini, S., Sivaji, M., Senthil, N., Nagarajan, P., Vellaikumar, S., Ganesan, K.N., Balagopal, R., Ravedndran, M. (2015). Simple protocol for maize single seed phytic acid estimation. *Annals of Plant and Soil Research*, *17*(SI), 391–395.
42. Ta, T.M.N., Hoang, C.H., Nguyen, T.M., Tran, T.T.T., Ton, N.M.N., Le, V.V.M. (2023). Effects of mulberry pomace addition and transglutaminase treatment on the quality of pasta enriched with antioxidants and dietary fiber. *Polish Journal of Food and Nutrition Sciences*, *73*(4), 301–310. https://doi.org/10.31883/pjfns/172244
43. Wang, W., Kang, L., Wei, H., Arora, R., Lee, Y.Y. (2011). Study on the decreased sugar yield in enzymatic hydrolysis of cellulosic substrate at high solid loading. *Applied Biochemistry and Biotechnology*, *164*(7), 1139–1149. https://doi.org/10.1007/s12010-011-9200-8

44. Zhu, K., Huang, S., Peng, W., Qian, H., Zhou, H. (2010). Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber. *Food Research International*, *43*(4), 943–948. https://doi.org/10.1016/j.foodres.2010.01.005

45. Žilić, S., Aktağ, I.G., Dodig, D., Gökmen, V. (2021). Investigations on the formation of Maillard reaction products in sweet cookies made of different cereals. *Food Research International*, *144*, art. no. 110352. https://doi.org/10.1016/j.foodres.2021.110352

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Comparative Study on the Incorporation of Lesser Mealworm (*Alphitobius diaperinus*) and House Cricket (*Acheta domesticus*) Powders into Shortbread Cookies: Effects on Physical, Chemical and Sensory Properties

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Edible insects have the potential to serve as a valuable and innovative source of nutrients. However, their incorporation can affect various product characteristics. This study aimed to evaluate the effect of using lesser mealworm larvae (LMP) and house cricket imago (HCP) powders in shortbread cookie recipe on their physical, sensory, and nutritional characteristics. The cookies prepared from wheat flour (control) and those with 10% and 20% (*w/w*) of wheat flour replaced by insect powders were analyzed. Additionally, the fat quality and sorption properties of the insect powders were evaluated to determine their impact on the storage stability of the cookies. The results indicated that the chemical composition of both insect powders influenced their sorption properties, contributing to their good storage stability Nevertheless, the changes caused by the incorporation of LMP were more pronounced than those caused by HCP. The insect powders improved the nutritional value of the cookies, notably increasing protein content (2.1 times for cookies with 20% LMP replacement) and essential fatty acid levels (3.3 times for cookies with 20% LMP replacement), compared to control. However, it diminished oxidative stability of the fat in cookies (with a 25.9% shorter induction time for 20% LMP cookies) and their sensory characteristics (primarily ratings of color, taste, and aroma were lower by 32.4–65.2%). Generally, the cookies with a 10% LMP replacement achieved consumer acceptability comparable to those with 20% HCP, suggesting that a lower level of LMP is preferable in the recipe compared to the HCP level. Overall, LMP could be deemed a promising ingredient for the pastry industry; however, further research is needed to enhance the sensory characteristics and shelf-life of products enriched with this insect powder.

Keywords: consumer acceptance, edible insects, fat quality, house cricket imago, lesser mealworm larvae

INTRODUCTION

With the global population surpassing 8 billion, the search for new alternative food sources has become imperative. This population growth is linked to improved quality of life, better access to healthcare, advancements in medicine, and enhanced hygiene. Consequently, scientists are compelled to find new food

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sources to meet the energy requirements of a growing population [Mariutti *et al*., 2021]. One such source can be edible insects, which are a staple food in many Asian countries, have been a kind of a traditional dish for many generations, and are considered a delicacy. In recent years, entomophagy (the consumption of insects) has garnered increasing interest in European countries, both from scientific perspectives (studying their nutritional composition, health effects, and consumer acceptance) and in terms of actual consumption [Menozzi *et al*., 2017].

It is estimated that nearly 2,000 insect species are considered edible worldwide. Specifically, Ramos-Elorduy [2009] identifies 2,086 edible species, Mistuhashi [2016] reports 2,141 species, and van Itterbeeck & Pelozuelo [2022] list 2,111 species. Insects can be consumed in various forms, including whole insects subjected to heat treatments (*e.g.*, boiling, roasting, frying, drying) or in powdered form (*e.g.*, as an ingredient in bread or pasta). However, a significant barrier to their adoption in Europe is the psychological aspect. European culture traditionally views insects as pests rather than as a viable food source. Consequently, the European population is mostly not ready to incorporate edible insects into their daily diet, with additional concerns regarding health safety and hygiene further impeding their acceptance.

Interest in edible insects is primarily a result of their high content (30–77 g/100 g) of complete, highly digestible (76.6–98%) protein [Kim *et al*., 2019]. Besides being a rich protein source, edible insects provide several other essential nutrients. The second largest component of edible insects is fat, which shows significant potential for use in the food industry. However, its application requires a detailed understanding of the chemical composition, as the nutritional value, technological, and functional properties of food products depend on it [Tzompa-Sosa *et al*., 2021]. The fat content in dry matter of edible insects varies by species. When analyzing 236 edible insects, Rumpold & Schlüter [2013] found that the average fat contents *per* order ranged from 13.41 g/100 g for *Orthoptera* (grasshoppers, crickets, and locusts), which are rich in protein, to 33.40 g/100 g for *Coleoptera* (beetles and bugs). Aguilera *et al*. [2021] reported even higher fat contents of 50.1 g/100 g in freeze-dried ant larvae and 40.6 g/100 g in mealworm larvae. The fat fraction in edible insects is composed mainly of triacylglycerols (approximately 80%), with smaller amounts of phospholipids (up to 20%), cholesterol, free fatty acids (FFA), and wax esters [da Silva Lucas *et al*., 2020]. Notably, insect-derived fat has a high nutritional value due to the presence of unsaturated essential fatty acids such as linoleic and α-linolenic acids [Udomsil *et al*., 2019]. The favorable fat profile of edible insects can significantly increase the polyunsaturated fatty acid content in enriched food products [Acosta-Estrada *et al*., 2021]. In addition to enhancing nutritional value, the incorporation of insect powder can influence the physical and sensory properties of food products [Kim *et al*., 2022]. Fats in baked products contribute to tenderness, flavor release, mouthfeel, and the reduction of gluten structure, as well as imparts functional properties. Due to their high-quality fat content, edible insects can replace some of the fat used in baking, thereby increasing the nutritional value of the final product [Delicato *et al*., 2020].

Given the high nutritional value of edible insects, a growing number of researchers has investigated the feasibility of incorporating insect powder to food products such as baked goods, bars, cakes, and cookies [Delicato *et al*., 2020; Kowalski *et al*., 2022a,b; Sriprablom *et al*., 2022]. Current research primarily focuses on using insects to increase the protein content of these products.

To the best of our knowledge, the lesser mealworm (*Alphitobius diaperinus*) is still scarcely utilized in food production, despite being a superior protein source comparable to the house cricket (*Acheta domesticus*), which is the most popular insect farmed exclusively for human consumption [Kurečka *et al*., 2021]. It is noteworthy that the European Commission has recently authorized the placing on the market of frozen, paste, dried, and powdered forms of lesser mealworm larvae (on 5 January 2023) [van Huis, 2023]. The literature indicates that this insect is characterized by a short life cycle and feeds on a varied organic matter, including agricultural and food by-products and wastes, making it relatively easy to farm and also that it contains bioactive compounds with antioxidant and antimicrobial properties that may elicit benefits to human health [Siddiqui *et al*., 2024]. To date, Roncolini *et al*. [2020] used lesser mealworm powder (10% to 30% wheat flour replacement, *w/w*) to produce wheat snacks. Ortolá *et al*. [2022] compared biscuits formulated with lesser mealworm and yellow mealworm flours, while Mazurek *et al*. [2023] and Skotnicka *et al*. [2022] compared the same types of flours as ingredients in wheat pancakes. In turn, Kowalski *et al*. [2022b; 2023] studied nut bars and sponge cakes with the addition of powders of three insects (yellow mealworm, house cricket, and lesser mealworm). Although these cited studies indicate an increase in the nutritional value of the final product, their findings are often inconclusive, highlighting the need for further research, particularly regarding product stability.

Therefore, this study aimed to compare the physical, sensory, and nutritional characteristics of shortbread cookies without and with the incorporation of insect powders from two edible insects: lesser mealworm larvae and house cricket imago. Additionally, the quality of the insect powder, including fatty acid composition, degrees of fat hydrolysis and oxidation, and sorption properties, was evaluated to help understand its impact on the stability of the enriched products.

MATERIALS AND METHODS

Materials and reagents

The primary raw materials were powders derived from the larvae of lesser mealworm (*Alphitobius diaperinus*) (Isaac Nutrition, Cologne, Germany) and the adult form (imago) of house cricket (*Acheta domesticus*) (SENS Food, London, United Kingdom), which were purchased from online stores. The other ingredients required to prepare the shortbread cookies, such as wheat flour type 550, butter (minimum milk fat content 82%), white sugar, and salt, were sourced from local stores in Olsztyn, Poland. These ingredients were characterized by their appropriate shelf-life and typical organoleptic characteristics.

All reagents used in the study, including acetic acid, chloroform, methanol, *n*-hexane, potassium hydroxide, sodium thiosulfate, sulfuric acid, potassium iodide, and zinc, were of the analytical grade and were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

Measurements of color parameters of the insect powders

The color of the insect powders was measured using digital image analysis, which employed a Nikon DXM 1200 digital camera (Amsterdam, Netherlands) with a resolution of 1,280×1,024 pixels (1.4 million pixels), KAISER RB HF lighting (Buchen, Germany) consisting of four lamps with a color temperature of 5,400 K, and a computer with an image capture card and LUCIA G software v. 4.80 (Prague, Czech Republic). The study used the CIE *L**, *a**, *b** color space, where *L** denotes brightness (values in the range of 0–100%, from black to white color), *a** denotes green (negative values) or red (positive values) color intensity (values from −120 to +120), and *b** denotes blue (negative values) or yellow (positive values) color intensity (values from −120 to +120). Samples of each powder were placed in a Petri dish under the camera lens, and 10 images were captured and subsequently analyzed using the LUCIA G software.

Analysis of the proximate composition of the insect powders

The contents of the following compounds were analyzed in the insect powders using the official methods of AOAC International [AOAC, 2000]: water content (method no. 930.15), protein content (method no. 990.03), fat content (method no. 920.39), ash content (method no. 942.05), and fiber content (method no. 962.09). The carbohydrate content was determined by difference, *i.e.*, the residual weight after subtracting amounts of water, protein, fat, ash, and fiber. The results were expressed in g *per* 100 g of insect powder.

Analysis of the sorption properties of the insect powders

Water activity (a_w) of the insect powders was measured using an AquaLab 4TE instrument (AS4 v. 2.14.0 2017 from Decagon Devices, Inc., Pullman, WA, USA) with an accuracy of ± 0.0003 at 20°C [Ruszkowska *et al*., 2022].

The assessment of sorption properties was carried out using the static method by determining sorption isotherms and the humidity equilibrium between the sample and the atmosphere with a controlled relative humidity, adjusted using saturated salt solutions. The samples were placed in a hygrostat with a_w ranging from 0.07 to 0.98 and stored for 30 days at 20°C±1°C. The mathematical descriptions of the sorption isotherms were derived using the Brunauer, Emmett, and Teller (BET) model within the a_w range of 0.07 to 0.33. Parameters such as monomolecular layer capacity (v_m), energy constant (c_e), and specific sorption area (SSA) were determined [Tańska *et al*., 2017]. The results were analyzed using Jandel-Table Curve 2D software v. 5.01 (Systat Software Inc., Palo Alto, CA, USA). The fit of the empirical data to the BET equation was characterized by the determination coefficient (R^2), the sum of squared deviations of the theoretical from empirical values (SKO), the values of standard errors (RMS) and the standard error of fit (FitStdErr).

Analysis of the fat quality of the insect powders

The fat quality in the insect powders was evaluated by determining the degree of hydrolysis (acid value) and oxidation (peroxide value), as well as the fatty acid composition. Firstly, fat was extracted from the powder using *n*-hexane in a Soxhlet apparatus [Tzompa-Sosa *et al*., 2014].

The acid and peroxide values of the extracted fat were determined according to AOCS Official Methods [AOCS, 2009] (methods Te 1a–64 and Cd 8b–90, respectively). Each powder was analyzed in triplicate.

The fatty acid composition of the extracted fat was determined following the procedure described by Mikołajczak *et al*. [2022]. Fatty acid methyl esters (FAME) were prepared by methylation at 70°C for 2 h with a mixture of methanol, chloroform and sulfuric acid in ratio of 100:100:1 (*v/v/v*). Zinc powder was then used to neutralize the sulfuric acid, and the solvents were evaporated under a stream of nitrogen. The obtained FAME was dissolved in *n*-hexane, centrifuged (5417R type centrifuge, Eppendorf AG, Hamburg, Germany) for 10 min at 25,000×*g*, and analyzed by gas chromatography mass spectrophotometry (GC-MS) using a QP2010 PLUS device (Shimadzu, Tokyo, Japan). A capillary column (BPX-70, 25 m × 0.22 mm × 0.25 µm, SGE Analytical Science, Ringwood, Australia) and helium at a flow rate of 1.3 mL/min were used for the FAME separation. The interface and ion source temperatures were set at 240°C, while column temperature was programmed in the range of 150–250°C. The electron energy of 70 eV was applied. Fatty acids were identified based on their mass spectra, and their contents were expressed as a percentage of total fatty acids based on peak areas. Each powder was analyzed in triplicate.

Preparation of shortbread cookies

The recipe of control cookies comprised 498.34 g of wheat flour, 332.23 g of butter, 166.11 g of sugar, and 3.32 g of salt. In other cookie variants, 10% or 20% (*w/w*) of wheat flour was replaced by the insect powder, with no changes to the other ingredients. Cold butter, previously cut into smaller pieces, was added to the bowl of the mixer along with the powdered sugar specified in the recipe. The ingredients were mixed for 5 min in a mixer (GM-2, Bydgoszcz, Poland). Subsequently, the pre-sifted wheat flour, or its mixture with the designated proportion of the insect powder, and salt, were added to the bowl and mixed again for 5 min. The kneaded dough was set aside to cool at 5°C for 30 min. After cooling, the dough was rolled out with a rolling pin into ribbons 5 mm thick. Star-shaped cutters with a diameter of 50 mm were used to shape the dough. Each batch of dough yielded 48–50 cookies. The cookies were baked in a convection-steam oven type XVC105 (UNOX, Sp.A., Cadoneghe, Italy) at 180°C for 10 min. After baking, the cookies were allowed to cool for 2 h at 21°C before testing. Two independent production runs were conducted for the analyses.

Analysis of the color parameters of the cookies

The color of the shortbread cookies was measured using digital image analysis, following the method described above for the color analysis of the insect powders with some modifications. The cookies were prepared by slicing 1 cm from the center of the cookie, and images (10 for each sample) of the cross-section were analyzed. Additionally, the total color differences (ΔE) were calculated using the formula (1):

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where: ΔL^*, Δa^*, and Δb^* represent the differences in the L^*, a^* and b^* parameters between the control cookies and the fortified cookies, respectively.

Analysis of the fat quality and oxidative stability of the cookies

The analyses of fat quality (degree of hydrolysis and oxidation, and fatty acid composition) in the shortbread cookies were performed as described above for the insect powders. Additionally, the oxidative stability of the cookies was evaluated using a 743 Rancimat (Metrom, Zofingen, Switzerland) eight-channel oxidative stability instrument. Cookie samples (2.5 g each) were placed in a reaction vessel within a thermostatic electric heating block. The temperature was set to 110°C, and the airflow rate was 20 L/h. Oxidative stability was expressed as induction time in hours. Each sample was analyzed in triplicate.

Analysis of the nutritional value of the cookies

The analyses of the nutritional value (content of protein, fat, ash, fiber, and water) of the shortbread cookies were performed as described above for the insect powders. Additionally, the total energy was calculated using conversion factors of 4 kcal/g for carbohydrates, 4 kcal/g for proteins, and 9 kcal/g for fat.

Analysis of the consumer acceptance of the cookies

A group of 41 randomly selected individuals, including 27 women and 14 men, from the Faculty of Food Science at the University of Warmia and Mazury in Olsztyn, Poland, participated in the consumer acceptance test of the cookies. The evaluation took place 24 h after baking at room temperature. Characteristics of the cookies were assessed using a structured linear scale, where 0 points represented the lowest rating and 10 points represented the highest rating. The following characteristics of the cookies were addressed in the evaluation: taste (0 – foreign, undetectable taste, 10 – peculiar, buttery), aroma (0 – extraneous odor, undetectable, 10 – sweet, buttery odor), brightness of the surface color (0 – dark color, 10 – light color), uniformity of the surface color (0 – non-uniform color, 10 – uniform color), consistency (0 – soft, non-crunchy, 10 – hard, crunchy), and texture (0 – very little force needed to break, 10 – very much force needed to break). All cookie samples were coded to prevent evaluators from identifying the type of cookie [Mieszkowska & Marzec, 2015].

Statistical analysis

All results were presented as mean values ± standard deviations. A one-way analysis of variance (ANOVA), followed by the Tukey test, was performed to analyze significant differences between the samples ($p \leq 0.05$). The analysis was performed using Statistica v. 13.3 software (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Color parameters and proximate composition of the insect powders

The color of products is a crucial determinant of consumer choice [Alhujaili *et al*., 2023]. In the case of unconventional ingredients, such as insect powders, color can contribute to either positive perception of a product or to its rejection, being an important element of food neophobia. The results of the insect powder color measurements are shown in **Table 1**. Statistical analysis revealed significant differences ($p \leq 0.05$) among the insect powders in all evaluated color parameters. The L^* value was approximately 25% higher in the lesser mealworm powder (LMP) compared to the house cricket powder (HCP). The positive values of a^* and b^* were measured, pointing to the presence of red and yellow colors, respectively. The color of LMP was less red (lower a^* value) and more yellow (higher b^* value) than the color of HCP. In contrast, house cricket powder studied by Singh [2020] showed lower L^* (47.8–52.3) and b^* (8.38–11.2) values and higher a^* values (3.73–4.28). Unfortunately, there is a lack of research on the color parameters of lesser mealworm powder to enable any conclusive comparisons.

When analyzing the differences between the proximate composition of the powders from the two types of insects, statistically significant differences ($p \leq 0.05$) were noted for all compounds (**Table 1**). Specifically, LMP had a higher water content

Table 1. Physical and chemical characteristics of lesser mealworm powder (LMP) and house cricket powder (HCP).

Characteristic	LMP	HCP
Color parameter		
L^*	70.87±2.42^a	56.83±0.85^b
a^*	1.71±0.38^b	2.25±0.09^a
b^*	21.32±0.91^a	14.40±0.22^b
Compound content (g/100 g)		
Water	5.66±0.06^a	3.54±0.04^b
Protein	59.55±0.56^b	65.44±0.75^a
Carbohydrate	2.24±0.24^b	3.84±0.07^a
Fat	25.28±0.17^a	18.10±0.24^b
Fiber	4.12±0.18^b	5.56±0.10^a
Ash	3.15±0.13^b	3.52±0.06^a

Results are shown as mean ± standard deviation (n=5 for color parameters and n=3 for compound contents). Different letters within the same row indicate significant differences between the powders ($p \leq 0.05$).

compared to HCP, which was likely attributed to differences in the chemical composition of the raw materials, the degree of processing, and the technological parameters of the drying process employed by the manufacturers. For comparison, Udomsil *et al*. [2019] reported a water content of 6.3% in the powder from dried adult house crickets and 3% in the powder from field crickets. In contrast, Osimani *et al*. [2018] showed a higher water content (9.40%) in HCP.

LMP had a nearly 40% higher fat content compared to HCP (**Table 1**). Conversely, HCP contained higher levels of protein, carbohydrates, fiber, and ash compared to LMP. Alike differences in the content of chemical compounds in powders from these insects were observed by Kowalski *et al*. [2022a, 2023]. Interestingly, the contents of compounds determined in this study differ from those reported by the cited authors, despite using insect powders produced by the same manufacturers. This discrepancy highlights the significant heterogeneity of insect powders, likely influenced by the insects' origin, processing conditions, and storage duration. Therefore, the future mass production of products containing edible insects may pose a challenge due to the unpredictable quality of these raw materials. Ensuring consistent quality is critical for the widespread acceptance and success of edible insect products.

Sorption properties of the insect powders

Water content and water activity are fundamental parameters that influence the chemical, physical, and microbiological product quality [Mathlouthi, 2001]. These parameters characterize a product's ability to adsorb and desorb water from the environment depending on the potential difference between the product and its environment [Mathlouthi, 2001] and they are critical for the storage stability of products. The water activity (a_w) of the insect powders is shown in **Table 2**. The a_w of LMP was found to be 0.383, which is 1.5 times higher than the a_w of HCP. The determined a_w values indicated that the insect powders had ensured microbiological stability, since a minimum a_w for yeasts and molds proliferation is 0.61, with filamentous fungi being the predominant microorganisms in food products [Tapia *et al*., 2020]. Marzoli *et al*. [2023] found similar values of a_w for house cricket powders stored at room temperature for one year.

Table 2. Water activity (a_w) and parameters of Brunauer, Emmett, and Teller (BET) model used to describe the sorption isotherms of lesser mealworm powder (LMP) and house cricket powder (HCP).

Characteristic	LMP	HCP
a_w	0.383±0.003[a]	0.254±0.002[b]
v_m	3.5929	3.2517
c_e	27.86	24.38
R^2	0.9719	0.9988
SKO	0.8485	0.1691
RSM (%)	1.4846	0.5298
FitStdErr	0.1029	0.0509
SSA (m^2/g)	126.23	114.25

Results of a_w are shown as mean ± standard deviation (n=3). Different letters within the row with a_w indicate significant differences between the powders ($p \leq 0.05$). v_m, monolayer capacity; c_e, energy constant; R^2, determination coefficient; SKO, sum of squared deviations of the theoretical from empirical values; RMS, standard error; FitStdErr, standard error of fit.; SSA, specific sorption area.

The sorption isotherms of the insect powders are shown in **Figure 1A**. Evaluating the course of the isotherms, it was found that they were characterized by a sigmoidal shape, showing similarity to type II isotherms according to the classification proposed by Brunauer [Mathlouthi, 2001]. Both isotherms demonstrated a continuous course over the entire range of a_w (0.07–0.98) (**Figure 1A**), suggesting no significant change in the structure determined by an increase in the degree of ordering of the individual components in the products. LMP sorption isotherm showed that the water desorption process occurred in the I area (a_w in the range of 0.07–0.33). In the case of HCP, the desorption process was in the narrower a_w range of 0.07–0.11. Analysis of the graphical course of sorption isotherms also showed that for areas II and III, a higher equilibrium water content was determined for LMP. In the environment with a_w of 0.75 (isotherm area II), the equilibrium water content for LMP was

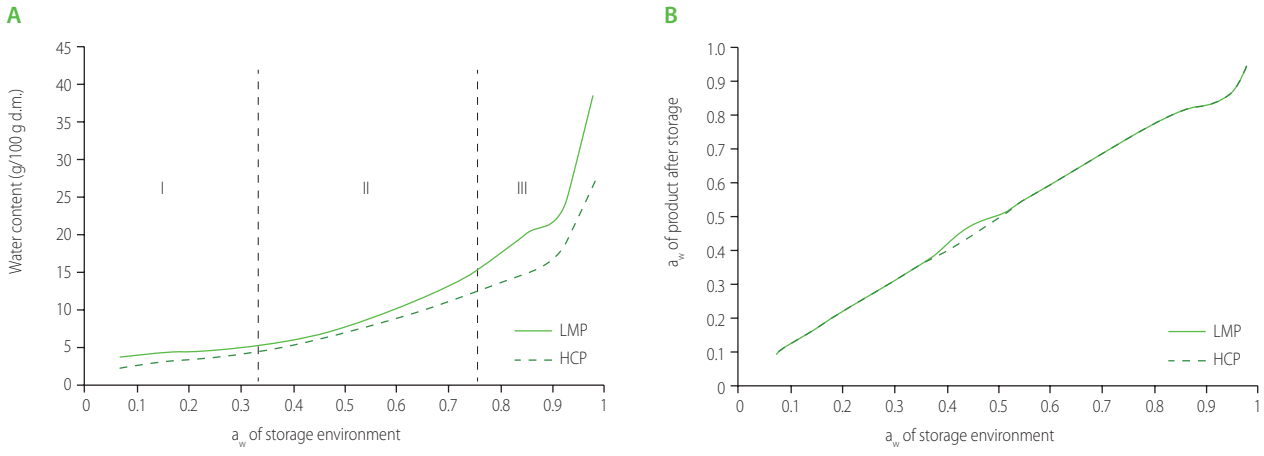

Figure 1. The sorption isotherm (**A**) and water activity, a_w (**B**) of lesser mealworm powder (LMP) and house cricket powder (HCP) after storage for 30 days at 20°C in an environment with a_w in the range of 0.07–0.98 (n=3). I, first isotherm area (a_w=0.07–0.33); II, second isotherm area (a_w=0.33–0.75); III, third isotherm area (a_w=0.75–0.98); d.m., dry matter.

15.38 g/100 g d.m., and in the environment with a_w of 0.98 (isotherm area III) it was 38.30 g/100 g d.m.

Based on the sorption isotherms, it can be hypothesized that the differences in the hygroscopicity of the tested products were mainly determined by their chemical composition. The HCP had a slightly higher protein content compared to the LMP, as well as a higher fiber content and a lower fat content (**Table 1**). These differences explain the higher equilibrium water content observed in the areas II and III of the sorption isotherm. Similar shapes of water sorption isotherms for bread flour (wheat) were found by Moreira *et al*. [2010] and for tapioca flour by Chisté *et al*. [2012].

The water activity of the insect powders after storage for 30 days in a hygrostat with constant relative humidity in the range of environmental a_w of 0.07–0.98 showed that the powders did not reach the level of environmental water activity (**Figure 1B**). However, the LMP reached higher water activity after 30 days of storage than the HCP. The differences were probably due to the different content of hydrophilic compounds in these products, which are capable of binding water [Nyangena *et al*., 2020].

To estimate the parameters of surface microstructure of the insect powders, the empirically determined isotherms of water sorption were transformed according to the BET model in the a_w range of 0.07–0.33. The parameters of the BET model are presented in **Table 2**. The capacity of the monolayer (v_m) is an indicator of the availability of polar sites of the adsorbent for water vapor, regardless of which compound is the source of hydrophilic groups [Ocieczek & Zieba, 2020]. A slightly higher v_m value was found for LMP, probably due to its higher protein content. Furthermore, considering the higher position of the LMP isotherm with regard to a_w of 0.11–0.33, it is likely that with the increase in water content in the environment, there were microstructural changes in the product, indicating that the shelf-life of the LMP was longer compared to that of the HCP. The development of the surface of the monomolecular layer protects food products due to the absorption of a certain amount of water [Mathlouthi, 2001]. The energy constant (c_e) reflects the difference between the enthalpy of monolayer desorption and the enthalpy of liquid adsorbent evaporation. The results obtained for the insect powders ($c_e \geq 2$) (**Table 2**) confirmed the sigmoidal shape of the sorption curve. Based on the SKO and RMS values, it was found that the parameters of the BET model described well the sorption process of the insect powders. The RMS error did not exceed 10%, indicating good fit of the model to the sorption data in the water activity range of 0.07–033. The specific sorption area (SSA) is one of the most important parameters characterizing the adsorbents. Among the studied insect powders, LMP had a higher SSA (126.23 m^2/g), which was probably due to differences in chemical composition (**Table 1**) and/or conditions of edible insect processing (especially temperature).

Fat quality of the insect powders

The major fatty acids identified in both LMP and HCP were oleic, linoleic, and palmitic acids (**Table 3**). Similar fatty acid profiles were obtained by Kowalski *et al*. [2022a] for both powders and by Roncolini *et al*. [2020] for LMP. The lipid fraction of LMP exhibited a specific composition, with saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) at comparable share in the total fatty acids (30.1% 34.5%, and 35.4%, respectively). Consequently, LMP may exhibit lower oxidative stability during storage or baking compared to HCP, which had a higher SFA content (39.8%) and a lower PUFA content (30.9%). Udomsil *et al*. [2019] reported that fatty acid composition varied in insect species, depending on many factors such as environmental conditions and their diet. Moreover, differences in fatty acid profiles can be attributed to the developmental stage and sex of the insect. For instance, Orkusz [2021] showed that the fat of house cricket larvae contained a higher percentage of unsaturated fatty acids (MUFAs and PUFAs) and lower percentage of SFAs compared to the fat of adult insect. In turn, Kulma *et al*. [2019] reported that female house crickets had a significantly higher lipid content and slightly higher MUFA levels than males. However, Sushchik *et al*. [2013] highlighted that the fatty acid profiles of whole bodies or specific tissues change throughout the development from egg to adult, with variations depending on species and diet. Nevertheless, the fat of most edible insects is primarily composed of triacylglycerols, with minor amounts of other lipids such as mono- and diacylglycerols, cholesterol, phytosterols, free fatty acids, and phospholipids [Mlček *et al*., 2019; Tzompa-Sosa *et al*., 2014]. Among phytosterols derived from the feed, stigmasterol,

Table 3. Fatty acid composition and quality parameters of fat extracted from lesser mealworm powder (LMP) and house cricket powder (HCP).

Parameter	LMP	HCP
Fatty acid composition (% of total fatty acids)		
Lauric acid (C12:0)	0.02±0.02^a	0.04±0.05^a
Myristic acid (C14:0)	0.49±0.01^b	0.69±0.01^a
Pentadecanoic acid (C15:0)	0.06±0.08^a	0.05±0.07^a
Palmitic acid (C16:0)	25.24±0.33^b	27.36±0.01^a
Palmitoleic acid (C16:1)	0.26±0.07^b	0.98±0.01^a
Margaric acid (C17:0)	0.45±0.01^a	0.28±0.01^b
Margaricoleic acid (C17:1)	0.05±0.01^a	0.02±0.01^a
Stearic acid (C18:0)	9.12±0.09^b	11.37±0.01^a
Oleic acid (C18:1)	34.22±0.15^a	27.40±0.07^b
Linoleic acid (C18:2*n*6)	27.51±0.36^b	29.68±0.33^a
α-Linolenic acid (C18:3*n*3)	2.53±0.08^a	1.18±0.01^b
Other fatty acids	0.10±0.06^b	0.97±0.11^a
Fat quality parameters		
Acid value (mg KOH/g)	3.21±0.04^a	2.81±0.01^b
Peroxide value (mEq O_2/kg)	1.56±0.03^b	2.05±0.05^a

Results are shown as mean ± standard deviation (n=3). Different letters within the same row indicate significant differences between the powders ($p \leq 0.05$).

β-sitosterol, and campesterol were determined in house cricket fat [Tzompa-Sosa *et al*., 2021], and stigmasterol and β-sitosterol in fat of mealworm and superworm larvae [Mlček *et al*., 2019]. Their metabolites including desmosterol, cholesterol, cholestan-3-ol, and cholest-7-en-3-ol as well as phospholipids such as phosphatidylcholine (a major phospholipid), phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, phosphatidylglycerol, hexosylceramides, and lactosylceramide were also identified in house cricket fat [Tzompa-Sosa *et al*., 2021]. It is worth noting that phosphatidylcholine is a major component of lecithin, which is used in foods as an antioxidant, taste preservative, and emulsifier.

It is proven that edible insects are rich in healthy lipids, including ω-3 and ω-6 fatty acids. Notably, the fatty acid composition of edible insects is considered more desirable than that of conventional foods [Udomsil *et al*., 2019]. The fatty acid composition of house crickets, in particular, is comparable to that of poultry or fish in terms of the content of unsaturated fatty acids [Rumpold & Schlüter, 2013; Tzompa-Sosa *et al*., 2021]. However, it should be noted that enriching food products with insect-derived ingredients may reduce product stability due to a higher PUFA content. On the other hand, edible insect powders are a source of bioactive compounds with antioxidant activity. Gharibzahedi & Altintas [2023] reported that lesser mealworm larvae oil extracted with *n*-hexane contained 0.134 mg/g tocopherols (mainly γ-tocopherol), 0.64 mg/g carotenoids, and 3.65 mg/g phenolic compounds.

Fat quality of the insect powders was assessed by determination of the acid value (AV), which measures the free fatty acid content and indicates the degree of fat hydrolysis, and the peroxide value (PV), which measures peroxide content and indicates the degree of fat oxidation (rancidity). For both indices, significant differences ($p \leq 0.05$) were found between the powders (**Table 3**). Fat in HCP was less hydrolyzed, while the fat in LMP was slightly less oxidized. Similar results of PV (0.96–2.21 mEq O_2/kg) were noted in house cricket fat extracted by Ugur *et al*. [2021] and Khatun *et al*. [2021]. Lower values for both fat quality indices (AV<2.3 mEq O_2/kg and PV<0.5 mg/kg) were found for the fat extracted from LMP by Gharibzahedi & Altintas [2023]. However, it was reported that the fat quality changed according to the manufacturing process of edible insect products. For instance, Singh *et al*. [2020] found that the fat from freezing house crickets at −20°C for 2 h 10 min showed a significantly higher acid value (13.8 mg KOH/100 g product) compared to the fat from house crickets placed in a sterile plastic bag for 3 h 1 min (4.72 mg KOH/100 g product). Also, Hurtado-Ribeira *et al*. [2023] demonstrated that processing factors, including slaughtering methods (blanching or freezing), drying techniques (oven-drying or freeze-drying), and defatting processes (mechanical pressing or supercritical CO_2 extraction), along with their interactions, significantly influenced the oxidation level of the resulting fat and defatted meal from black soldier fly larvae. At present, there are no specific regulations or standards stipulating the quality parameters of fats produced from edible insects. Therefore, the established maximum PV and AV for fish oil (3 mg KOH/g and 10 mEq O_2/kg, respectively) and unrefined edible oils (4 mg KOH/g and 10 mEq O_2/kg, respectively) are used by researchers as reference points for the quality of edible insect fat [Hurtado-Ribeira *et al*., 2023; Khatun *et al*., 2021; Ugur *et al*., 2021].

Effect of the insect powders on the color of the shortbread cookies

The results of measuring the surface color of the shortbread cookies are shown in **Table 4**, and the cookies appearance is presented in **Figure 2**. The incorporation of the insect powders, from both house cricket imago and lesser mealworm larvae to cookie recipes resulted in decreased cookie lightness. The color of the control cookies had the highest L^* value (82.93), while the lowest L^* value (70.46) was observed for the cookies with 20% HCP. All samples were characterized by negative a^* values, indicating the presence of green hues. The lowest value of this parameter (−0.22) was measured for the cookies with the highest HCP percentage in the recipe, showing a shift towards red. The values of the b^* parameter were positive, reflecting yellowness in the sample color. The lowest b^* value (33.93) was recorded for the control cookies, while the highest b^* value (45.08) was measured for the cookies with 20% HCP. The total color differences (ΔE) between the control cookies and the cookies with insect powders ranged from 3.94 to 16.97. The cookies with 10% LMP were the most similar in color to the control cookies, but doubling the amount of this insect powder significantly increased their ΔE. For cookies with HCP, the difference in ΔE depending on the amount of this insect powder in cookie recipe was smaller than for cookies with LMP.

The effect of insect additions on the color of wheat products has been confirmed by other researchers. The study by Zielińska *et al*. [2021] showed that the incorporation of 2–10%

Table 4. Color parameters of cookies prepared from wheat flour (control) and from wheat flour replaced by 10% and 20% (*w/w*) of lesser mealworm powder (10% LMP and 20% LMP, respectively), and by 10% and 20% (*w/w*) of house cricket powder (10% HCP and 20% HCP, respectively).

Parameter	10% LMP	20% LMP	10% HCP	20% HCP	Control
L^*	80.07±5.10^{a}	71.51±3.45^{bc}	74.02±4.95^{ab}	70.46±1.25^{c}	82.93±4.10^{a}
a^*	−1.70±0.33^{b}	−1.42±0.38^{b}	−0.88±0.28^{c}	−0.22±0.24^{d}	−3.09±0.71^{a}
b^*	36.26±2.89^{c}	44.41±2.50^{a}	40.59±2.42^{b}	45.08±1.84^{a}	33.93±1.40^{d}
ΔE	3.94±1.83^{c}	15.59±1.32^{a}	11.34±1.40^{b}	16.97±2.92^{a}	–

Results are shown as mean ± standard deviation (n=10). Different letters within the same row indicate significant differences between the cookies ($p \leq 0.05$).

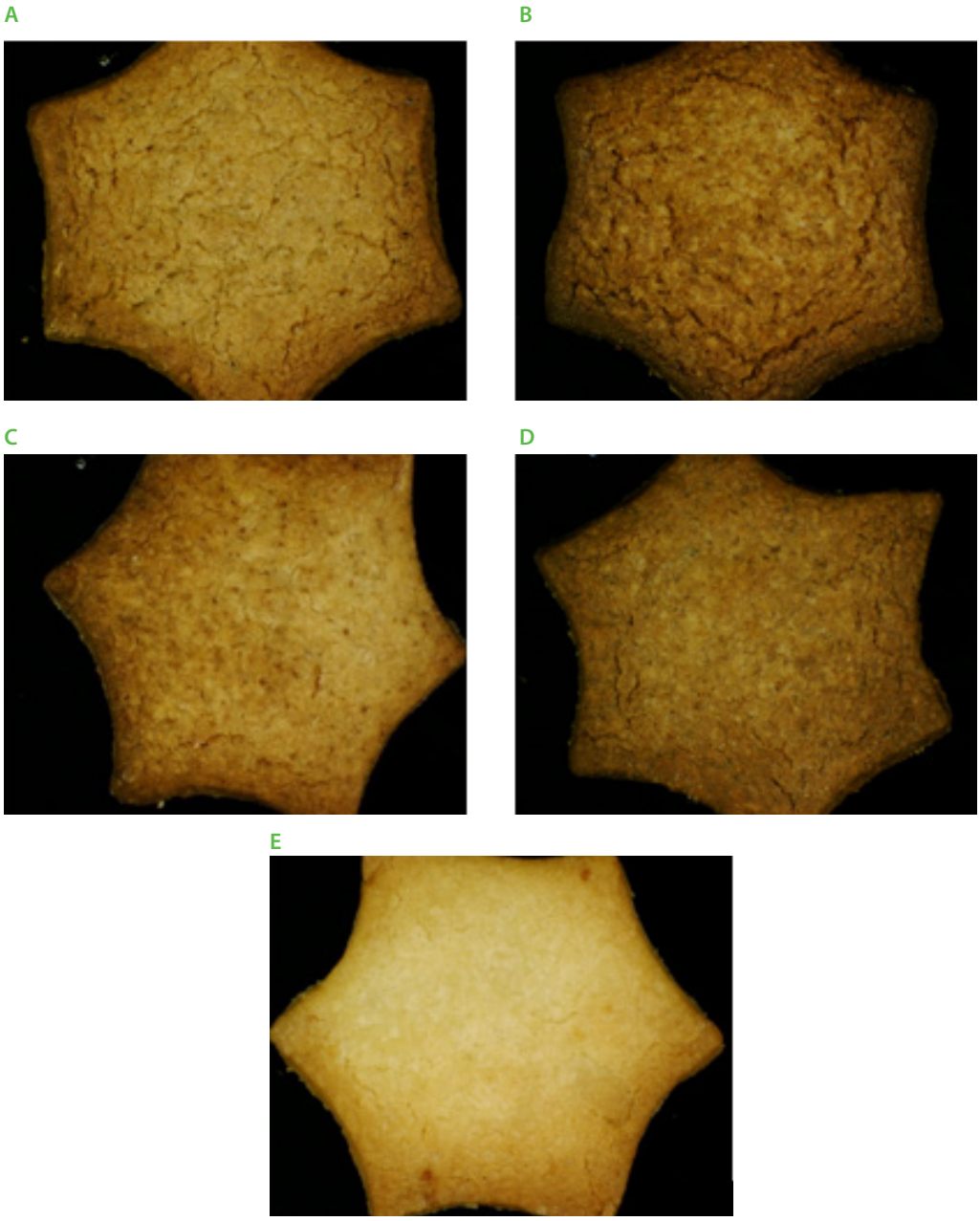

Figure 2. Images of cookies prepared from wheat flour replaced by 10% (**A**) and 20% (**B**) of lesser mealworm powder (*w/w*), and by 10% (**C**) and 20% (**D**) of house cricket powder (*w/w*), as well as control cookies from wheat flour (**E**).

of ground lyophilized insects (mealworm larvae and adult tropical house crickets) into muffins noticeably decreased their *b** value, while changes in the *a** parameter were insignificant. Pauter *et al*. [2018] found that increasing the amount of cricket powder (from 5 to 10%) shifted the green/red (*a**) and blue/yellow (*b**) color balances towards green and blue, respectively. In turn, Sriprablom *et al*. [2022] noted that higher levels of insect powder substitution (0–30%) significantly increased the redness and yellowness of the cookies. Nevertheless, the cited studies indicated that the lightness decreased with increased insect powder addition. A darker color in wheat products containing insects was also observed by González *et al*. [2019] (bread), Zielińska & Pankiewicz [2020] (biscuits), Duda *et al*. [2019] (pasta), and Pauter *et al*. [2018] (muffins). This decrease can be attributed to the darker color of insect powders compared to wheat flour. Furthermore, samples with a higher amount of protein supplied from the insect powder may have intensified the Maillard reaction occurring at high processing temperatures, resulting in reduced lightness of the baked goods [Sriprablom *et al*., 2022; Zielińska *et al*., 2021].

■ Effect of the insect powders on the fat quality and oxidative stability of the shortbread cookies

Since insect powders are a rich source of fat, it is assumed that their addition alters the fatty acid profile of the final products. Due to the high shares of MUFAs and PUFAs, incorporating both powders increased the contents of oleic, linoleic, and α-linolenic acids in all enriched cookies compared to the control cookies

(Table 5). Specifically, there was a 3.3-fold increase in PUFAs in the cookies with 20% LMP. The higher percentages of oleic acid and α-linolenic acid in the LMP than in the HCP (Table 3) resulted in a greater proportion of these acids in the cookies with LMP compared to those with HCP (Table 5). Our results were consistent with those reported by Smarzyński *et al*. [2021] who observed a slight increase in MUFAs and PUFAs in cookies with 10% HCP. It can be concluded that the use of insect powder significantly enhances the nutritional value of cookies by diversifying the lipid profile and increasing the proportion of PUFAs. On the other hand, it should be noted that a higher proportion of PUFAs can negatively affect the shelf-life of cookies and accelerate oxidation processes. This hypothesis was confirmed by the Rancimat test results (Table 5). The least oxidatively stable were the cookies with 20% LMP. An induction time for these cookies was approximately 1.3 times lower compared to the control cookies (83 h *vs*. 112 h). Among the cookies with edible insect powders, the longest induction time (102 h) was obtained for those with 10% HCP. Generally, increasing the proportions of oleic and linoleic acids by incorporating edible insect powders correlated with lower oxidative stability of the enriched cookies. Therefore, incorporating natural antioxidant ingredients (*e.g.*, cocoa or spices) or utilizing specialized packaging (*e.g.*, vacuum or inert gas-filled) should be considered during product formulation and shelf-life projection.

Effect of the insect powders on the nutritional value of the shortbread cookies

The nutritional value of the control and enriched shortbread cookies is shown in Table 6. The energy values of all types of cookies were similar, in the range of 513–525 kcal/100 g. However, compared to the control cookies, the protein content increased by about 50% in the cookies with 10% LMP and 10% HCP, and by 113% and 100% in those with 20% LMP and 20% HCP, respectively. In another study, a 10% addition of cricket powder increased the protein content in biscuits by 41% [Smarzyński *et al*., 2021] and by 40.2% in cookies [Bawa *et al*., 2020]. In addition to increasing the protein content of the product, insect powder also improves the nutritional value of protein due to the full range of essential amino acids it contains [Kowalski *et al*., 2022a].

The incorporation of insect powders to the cookies resulted in a significant ($p \leq 0.05$) increase in their fat content, with a greater

Table 5. Fatty acid composition and quality parameters of fat of cookies prepared from wheat flour (control) and from wheat flour replaced by 10% and 20% (*w/w*) of lesser mealworm powder (10% LMP and 20% LMP, respectively), and by 10% and 20% (*w/w*) of house cricket powder (10% HCP and 20% HCP).

Parameter	10% LMP	20% LMP	10% HCP	20% HCP	Control
Fatty acid composition (% of total fatty acids)					
Caprylic acid	1.14±0.11^{a}	1.03±0.15^{a}	1.11±0.10^{a}	1.06±0.20^{a}	1.68±0.07^{a}
Capric acid	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.07±0.10^{a}
Lauric acid	2.37±0.02^{a}	2.57±0.12^{a}	2.31±0.06^{a}	2.30±0.12^{a}	2.84±0.05^{a}
Myristic acid	9.02±0.21^{a}	8.28±0.16^{c}	10.05±0.16^{ab}	7.96±0.14^{ab}	11.15±0.00^{a}
Myristoleic acid	1.05±0.03^{a}	1.05±0.02^{b}	1.08±0.01^{a}	1.05±0.02^{a}	1.15±0.03^{a}
Pentadecanoic acid	1.22±0.03^{ab}	1.14±0.02^{b}	1.20±0.02^{ab}	1.19±0.00^{ab}	1.25±0.02^{a}
Palmitic acid	34.36±0.40^{a}	31.84±0.27^{a}	34.86±0.14^{a}	33.70±0.18^{a}	36.62±0.25^{a}
Palmitoleic acid	2.08±0.12^{a}	1.69±0.13^{a}	2.09±0.11^{a}	2.07±0.10^{a}	2.16±0.01^{a}
Margaric acid	0.54±0.02^{a}	0.31±0.02^{a}	0.66±0.01^{a}	0.33±0.04^{a}	0.67±0.05^{a}
Margaricoleic acid	0.30±0.00^{a}	0.16±0.22^{a}	0.39±0.27^{a}	0.30±0.00^{a}	0.39±0.06^{a}
Stearic acid	11.50±0.13^{b}	11.46±0.18^{b}	11.84±0.20^{b}	11.94±0.15^{b}	11.58±0.23^{a}
Oleic acid	28.42±0.35^{a}	28.30±0.40^{a}	26.25±0.42^{b}	26.96±0.30^{b}	25.40±0.15^{c}
Linoleic acid	7.24±0.13^{bc}	10.80±0.18^{a}	7.14±0.02^{c}	9.54±0.08^{b}	3.61±0.13^{d}
α-Linolenic acid	0.66±0.06^{c}	1.15±0.04^{a}	0.57±0.02^{c}	0.97±0.00^{b}	0.00±0.04^{d}
Other fatty acids	0.20±0.02^{b}	0.22±0.04^{a}	0.44±0.05^{a}	0.63±0.03^{a}	1.43±0.08^{a}
Fat quality parameters					
Acid value (mg KOH/g)	2.81±0.12^{a}	2.31±0.06^{b}	2.40±0.03^{b}	1.90±0.06^{c}	2.81±0.11^{a}
Peroxide value (mEq O_2/kg)	12.52±0.05^{b}	11.20±0.13^{c}	11.14±0.04^{c}	10.12±0.06^{d}	13.02±0.02^{a}
Induction time at 110°C (h)	96±0.4^{c}	83±0.5^{cd}	102±0.3^{b}	98±0.2^{d}	112±0.3^{a}

Results are shown as mean ± standard deviation (n=3). Different letters within the same row indicate significant differences between the cookies ($p \leq 0.05$).

increase noted in the cookies with 20% LMP and 20% HCP, due to the higher fat content of the powders compared to wheat flour (**Table 1**).

Consumer acceptance of the shortbread cookies with the insect powders

Consumer acceptance is a crucial factor when introducing new products to the food market [Hassoun *et al*. 2022]. The results of consumer acceptance evaluation of the shortbread cookies with the insect powders are presented in **Table 7**. This evaluation included the assessment of taste, aroma, surface color brightness and uniformity, consistency, and texture. The shortbread cookies without the insect powder were rated as follows: taste (8.07), aroma (7.54), surface color brightness (9.41), surface color uniformity (8.22), consistency (6.78), and texture (3.80). According to the evaluators, the taste of the control cookies was buttery, characteristic of the raw materials used, and the aroma was sweet and buttery. In addition, the color of the control cookies was the brightest and most uniform. The scores given to the enriched cookies varied and were dependent on both insect type and powder amount added. As the amount of the insect powder in the cookies increased, the scores for taste, aroma, and color decreased; however, the observed differences were not statistically significant ($p>0.05$). Osimani *et al.* [2018] and Roncolini *et al*. [2019] also demonstrated that bread with cricket and mealworm powders had lower consumer acceptance compared to control bread without insect powder incorporation. In a study by Zielińska *et al*. [2021], lower overall acceptability of muffins with the addition of mealworm larvae and banded cricket powders compared to control muffins was due to lower color, consistency, smell, and taste ratings. González *et al*. [2019] highlighted that color changes in particular have a significant impact on consumer acceptance of bakery products. Pauter *et al.* [2018] reported that changes in the color of muffins containing cricket powder were perceived by consumers as unattractive and overly dark. On the other hand, some researchers state that the darker color of bakery and confectionery products is perceived by consumers as typical of health-promoting products, such as those obtained from whole-grain flours [Bawa *et al*., 2020; González *et al*., 2019; Pauter *et al*., 2018]. In the current study, the cookies with LMP received lower scores for surface color compared to cookies with HCP (**Table 7**). This lower rating could be attributed to the higher content of sugars in LMP, which contributes to Maillard reactions during thermal processing, resulting in a less acceptable color.

Edible insects remain a controversial food ingredient, and despite their increasing acceptance, they still have their opponents. It is pointed out that the knowledge of insect content in a food product can lead to lower consumer ratings [Alhujaili *et al*., 2023]. In addition, insects have a specific taste and aroma, which may not be acceptable to some consumers [Castro & Chambers, 2019]. Therefore, the percentage of insect powder in the final product is crucial. In this study, as the wheat flour replacement

Table 6. Nutritional value of cookies prepared from wheat flour (control) and from wheat flour replaced by 10% and 20% (*w/w*) of lesser mealworm powder (10% LMP and 20% LMP, respectively), and by 10% and 20% (*w/w*) of house cricket powder (10% HCP and 20% HCP, respectively).

Energy value/Nutrient content in 100 g	10% LMP	20% LMP	10% HCP	20% HCP	Control
Energy value (kcal)	519.5±0.9^{b}	525.1±0.3^{a}	516.0±0.5^{c}	518.5±0.4^{b}	513.0±0.8^{d}
Protein (g)	7.9±0.34^{c}	10.5±0.16^{b}	8.3±0.19^{c}	11.3±0.22^{a}	5.3±0.27^{d}
Carbohydrate (g)	57.4±0.31^{b}	53.5±0.19^{d}	56.8±0.32^{c}	52.4±0.26^{e}	61.3±0.42^{a}
Fat (g)	28.7±0.23^{b}	29.9±0.15^{a}	28.4±0.08^{b}	29.3±0.14^{a}	27.4±0.17^{c}
Ash (g)	0.9±0.02^{c}	1.1±0.02^{b}	1.1±0.02^{b}	1.4±0.02^{a}	0.8±0.02^{c}
Fiber (g)	1.5±0.02^{c}	1.6±0.03^{bc}	1.7±0.01^{b}	2.0±0.02^{a}	1.3±0.02^{d}
Water (g)	3.6±0.01^{c}	3.4±0.05^{d}	3.7±0.03^{b}	3.6±0.03^{bc}	3.9±0.02^{a}

Results are shown as mean ± standard deviation ($n=3$). Different letters within the same row indicate significant differences between the cookies ($p\leq0.05$).

Table 7. The consumer acceptability (rating on the linear scale from 1 to 9) of cookies prepared from wheat flour (control) and from wheat flour replaced by 10% and 20% (*w/w*) of lesser mealworm powder (10% LMP and 20% LMP, respectively) and by 10% and 20% (*w/w*) of house cricket powder (10% HCP and 20 HCP, respectively).

Characteristic	10% LMP	20% LMP	10% HCP	20% HCP	Control
Taste	5.27±2.86^{b}	4.71±2.69^{b}	6.29±2.43^{ab}	5.49±2.23^{b}	8.07±1.89^{a}
Aroma	4.93±2.23^{b}	5.10±2.59^{b}	5.95±2.67^{ab}	5.59±2.44^{b}	7.54±2.45^{a}
Brightness of surface color	3.71±1.93^{bc}	3.27±1.55^{bc}	5.32±2.07^{b}	2.56±1.92^{c}	9.41±0.77^{a}
Uniformity of surface color	4.80±2.59^{cd}	4.66±2.67^{d}	6.10±2.14^{b}	5.15±2.48^{c}	8.22±1.98^{a}
Consistency	5.85±2.29^{ab}	4.73±2.24^{b}	6.10±1.74^{ab}	5.93±2.09^{ab}	6.78±2.26^{a}
Texture	3.63±2.08^{a}	2.93±2.00^{a}	3.80±2.23^{a}	3.61±2.26^{a}	3.80±2.20^{a}

Results are shown as mean ± standard deviation ($n=41$). Different letters within the same row indicate significant differences between the cookies ($p\leq0.05$).

by the insect powder increased in the cookie recipes, the sensory evaluation scores decreased. However, these differences were not statistically significant ($p>0.05$). Kowalski *et al*. [2022a] showed that only a 30% addition of house cricket powder to bread significantly affected its external appearance, while bread with 10% insect powder achieved full consumer acceptance. The attitude towards edible insects and psychological aspects are also important. In Poland, edible insects are still treated as a new and controversial product. Castro Delgado *et al*. [2020] conducted sensory studies in Mexico, Spain, and the USA on cookies with 15% and 30% house cricket powder. They reported that Mexican consumers indicated cookies with 30% cricket powder as acceptable as control cookies, which was probably due to greater familiarity and acceptance of edible insects, which ultimately did not affect the psychological perception of taste and aroma by the consumers. Considering the obtained results, further studies may explore the incorporation of aromatic spices or fruits to enhance the sensory properties of the cookies, particularly their taste and aroma.

CONCLUSIONS

The present study discussed the potential application of lesser mealworm and house cricket powders in the production of shortbread cookies. The findings indicated that both insect powders enhanced the nutritional value of the cookies, particularly by increasing protein content and improving the fatty acid profile. The incorporation of lesser mealworm powder enriched the cookies with mono- and polyunsaturated fatty acids, notably oleic, linoleic, and α-linolenic acids. The increases in linoleic and α-linolenic acids were more pronounced than those observed with house cricket powder. Neither of the edible insect powders increased fat hydrolysis and oxidation levels in the baked cookies compared to the control products. However, the cookies containing lesser mealworm powder exhibited lower oxidative stability, as indicated by a shorter induction time, compared to those made with house cricket powder, suggesting a potentially shorter shelf-life for this innovative product. Despite this, the sorption properties of both insect powders indicated parameters that could ensure microbiological stability. In turn, consumer acceptance decreased with edible insect powder incorporation in the cookies. Nevertheless, the scores given for individual characteristics generally indicated an acceptable quality for the shortbread cookies produced.

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The study received no external funding.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

ADDITIONAL INFORMATION

All procedures for sensory evaluation were carried out in accordance with relevant laws and institutional guidelines of the Committee for Research Ethics of the University of Warmia and Mazury in Olsztyn. All individual participants took part in the study voluntarily knowing its purpose and scope. Participants gave informed consent that they were aware their responses were confidential. They were able to withdraw from the survey at any time without giving a reason.

All data generated or analyzed during this study are included in this published article.

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REFERENCES

1. AOAC (2000). *Official Methods of Analysis* (17th edition). The Association of Official Analytical Chemists International. Gaithersburg, MD, USA.
2. AOCS (2009). *Official Methods and Recommended Practices of the AOCS* (6th edition). American Oil Chemists's Society. Champaign, IL, USA.
3. Acosta-Estrada, B.A., Reyes, A., Rosell, C.M., Rodrigo, D., Ibarra-Herrera, C.C. (2021). Benefits and challenges in the incorporation of insects in food products. *Frontiers in Nutrition*, *8*, art. no. 687712. **https://doi.org/10.3389/fnut.2021.687712**
4. Aguilera, Y., Pastrana, I., Rebollo-Hernanz, M., Benitez, V., Álvarez-Rivera, G., Viejo, J.L., Martín-Cabrejas, M.A. (2021). Investigating edible insects as a sustainable food source: Nutritional value and techno-functional and physiological properties. *Food & Function*, *12*(14), 6309-6322. **https://doi.org/10.1039/d0fo03291c**
5. Alhujaili, A., Nocella, G., Macready, A. (2023). Insects as food: Consumers' acceptance and marketing. *Foods*, *12*(4), art. no. 886. **https://doi.org/10.3390/foods12040886**
6. Bawa, M., Songsermpong, S., Kaewtapee, C., Chanput, W. (2020). Nutritional, sensory, and texture quality of bread and cookie enriched with house cricket (*Acheta domesticus*) powder. *Journal of Food Processing and Preservation*, *44*(8), art. no. e14601. **https://doi.org/10.1111/jfpp.14601**
7. Castro Delgado, M., Chambers IV, E., Carbonell-Barrachina, A., Noguera Artiaga, L., Vidal Quintanar, R., Burgos Hernandez, A. (2020). Consumer acceptability in the USA, Mexico, and Spain of chocolate chip cookies made with partial insect powder replacement. *Journal of Food Science*, *85*(6), 1621-1628. **https://doi.org/10.1111/1750-3841.15175**
8. Castro, M., Chambers, E. (2019). Consumer avoidance of insect containing foods: primary emotions, perceptions and sensory characteristics driving consumers considerations. *Foods*, *8*(8), art. no. 351. **http://doi.org/10.3390/foods8080351**
9. Chisté, R.C., Silva, P.A., Lopes, A.S., da Silva Pena, R. (2012). Sorption isotherms of tapioca flour. *International Journal of Food Science & Technology*, *47*(4), 870-874. **https://doi.org/10.1111/j.1365-2621.2011.02900.x**
10. da Silva Lucas, A.J., de Oliveira, L.M., Da Rocha, M., Prentice, C. (2020). Edible insects: An alternative of nutritional, functional and bioactive compounds. *Food Chemistry*, *311*, art. no. e126022. **https://doi.org/10.1016/j.foodchem.2019.126022**
11. Delicato, C., Schouteten, J.J., Dewettinck, K., Gellynck, X., Tzompa-Sosa, D.A. (2020). Consumers' perception of bakery products with insect fat as partial butter replacement. *Food Quality and Preference*, *79*, art. no. e103755. **https://doi.org/10.1016/j.foodqual.2019.103755**
12. Duda, A., Adamczak, J., Chełmińska, P., Juszkiewicz, J., Kowalczewski, P. (2019). Quality and nutritional/textural properties of durum wheat pasta enriched with cricket powder. *Foods*, *8*(2), art. 46. **https://doi.org/10.3390/foods8020046**
13. Gharibzahedi, S.M.T., Altintas, Z. (2023). Lesser mealworm (*Alphitobius diaperinus* L.) larvae oils extracted by pure and binary mixed organic solvents: Physicochemical and antioxidant properties, fatty acid composition, and lipid quality indices. *Food Chemistry*, *408*, art. no. e135209. **https://doi.org/10.1016/j.foodchem.2022.135209**
14. González, C.M., Garzón, R., Rosell, C.M. (2019). Insects as ingredients for bakery goods. A comparison study of *H. illucens*, *A. domestica* and *T. molitor* flours. *Innovative Food Science & Emerging Technologies*, *51*, 205-210. **https://doi.org/10.1016/j.ifset.2018.03.021**
15. Hassoun, A., Cropotova, J., Trif, M., Rusu, A.V., Bobiş, O., Nayik, G.A., Jagdale, Y.D., Saeed, F., Afzaal, M., Mostashari, P., Khaneghah, A.M., Regenstein, J.M. (2022). Consumer acceptance of new food trends resulting from the fourth industrial revolution technologies: A narrative review of literature and future perspectives. *Frontiers in Nutrition*, *9* art. no. 972154. **https://doi.org/10.3389/fnut.2022.972154**

16. Hurtado-Ribeira, R., Hernández, D.M., Villanueva-Bermejo, D., García-Risco, M.R., Hernández, M.D., Vázquez, L., Fornari, T., Martin, D. (2023). The interaction of slaughtering, drying, and defatting methods differently affects oxidative quality of the fat from black soldier fly (*Hermetia illucens*) larvae. *Insects*, *14*(4), art. no. 368.
https://doi.org/10.3390/insects14040368
17. Khatun, H., Claes, J., Smets, R., De Winne, A., Akhtaruzzaman, M., Van Der Borght, M. (2021). Characterization of freeze-dried, oven-dried and blanched house crickets (*Acheta domesticus*) and Jamaican field crickets (*Gryllus assimilis*) by means of their physicochemical properties and volatile compounds. *European Food Research and Technology*, *247*, 1291-1305.
https://doi.org/10.1007/s00217-021-03709-x
18. Kim, T.-K., Cha, J.Y., Yong, H.I., Jang, H.W., Jung, S., Choi, Y.S. (2022). Application of edible insects as novel protein sources and strategies for improving their processing. *Food Science of Animal Resources*, *42*(3), 372-388.
https://doi.org/10.5851/kosfa.2022.e10
19. Kim, T.K., Yong, H.I., Kim, Y.-B., Kim, H.-W., Choi, C.-S. (2019). Edible insects as a protein source: a review of public perception, processing technology, and research trends. *Food Science of Animal Resources*, *39*(4), 521-540.
https://doi.org/10.5851/kosfa.2019.e53
20. Kowalski, S., Mikulec, A., Mickowska, B., Skotnicka, M., Mazurek A. (2022a). Wheat bread supplementation with various edible insect flours. Influence of chemical composition on nutritional and technological aspects. *LWT – Food Science and Technology*, *159*, art. no. e113220.
https://doi.org/10.1016/j.lwt.2022.113220
21. Kowalski, S., Oracz, J., Skotnicka, M., Mikulec, A., Gumul, D., Mickowska, B., Mazurek, A., Sabat, R., Wywrocka-Gurhul, A., Żyżelewicz, D. (2022b). Chemical composition, nutritional value, and acceptance of nut bars with the addition of edible insect powder. *Molecules*, *27*(23), art. no. 8472.
https://doi.org/10.3390/molecules27238472
22. Kowalski, S., Gumul, D., Oracz, J., Rosicka-Kaczmarek, J., Mikulec, A., Mickowska, B., Skotnicka, M., Zborowski, M. (2023). Chemical composition, antioxidant properties and sensory aspects of sponge cakes supplemented with edible insect flours. *Antioxidants*, *12*(11), art. no. 1912.
https://doi.org/10.3390/antiox12111912
23. Kulma, M., Kouřimská, L., Plachý, V., Božik, M., Adámková, A., Vrabec, V. (2019). Effect of sex on the nutritional value of house cricket, *Acheta domestica* L. *Food Chemistry, 272*, 267-272.
https://doi.org/10.1016/j.foodchem.2018.08.049
24. Kurečka, M., Kulma, M., Petříčková, D., Plachý, V., Kouřimská, L. (2021). Larvae and pupae of *Alphitobius diaperinus* as promising protein alternatives. *European Food Research and Technology*, *247*, 2527-2532.
https://doi.org/10.1007/s00217-021-03807-w
25. Mariutti, L.R.B., Sarmento Rebelo, K., Bisconsin-Junior, A., Santos de Morais, J., Magnani, M., Maldonade, I.R., Cazarin, C.B.C. (2021). The use of alternative food sources to improve health and guarantee access and food intake. *Food Research International*, *149*, art. no. 110709.
https://doi.org/10.1016/j.foodres.2021.110709
26. Marzoli, F., Tata, A., Zacometti, C., Malabusini, S., Jucker, C., Piro, R., Ricci, A., Belluco, S. (2023). Microbial and chemical stability of *Acheta domesticus* powder during one year storage period at room temperature. *Frontiers in Sustainable Food Systems*, *7*, art. no. e1179088.
https://doi.org/10.3389/fsufs.2023.1179088
27. Mathlouthi, M. (2001). Water content, water activity, water structure and the stability of foodstuffs. *Food Control*, *12*(7), 409-417.
https://doi.org/10.1016/S0956-7135(01)00032-9
28. Mazurek, A., Palka, A., Skotnicka, M., Kowalski, S. (2022). Consumer attitudes and acceptability of wheat pancakes with the addition of edible insects: Mealworm (*Tenebrio molitor*), buffalo worm (*Alphitobius diaperinus*), and cricket (*Acheta domesticus*). *Foods*, *12*(1), art. no. 1.
https://doi.org/10.3390/foods12010001
29. Menozzi, D., Sogari, G., Veneziani, M., Simoni, E., Mora, C. (2017). Eating novel foods: An application of the Theory of Planned Behaviour to predict the consumption of an insect-based product. *Food Quality and Preference*, *59*, 27-34.
https://doi.org/10.1016/j.foodqual.2017.02.001
30. Mieszkowska, A., Marzec, A. (2015). Structure analysis of short-dough biscuits and its correlation with sensory discriminants. *Journal of Texture Studies*, *46*(5), 313-320.
https://doi.org/10.1111/jtxs.12130
31. Mikołajczak, N., Tańska, M., Ogrodowska, D., Czaplicki, S. (2022). Efficacy of canolol and guaiacol in the protection of cold-pressed oils being a dietary source linoleic acid against oxidative deterioration. *Food Chemistry*, *393*, art. no. e133390.
https://doi.org/10.1016/j.foodchem.2022.133390
32. Mistuhashi, J. (2016). Edible Insects of the World (1st edition). Taylor & Francis Group: Boca Raton, FL, USA. ISBN 9781315367927.
https://doi.org/10.1201/9781315367927
33. Mlček, J., Adámková, A., Adámek, M., Borkovcová, M., Bednářová, M., Knížková, I. (2019). Fat from Tenebrionidae bugs – sterols content, fatty acid profiles, and cardiovascular risk indexes. *Polish Journal of Food and Nutrition Sciences*, *69*(3), 247—254.
https://doi.org/10.31883/pjfns/109666
34. Moreira, R., Chenlo, F., Torres, M.D., Prieto, D.M. (2010). Water adsorption and desorption isotherms of chestnut and wheat flours. *Industrial Crops and Products*, *32*(3), 252–257.
https://doi.org/10.1016/j.indcrop.2010.04.021
35. Nyangena, D.N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., Nakimbugwe, D., Fiaboe, K.K. (2020). Effects of traditional processing techniques on the nutritional and microbiological quality of four edible insect species used for food and feed in East Africa. *Foods*, *9*(5), art. no. 574.
https://doi.org/10.3390/foods9050574
36. Ocieczek, A., Zieba, M. (2020). Comparison of the sorption properties of fruit powder shampoos using the BET, GAB, and Peleg models. *ACS Omega*, *5*(24), 14354-14359.
https://doi.org/10.1021/acsomega.0c00851
37. Orkusz, A. (2021). Edible insects *versus* meat – Nutritional comparison: Knowledge of their composition is the key to good health. *Nutrients*, *13*(4), art. no. 1207.
https://doi.org/10.3390/nu13041207
38. Ortolá, M.D., Martínez-Catalá, M., Yuste Del Carmen, A., Castelló, M.L. (2022). Physicochemical and sensory properties of biscuits formulated with *Tenebrio molitor* and *Alphitobius diaperinus* flours. *Journal of Texture Studies*, *53*(4), 540-549.
https://doi.org/10.1111/jtxs.12692
39. Osimani, A., Milanović, V., Cardinali, F., Roncolini, A., Garofalo, C., Clementi, F., Pasquini, M., Mozzon, M., Foligni, R., Raffaelli, N., Zamporlini, F., Aquilanti, L. (2018). Bread enriched with cricket powder (*Acheta d*omesticus): A technological, microbiological and nutritional evaluation. *Innovative Food Science and Emerging Technologies, 48*, 150–163.
http://doi.org/10.1016/j.ifset.2018.06.007
40. Pauter, P., Różańska, M., Wiza, P., Dworczak, S., Grobelna, N., Sarbak, P., Kowalczewski, P. (2018). Effects of the replacement of wheat flour with cricket powder on the characteristics of muffins. *Acta Scientiarum Polonorum Technologia Alimentaria*, *17*(3), 227-233.
https://doi.org/10.17306/j.afs.0570
41. Ramos-Elorduy, J. (2009). Anthropo-entomophagy: Cultures, evolution and sustainability. *Entomological Research*, *39*(5), 271–288.
https://doi.org/10.1111/j.1748-5967.2009.00238.x
42. Roncolini, A., Milanović, B., Cardinali, F., Osimani, A., Garofalo, C., Sabbatini, R., Clementi, F., Pasquini, M., Mozzon, M., Foligni, R., Raffaelli, N., Zamporlini, F., Minazzato, G., Trombetta, M. F., Van Buitenen, A., Van Campenhout, L., Aguilanti, L. (2019). Protein fortification with mealworm (*Tenebrio molitor L.)* powder: Effect on textural, microbiological, nutritional and sensory features of bread. *PLoS One*, *14*(2), 1–29.
https://doi.org/10.1371/journal.pone.0211747
43. Roncolini, A., Milanović, V., Aquilanti, L., Cardinali, F., Garofalo, C., Sabbatini, R., Clementi, F., Belleggia, L., Pasquini, M., Mozzon, M., Foligni, R., Trombetta, M.F., Haouet, M.N., Altissimi, M.S., Di Bella, S., Piersanti, A., Griffoni, F., Reale, A., Niro, S., Osimani, A. (2020). Lesser mealworm (*Alphitobius diaperinus*) powder as a novel baking ingredient for manufacturing high-protein, mineral-dense snacks. *Food Research International*, *131*, art. no. e109031.
https://doi.org/10.1016/j.foodres.2020.109031
44. Rumpold, B.A., Schlüter, O.K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research*, *57*(5), 802-823.
https://doi.org/10.1002/mnfr.201200735
45. Ruszkowska, M., Tańska, M., Kowalczewski, P.Ł. (2022). Extruded corn snacks with cricket powder. Impact on physical parameters and consumer acceptance. *Sustainability*, *14*(24), art. no. e16578.
https://doi.org/10.3390/su142416578
46. Siddiqui, S.A., Wu, Y.S., Vijeepallam, K., Batumalaie, K., Hatta, M.M., Lutuf, H., Castro-Muñoz, R., Fernando, I., Ibrahim, S.A. (2024). *Alphitobius diaperinus* larvae (lesser mealworm) as human food – An approval of the European Commission – A critical review. *Journal of Insects as Food and Feed*, 1(aop), 1-40.
https://doi.org/10.1163/23524588-20240133
47. Singh, Y., Cullere, M., Kovitadhi, A., Chundang, P., Dalle Zotte, A. (2020). Effect of different killing methods on physicochemical traits, nutritional characteristics, *in vitro* human digestibility and oxidative stability during storage of the house cricket (*Acheta domesticus* L.). *Innovative Food Science and Emerging Technologies*, *65*, art. no. 102444.
http://doi.org/10.1016/j.ifset.2020.102444
48. Skotnicka, M., Mazurek, A., Karwowska, K., Folwarski, M. (2022). Satiety of edible insect-based food products as a component of body weight control. *Nutrients 14*(10), art. no. 2147.
https://doi.org/10.3390/nu14102147
49. Smarzyński, K., Sarbak, P., Kowalczewski, P.L., Różańska, M.B., Rybicka, I., Polanowska, K., Baranowska, H.M. (2021). Low-field NMR study of shortcake

biscuits with cricket powder, and their nutritional and physical characteristics. *Molecules, 26*(17), art. no. 5417.
https://doi.org/10.3390/molecules26175417

50. Sriprablom, J., Kitthawee, S., Suphantharika, M. (2022). Functional and physicochemical properties of cookies enriched with edible insect (*Tenebrio molitor* and *Zophobas atratus*) powders. *Journal of Food Measurement and Characterization*, *16*(3), 2181-2190.
https://doi.org/10.1007/s11694-022-01324-2
51. Sushchik, N.N., Yurchenko, Y.A., Gladyshev, M.I., Belevich, O.E., Kalachova, G.S., Kolmakova, A.A. (2013). Comparison of fatty acid contents and composition in major lipid classes of larvae and adults of mosquitoes (Diptera: *Culicidae*) from a steppe region. *Insect Science*, *20*(5), 585-600.
https://doi.org/10.1111/j.1744-7917.2012.01582.x
52. Tańska, M., Konopka, I., Ruszkowska, M. (2017). Sensory, physico-chemical and water sorption properties of corn extrudates enriched with spirulina. *Plant Foods Human Nutrition*, *72*, 250-257.
https://doi.org/10.1007/s11130-017-0628-z
53. Tapia, M.S., Alzamora, S.M., Chirife, J. (2020). Effects of water activity (aw) on microbial stability as a hurdle in food preservation. In G.V. Barbosa-Cánovas, A.J. Fontana Jr., S.J. Schmidt, T.P. Labuza (Eds.), *Water Activity in Foods: Fundamentals and Applications*. John Wiley & Sons, Inc., pp. 323-355.
https://doi.org/10.1002/9781118765982.ch14
54. Tzompa-Sosa, D.A., Yi, L., van Valenberg, H.J., van Boekel, M.A., Lakemond, C.M. (2014). Insect lipid profile: aqueous versus organic solvent-based extraction methods. *Food Research International*, *62*, 1087-1094.
https://doi.org/10.1016/j.foodres.2014.05.052
55. Tzompa-Sosa, D.A., Dewettinck, K., Provijn, P., Brouwers, J.F., de Meulenear, B., Oonicx, D.G.A.B. (2021). Lipidome of cricket species used as food. *Food Chemistry*, *349*, art. no. e129077.
https://doi.org/10.1016/j.foodchem.2021.129077
56. Udomsil, N., Imsoonthornruksa, S., Gosalawit, C., Ketudat-Cairns, M. (2019). Nutritional values and functional properties of house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*). *Food Science and Technology Research*, *25*(4), 597-605.
https://doi.org/10.3136/fstr.25.597
57. Ugur, A.E., Bolat, B., Oztop, M.H., Alpas, H. (2021). Effects of high hydrostatic pressure (HHP) processing and temperature on physicochemical characterization of insect oils extracted from *Acheta domesticus* (house cricket) and *Tenebrio molitor* (yellow mealworm). *Waste and Biomass Valorization*, *12*, 4277-4286.
https://doi.org/10.1007/s12649-020-01302-z
58. van Itterbeeck, J., Pelozuelo, L. (2022). How many edible insect species are there? A not so simple question. *Diversity*, *14*(2), art. no. 143.
https://doi.org/10.3390/d14020143
59. van Huis, A. (2023). Prospects for insects as human food. *Journal of Consumer Protection and Food Safety*, *18*, 105-106.
https://doi.org/10.1007/s00003-023-01438-9
60. Zielińska, E., Pankiewicz, U., Sujka, M. (2021). Nutritional, physiochemical, and biological value of muffins enriched with edible insects flour. *Antioxidants*, *10*(7), art. no. 1122.
https://doi.org/10.3390/antiox10071122
61. Zielińska, E., Pankiewicz, U. (2020). Nutritional, physiochemical, and antioxidative characteristics of shortcake biscuits enriched with *Tenebrio molitor* flour. *Molecules*, *25*(23), art. no. 5629.
https://doi.org/10.3390/molecules25235629

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Unlocking the Potential of Buckwheat Hulls, Sprouts, and Extracts: Innovative Food Product Development, Bioactive Compounds, and Health Benefits – a Review

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This comprehensive review explores the underutilized buckwheat hulls, sprouts, and grain and sprout extracts, concentrating on their nutritional characteristics, health advantages, and possible uses in developing functional food products. Buckwheat, a pseudocereal, is emphasized for its impressive nutritional content, including high levels of dietary fiber, essential minerals, and vitamins, as well as bioactive compound content, such as phenolic acids and flavonoids mainly rutin. The paper discusses the significant antioxidant and antimicrobial properties of buckwheat hulls, sprouts, and extracts, which contribute to their utility in creating healthier, functional food products. Buckwheat sprouts are noted for their enhanced levels of antioxidants and nutrients compared to mature grains. Meanwhile, buckwheat hulls, traditionally seen as by-products, are identified as sources of dietary fiber and flavonoids, suitable for use in dietary supplements and functional foods. The extracts from these parts are rich in bioactive compounds that offer health-promoting effects. The possible effects of addition of buckwheat hulls, sprouts, and extracts to food products in terms of nutritional, textural, and sensory properties are also discussed. The review underscores the need for further research to optimize the use of buckwheat less-utilized parts and to better understand their health impacts. By highlighting the novel uses and health benefits of buckwheat hulls, sprouts, and extracts, the review contributes to the growing field of sustainable food practices and the development of functional foods.

Keywords: buckwheat derivatives, buckwheat by-products, health benefits, zero-waste food industry

INTRODUCTION

Buckwheat, a pseudocereal distinguished by its impressive nutritional profile and adaptability, has been gaining attention not only for its grains but also for its underutilized parts such as sprouts and hulls, and extracts from these parts and grains. Unlike true cereals, buckwheat is related to sorrel and rhubarb, making it a valuable crop for both human consumption and sustainable agriculture practices, abundant in compounds with antioxidant, antimicrobial, and anti-inflammatory properties like phenolic acids and flavonoids (mainly rutin) [Dębski *et al*., 2021; Mazahir *et al*., 2022]. Buckwheat, replete with substantial amounts of dietary fiber, crucial minerals, and vitamins, functions as an indispensable ingredient across various culinary contexts. Furthermore, it acts as a vital link in addressing nutrient deficiencies and enhancing food security [Jha *et al*., 2024]. The presence of slowly digestible proteins and starches in buckwheat underscores the nutrition value of its derived products [Džafić & Oručević Žuljević, 2022; Kreft *et al*., 2022]. Its sprouts are rich in bioactive compounds [Atambayeva

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et al., 2023; Zhang *et al*., 2015], while the hulls, often discarded as waste, have potential uses in food technology and other industries [Kan *et al*., 2023; Zhang *et al*., 2023]. Buckwheat extracts have also been studied for their antioxidant and functional properties [Hęś *et al*., 2017]. The exploration of these buckwheat parts and extracts aligns with the global trend towards utilizing whole plants to minimize waste and maximize health benefits, marking an innovative shift in food product development.

The potential of buckwheat sprouts, hulls, and extracts in creating new, health-oriented food products is vast, yet underexplored. While the grains of buckwheat have been extensively studied, there remains a significant knowledge gap concerning the optimal use of its other derivatives. Sprouts, which emerge during the germination process, are known for their enhanced levels of antioxidants and nutrients compared to mature grains [Molska *et al*., 2022a; Shreeja *et al*., 2021]. Hulls, traditionally seen as by-products, contain valuable fibers and flavonoids, offering opportunities for use in dietary supplements and as functional food ingredients [Gutiérrez *et al*., 2023; Zhang *et al*., 2023]. Meanwhile, the bioactive compounds in buckwheat extracts, including rutin and quercetin, have demonstrated health-promoting effects, yet their integration into everyday food products is still in its infancy. Moreover, our literature search revealed that information on green buckwheat (thermally untreated) grain, as well as its sprouts and hulls, is notably limited. Investigating green buckwheat and its derivatives could prove economically beneficial since they require no treatment, thus lowering production costs. Also, the abundant bioactive compounds present in green buckwheat offer extensive research opportunities, potentially leading to innovative applications in nutrition and health sciences. This underexplored area promises substantial economic and scientific value, urging further exploration.

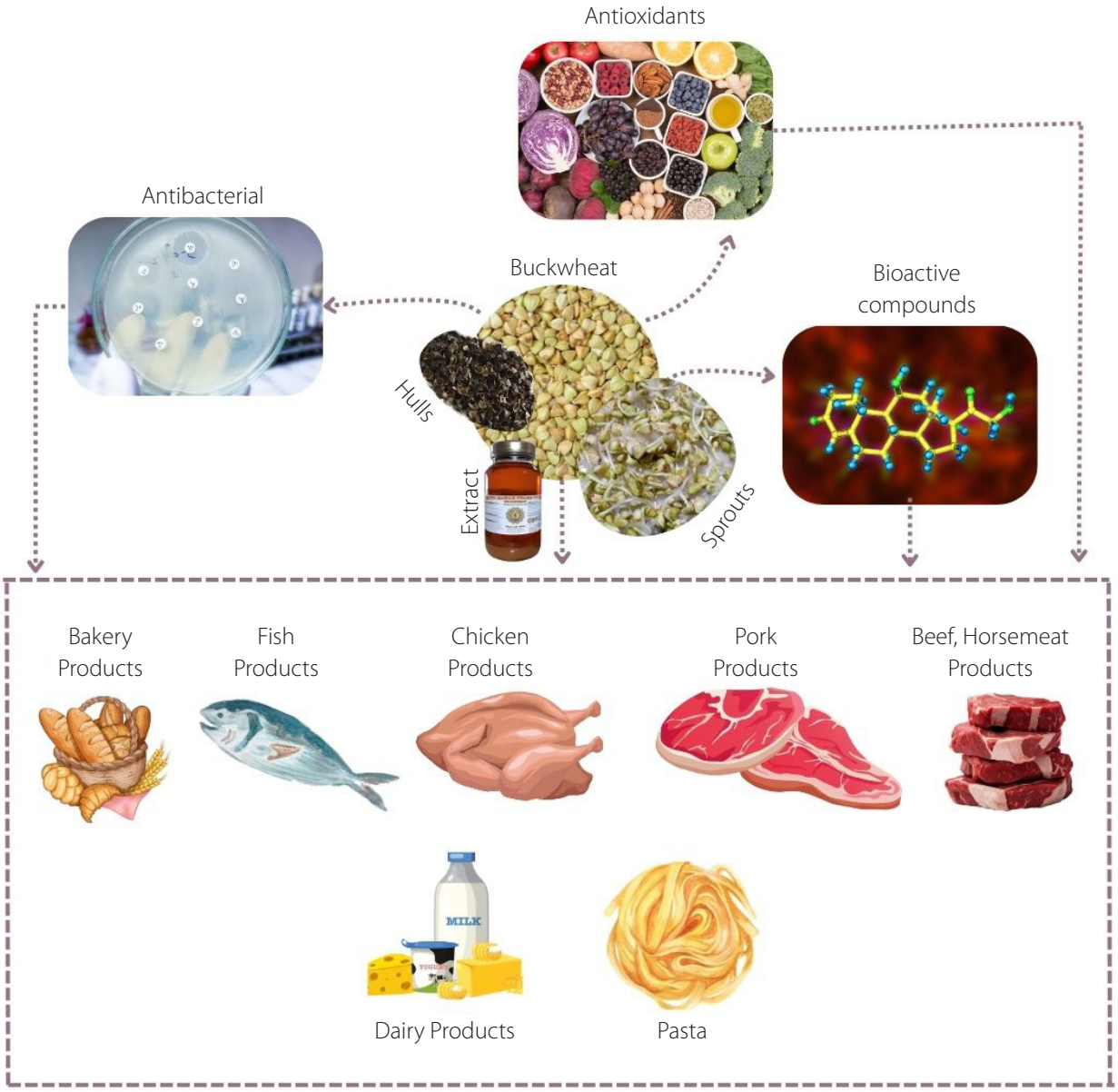

Figure 1. Integration of buckwheat hulls, sprouts, and extracts into diverse food categories for enhanced health benefits.

This review aims to consolidate existing research on the nutritional and bioactive profiles of buckwheat sprouts, hulls, and extracts, highlighting innovative approaches in food product development (**Figure 1**). By examining how these components have been used in various food matrices, the review will shed light on their functional benefits and potential health impacts. Moreover, it will discuss the technological challenges and opportunities in harnessing these parts of buckwheat and commercial application in food industries. This comprehensive synthesis will not only fill the existing knowledge gaps but also stimulate further research and development in this promising area.

The contribution of this review to the current scientific knowledge is twofold. Firstly, it provides a systematic overview of the potential applications and health benefits of buckwheat lesser-utilized parts and extracts, thus broadening the scope of uses for this versatile plant. Secondly, it encourages the adoption of sustainable practices in the food industry by promoting the use of agricultural by-products. This aligns with the global objectives of reducing food waste and enhancing nutritional quality in food production. Through this review, investors in the food industry, researchers, and consumers will gain a deeper understanding of the innovative potential that buckwheat sprouts, hulls, and extracts hold, steering the future direction of food product development towards more sustainable and health-conscious choices.

LITERATURE SEARCH AND STUDY SELECTION CRITERIA

This review adhered to the PRISMA guidelines [Moher *et al*., 2009; Rethlefsen *et al*., 2021] and aimed to conduct a thorough and systematic literature search using several databases, such as PubMed, Scopus, Web of Science SCIE, and Google Scholar. Keywords such as "buckwheat sprouts", "buckwheat hulls", "buckwheat extracts", "bioactive compounds", "health benefits", "flavonoids", "nutritional value", "anti-inflammatory effects", "anti-cancer potential", "functional foods", "dietary fiber", "protein content", "cardiovascular health", "mineral content", and "antimicrobial activity" were employed to locate relevant studies published in peer-reviewed journals. Both original research articles and review papers were included to ensure thorough coverage of the topic. Furthermore, references cited in the chosen articles were examined to uncover additional relevant research. The criteria for inclusion were specifically targeted at studies published in English, which investigated the nutritional makeup, bioactive compounds, and health-related outcomes associated with buckwheat sprouts, hulls, and extracts.

Studies were considered if they met the following criteria: (i) involved samples of buckwheat hulls, sprouts, or extracts; and (ii) analyzed nutrient and bioactive compounds for the development of functional foods and their health benefits.

After searching the following electronic databases PubMed, Scopus, Web of Science SCIE, and Google Scholar, 870 potentially relevant citations were identified, and after removing 51 duplicates and 557 citations for other reasons, 262 abstracts and titles were evaluated according to inclusion and exclusion criteria. Full texts and reference lists of 186 studies were evaluated, among which 120 studies met the criteria and were selected for inclusion in this review. In these studies, buckwheat hulls, sprouts, and some of their extracts and grain extracts have been used as additional ingredients in functional foods development, while their health benefits were studied in animals, and their bioactive compounds were identified in hulls, sprouts, and extracts, which we discuss in detail in the later sections.

NUTRITIONAL AND FUNCTIONAL INSIGHTS OF BUCKWHEAT HULLS, SPROUTS, AND EXTRACTS

Macronutrients (protein, fiber, carbohydrate, fat)

While buckwheat may not offer as much protein as other pseudocereals, like amaranth (139 mg/g dry weight, dw) and quinoa (165 mg/g dw) [Ahmed *et al*., 2014], it typically contains more protein than rice (6.8 g/100g grain), wheat (11.8 g/100g), and maize (9.4 g/100g) [Pirzadah & Malik, 2020]. Guo *et al*. [2007] reported that the average protein content in buckwheat is 12.94 g/100 g. The slower buckwheat protein digestibility, possibly due to polyphenol binding, is balanced by a highly beneficial amino acid composition that effectively meets essential biological needs [Luthar *et al*., 2021; Zhu, 2021]. Although buckwheat grains contain relatively low protein levels (~12 g/100 g), with these levels being higher than in most cereals, yet significantly lesser than in leguminous plants such as soybean meal (~51 g/100 g), it distinguishes itself through an abundant presence of lysine and arginine, two amino acids indispensable to human health [Ahmed *et al*., 2014; Dziadek *et al*., 2016; Jin *et al*., 2022; Luthar *et al*., 2021].

Buckwheat hulls

Buckwheat hulls, often considered a by-product in the production of buckwheat dehulled grains, have a modest nutritional profile, particularly in terms of crude protein and crude fat content (**Table 1**, **Figure 2**). Significant variability was observed among different varieties of buckwheat in terms of their hull protein content, ranging from 3.0 g/100 g to 6.5 g/100 g among 10 cultivars, with the average of 4.7 g/100 g [Lu *et al*., 2013]. Despite this low level, buckwheat proteins still offer all the essential amino acids, contributing 1.04 g/100 g dw for essential amino acids and 1.53 g/100 g dw for non-essential amino acids [Zhang *et al*., 2023]. However, these amounts are not sufficient to fulfil dietary requirements on their own.

In terms of crude fat, buckwheat hulls contain even smaller quantities (<1 g/100 g), compared to dehulled seed (~2.5 g/100 g), which varies depending on the cultivar [Ahmed *et al*., 2014; Dziadek *et al*., 2016; Matseychik *et al*., 2021; Zhang *et al*., 2023]. This minimal fat content comprises primarily of unsaturated fatty acids [Dziadek *et al*., 2016; Zhang *et al*., 2023]. The low-fat content is consistent with the composition of most plant hulls, which are primarily designed to protect the seed rather than store nutrients.

Buckwheat hulls were identified as a particularly rich source of total carbohydrates, with an average content of 92.02 g/100 g across six different cultivars/strains, as reported in a study by Dziadek *et al*. [2016]. The carbohydrates of buckwheat hulls are mostly

Table 1. Proximate composition of buckwheat hulls and sprouts.

Index	Hulls	Sprouts	Reference
Crude protein	on average 5.42 g/100 g	-	Dziadek *et al.* [2016]
	on average 4.7 g/100 g		Lu *et al.* [2013]
	4.05 g/100g		Zhang *et al.* [2023]
	4.83 g/100g		Matseychik *et al.* [2021]
		18.75 g/100 g fw	Sturza *et al.* [2020]
		24.3 g/100 g db	Kim *et al.* [2001]
		21.82 g/100 g dw	Lee & Kim [2008]
		20.8 g/100 g db	Kim *et al.* [2005]
		16.3 g/100 g dw	Peng *et al.* [2009]
		14.4 g/100 g dw	Molska *et al.* [2022b]
	on average 57.5 g/kg dm		Biel & Maciorowski [2013]
Crude fat	on average 0.59 g/100 g		Dziadek *et al.* [2016]
	trace		Matseychik *et al.* 2021
	0.4-0.9 g/100 g		Ahmed *et al.* [2014]
	0.13 g/100 g		Zhang *et al.* [2023]
		2.98 g/100 g dw	Lee & Kim [2008]
		25.26 mg/g	Zhang *et al.* [2015]
		1.3 g/100 g db	Kim *et al.* [2005]
		2.5 g/100 g dw	Peng *et al.* [2009]
		5.54 g/100 g fw	Sturza *et al.* [2020]
Total carbohydrates	on average 92.02 g/100 g		Dziadek *et al.* [2016]
	41.31 g/100 g		Matseychik *et al.* [2021]
		71.42 g/100 g dw	Lee & Kim [2008]
Ash	on average 1.97 g/100 g		Dziadek *et al.* [2016]
	on average 1.94 g/100 g		Lu *et al.* [2013]
	1.7 g/100 g		Zhang *et al.* [2023]
	1.49 g/100 g		Matseychik *et al.* [2021]
	on average 21 g/kg		Biel & Maciorowski [2013]
		2.53 g/100 g fw	Sturza *et al.* [2020]
	6.82 g/100 g		Matseychik *et al.* [2021]
		3.21 g/100 g db	Kim *et al.* [2001]
		3.78 g/100 g dw	Lee & Kim [2008]
		2.6 g/100 g db	Kim *et al.* [2005]
Starch	on average 1.20 g/100 g		Dziadek *et al.* [2016]
	2.55 g/100 g		Zhang *et al.* [2023]
		61.3 g/100 g dw	Peng *et al.* [2009]
Dietary fibre	on average 79.11 g/100 g		Dziadek *et al.* [2016]
	on average 80.6 g/100 g		Lu *et al.* [2013]
	31.31 g/100 g		Zhang *et al.* [2023]

Table 1 continued. Proximate composition of buckwheat hulls and sprouts.

Index	Hulls	Sprouts	Reference
	40.01 g/100 g		Matseychik *et al.* [2021]
	91.18 g/100 g		Dziedzic *et al.* [2012]
Crude fibre	average of 511.13 g/kg		Biel & Maciorowski [2013]
		4.67 g/100 g fw	Sturza *et al.* [2020]
		8.59 g/100 g db	Kim *et al.* [2001]
		16.11 g/100 g dw	Molska *et al.* [2022b]
		4.2 g/100 g dw	Peng *et al.* [2009]
Soluble dietary fibre		41.2–43.3 mg/g	Wu *et al.* [2023]

db, dry basis; dw, dry weight; fw, fresh weight.

in the form of insoluble non-starch polysaccharides (31.1 g/100 g), which contribute to their high fiber content [Zhang *et al.*, 2023]. The fiber content in buckwheat hulls is primarily insoluble and comprises significant amounts of xylose (15.78%), glucose (9.67%), and uronic acid (3.68%) relative to the total content in the hull [Zhang *et al.*, 2023]. This composition indicates a rich presence of hemicellulose-based fractions like xylan, xyloglucan, arabinoxylan, or galactoxyloglucan, alongside pectin-type polysaccharides, which are essential for digestive health [Matseychik *et al.*, 2021; Zhang *et al.*, 2023]. As well, the soluble fibre in the buckwheat hulls was also mainly composed of 0.06% xylose, 0.09% glucose, and 0.09% uronic acid [Zhang *et al.*, 2023].

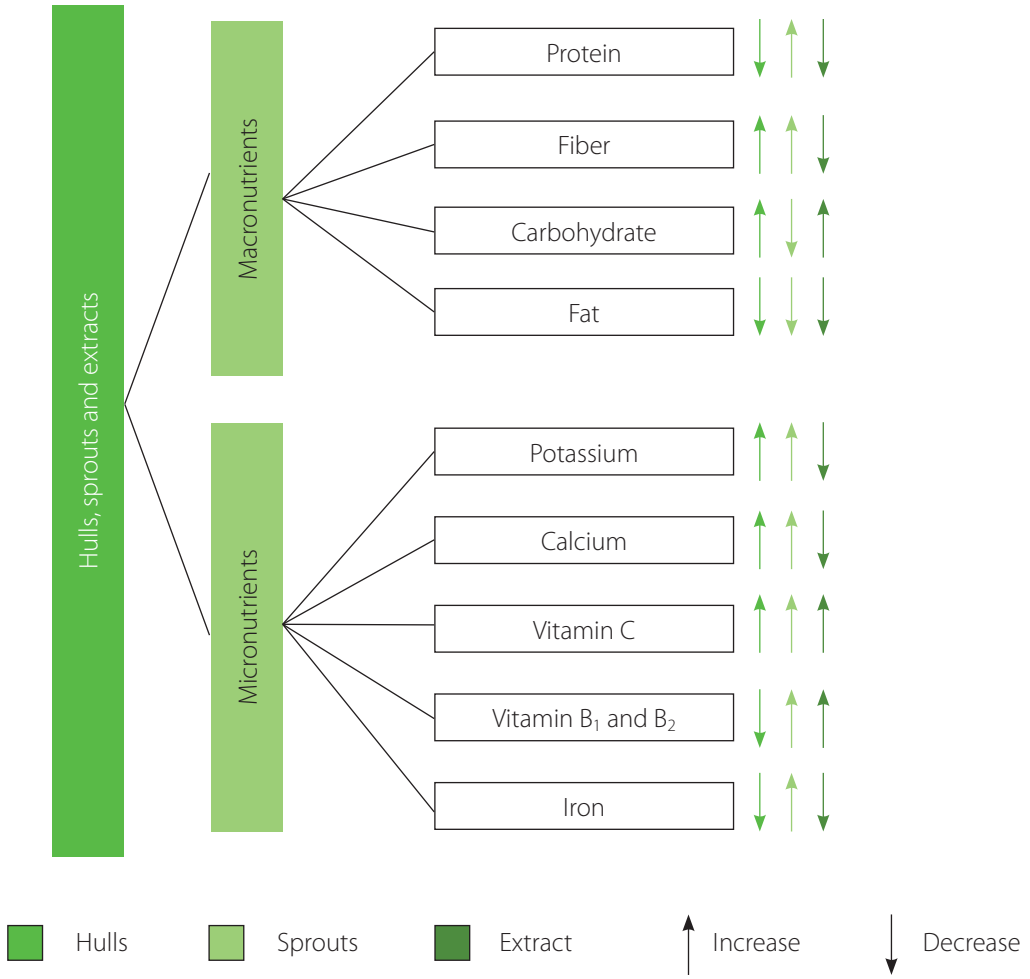

Figure 2. Nutritional compounds of buckwheat hulls, sprouts, and extracts.

Buckwheat sprouts

The buckwheat sprouts as a new vegetable were introduced for the first time by Kim *et al.* [2001, 2004] and since then, these sprouts have gained popularity in many countries, primarily consumed as they are, cherished for their soft yet slightly crispy texture and appealing fragrance. Buckwheat sprouts are noted for their elevated levels of protein, minerals, and crude fiber, exceeding those found in grains and hulls. Protein content varies under different sprouting conditions, as evidenced by various studies: Sturza *et al*. [2020] recorded 18.75 g/100 g fresh weight (fw), Kim *et al*. [2001] found 24.3 g/100 g dw (on the 8th day of germination), Lee & Kim [2008] determined 21.82 g/100 g dw (on the 7th day of germination), Kim *et al*. [2005] noted 20.8 g/100 g, and Peng *et al*. [2009] reported 16.3 g/100 g dw. Additionally, buckwheat sprouts modified with a probiotic yeast strain demonstrated a total protein content increase of approximately 22%, rising from 11.6 to 14.4 g/100 g [Molska *et al*., 2022b]. This augmentation is largely attributable to an increase in glutelins, which, along with globulins, form the major protein fractions in buckwheat [Molska *et al*., 2022b]. The protein found in these sprouts is of a notably high quality, featuring a comprehensive spectrum of essential amino acids such as valine, tyrosine, and lysine [Kim *et al*., 2001, 2004; Woo *et al*., 2013]. Also, modifications using yeast have led to an increase in methionine content, according to Molska *et al*. [2022b]. The protein content in sprouts can be higher than in unsprouted buckwheat seeds due to changes in seed chemistry that occur during germination and the breakdown by proteases of insoluble storage proteins into soluble peptides and then by hydrolases to generate amino acids, basic sugars, and unsaturated fatty acids, thereby enhancing the digestibility of nutrients in grains [Ali & Elozeiri, 2017; Guzmán-Ortiz *et al*., 2019; Zhou *et al*., 2016]. The different innovative technologies such as microwave, magnetic, electromagnetic, ultrasonic, and light (visible and ultraviolet) applied for seed germinating enhance the bioavailability of proteins and increase the levels of not only essential amino acids, such as glutamic acid and aspartic acid, but also accumulation of active compounds such as flavonoids [Wang *et al*., 2019]. This makes buckwheat sprouts a highly nutritious option for inclusion in healthy foods, offering a rich source of plant-based protein that supports muscle growth, repair, and overall health. Their high protein content, coupled with other nutritional benefits, positions buckwheat sprouts as a superior ingredient in the development of functional and fortified food products [Kim *et al*., 2001, 2004; Sturza *et al*., 2020].

The fat content in buckwheat is relatively low, typically ranging from 1 to 3 g/100 g of dw [Ahmed *et al*., 2014]. During the germination process, fats and carbohydrates are broken down to supply energy for seed growth, resulting in decreased levels of these macronutrients. According to a study by Zhang *et al*. [2015], the crude fat content in buckwheat was observed to decline from 30.68 mg/g in ungerminated seeds to 25.26 mg/g after germinating for 72 h. Despite the low quantity, the fat in buckwheat sprouts is rich in essential fatty acids, particularly linoleic acid, and α-linolenic acid, with unsaturated fatty acids comprising over 83% of the total lipid content, which are important for maintaining heart health and supporting immune function [Kim *et al*., 2004; Molska *et al*., 2020; Shahidi & Ambigaipalan, 2018; Zhou *et al*., 2015].

Buckwheat sprouts offer a higher crude fiber content at 8.59 g/100 g (on the 8th day of germination), surpassing that of unsprouted seeds which contain 3.82 g/100 g, according to Kim *et al*. [2001]. Furthermore, Sturza *et al*. [2020] highlighted that sprouted buckwheat flour had the richest fiber composition at 4.67 g/100 g when compared to regular buckwheat at 4.08 g/100 g and wheat flour at only 1.14 g/100 g. The crude fiber in these products includes a variety of fibrous materials such as cellulose, lignin, and hemicellulose, and its composition can vary depending on the buckwheat cultivar [Witkowicz *et al*., 2019]. Dietary fiber is categorized into two types: insoluble dietary fiber (IDF) and soluble dietary fiber (SDF). IDF mainly includes cellulose, hemicellulose, and lignin, substances that do not dissolve in water. Conversely, SDF consists of water-soluble components like pectin and gums [Guan *et al*., 2021]. Molska *et al*. [2022a] found that probiotic-rich buckwheat sprouts had the highest content of total dietary fiber (16.11 g/100 g), while the lowest content was found in seeds (11.37 g/100 g) and the dominant dietary fiber fraction in probiotic-rich sprouts was soluble dietary fiber. Kim *et al*. [2009a] found that the thermomechanical extrusion process altered the balance between soluble and insoluble dietary fibers, favoring an increase in SDF. Consequently, SDF content in sprouts rose from 9.0 g/100 g to 12.4 g/100 g, while IDF content decreased from 15.3 g/100 g to 10.5 g/100 g. SDF yields from common and tartary buckwheat sprouts were comparably measured at 43.3 mg/g and 41.2 mg/g, respectively, indicating that germination enhances the SDF content in buckwheat seeds [Wu *et al*., 2023]. The high fiber content helps in promoting satiety, reducing cholesterol levels, and supporting overall gut health, making buckwheat sprouts an ideal ingredient for functional foods aimed at improving digestive wellness [Kim *et al*., 2004].

Buckwheat extracts

Buckwheat extracts, typically obtained from the seeds, sprouts, or hulls of buckwheat, are concentrated sources of bioactive compounds, therefore they are relatively low in macronutrients such as crude protein and crude fat (**Figure 2**). Unlike many common plant protein sources where globulin is the most abundant protein (60–80%), albumin (20.99%), globulin (12.80%), and glutelin (13.31%) are the predominant fractions in buckwheat protein [Hua *et al*., 2024]. The proteins tend to aggregate and precipitate out of solution, making their extraction less efficient [Yang *et al*., 2021]. This aggregation and precipitation of the large protein molecules is the primary reason for the low protein content observed in buckwheat extracts.

As mentioned in the previous section, buckwheat seeds and sprouts, particularly those of the species such as *Fagopyrum esculentum* and *Fagopyrum tataricum*, are recognized for their low crude fat content. Therefore, the fat present in buckwheat grain, hull and seed extracts is in trace amounts unless specifically targeted for extraction.

Lack of proteins and lipids in extracts makes them ideal for use in supplements and functional foods where high-intensity natural bioactives are desired without additional calories from macronutrients. The main bulk substances of buckwheat extracts are carbohydrates, primarily in the form of starch and various soluble carbohydrates like fagopyritols [Ahmed *et al*., 2014; Zhang *et al*., 2023; Zieliński *et al*., 2019]. Fagopyritols, which are galactosyl derivatives of D-chiro-inositol, play a crucial role in the maturation of buckwheat seeds [Zieliński *et al*., 2019]. The carbohydrate content in buckwheat extracts can vary depending on the extraction and processing methods used.

Micronutrients (mineral and vitamin composition)

Buckwheat hulls and sprouts each offer distinct profiles of minerals and vitamins [Dziadek *et al*., 2016; Witkowicz & Biel, 2022]. The content of individual minerals in both hulls and sprouts, as well as extracts can vary depending on the plant cultivar, grain processing, and extraction methods (**Table 2**). While buckwheat extracts are rich in bioactive compounds and they do not serve as significant sources of traditional nutrients such as vitamins and minerals.

Buckwheat hulls

Buckwheat hulls contain trace amounts of magnesium, calcium, and phosphorus [Dziadek *et al*., 2016; Ikeda *et al*., 1999; Kim *et al*., 2005; Matseychik *et al*., 2021; Sytar *et al*., 2016; Witkowicz & Biel, 2022]. Although the contents of these minerals in the hulls are relatively low, they still contribute to the overall mineral intake when included in the diet. Studies have shown that the hulls are particularly high in calcium, making them beneficial for bone health [Ikeda *et al*., 1999]. In addition, they contain minerals such as iron, zinc, and manganese, which are crucial for various metabolic processes and maintaining overall health [Yilmaz *et al*., 2020]. The presence of these minerals has led buckwheat hulls to be considered as a valuable ingredient for developing fortified foods such as noodles, yogurt, or tea [Ikeda *et al*., 1999; Liu *et al*., 2022; Zielińska *et al*., 2013; Znamirowska *et al.,* 2020]. Buckwheat hulls, while primarily valued for their high fiber content, also contain important vitamins, albeit in smaller quantities compared to other parts of the buckwheat plant. Thus, Kuznetsova *et al*. [2020] documented the mineral and vitamin content in buckwheat hull across various cultivars, revealing a range of contents. Iron was determined between 38.32 and 77.85 mg/kg, zinc from 10.36 to 18.54 mg/kg, copper from 1.18 to 4.66 mg/kg, and manganese from 2.95 to 4.96 mg/kg. The hulls also contained significant levels of vitamins, with vitamin B_1 (thiamine) at 4.6 mg/g, B_3 (niacin and niacinamide) at 17.6 mg/g, B_5 (pantothenic acid) at 10.2 mg/g, and vitamin C (ascorbic acid) at 42.5 mg/g. The presence of these vitamins contributes to the overall nutritional profile of buckwheat hulls, supporting metabolic processes and antioxidant defences. Nandan *et al*. [2024] found that the mineral distribution in buckwheat hull was 3.40–4.20 g/100 g.

Buckwheat sprouts

Buckwheat sprouts are highly nutritious, with a mineral profile that surpasses that of the grains [Ikeda *et al*., 1999; Pongrac *et al*., 2016]. The sprouts are particularly rich in magnesium, phosphorus, potassium, calcium, and molybdenum [Kim *et al*., 2001, 2005; Witkowicz & Biel, 2022]. Kim *et al*. [2005] reported that buckwheat sprouts contained significant levels of calcium at 152.0 mg/100 g, zinc at 9.9 mg/100 g, magnesium at 485.0 mg/100 g, and iron at 5.4 mg/100 g on a dry basis. Also, the vitamin content was determined, revealing vitamin A at 1,180 IU/100 g, vitamin C at 203 mg/100 g, and vitamin E at 32.1 mg/100 g on a dry basis. Mentioned minerals play crucial roles in numerous physiological processes, such as muscle and nerve activity, maintaining bone health, and generating energy. Sprouts evolve from grains through imbibition, germination, and various seedling development stages. During this process, they lose dry matter (primarily nonfibrous carbohydrates) due to respiration, while water content and mineral uptake from newly developed roots increase. These changes enhance the content of minerals in sprouts, leading to higher mineral contents in both tartary and common buckwheat sprouts compared to their grain forms [Pongrac *et al*., 2016]. Lee *et al*. [2006] reported higher contents of magnesium, phosphorus, potassium, and iron, but lower contents of calcium and zinc in tartary buckwheat sprouts. The contents of these minerals make buckwheat sprouts a nutrient-dense food. Buckwheat sprouts are also rich in vitamins. They are particularly high in vitamin C, with levels increasing significantly during the germination process, reaching up to 171.5 mg/100 g at the end of sprouting [Kim *et al*., 2004]. Furthermore, buckwheat sprouts have abundant vitamins B_1 and B_6 [Kim *et al*., 2004]. The high vitamin content, especially of vitamin C, makes buckwheat sprouts an excellent choice for boosting the nutritional value of various food products such as sprouts as a functional food [Kim *et al*., 2001, 2007b], spices [Serikbaeva *et al*., 2021], or bread [Xu *et al*., 2014].

ANTIOXIDATIVE AND ANTIMICROBIAL CAPACITIES OF BUCKWHEAT HULLS, SPROUTS, AND EXTRACTS

Buckwheat contains various bioactive compounds, such as flavonoids (vitexin, isovitexin, isoorientin, orientin, rutin, isoquercetin, and quercetin), phenolic acids (ferulic, vanillic, protocatechuic, and gallic acids), and carotenoids [Cui *et al*., 2020; Lim *et al*., 2012; Park *et al*., 2019]. These compounds contribute to numerous health benefits through their antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and cardiovascular support properties, as well as promote gastrointestinal health, bone health, and antiaging effects (**Figure 3**). Among the bioactivities caused by phenolic compounds, the antioxidant and antimicrobial potential of buckwheat sprouts and hulls, as well as extracts from these parts of the plant and from grains, is often highlighted in terms of the use of various forms of buckwheat as a valuable ingredient in the development of functional foods.

Antioxidant potential of buckwheat hulls, sprouts, and extracts

Buckwheat hulls

Buckwheat hulls are recognized for their potent antioxidant effects, attributed to abundance of phenolic compounds such as quercetin, rutin, and protocatechuic acid. According to Dziadek *et*

Table 2. Mineral content of buckwheat hulls and sprouts.

Mineral	Hulls	Sprouts	Reference
Calcium	-	1,118 mg/100g db	Kim *et al.* [2001]
	-	3.9 g/kg dm	Witkowicz & Biel [2022]
	-	152.0 mg/100 g	Kim *et al.* [2005]
	-	8,410 mg/kg dw	Pongrac *et al.* [2016]
	260.0 mg/100 g	-	Matseychik *et al.* [2021]
	97.4 mg/100 g dw	-	Ikeda *et al.* [1999]
Magnesium	-	804.0 mg/100 g	Kim *et al.* [2001]
	-	5.5 g/kg dm	Witkowicz & Biel [2022]
	-	5,470 mg/kg dw	Pongrac *et al.* [2016]
	-	485.0 mg/100 g	Kim *et al.* [2005]
	112 mg/100 g dw	-	Ikeda *et al.* [1999]
Phosphorous	-	12.1 g/kg dm	Witkowicz & Biel [2022]
	-	7,930 mg/kg dw	Pongrac *et al.* [2016]
	127 mg/100 g dw		Ikeda *et al.* [1999]
Potassium	-	1,798 mg/100 g db	Kim *et al.* [2001]
	-	11.7 g/kg dm	Witkowicz & Biel [2022]
	-	7,290 mg/kg dw	Pongrac *et al.* [2016]
	840.0 mg/100 g	-	Matseychik *et al.* [2021]
	1,267 mg/kg dw	-	Ikeda *et al.* [1999]
Sodium	-	134.9 mg/100 g db	Kim *et al.* [2001]
	-	0.7 g/kg dm	Witkowicz & Biel [2022]
	1,000.0 mg/100 g	-	Matseychik *et al.* [2021]
Zinc	-	10.5 mg/100 g db	Kim *et al.* [2001]
	-	9.9 mg/100 g	Kim *et al.* [2005]
	-	130 mg/kg dw	Pongrac *et al.* [2016]
	-	48.5 mg/kg dm	Witkowicz & Biel [2022]
	varied from 10.36 to 18.54 mg/kg	-	Kuznetsova *et al.* [2020]
	1.24 mg/kg dw	-	Ikeda *et al.* [1999]
Copper	-	5.8 mg/100 g db	Kim *et al.* [2001]
	-	9.0 mg/kg dm	Witkowicz & Biel [2022]
		10.8 mg/kg dw	Pongrac *et al.* [2016]
	varied from 1.18 to 4.66 mg/kg	-	Kuznetsova *et al.* [2020]
	0.63 mg/kg dw	-	Ikeda *et al.* [1999]
Manganese	-	2.7 mg/100 g db	Kim *et al.* [2001]
	-	22.4 mg/kg dm	Witkowicz & Biel [2022]
	-	21.1 mg/kg dw	Pongrac *et al.* [2016]
	varied from 2.95 to 4.96 mg/kg	-	Kuznetsova *et al.* [2020]
	9.16 mg/kg dw	-	Ikeda *et al.* [1999]

Table 2 continued. Mineral content of buckwheat hulls and sprouts.

Mineral	Hulls	Sprouts	Reference
Iron	-	20.6 mg/100 g db	Kim *et al.* [2001]
	-	50.3 mg/kg dm	Witkowicz & Biel [2022]
	-	5.4 mg/100 g	Kim *et al.* [2005]
	-	70.7 mg/kg dw	Pongrac *et al.* [2016]
	48.0 mg/100g	-	Matseychik *et al.* [2021]
	varied from 38.32 to 77.85 mg/kg	-	Kuznetsova *et al.* [2020]
Molybdenum	-	45.7 mg/kg dm	Witkowicz & Biel [2022]
	-	1.89 mg/kg dw	Pongrac *et al.* [2016]

dm, dry matter; db, dry basis; dw, dry weight.

al. [2016], the total phenolic content in these hulls varies between 434.06 and 525 mg/100 g, dependent on the cultivar or strain. Zhang *et al.* [2023] identified protocatechuic acid (390.71 mg/kg) as the most prevalent metabolite in buckwheat hulls, present in both free (184.81 mg/kg) and bound (205.91 mg/kg) forms. Further, an analysis by Zhang *et al.* [2017] identified seven distinct flavonoids in buckwheat hulls — orientin, isoorientin, vitexin, isovitexin, hyperin, rutin, and quercetin. The levels of vitexin/isovitexin, hyperin, and rutin in buckwheat hulls showed considerable variation across the eight cultivars tested [Zhang *et al.*, 2017]. The content of vitexin and isovitexin ranged from 101.65 to 188.78 mg/100 g, hyperin content varied from 53.55 to 274.10 mg/100 g, while rutin content ranged from 62.43 to 173.57 mg/100 g [Zhang *et al.*, 2017]. According to results given by Cui *et al.* [2020], rutin, isoorientin, vitexin, and hyperoside showed varied efficacy in scavenging different free radicals ($^{\bullet}OH$, $O_2^{\bullet-}$, $DPPH^{\bullet}$) and only rutin showed excellent total antioxidant capacity (T-AOC). Rutin was particularly notable as a predominant flavonoid in buckwheat hulls, a finding supported by several studies [Cui *et al.*, 2020; Park *et al.*, 2019; Sedej *et al.*, 2012; Zhang *et al.*, 2017]. These bioactive compounds through their antioxidant activity offered numerous health advantages such as lowering cholesterol levels [Lin *et al.*, 2008], reducing blood pressure, diminishing inflammatory responses [Al-Khayri *et al.*, 2022; Qing *et al.*, 2023; Tsai *et al.*, 2012], and aiding in the management of diabetes and obesity [Cai *et al.*, 2023; Zhao *et al.*, 2018]. Phenolic antioxidants found in buckwheat hulls have been shown to decrease the formation of reactive oxygen species (ROS) and malondialdehyde (MDA) or increase catalase (CAT) activity on cellular-based assays [Cui *et al.*, 2020]. Although the existing evidence is promising, further investigations are required to clarify the full scope of buckwheat hull effects.

Buckwheat sprouts

Buckwheat sprouts are particularly rich in antioxidants, including rutin, quercetin, and various phenolic acids such as chlorogenic, caffeic, ferulic, and gallic acids [Ji *et al.*, 2016; Mansur *et al.*, 2022; Molska *et al.*, 2022a]. The total phenolic content of buckwheat sprouts is higher than that of seeds [Aloo *et al.*, 2021; Molska *et al.*, 2022a]. Three phenolic acids (caffeoyl-glucoside,

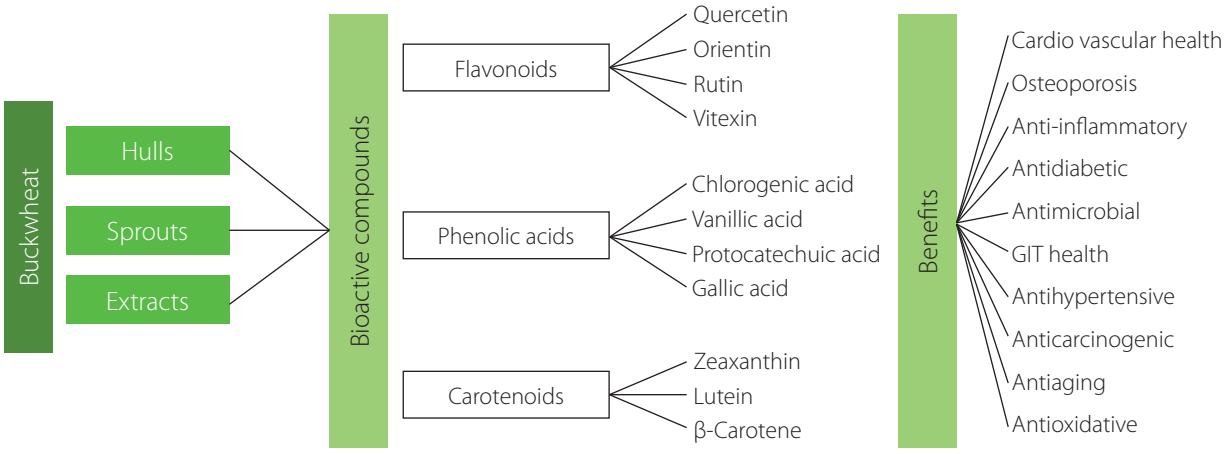

Figure 3. Bioactive compounds of buckwheat hulls, sprouts, and extracts and their potential health benefits.

caffeoyl-rhamnopyranosyl-glucopyranosyl-glucopyranoside, and caffeoyl-rhamnopyranosyl-glucopyranosyl) were identified in the sprouts [Molska *et al*., 2022a]. Caffeoyl-rhamnopyranosyl-glucopyranosyl was the dominant phenolic acid; its content in control sprouts was 92.04 µg/g dw and in probiotic-rich sprouts it was 118.23 µg/g dw. The caffeoyl content was 53.23 µg/g dw in control sprouts, and 55.48 µg/g dw in probiotic-rich sprouts. It is also important to note that the specific contributions of flavonoid content, specifically rutin, orientin, and epicatechin uniquely contribute to the overall antioxidant activity, illustrating a complex relationship between specific flavonoid levels and the antioxidant capacity in both common and tartary buckwheat sprouts [Rauf *et al*., 2019; Wiczkowski *et al*. 2014; Zych-Wężyk & Krzepiłko, 2012]. Significant increases in specific flavonoids have been documented during the sprouting process; for example, Borgonovi *et al*. [2023] observed that rutin content soared from 9.91 µg/g dw in buckwheat grain to 23.01 µg/g dw in grains sprouting for 72 days. Similarly, quercetin content increased from 3.39 µg/g dw in the unsprouting grain to 28.12 µg/g dw in grains sprouting over the same period. As the germination continued, the levels of rutin and quercetin progressively increased, enhancing the sprouts' capacity to reduce oxidation and scavenge free radicals [Borgonovi *et al*., 2023].

During sprouting, the phenolic content in buckwheat increases, leading to enhanced antioxidant capacity. The ferric reducing antioxidant power (FRAP) of grains was shown to increase significantly from 9.91 µmol Trolox eq/g dw in unsprouting grains to 17.98 µmol Trolox eq/g dw in the grains germinating for 72 h [Borgonovi *et al*., 2023]. Meanwhile, the total antioxidant capacity (TAC) showed a notable increase only after 72 h of sprouting, from 26.21 to 46.99 µmol Trolox eq/g dw. Also, the antioxidant potential measured by ABTS assay was at 12.63 mg Trolox eq/g (control sprouts) and 19.78 mg Trolox eq/g (probiotic-rich sprouts) before digestion (*in vitro*), and at 9.13 mg Trolox eq/g (control sprouts) and 11.05 mg Trolox eq/g (probiotic-rich sprouts) after digestion, with genetically-modified sprouts showing the highest activity [Molska *et al*., 2022a]. In terms of reduction power, the modified sprouts exhibited the highest value at 31.15 mg Trolox eq/g, significantly surpassing that of the seeds [Molska *et al*., 2022a]. Liu *et al*. [2008] analyzed the reducing power of ethanol extracts from both common and tartary buckwheat sprouts and found that the reducing power, measured in absorbance at 700 nm, was greater in the tartary buckwheat sprouts extracts, with a peak value of approximately 0.35, compared to common buckwheat sprouts, which showed a maximum of about 0.25. This suggests a superior antioxidant capability of the tartary buckwheat sprout extract at the concentration of 5 mg/mL.

In the investigation conducted by Aloo *et al*. [2021], the focus was on evaluating the impact of sprouting on alfalfa and buckwheat seeds in terms of their antioxidant, antidiabetic, and antiobesity capabilities, as well as alterations in their metabolite compositions. The findings demonstrated that buckwheat sprouts outperformed the rest, showcasing the strongest ability to scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, followed in effectiveness by alfalfa sprouts, buckwheat seeds, and finally alfalfa seeds.

Utilizing advanced analytical techniques, such as liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS), the investigation into anthocyanin profiles spanned various varieties and breeding lines of common and tartary buckwheat sprouts [Kim *et al*., 2007b]. This research uncovered the presence of four distinct anthocyanins in common buckwheat sprouts and two in tartary buckwheat sprouts. Notably, the Hokkai T10 variety emerged with the highest anthocyanin content, positioning it as an exceptional candidate for "Moyashi" type sprouts. Anthocyanins are known for their antioxidant activity; therefore, it seems that this group of phenolic compounds may also contribute to the antioxidant potential of buckwheat sprouts.

Buckwheat extracts

Sun & Ho [2005] found that extracts from buckwheat whole grains, using solvents such as acetone, butanol, ethanol, ethyl acetate, and methanol, exhibited significant antioxidant effects. Notably, the methanolic extract was the most effective, showing a high antioxidant activity coefficient of 627 at 200 mg/L, measured *via* the carotene-bleaching method. In contrast, acetone extracts had the highest total phenolic content at 3.4 g catechin eq/100 g and displayed the strongest scavenging activity, at 78.6%, at a concentration of 0.1 mg/mL, according to the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay. Li *et al*. [2013] demonstrated that extracts from buckwheat hulls had a higher total phenolic content (TPC) and antioxidant capacity than those derived from buckwheat flour. Common buckwheat (*F. esculentum* Möench) hull extract exhibited the highest reducing power and DPPH radical scavenging activity with the average EC_{50} 84.54 µg/mL and IC_{50} 11.54 µg/mL, respectively compared to flour extract which showed the lowest TPC, reducing power and DPPH radical scavenging activity. Further research by Hęś *et al*. [2012] revealed that extracts from buckwheat hulls, especially those obtained using methanol as a solvent, had significantly high DPPH radical scavenging activity, with methanol extracts displaying double the activity of acetone extract and eight times the activity of water extract. These findings suggest that methanol is an effective solvent for extracting antioxidants from buckwheat and that these extracts show great potential as food additives to replace artificial antioxidants. This is also confirmed by the fact that buckwheat hull extracts were able to significantly reduce the total oxidation rate of bulk oil and oil-in-water and water-in-oil emulsions [Lee *et al*., 2022], *i.e.*, model systems corresponding to food products. The authors concluded that flavonoid glycosides and methylated phenolics were mainly responsible for the reduction in the emulsion oxidation rate.

Antimicrobial properties of buckwheat hulls, sprouts, and extracts

The rising prevalence of bacterial resistance to current antibiotics has become a serious concern, necessitating the search for novel classes of antibacterial agents, particularly from natural sources. Buckwheat hulls, sprouts, and extracts are known not just for their nutritional and antioxidant properties but also for

Table 3. Antimicrobial activity of buckwheat hull and sprout extracts.

Extracted material	Extract concentration	Diameter of inhibition zone (mm)							Reference
		Gram-positive				Gram-negative			
		Staphylococcus aureus	*Bacillus subtilis*	*Bacillus cereus*	*Enterococcus faecalis*	*Salmonella choleraesuis*	*Proteus mirabilis*	*Escherichia coli*	
Hull	100 mg/mL	12.6	-	13.9	13.6	11.3	11.0	10.6	Čabarkapa *et al.* [2008]
	50 mg/mL	11.6	-	13.3	13.3	10.0	10.33	9.6	
	500 µg	9	-	-	-	-	-	-	Cho *et al.* [2006]
Sprouted grain	Not mentioned	3.7	3.6	-	-	-	-	1.5	Zhou *et al.* [2011a]

their antimicrobial capabilities, attributed to their high content of flavonoids like rutin and quercetin, making them valuable in potential therapeutic applications. The crude methanol extract from tartary buckwheat sprouts showed significant inhibitory activity against various bacteria, impacting both Gram-negative strains (*Pseudomonas lachrymans* and *Salmonella typhimurium*) and Gram-positive strains (*Bacillus subtilis*, *Scaphirhynchus albus*, and *Staphylococcus aureus*), with minimum inhibitory concentration (MIC) values varying between 0.8 mg/mL and 3.2 mg/mL [Zhong *et al*., 2022]. Among the six primary flavonoids analyzed for their antibacterial properties — including isoorientin, vitexin, isovitexin, rutin, quercetin, and kaempferol — quercetin emerged as the most effective, demonstrating robust antibacterial activity against all the tested bacteria except for *E. coli* and *S. epidermidis* [Zhong *et al*., 2022]. Moreover, buckwheat hull extracts have shown effective antimicrobial activity against a range of pathogens (**Table 3**). Cho *et al.* [2006] identified three antimicrobial compounds in methanol extracts of buckwheat (*F. esculentum*) hulls using mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopic techniques. Their structures were elucidated as 6,7-dihydroxy-3,7-dimethyl-octa-2(*Z*),4(*E*)-dienoic acid, 6,7-dihydroxy-3,7-dimethyl-octa-2(*E*),4(*E*)-dienoic acid, and 4,7-dihydroxy-3,7-dimethyl-octa-2(*E*),5(*E*)-dienoic acid. At a concentration of 500 µg, these compounds exhibited antimicrobial activity against *S. aureus* as assayed by the paper disc method. Research detailed in a study by Čabarkapa *et al.* [2008] demonstrated that buckwheat hull extracts exhibited notable antimicrobial activity against both Gram-positive and Gram-negative bacteria, more so at higher concentrations when determined by the disk diffusion method. At 100 mg/mL, the extracts showed significant inhibition against Gram-positive bacteria like *S. aureus*, *Bacillus cereus*, and *Enterococcus faecalis*, in contrast to weaker effects against Gram-negative bacteria such as *Salmonella choleraesuis*, *Proteus mirabilis*, and *Escherichia coli*. Also, ethanolic extracts from buckwheat hulls have been found to inhibit the growth of *Aspergillus flavus* and reduce aflatoxin production [Nobili *et al*., 2019]. Currently, there are insufficient studies on the use of controlled delivery systems in real food applications, particularly lacking comparisons with direct additions of similar quantities of antimicrobial plant phenolics or extracts. Such comparisons are crucial to determine the value added by controlled delivery systems, considering the extra costs associated with their formulation and development. This gap in research highlights the need for more comprehensive studies to assess the effectiveness and cost-efficiency of these innovative delivery methods in food technology.

HEALTH-RELATED PROPERTIES OF BUCKWHEAT HULLS, SPROUTS, AND EXTRACTS

The consumption of buckwheat and its sprouts, moreover different food products with buckwheat hull and extracts, can provide health benefits. The antioxidant (discussed above) [Borgonovi *et al*., 2023; Cui *et al*., 2020; Mazahir *et al*., 2022], anti-cancer [Kim *et al*., 2007a], anti-inflammatory and anti-hypertensive [Karki *et al*., 2013; Koyama *et al*., 2013] properties of buckwheat sprouts, hulls and their extracts were reported. Researches have also highlighted the anti-obesity and anti-diabetic effects of buckwheat grain and bran extracts and sprouts [Aloo *et al*., 2021; Hosaka *et al*., 2014; Lee *et al*., 2017]. Including buckwheat hulls, sprouts, and extracts in diets offers extensive health advantages, which are summarized in **Table 4**.

Kim *et al.* [2007a] extracted buckwheat hull with 70% ethanol, then fractionated it stepwise. Hexane and ethyl acetate fractions showed significant inhibition against human carcinoma cells including human breast adenocarcinoma (MCF-7), human hepatocellular carcinoma (Hep3B), and human lung adenocarcinoma (A549). All samples, except the aqueous fraction, demonstrated anticancer effects, with inhibition rates above 20% in sarcoma-180 implanted mice, suggesting potent anticancer properties. Similarly, tartary buckwheat sprout extract rich in flavonoids showed strong inhibitory activity for the growth of MCF-7 and human gastric cancer cell line (MGC80-3) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [Zhou *et al*., 2011a, b; 2019]. Flow cytometry confirmed apoptosis and cell cycle arrest. Also, the extracts inhibited angiogenesis in a chick chorioallantoic membrane (CAM) assay, suggesting their potential for antitumor therapy or functional food additives. Moreover, buckwheat hulls have shown promising anticancer properties post-digestion in simulated gastrointestinal conditions, significantly reducing the growth of human colon adenocarcinoma cells (HT-29) [Dziedzic *et al*., 2018].

Table 4. Health benefits of buckwheat hulls, sprouts, and extracts.

Type of diseases	Buckwheat added way	Model of study	Effect of study	Reference
Obesity and diabetes	Tartary buckwheat sprouts	Rats fed a diet with tartary buckwheat sprout powder	• Lower plasma cholesterol levels • Increased fecal bile acid excretion	Kuwabara *et al.* [2007]
	Buckwheat sprouts	Hamsters	Reduced liver/body weight ratios, serum triglycerides, LDL cholesterol	Lin *et al.* [2008]
	Buckwheat hull extracts rich in flavonoids	Type 2 diabetic rats	• Alleviated insulin resistance • Lowered blood glucose levels and enhanced oxidative stress responses	Wang *et al.* [2021]
Atherosclerosis	Buckwheat sprouts diet	32 Wistar rats	A statistically significant decrease of fat digestibility in the groups fed a high-fat diet	Molska *et al.* [2023]
Alcohol abuse	Rutin-enriched tartary buckwheat flour extracts	Single oral dose in rats	Helped protect the liver from damage caused by repeated ethanol exposure	Jin *et al.* [2020]
Cancer	Buckwheat hull extract	Study *in vitro*, SRB assay	• Hexane and ethyl acetate fractions showed higher inhibition effects against MCF-7 cells and Hep3B cells • The ethyl acetate fraction yielded the highest inhibition rate against A549 cells • Decreases tumor formation in sarcoma-180 implanted mice	Kim *et al.* [2007a]
	Sprout extract rich in flavonoids	Study *in vivo*, CAM assay	Significant inhibitory activity on the growth of MCF-7 cancer cells	Zhou *et al.* [2011b]
	Sprout extract rich in flavonoids	Study *in vitro*	Showed anti-tumor activity against MGC80-3	Zhou *et al.* [2019]
Hypertension, dyslipidemia, hyperglycemia	Germinated buckwheat extracts	Rats	• Lowered systolic blood pressure • Reduced oxidative damage in aortic endothelial cells	Kim *et al.* [2009b]

CAM, Chick chorioallantoic membrane assay; MGC80-3, human gastric cancer cell line; MCF-7, human breast adenocarcinoma; Hep3B, human hepatocellular carcinoma; A549, human lung adenocarcinoma; LDL, low-density lipoprotein; SRB, sulforhodamine B.

Rutin-enriched extracts from tartary buckwheat flour were produced using hydrothermal treatment and their pharmacokinetic characteristics were assessed after a single oral dose in rats [Jin *et al*., 2020]. The findings indicated that these rutin-enriched extracts were absorbed more effectively and remained in the bloodstream longer compared to native tartary buckwheat flour extract or standard rutin formulations. Moreover, their antioxidant properties helped protect the liver from damage caused by repeated ethanol exposure. A study quantifying rutin in raw buckwheat extracts and germinated buckwheat extracts (RBE and GBE) through high-performance liquid chromatography (HPLC) assessed their impacts on body weight, systolic blood pressure (SBP), and nitrotyrosine levels in hypertensive and normotensive rats [Kim *et al*., 2009b]. It was found that GBE, which contained higher levels of rutin, lowered SBP more effectively than RBE. Additionally, both extracts demonstrated a reduction in oxidative damage within aortic endothelial cells, underscoring GBE's potential for antihypertensive effects and vascular protection.

An investigation into the effects of different buckwheat sprouts on cholesterol metabolism in rats revealed that diets enriched with tartary buckwheat sprout powder significantly reduced plasma cholesterol levels and increased fecal bile acid excretion compared to control groups [Kuwabara *et al*., 2007]. These findings suggest that tartary buckwheat sprouts may enhance cholesterol metabolism by promoting the upregulation of hepatic mRNA expression and boosting the excretion of fecal matter.

In another work, Lin *et al*. [2008] discovered that contents of total phenolics, quercetin, and L-ascorbic acid in buckwheat sprouts peaked on day 8, while on day 10, the highest levels of oxalic, malic, tartaric, citric acids, rutin, and γ-aminobutyric acid was found. Ethanolic extract of sprouts after 8 days of germination exhibited potent antioxidant properties and moderate Fe^{2+}-chelating ability. Supplementing Syrian hamsters' diets with these sprouts significantly enhanced their health markers, reducing liver/body weight ratios, serum triglycerides, LDL cholesterol, and improving feed efficiency, thus underscoring their nutritional value.

A study by Molska *et al*. [2023] was conducted to evaluate the effects of incorporating buckwheat sprouts (*F. esculentum* Moench), modified by the probiotic yeast strain *Saccharomyces cerevisiae* var. *boulardii*, into a high-fat (atherogenic) diet on rat morphology and digestibility parameters. The findings indicated a statistically significant reduction in fat digestibility among the groups consuming the diet supplemented with these sprouts, highlighting a novel application for buckwheat sprouts in dietary interventions.

Flavonoids extracted from common buckwheat hulls significantly alleviate insulin resistance, thus lowering blood glucose levels and enhancing oxidative stress responses in type 2 diabetic rats [Wang *et al*., 2021]. Notably, treatment with these buckwheat

hull flavonoids also improved symptoms of diabetes-related liver damage. This improvement was evident through the reduction of liver fat and decreased levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are markers of liver health. These findings highlight the potent antioxidant and hepatoprotective capabilities of buckwheat hull flavonoids, suggesting their potential as valuable nutraceuticals and dietary supplements for preventing and/or treating liver disorders.

EFFECT OF BUCKWHEAT HULLS, SPROUTS, AND EXTRACTS ADDITION ON THE NUTRITIONAL, TEXTURAL, AND SENSORY PROPERTIES OF FOOD PRODUCTS

Research has delved into the utilization of buckwheat hulls, sprouts, and their respective extracts as potential functional ingredients in food production [Wronkowska *et al*., 2023; Yang *et al*., 2019; Zhang *et al*., 2023; Znamirowska *et al*., 2020]. Such studies have methodically explored the impact these additions have on the nutritional value, textural characteristics, and organoleptic qualities of food products (**Table 5**).

Meat products

In response to growing health concerns associated with meat consumption, there is an increasing interest in enhancing the nutritional profile of meat products. This is being addressed by incorporating various nutritious ingredients into meat products. Such enhancements aim to improve the overall health benefits of meat consumption, making these products more attractive to health-conscious consumers. This approach not only diversifies the nutritional content, potentially reducing the risks linked to high-meat diets but also aligns with consumer trends favoring functional foods that support a healthier lifestyle [Geiker *et al*., 2021]. Adding dietary fiber sources, such as buckwheat and its derivatives, offers a promising approach. Various studies have aimed to examine the effects of adding buckwheat sprouts, hulls, and extracts, to meat products including pork, beef, horsemeat, and poultry products to improve their nutritional value, texture parameters, and taste (**Table 5**).

A study by Salejda *et al*. [2022] showed that adding 3% hulls to frankfurter sausages increased cooking losses, enhanced firmness, enriched the amino acid profile, increased total amino acid content (from 161.8 to 228.0 mg/kg), and elevated essential mineral levels, proving buckwheat hull efficacy as a sustainable, nutritional meat additive. Buckwheat extracts, derived from grains or sprouts, as concentrated sources of phenolic compounds added to pork products have been shown to effectively inhibit lipid oxidation and microbial growth [Hęś *et al*., 2017; Pietrzak *et al*., 2022]. For example, aqueous and ethanolic extracts of buckwheat hulls reduced the formation of thiobarbituric acid reactive substances (TBARS) and extended the induction time of lipid oxidation of chicken meatballs during 14 days of refrigerated storage, while improving their microbiological stability [Pietrzak *et al*., 2022]. These extracts also helped in maintaining the sensory qualities of the chicken meatballs. A study by Hęś *et al*. [2017] has shown that frozen pork meatballs enriched with buckwheat hull extract (additive at the level 0.5%) demonstrated lower peroxide content and TBARS values after 180 days of storage compared to those treated with a synthetic antioxidant like butylated hydroxytoluene, BHT (additive at the level 0.02%). These studies highlight the potential of buckwheat hull extracts in extending meat products' shelf life and preserving their quality by mitigating lipid oxidation.

In our latest research [Atambayeva *et al*., 2023], we explored the benefits of incorporating ground green buckwheat (untreated buckwheat) sprouts into horsemeat and chicken patties, aiming to improve their quality, extend their shelf life, and boost their safety profile. Adding ground green buckwheat sprout to the patties significantly improved nutritional values, including protein and lipid content, cooking performance, and moisture and lipid preservation, alongside enhancing antioxidant properties, as seen in increased total phenolic content and DPPH radical scavenging activity. Notably, ground green buckwheat sprout application slightly altered color parameters (L^*, a^*, b^*), indicating changes in appearance. Sensory evaluation also revealed that patties were more acceptable and juicier to consumers.

Bakery products and pasta

The addition of buckwheat hulls, sprouts, and extracts to bakery products can significantly enhance their nutrient and bioactive compound profiles and provide unique flavors and textures. Each form of buckwheat brings specific characteristics and benefits when incorporated into baked goods such as breads, muffins, and cookies (**Table 5**). Liu *et al*. [2022] and Zhang *et al*. [2017] have documented the significant nutritional properties of buckwheat hull, noting its richness in bioactive compounds compared to buckwheat flour. Furthermore, Dziadek *et al*. [2016] conducted a study on six different cultivars and strains of common buckwheat, including whole seeds, dehulled seeds, and hulls, finding that the hulls contained the highest levels of dietary fiber and total phenolics, as well as exhibited the greatest antioxidant activity among the samples tested. Also, it has been shown that common bakery products can benefit from the inclusion of buckwheat flour or hull, which not only enhances their antioxidative potential but also improves sensory and storage properties [Wronkowska *et al*., 2019, 2023]. Phenolics of buckwheat hulls provide or enhance antioxidant capacity. Their addition can also help in reducing acrylamide formation during baking, a compound that poses health risks due to its potential carcinogenic effects [Teng *et al*., 2018]. Phenolic extracts from both common buckwheat (*F. esculentum* Moench) and tartary buckwheat (*F. tataricum* Gaertn.) have been incorporated into breadmaking processes and specifically, these extracts, sourced from the seeds and sprouts of tartary and common buckwheat resulted in notable reductions in acrylamide content [Melini *et al*., 2024]. The reductions were quantified as 23.5% for tartary buckwheat seeds, 27.3% for tartary buckwheat sprouts, 17.0% for common buckwheat seeds, and 16.7% for common buckwheat sprouts, enhancing the health benefits of the breads produced. Moreover, the antioxidant properties of the phenolic compounds

Table 5. Effect of buckwheat, buckwheat sprouts, hulls, and their extracts on food properties.

Food product	Type of buckwheat	Buckwheat incorporation: type and level	Type of effect	Reference
Meat products				
Pork + Chicken (meatballs)	*Fagopyrum esculentum* Moench	Buckwheat hulls: extract	• Did not affect the total colour difference parameter; • Achieved high acceptability; • The total plate count was lower during 14 days of storage; • The effective inhibition of lipid oxidation processes.	Pietrzak *et al.* [2022]
		Buckwheat hulls: extract	• Controlled peroxide and TBARS values • A higher free radical scavenging activity • Higher Fe(II) ion chelating ability • Prolonged shelf life	Hęś *et al.* [2017]
Pork (frankfurter-type sausages)	*Fagopyrum esculentum* Moench	Buckwheat hulls: 3%	• Buckwheat hulls (3%): more cooking, less storage loss. • Sausages' the firmness increased after two weeks' storage • Increased the content of manganese, calcium, potassium and magnesium	Salejda *et al.* [2022]
Horsemeat (patties)	*Fagopyrum esculentum* Moench	Ground green buckwheat sprouts: 5%	• Enriched the contents of protein and fat, cooking efficiency, retention of moisture and fat, total phenolic levels, and DPPH radical scavenging capacity • Green buckwheat sprouts maintained their color throughout the storage period	Atambayeva *et al.* [2023]
		Buckwheat extract: 0.5% and 1%	• Improved horse-meat product oxidative stability, quality, sensory, and color	Uzakov *et al.* [2020]
Bakery products				
Bread/rolls	*Fagopyrum esculentum* Moench	Adding 3% of buckwheat hull to wheat flour	• Reduction in GSH and GSSG content • Increased of content of α-, β-,γ-, and δ-tocopherols • Increase in the value of the antioxidant capacity after the baking process	Wronkowska *et al.* [2023]
		Buckwheat hull: mixed rye/wheat flour with 4% of roasted buckwheat hull, wheat flour with 3% of raw buckwheat hull	• Bread containing 4% roasted buckwheat hulls exhibited the highest levels of TPC and AC • Showed positive effects on sensory qualities, consumer acceptance, and microbial qualities after storage	Bączek *et al.* [2023]
	Tartary buckwheat, common buckwheat	Seed and sprout extracts from tartary and common buckwheat	• Reduced acrylamide level in bread • All four buckwheat extracts reduced acrylamide levels in the asparagine/glucose system • Did not affect the crust color, aroma, taste, crumb appearance, and hardness of the bread.	Jing *et al.* [2019]
	Fagopyrum esculentum	Buckwheat hull	• Reduced baking loss and increased firmness • Prevented amylopectin retrogradation and starch recrystallization • Retained more moisture and reduced staling	Wang *et al.* [2023]
		Buckwheat sprouts flour (10%)	• Increased total antioxidant activity • Enhances nutritional content without compromising texture or taste	Sturza *et al.* [2020]
	Fagopyrum tataricum (Xinong 9940)	Tartary buckwheat sprouts	• Higher bioactive compounds and functional value • The optimal addition is 8% of sprouts flour to achieve the acceptability of consumers	Xu *et al.* [2014]
	Common buckwheat	Mix of buckwheat flour and buckwheat sprouts: 10–30 % addition to wheat flour	• Ingredient to produce reconstituted rice • Contained higher levels of flavonoids, other phenolics, and flavor compounds	Kang *et al.* [2024]
Other products				
Fish products	*Fagopyrum tataricum* Gaertn	Tartary buckwheat: extract 0.5% to 1.5% (*w/v*) addition to chitosan	• Preserved quality and exhibited an extended shelf life at 0°C. • Offers potential for application in coatings	Yang *et al.* [2019]
Pasta	Common buckwheat	Ground buckwheat hull	• Exhibited a reduction in the optimal cooking time • An increase in weight index and cooking loss • Enhancement in total phenolic content and antioxidant activity	Sujka *et al.* [2022]
Noodles	Fagopyrum esculentum	Mixed dough with 1–5 grams buckwheat bran/hull in 100 g wheat.	• Improved properties of dough and characteristic of noodles • Improved rheological and tensile properties	Liu *et al.* [2022]

Table 5 continued. Effect of buckwheat, buckwheat sprouts, hulls, and their extracts on food properties.

Food product	Type of buckwheat	Buckwheat incorporation: type and level	Type of effect	Reference
Yogurt	Common buckwheat	Micronized buckwheat hulls	• Decreased total acidity and syneresis • Reduced the colour brightness and increased the intensity of the red and yellow colours • Demonstrated the beneficial effect on *L. bulgaricus*	Znamirowska *et al.* [2020]
Chocolate cream		Ground buckwheat hull	• Increased antioxidant activity • Increased fibers content in desserts	Matseychik *et al.* [2021]
Spice		Buckwheat sprouts	• Increased protein content by 1.38 times • Reduced mass fraction of carbohydrates by 1.57 times • Reduced the mass fraction of fat by 2 times	Serikbaeva *et al.* [2021]
Tea	*Fagopyrum esculentum* Moench	Buckwheat hulls	• Showed lower antioxidant capacity and inhibitory activity	Zielińska *et al.* [2013]

TBARS, Thiobarbituric acid reactive substances; DPPH radical, 2,2-diphenyl-1-picrylhydrazyl radical; GSH and GSSG, glutathione in reduced (GSH) and oxidized (GSSG) form; TPC, total phenolic content; AC, antioxidant capacity.

in buckwheat hulls have been shown to contribute to improved shelf life and nutritional value of the bakery products without adversely affecting their sensory qualities like taste and texture [Jing *et al*., 2019]. However, it is important to note that excessive inclusion of hulls could lead to a denser, dryer product; hence their amount needs to be balanced to maintain product quality.

Raw and roasted buckwheat hulls markedly raised the content of bioavailable phenolic compounds and antioxidant capacity of the baked goods [Bączek *et al*., 2023; Wronkowska *et al*., 2023]. Incorporating raw and roasted buckwheat hull into bakery products notably improved their measured parameters over control samples; notably, before digestion, bread containing 4% roasted buckwheat hull exhibited the highest TPC at 1.80 mg gallic acid eq/g dry matter (dm), and after *in vitro* digestion, the soluble fraction of the examined bakery products showed a 75–90% higher TPC and antioxidant capacity compared to the insoluble fractions [Bączek *et al*., 2023]. Wronkowska *et al*. [2023] observed an increase in contents of α-, β-, γ-, and δ-tocopherols during the production stages of wheat rolls, with the highest levels found in rolls containing 3% buckwheat hull. However, there was a significant reduction in the content of glutathione in reduced (GSH) and oxidized (GSSG) form during baking. The authors concluded that the rise in antioxidant capacity post-baking might be due to the formation of new antioxidant compounds.

The effect of the size of buckwheat hull powder particles on bread quality, comparing tissue-scale (500–100 μm) to cell-scale (50–10 μm) powders, was examined by Wang *et al*. [2023]. The study used a 3% (*w/w*) content of buckwheat hull in the bread formulation and authors found that while tissue-scale buckwheat hull powder minimally affected loaf volume and crumb firmness, the cell-scale buckwheat hull powder significantly decreased volume, reduced baking loss, and increased firmness. Also, the cell-scale buckwheat hull powder better prevented amylopectin retrogradation and starch recrystallization, retained more moisture, and reduced staling compared to the tissue-scale buckwheat hull powder.

Fresh bread's sensory qualities and acceptability were improved when 0.3–0.5% buckwheat hull hemicelluloses were added to bread wheat flour [Hromádková *et al*., 2007]. Buckwheat hull hemicelluloses reduced crumb hardness during storage, yielding a softer, more elastic bread compared to the control. Over time, buckwheat hull hemicelluloses enhanced breads maintained superior softness and elasticity, suggesting buckwheat hull hemicellulose potential to improve bread made from medium-quality wheat flours.

Sturza *et al*. [2020] found that the addition of sprouted buckwheat flour (10%) and buckwheat grain flour (20%) into wheat flour in buns increased total antioxidant activity by 59.04% compared with non-sprouted buckwheat flour, and results showed that replacing wheat flour with 20% buckwheat and 10% sprout flours enhanced the nutritional content without compromising texture or taste, receiving high consumer ratings. Similarly, an optimal addition of 8% was found in incorporating tartary buckwheat sprouts into Chinese steamed bread and was shown to optimize both its functional qualities (texture, taste, structural integrity, and overall quality) and consumer satisfaction [Xu *et al*., 2014].

Pasta is esteemed not only for its delightful culinary attributes but also as an essential element of a nutritious diet, thanks to its healthful and gastronomic properties. In an exploration of alternative pasta formulations, the substitution of semolina with ground buckwheat hulls was examined, leading to an increase in fiber content from 4.31% to 14.15% when 20% hulls were incorporated [Sujka *et al*., 2022]. This adjustment notably decreased cooking times and raised both the weight index and cooking loss, while significantly enriching the phenolic content and antioxidant activities. However, the use of buckwheat hulls exceeding 10% resulted in the pasta acquiring an unfavorable aroma and taste. Despite these sensory challenges, such modifications hold promise for boosting the nutritional value of products. Liu *et al*. [2022] compared the properties of dough made from wheat flour with addition of buckwheat bran or buckwheat hull and analyzed the quality of noodles from both types of doughs. Due

to a higher fibre content in doughs made from enriched flours (both additives), their starch pasting properties were declined compared to the control without additives; however, when 4% buckwheat bran or hull was used, the hardness and chewiness of dough was acceptable; gluten network was still formed. Moreover, the cooking loss of noodles with buckwheat bran was lower than of the product with buckwheat hulls.

Other products

Incorporating buckwheat hulls into dairy products such as yogurt can enhance their nutritional profile and functional properties. Adding buckwheat hulls to yogurt has been shown to decrease total acidity and reduce syneresis, thereby improving texture and stability [Znamirowska *et al*., 2020]. Moreover, the phenolics of buckwheat hulls enhance the antioxidant capacity of the yogurt and can contribute to improved shelf life and health benefits. This is because phenolic compounds are well-recognized for their dual role in enhancing the longevity of perishable items and contributing health advantages, which supports the formulation of foods free from synthetic additives [Martillanes *et al*., 2017; Matsumura *et al*., 2023]. The addition of buckwheat hulls positively affected the microbiological properties of yogurt, promoting the growth of beneficial bacteria like *Lactobacillus bulgaricus* [Znamirowska *et al*., 2020].

Using buckwheat by-products, specifically hull fine powder and melanin, in dessert formulations like chocolate cream and honeysuckle mousse enhanced their sensory appeal and physicochemical properties, including antioxidant capacity [Matseychik *et al*., 2021]. Sensory evaluations and antioxidant contents determined optimal inclusion rates at 1.5 g and 0.037 g *per* serving, respectively. These additions not only boosted antioxidant intake, covering over 15% of daily needs, but also increased dietary fiber content, making these desserts functional products.

An optimized blend of sprouted buckwheat, wheat, black rice, and purple potato flours was developed to create reconstituted rice with enhanced flavor and a reduced glycemic index (GI) [Kang *et al*., 2024]. This innovative formulation produced rice with distinctive colors and a robust cereal flavor, enriched with a superior nutritional profile and medium GI values, offering significant benefits for blood glucose management.

Research into the influence of temperature and germination duration on vitamin enrichment in buckwheat led to its potential use in a novel seasoning [Serikbaeva *et al*., 2021]. Optimal sprouting conditions – (21.5°C for three days using the Bogatyr variety) – resulted in the highest levels of vitamins B, E, and C at 4.591 mg/100 g. A seasoning blend incorporating 30% of these sprouted buckwheat grains excelled in protein, vitamin content, and both micro- and macronutrient levels, additionally exhibiting a 25% enhancement in antioxidant activity.

Yang *et al*. [2019] found that mixtures of tartary buckwheat extract and chitosan were more effective in extending the shelf life of coated tilapia (*Oreochromis niloticus*) fillets compared to chitosan only during storage at 0°C for 18 days. The shelf life has been extended from 6 days for fish coated with chitosan to up to 15 days when the coating with the addition of tartary buckwheat extract was used.

STRENGTHS AND LIMITATIONS OF CURRENT REVIEW

This systematic review offers a first direct comparison of the properties of buckwheat sprouts, hulls, and extracts, their health benefits, and their use in developing new functional foods under a zero-waste principle. However, there are limitations to note. Despite our comprehensive search strategy, not all online databases were covered, and some articles, particularly non-English ones, may have been overlooked. Also, the high variability in study designs, such as different parts of the plant used, extraction methods, and units of measure, made it difficult to quantitatively synthesize the data, leading us to focus primarily on a narrative summary of the most pertinent findings.

CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

Buckwheat hulls, sprouts, and extracts are rich in carbohydrates, proteins, fiber, and bioactive compounds, with sprouts and extracts notably high in flavonoids, mainly rutin. Although often discarded, buckwheat hulls are a valuable source of dietary fiber and carbohydrates. The presence of bioactive compounds across all buckwheat derivatives contributes significantly to their health-promoting qualities, making them vital for inclusion in human diets. These derivatives are particularly beneficial for individuals with celiac disease, offering a safe, gluten-free food option. Nonetheless, the impact of food processing on the nutritional quality of buckwheat products poses a challenge to the industry, necessitating a careful approach to the transformation of raw materials.

Future research on buckwheat hulls, sprouts, and extracts holds immense potential for advancing sustainable food production and zero-waste principles in the food industry. Particularly, the exploration of buckwheat hulls as a value-added ingredient in various food products can significantly contribute to waste reduction and resource efficiency. Studies can focus on optimizing processing methods to enhance the nutritional and functional properties of buckwheat hulls, enabling their broader use in foods like bakery products, beverages, and dietary supplements. Moreover, research into the bioactive components of green (thermally untreated) buckwheat sprouts and extracts could lead to innovative applications in nutraceuticals and functional foods, further extending their health benefits. Such investigations are vital not only for maximizing the utility of all buckwheat plant components but also for promoting sustainability through the adoption of a no-waste approach in food processing and product development. This aligns with global efforts to achieve more sustainable food systems by reducing food waste and enhancing the nutritional value of food products. Thus, the findings from this review, highlighting the rich bioactive profiles of buckwheat hulls, sprouts, and extracts provide functional food developers with valuable insights into creating products that are both nutritious and aligned with health-conscious

consumer preferences. Utilizing these components can help in formulating foods that not only meet daily nutritional needs but also offer targeted health benefits such as enhanced cardiovascular health, improved digestive function, and potent antioxidant protection.

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CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors.

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REFERENCES

1. Ahmed, A., Khalid, N., Ahmad, A., Abbasi, N.A., Latif, M.S.Z., Randhawa, M.A. (2014). Phytochemicals and biofunctional properties of buckwheat: A review. *Journal of Agricultural Science*, *152*(3), 349–369. https://doi.org/10.1017/s0021859613000166
2. Ali, A.S., Elozeiri, A.A. (2017). Metabolic processes during seed germination. In J.C. Jimenez-Lopez (Ed.). *Advances in Seed Biology*. IntechOpen Lid, London, United Kingdom. https://doi.org/10.5772/intechopen.70653
3. Al-Khayri, J.M., Sahana, G.R., Nagella, P., Joseph, B.V., Alessa, F.M., Al-Mssallem, M.Q. (2022). Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*, *27*(9), art. no. 2901. https://doi.org/10.3390/molecules27092901
4. Aloo, S.O., Ofosu, F.K., Oh, D.H. (2021). Effect of germination on alfalfa and buckwheat: Phytochemical profiling by UHPLC-ESI-QTOF-MS/MS, bioactive compounds, and *in-vitro* studies of their diabetes and obesity-related functions. *Antioxidants*, *10*(10), art. no. 1613. https://doi.org/10.3390/antiox10101613
5. Atambayeva, Zh., Nurgazezova, A., Assirzhanova, Zh., Urazbayev, Zh., Kambarova, A., Dautova, A., Idyryshev, B., Sviderskaya, D., Kaygusuz, M. (2023). Nutritional, physicochemical, textural and sensory characterization of horsemeat patties as affected by whole germinated green buckwheat and its flour. *International Journal of Food Properties 26*(1), 600-613. https://doi.org/10.1080/10942912.2023.2174552
6. Bączek, N., Haros, C.M., Wronkowska, M. (2023). Buckwheat hull, a valuable bakery product ingredient: assessment of bioaccessible phenolics and antioxidant capacity. *European Food Research and Technology*, *249*(2), 353–358. https://doi.org/10.1007/s00217-022-04120-w
7. Biel, W., Maciorowski, R. (2013). Evaluation of chemical composition and nutritional quality of buckwheat groat, bran and hull (*Fagopyrum Esculentum* Moench L.). *Italian Journal of Food Science*, *25*(4), 384–389.
8. Borgonovi, S.M., Chiarello, E., Pasini, F., Picone, G., Marzocchi, S., Capozzi, F., Bordoni, A., Barbiroli, A., Marti, A., Iametti, S., Di Nunzio, M. (2023). Effect of Sprouting on biomolecular and antioxidant features of common buckwheat (*Fagopyrum esculentum*). *Foods*, *12*(10), art. no. 2047. https://doi.org/10.3390/foods12102047
9. Čabarkapa, I.S., Sedej, I.J., Sakač, M.B., Šarić, L.Č., Plavšić, D. (2008). Antimicrobial activity of buckwheat (*Fagopyrum esculentum* Moench) hulls extract. *Food Processing, Quality and Safety*, *35*(4), 159-163.
10. Cai, C., Cheng, W., Shi, T., Liao, Y., Zhou, M., Liao, Z. (2023). Rutin alleviates colon lesions and regulates gut microbiota in diabetic mice. *Scientific Reports*, *13*(1), art. no. 4897. https://doi.org/10.1038/s41598-023-31647-z
11. Cho, J., Moon, J., Kim, H., Ma, S., Kim, S., Jang, M., Kawazoe, K., Takaishi, Y., Park, K. (2006). Isolation and structural elucidation of antimicrobial compounds from buckwheat hull. *Journal of Microbiology and Biotechnology*, *16*, 538-542.
12. Cui, Y., Zhao, Z., Liu, Z., Liu, J., Piao, C., Liu, D. (2020). Purification and identification of buckwheat hull flavonoids and its comparative evaluation on antioxidant and cytoprotective activity *in vitro*. *Food Science & Nutrition*, *8*(7), 3882-3892. https://doi.org/10.1002/fsn3.1683
13. Dębski, H., Wiczkowski, W., Szawara-Nowak, D., Horbowicz, M. (2021). Elicitation with sodium silicate and iron chelate affects the contents of phenolic compounds and minerals in buckwheat sprouts. *Polish Journal of Food and Nutrition Sciences*, *71*(1), 21-28. https://doi.org/10.31883/pjfns/131061
14. Džafić, A., Oručević Žuljević, S. (2022). The Importance of buckwheat as a pseudocereal: Content and stability of its main bioactive components. In V. Y. Waisundara (Ed.), *Pseudocereals*. IntechOpen Ltd, London, United Kingdom. https://doi.org/10.5772/intechopen.102570
15. Dziadek, K., Kopeć, A., Pastucha, E., Piątkowska, E., Leszczyńska, T., Pisulewska, E., Witkowicz, R., Francik, R. (2016). Basic chemical composition and bioactive compounds content in selected cultivars of buckwheat whole seeds, dehulled seeds and hulls. *Journal of Cereal Science*, *69*, 1-8. https://doi.org/10.1016/j.jcs.2016.02.004
16. Dziedzic, K., Górecka, D., Kucharska, M., Przybylska, B. (2012). Influence of technological process during buckwheat groats production on dietary fibre content and sorption of bile acids. *Food Research International*, *47*(2), 279–283. http://dx.doi.org/10.1016/j.foodres.2011.07.020
17. Dziedzic, K., Górecka, D., Szwengiel, A., Olejnik, A., Rychlik, J., Kreft, I., Drożdżyńska, A., Walkowiak, J. (2018). The cytotoxic effect of artificially digested buckwheat products on HT-29 colon cancer cells. *Journal of Cereal Science*, *83*, 68-73. https://doi.org/10.1016/j.jcs.2018.07.020
18. Geiker, N.R.W., Bertram, H.C., Mejborn, H., Dragsted, L.O., Kristensen, L., Carrascal, J.R., Bügel, S., Astrup, A. (2021). Meat and human health – Current knowledge and research gaps. *Foods*, *10*(7), art. no. 1556. https://doi.org/10.3390/foods10071556
19. Guan, Z.W., Yu, E.Z., Feng, Q. (2021). Soluble dietary fiber, one of the most important nutrients for the gut microbiota. *Molecules*, *26*(22), art. no. 6802. https://doi.org/10.3390/molecules26226802
20. Guo, Y.Z., Chen, Q.F., Yang, L.Y., Huang, Y.H. (2007). Analyses of the seed protein contents on the cultivated and wild buckwheat *Fagopyrum esculentum* resources. *Genetic Resources and Crop Evolution*, *54*, 1465-1472. https://doi.org/10.1007/s10722-006-9135-z
21. Gutiérrez, Á.L., Villanueva, M., Rico, D., Harasym, J., Ronda, F., Martín-Diana, A.B., Caballero, P.A. (2023). Valorisation of buckwheat by-product as a health-promoting ingredient rich in fibre for the formulation of gluten-free bread. *Foods*, *12*(14), art. no. 2781. https://doi.org/10.3390/foods12142781
22. Guzmán-Ortiz, F.A., Castro-Rosas, J., Gómez-Aldapa, C.A., Mora-Escobedo, R., Rojas-León, A., Rodríguez-Marín, M.L., Falfán-Cortés, R.N., Román-Gutiérrez, A.D. (2019). Enzyme activity during germination of different cereals: A review. *Food Reviews International*, *35*(3), 177–200. https://doi.org/10.1080/87559129.2018.1514623
23. Hęś, M., Górecka, D., Dziedzic, K. (2012). Antioxidant properties of extracts from buckwheat by-products. *Acta Scientiarum Polonorum Technologia Alimentaria*, *11*(2), 167-174.
24. Hęś, M., Szwengiel, A., Dziedzic, K., Thanh-Blicharz, J.L., Kmiecik, D., Górecka, D. (2017). The effect of buckwheat hull extract on lipid oxidation in frozen-stored meat products. *Journal of Food Science*, *82*(4), 882–889. https://doi.org/10.1111/1750-3841.13682
25. Hosaka, T., Sasaga, S., Yamasaka, Y., Nii, Y., Edazawa, K., Tsutsumi, R., Shuto, E., Okahisa, N., Iwata, S., Tomotake, H., Sakai, T. (2014). Treatment with buckwheat bran extract prevents the elevation of serum triglyceride levels and fatty liver in KK-A^{y} mice. *Journal of Medical Investigation*, *61*(3.4), 345–352. https://doi.org/10.2152/jmi.61.345
26. Hromádková, Z., Stavová, A., Ebringerová, A., Hirsch, J. (2007). Effect of buckwheat hull hemicelluloses addition on the bread-making quality of wheat flour. *Journal of Food and Nutrition Research*, *46*(4), 158-166.
27. Hua, X.Y., Sim, S.Y.J., Henry, C.J., Chiang, J.H. (2024). The extraction of buckwheat protein and its interaction with kappa-carrageenan: Textural, rheological, microstructural, and chemical properties. *International Journal of Biological Macromolecules*, *260*(Part 1), art. no. 129427. https://doi.org/10.1016/j.ijbiomac.2024.129427
28. Ikeda, S., Yamashita, Y., Kreft, I. (1999). Mineral composition of buckwheat by-products and its processing characteristics to konjak preparation. *Fagopyrum*, *16*, 89-94.
29. Jha, R., Zhang, K., He, Y., Mendler-Drienyovszki, N., Magyar-Tábori, K., Quinet, M., Germ, M., Kreft, I., Meglič, V., Ikeda, K., Chapman, M.A., Janovská, D., Podolska, G., Woo, S.-H., Bruno, S., Georgiev, M.I., Chrungoo, N., Betekhtin, A., Zhou, M. (2024). Global nutritional challenges and opportunities: Buckwheat, a potential bridge between nutrient deficiency and food security. *Trends in Food Science & Technology*, *145*, art. no. 104365, https://doi.org/10.1016/j.tifs.2024.104365
30. Ji, H., Tang, W., Zhou, X., Wu, Y. (2016). Combined effects of blue and ultraviolet lights on the accumulation of flavonoids in tartary buckwheat sprouts. *Polish Journal of Food and Nutrition Sciences*, *66*(2), 93-98. https://doi.org/10.1515/pjfns-2015-0042

31. Jin, H.R., Lee, S., Choi, S.J. (2020). Pharmacokinetics and protective effects of tartary buckwheat flour extracts against ethanol-induced liver injury in rats. *Antioxidants*, *9*(10), art. no. 913. https://doi.org/10.3390/antiox9100913
32. Jin, J., Ohanenye, I.C., Udenigwe, C.C. (2022). Buckwheat proteins: Functionality, safety, bioactivity, and prospects as alternative plant-based proteins in the food industry. *Critical Reviews in Food Science and Nutrition*, *62*(7), 1752–1764. https://doi.org/10.1080/10408398.2020.1847027
33. Jing, Y., Li, X., Hu, X., Ma, Z., Liu, L., Ma, X. (2019). Effect of buckwheat extracts on acrylamide formation and the quality of bread. *Journal of the Science of Food and Agriculture*, *99*(14), 6482-6489. https://doi.org/10.1002/jsfa.9927
34. Kan, J., Cao, M., Chen, C., Gao, M., Zong, S., Zhang, J., Liu, J., Tang, C., Jin, C. (2023). *In vitro* antioxidant and lipid-lowering properties of free and bound phenolic compounds from buckwheat hulls. *Food Bioscience*, *53*, art. no. 102725. https://doi.org/10.1016/j.fbio.2023.102725
35. Kang, L., Luo, J., Su, Z., Zhou, L., Xie, Q., Li, G. (2024). Effect of sprouted buckwheat on glycemic index and quality of reconstituted rice. *Foods, 13*(8), art. no. 1148. https://doi.org/10.3390/foods13081148
36. Karki, R., Park, C.H., Kim, D.W. (2013). Extract of buckwheat sprouts scavenges oxidation and inhibits pro-inflammatory mediators in lipopolysaccharide-stimulated macrophages (RAW264.7). *Journal of Integrative Medicine, 11*(4), 246-252. https://doi.org/10.3736/jintegrmed2013036
37. Kim, D.E., Hong, S.Y., Kang, W.S., C.Y., Yu, Lee, B.G., Chung, I.M., Lim, J.D. (2009a). Influence of extrusion on dietary fiber profile and bioactive compound in different parts of tartary buckwheat (*Fagopyrum tataricum*). *Korean Journal of Medicinal Crop Science*, *17*(6), 379-387.
38. Kim, D.W., Hwang, I.K., Lim, S.S., Yoo, K.Y., Li, H., Kim, Y.S., Kwon, D.Y., Moon, W.K., Kim, D.W., Won, M.H. (2009b). Germinated buckwheat extract decreases blood pressure and nitrotyrosine immunoreactivity in aortic endothelial cells in spontaneously hypertensive rats. *Phytotherapy Research*, *23*(7), 993-998. https://doi.org/10.1002/ptr.2739
39. Kim, S., Kim, S., Park, C. (2004). Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International*, *37*(4), 319-327. https://doi.org/10.1016/J.FOODRES.2003.12.008
40. Kim, S.H., Cui, C.B., Kang, I.J., Kim, S.Y., Ham, S.S. (2007a). Cytotoxic effect of buckwheat (*Fagopyrum esculentum* Moench) hull against cancer cells. *Journal of Medicinal Food*, *10*(2), 232-238. https://doi.org/10.1089/jmf.2006.1089
41. Kim, S.J., Maeda, T., Sarker, M.Z.I., Takigawa, S., Matsuura-Endo, C., Yamauchi, H., Mukasa, Y., Saito, K., Hashimoto, N., Noda, T., Saito, T., Suzuki, T. (2007b). Identification of anthocyanins in the sprouts of buckwheat. *Journal of Agricultural and Food Chemistry*, *55*(15), 6314-6318. https://doi.org/10.1021/jf0704716
42. Kim, S.L., Son, Y.K., Hwang, J.J., Kim, S.K., Hur, H.S., Park, C.H. (2001). Development and utilization of buckwheat sprouts as functional vegetables. *Fagopyrum*, *18*, 49–54.
43. Kim, Y.S., Kim, J.G., Lee, Y.S., Kang, I.J. (2005). Comparison of the chemical components of buckwheat seed and sprout. *Journal of the Korean Society of Food Science and Nutrition*, *34*(1), 81-86 (in Korean). https://doi.org/10.3746/jkfn.2005.34.1.081
44. Koyama, M., Naramoto, K., Nakajima, T., Aoyama, T., Watanabe, M., Nakamura, K. (2013). Purification and identification of antihypertensive peptides from fermented buckwheat sprouts. *Journal of Agricultural and Food Chemistry*, *61*(12), 3013–3021. https://doi.org/10.1021/jf305157y
45. Kreft, I., Germ, M., Golob, A., Vombergar, B., Bonafaccia, F., Luthar, Z. (2022). Impact of rutin and other phenolic substances on the digestibility of buckwheat grain metabolites. *International Journal of Molecular Sciences*, *23*(7), art. no. 3923. https://doi.org/10.3390/ijms23073923
46. Kuwabara, T., Han, K.H., Hashimoto, N., Yamauchi, H., Shimada, K.I., Sekikawa, M., Fukushima, M. (2007). Tartary buckwheat sprout powder lowers plasma cholesterol level in rats. *Journal of Nutritional Science and Vitaminology*, *53*(6), 501-507. https://doi.org/10.3177/jnsv.53.501
47. Kuznetsova, E., Uchasov, D., Kuznetsova, O., Elena Kuznetsova, Bychkova, T., Brindza, J. (2020). The use of high-performance liquid chromatography (HPLC) to assess the antioxidant activity of buckwheat husk and indicators of the oxidant-antioxidant system of laboratory animals. In SPBPU DTMIS'20: *Proceedings of Peter the Great St. Petersburg Polytechnic University International Scientific Conference "Digital Transformation on Manufacturing, Infrastructure and Service"*, November 18–19, Saint – Petersburg, Russia. ACM, New York, NY, USA, art. no. 100. https://doi.org/10.1145/3446434.3446497
48. Lee, E.H., Kim, C.J. (2008). Nutritional changes of buckwheat during germination. *Korean Journal of Food Culture*, *23*, 121–129.
49. Lee, H., Lim, T., Kim, J., Kim, R.H., Hwang, K.T. (2022). Phenolics in buckwheat hull extracts and their antioxidant activities on bulk oil and emulsions. *Journal of Food Science*, *87*(7), 2831-2846. https://doi.org/10.1111/1750-3841.16175
50. Lee, H.S., Park, C.H., Park, B.J., Kwon, S.M., Chang, K.J., Kim, S.L. (2006). Rutin, catechin derivatives, and chemical components of tartary buckwheat (*Fagopyrum tataricum* Gaerth.) Sprouts. *Korean Journal of Crop Science*, *51*(S), 277-282.
51. Lee, S.G., Lee, D., Kang, H. (2017). Hypocholesterolemic effect of tartary buckwheat (*F. tataricum* Gaertn.) extract from high fat diet mice. *Biomedical Science Letters*, *23*, 34-38. https://doi.org/10.15616/BSL.2017.23.1.34
52. Li, F., Yuan, Y., Yang, X., Tao, S., Ming, J. (2013). Phenolic profiles and antioxidant activity of buckwheat (*Fagopyrum esculentum* Möench and *Fagopyrum tartaricum* L. Gaerth) hulls, brans and flours. *Journal of Integrative Agriculture*, *12*(9), 1684-1693. https://doi.org/10.1016/S2095-3119(13)60371-8
53. Lim, J.H., Park, K.J., Kim, B.K., Jeong, J.W., Kim, H.J. (2012). Effect of salinity stress on phenolic compounds and carotenoids in buckwheat (*Fagopyrum esculentum* M.) sprout. *Food Chemistry*, *135*(3), 1065–1070. https://doi.org/10.1016/j.foodchem.2012.05.068
54. Lin, L.Y., Peng, C.C., Yang, Y.L., Peng, R.Y. (2008). Optimization of bioactive compounds in buckwheat sprouts and their effect on blood cholesterol in hamsters. *Journal of Agricultural and Food Chemistry*, *56*(4), 1216-1223. https://doi.org/10.1021/jf072886x
55. Liu, C., Chen, Y., Yang, J., Chiang, B. (2008). Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. *Journal of Agricultural and Food Chemistry*, *56*(1), 173-178. https://doi.org/10.1021/JF072347S
56. Liu, D., Song, S., Tao, L., Yu, L., Wang, J. (2022). Effects of common buckwheat bran on wheat dough properties and noodle quality compared with common buckwheat hull. *LWT – Food Science and Technology*, *155*, art. no. 112971. https://doi.org/10.1016/j.lwt.2021.112971
57. Lu, L., Murphy, K., Baik, B.K. (2013). Genotypic variation in nutritional composition of buckwheat groats and husks. *Cereal Chemistry*, *90*(2), 132-137. https://doi.org/10.1094/CCHEM-07-12-0090-R
58. Luthar, Z., Zhou, M., Golob, A., Germ, M. (2021). Breeding buckwheat for increased levels and improved quality of protein. *Plants (Basel)*, *10*(1), art. no. 14. https://doi.org/10.3390/plants10010014
59. Mansur, A.R., Lee, S.G., Lee, B.H., Han, S.G., Choi, S.W., Song, W.J., Nam, T.G. (2022). Phenolic compounds in common buckwheat sprouts: composition, isolation, analysis and bioactivities. *Food Science and Biotechnology*, *31*(8), 935-956. https://doi.org/10.1007/s10068-022-01056-5
60. Martillanes, S., Rocha-Pimienta, J., Cabrera-Bañegil, M., Martín-Vertedor, D., Delgado-Adámez, J. (2017). Application of phenolic compounds for food preservation: Food additive and active packaging. In M. Soto-Hernandez, M. Palma-Tenango, M. del Rosario Garcia-Mateos (Eds.), *Phenolic Compounds — Biological Activity*; IntechOpen Ltd, London, United Kingdom, pp. 39–58. https://doi.org/10.5772/66885
61. Matseychik, I.V., Korpacheva, S.M., Lomovsky, I.O., Serasutdinova, K.R. (2021). Influence of buckwheat by-products on the antioxidant activity of functional desserts. *IOP Conference Series: Earth and Environmental Science*, *640*, art. no. 22038. https://doi.org/10.1088/1755-1315/640/2/022038
62. Matsumura, Y., Kitabatake, M., Kayano, S.I., Ito, T. (2023). Dietary phenolic compounds: their health benefits and association with the gut microbiota. *Antioxidants*, *12*(4), art. no. 880. https://doi.org/10.3390/antiox12040880
63. Mazahir, M., Ahmed, A., Ahmad, A., Ahmad, M.S., Khan, M.A., Manzoor, M.F. (2022). Extraction and determination of bioactive compounds and antioxidant activity of buckwheat seed milling fractions. *Food Science & Technology*, *42*, art. no. e81721. https://doi.org/10.1590/fst.81721
64. Melini, V., Vescovo, D., Melini, F., Raffo, A. (2024). Bakery product enrichment with phenolic compounds as an unexplored strategy for the control of the Maillard reaction. *Applied Sciences*, *14*(6), art. no. 2647. https://doi.org/10.3390/app14062647
65. Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. (2009). The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine*, *6*(7), art. no. e1000097. https://doi.org/10.1371/journal.pmed.1000097
66. Molska, M., Reguła, J., Kapusta, I., Świeca, M. (2022a). Analysis of phenolic compounds in buckwheat (*Fagopyrum Esculentum* Moench) sprouts modified with probiotic yeast. *Molecules*, *27*(22), art. no. 7773. https://doi.org/10.3390/molecules27227773

67. Molska, M., Regula, J., Rudzińska, M., Świeca, M. (2020). Fatty acid profile, atherogenic and thrombogenic health lipid indices of lyophilized buckwheat sprouts modified with the addition of *Saccharomyces cerevisae* var. *Boulardii*. *Acta Scientiarum Polonorum Technologia Alimentaria*, *19*(4), 483–490. http://dx.doi.org/10.17306/J.AFS.2020.0866
68. Molska, M., Reguła, J., Świeca, M. (2023). Adding modified buckwheat sprouts to an atherogenic diet — the effect on selected nutritional parameters in rats. *Plant Foods for Human Nutrition*, *78*, 279–285. https://doi.org/10.1007/s11130-023-01047-9
69. Molska, M., Reguła, J., Zielińska-Dawidziak, M., Tomczak, A., Świeca, M. (2022b). Starch and protein analysis in buckwheat (*Fagopyrum esculentum* Moench) sprouts enriched with probiotic yeast. *LWT – Food Science and Technology, 168*, art. no. 113903. https://doi.org/10.1016/j.lwt.2022.113903
70. Nandan, A., Koirala, P., Tripathi, A.D., Vikranta, U., Shah, K., Gupta, A.J., Agarwal, A., Nirmal, N. (2024). Nutritional and functional perspectives of pseudocereals, *Food Chemistry, 448*, art. no. 139072. https://doi.org/10.1016/j.foodchem.2024.139072
71. Nobili, C., De Acutis, A., Reverberi, M., Bello, C., Leone, G.P., Palumbo, D., Natella, F., Procacci, S., Zjalic, S., Brunori, A. (2019). Buckwheat hull extracts inhibit *Aspergillus flavus* growth and AFB1 biosynthesis. *Frontiers in Microbiology*, *10*, art. no. 01997. https://doi.org/10.3389/fmicb.2019.01997
72. Park, B.I., Kim, J., Lee, K., Lim, T., Hwang, K.T. (2019). Flavonoids in common and tartary buckwheat hull extracts and antioxidant activity of the extracts against lipids in mayonnaise. *Journal of Food Science and Technology*, *56*(5), 2712-2720. https://doi.org/10.1007/s13197-019-03761-2
73. Peng, C.C., Chen, K.C., Yang, Y.L., Lin, L.Y., Peng, R.Y. (2009). Aqua-culture improved buckwheat sprouts with more abundant precious nutrients and hypolipidemic activity. *International Journal of Food Sciences and Nutrition*, *60*(Suppl. 1), 232-245. https://doi.org/10.1080/09637480903018824
74. Pietrzak, D., Zwolan, A., Chmiel, M., Adamczak, L., Cegiełka, A., Hać-Szymańczuk, E., Ostrowska-Ligęza, E., Florowski, T., Oszmiański, J. (2022). The effects of extracts from buckwheat hulls on the quality characteristics of chicken meatballs during refrigerated storage. *Applied Sciences*, *12*(19) art. no. 9612. https://doi.org/10.3390/app12199612
75. Pirzadah, T.B., Malik, B. (2020). Pseudocereals as super foods of 21st century: Recent technological interventions. *Journal of Agriculture and Food Research*, *2*, art. no. 100052, https://doi.org/10.1016/j.jafr.2020.100052
76. Pongrac, P., Vogel-Mikuš, K., Potisek, M., Kovačec, E., Budič, B., Kump, P., Regvar, M., Kreft, I. (2016). Chapter 20 – Mineral and trace element composition and importance for nutritional value of buckwheat grain, groats, and sprouts. In M. Zhou, S.H. Woo, G. Wieslander, I. Kreft, N. Chrungoo (Eds.), *Molecular Breeding and Nutritional Aspects of Buckwheat*. Academic Press, Cambridge, Massachusetts, United States, pp. 261–271. https://doi.org/10.1016/b978-0-12-803692-1.00020-1
77. Qing, L., Li, S., Yan, S., Wu, C., Yan, X., He, Z., Chen, Q., Huang, M., Shen, C., Wang, S., Cao, M., Zhao, J. (2023). Anti-*Helicobacter pylori* activity of *Fagopyrum Tataricum* (L.) Gaertn. bran flavonoids extract and its effect on *Helicobacter pylori*-induced inflammatory response. *Food Science & Nutrition*, *11*(6), 3394-3403. https://doi.org/10.1002/fsn3.3329
78. Rauf, M., Yoon, H., Lee, S., Hyun, D.Y., Lee, M., Oh, S., Choi, Y.M. (2019). Evaluation of sprout growth traits and flavonoid content in common and tartary buckwheat germplasms. *Plant Breeding and Biotechnology*, *7*, 375-385. https://doi.org/10.9787/PBB.2019.7.4.375
79. Rethlefsen, M.L., Kirtley, S., Waffenschmidt, S., Ayala, A.P., Moher, D., Page, M.J., Koffel, J.B., PRISMA-S Group. (2021). PRISMA-S: An extension to the PRISMA Statement for Reporting Literature Searches in Systematic Reviews. *Systematic Reviews*, *10*, art. no. 39. https://doi.org/10.1186/s13643-020-01542-z
80. Salejda, A.M., Olender, K., Zielińska-Dawidziak, M., Mazur, M., Szperlik, J., Miedzianka, J., Zawiślak, I., Kolniak-Ostek, J., Szmaja, A. (2022). Frankfurter-type sausage enriched with buckwheat by-product as a source of bioactive compounds. *Foods*, *11*(5), art. no. 674. https://doi.org/10.3390/foods11050674
81. Sedej, I., Sakač, M., Mandić, A., Mišan, A., Tumbas, V., Čanadanović-Brunet, J. (2012). Buckwheat (*Fagopyrum esculentum* Moench) grain and fractions: Antioxidant compounds and activities. *Journal of Food Science*, *77*(9), C954-C959. https://doi.org/10.1111/j.1750-3841.2012.02867.x
82. Serikbaeva, A., Tnymbaeva, B., Mardar, M., Tkachenko, N., Ibraimova, S., Uazhanova, R. (2021). Determining optimal process parameters for sprouting buckwheat as a base for a food seasoning of improved quality. *Eastern-European Journal of Enterprise Technologies*, *4*(11(112)), 6-16. https://doi.org/10.15587/1729-4061.2021.237369
83. Shahidi., F, Ambigaipalan, P. (2018). Omega-3 polyunsaturated fatty acids and their health benefits. *Annual Review of Food Science and Technology*, *9*, 345-81. https://doi.org/10.1146/annurev-food-111317-095850
84. Shreeja, K., Devi, S., Suneetha, W.J., Prabhakar, B.N. (2021). Effect of germination on nutritional composition of common buckwheat (*Fagopyrum Esculentum* Moench). *International Research Journal of Pure and Applied Chemistry*, *22*(1), 1-7. https://doi.org/10.9734/irjpac/2021/v22i130350
85. Sturza, A., Păucean, A., Chiș, M.S., Mureșan, V., Vodnar, D.C., Man, S.M., Urcan, A.C., Rusu, I.E., Fostoc, G., Muste, S. (2020). Influence of buckwheat and buckwheat sprouts flours on the nutritional and textural parameters of wheat buns. *Applied Sciences*, *10*(22), art. no. 7969. https://doi.org/10.3390/app10227969
86. Sujka, K., Cacak-Pietrzak, G., Sułek, A., Murgrabia, K., Dziki, D. (2022). Buckwheat hull-enriched pasta: Ohysicochemical and sensory properties. *Molecules*, *27*(13), art. no. 4065. https://doi.org/10.3390/molecules27134065
87. Sun, T., Ho, C. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry*, *90*(4), 743-749. https://doi.org/10.1016/J.FOODCHEM.2004.04.035
88. Sytar, O., Brestic, M., Zivcak, M., Tran, L.S. (2016). The contribution of buckwheat genetic resources to health and dietary diversity. *Current Genomics*, *17*(3), 193-206. https://doi.org/10.2174/1389202917666160202215425
89. Teng, J., Hu, X., Tao, N., Wang, M. (2018). Impact and inhibitory mechanism of phenolic compounds on the formation of toxic Maillard reaction products in food. *Frontiers of Agricultural Science and Engineering*, *5*(3), 321-329. https://doi.org/10.15302/J-FASE-2017182
90. Tsai, H., Deng, H., Tsai, S., Hsu, Y. (2012). Bioactivity comparison of extracts from various parts of common and tartary buckwheats: Evaluation of the antioxidant- and angiotensin-converting enzyme inhibitory activities. *Chemistry Central Journal*, *6*, art. no. 78. https://doi.org/10.1186/1752-153X-6-78
91. Uzakov, Y., Kaldarbekova, M., Kuznetsova, O. (2020). Improved technology for new-generation Kazakh national meat products. *Foods and Raw Materials*, *8*(1), 76–83. https://doi.org/10.21603/2308-4057-2020-1-76-83
92. Wang, H., Liu, S., Cui, Y., Wang, Y., Guo, Y., Wang, X., Liu, J., Piao, C. (2021). Hepatoprotective effects of flavonoids from common buckwheat hulls in type 2 diabetic rats and HepG2 cells. *Food Science & Nutrition*, *9*(9), 4793-4802. https://doi.org/10.1002/fsn3.2390
93. Wang, J., Ma, H., Wang, S. (2019). Application of ultrasound, microwaves, and magnetic fields techniques in the germination of cereals. *Food Science and Technology Research*, *25*(4), 489-497. https://doi.org/10.3136/fstr.25.489
94. Wang, L., Li, Y., Guo, Z., Wang, H., Wang, A., Li, Z., Chen, Y., Qiu, J. (2023). Effect of buckwheat hull particle-size on bread staling quality. *Food Chemistry*, *405*(Part A), art. no. 134851. https://doi.org/10.1016/j.foodchem.2022.134851
95. Wiczkowski, W., Szawara-Nowak, D., Dębski, H., Mitrus, J., Horbowicz, M. (2014). Comparison of flavonoids profile in sprouts of common buckwheat cultivars and wild tartary buckwheat. *International Journal of Food Science & Technology*, *49*(9), 1977-1984. https://doi.org/10.1111/ijfs.12484
96. Witkowicz, R., Biel, W. (2022). A novel method for analyzing mineral ratio profiles of treated buckwheat sprouts (*Fagopyrum esculentum* Moench). *Journal of Food Composition and Analysis*, *114*, art. no. 104800. https://doi.org/10.1016/j.jfca.2022.104800
97. Witkowicz, R., Biel, W., Chłopicka, J., Galanty, A., Gleń-Karolczyk, K., Skrzypek, E., Krupa, M. (2019). Biostimulants and Microorganisms Boost the Nutritional Composition of Buckwheat (*Fagopyrum esculentum* Moench) Sprouts. *Agronomy*, 9, 469. https://doi.org/10.3390/agronomy9080469
98. Woo, S.H., Kamal, A.H.M., Park, S.M., Kwon, S.O., Park, S.U., Roy, S.K., Lee, J.Y., Choi, J.S. (2013). Relative distribution of free amino acids in buckwheat. *Food Science and Biotechnology*, *22*, 665–669. https://doi.org/10.1007/s10068-013-0129-2
99. Wronkowska, M., Bączek, N., Honke, J., Topolska, J., Wiczkowski, W., Zieliński, H. (2023). Wheat roll enhanced by buckwheat hull, a new functional food: focus on the retention of bioactive compounds. *Molecules*, *28*(11), art. no. 4565. https://doi.org/10.3390/molecules28114565
100. Wronkowska, M., Zieliński, H., Szmatowicz, B., Ostaszyk, A., Lamparski, G., Majkowska, A. (2019). Effect of roasted buckwheat flour and hull enrichment on the sensory qualities, acceptance and safety of innovative mixed rye/wheat and wheat bakery products. *Journal of Food Processing and Preservation*, *43*, art. no. e14025. http://dx.doi.org/10.1111/jfpp.14025

101. Wu, D.T., Wang, J., Li, J., Hu, J.L., Yan, H., Zhao, J., Zou, L., Hu, Y.C. (2023). Physicochemical properties and biological functions of soluble dietary fibers isolated from common and Tartary buckwheat sprouts. *LWT – Food Science and Technology*, *183*, art. no. 114944. https://doi.org/10.1016/j.lwt.2023.114944
102. Xu, F.Y., Gao, Q.H., Ma, Y.J., Guo, X.D., Wang, M. (2014). Tartary buckwheat flour and sprouts steamed bread. *Journal of Food Quality*, *37*, 318-328. https://doi.org/10.1111/jfq.12101
103. Yang, J., Zamani, S., Liang, L., Chen, L. (2021). Extraction methods significantly impact pea protein composition, structure and gelling properties. *Food Hydrocolloids*, *117*, art. no. 106678, https://doi.org/10.1016/j.foodhyd.2021.106678
104. Yang, X., Zhou, Y., Wang, B., Wang, F., Han, P., Li, L. (2019). Tartary buckwheat extract and chitosan coated tilapia (*Oreochromis niloticus*) fillets determine their shelf life. *Journal of Food Science*, *84*(6), 1288–1296. https://doi.org/10.1111/1750-3841.14649
105. Yilmaz, H.Ö., Ayhan, N.Y., Meriç, Ç.S. (2020). Buckwheat: A useful food and its effects on human health. *Current Nutrition & Food Science*, *16*(1), 29-34. https://doi.org/10.2174/1573401314666180910140021
106. Zhang, G., Xu, Z., Gao, Y., Huang, X., Zou, Y., Yang, T. (2015). Effects of germination on the nutritional properties, phenolic profiles, and antioxidant activities of buckwheat. *Journal of Food Science*, *80*(5), H1111-H1119. https://doi.org/10.1111/1750-3841.12830
107. Zhang, W., Zhu, Y., Liu, Q., Bao, J., Liu, Q. (2017). Identification and quantification of polyphenols in hull, bran and endosperm of common buckwheat (*Fagopyrum esculentum*) seeds. *Journal of Functional Foods*, *38*(Part A), 363-369. https://doi.org/10.1016/j.jff.2017.09.024
108. Zhang, Z., Fan, S., Duncan, G.J., Morris, A., Henderson, D., Morrice, P., Russell, W.R., Duncan, S.H., Neacsu, M. (2023). Buckwheat (*Fagopyrum esculentum*) hulls are a rich source of fermentable dietary fibre and bioactive phytochemicals. *International Journal of Molecular Sciences*, *24*(12), art. no. 16310. https://doi.org/10.3390/ijms242216310
109. Zhao, Z.Y., Piao, C.H., Wang, Y.H., Liu, J.M., Yu, H.S., Dai, W.C., Tang, Y.F., Wang, J., Liu, D.L. (2018). Isolation and anti-diabetic activity in vitro of flavonoids from buckwheat hull. *Food Science*, *39*(3), 21-27 (in Chinese: English abstract). https://doi.org/10.7506/spkx1002-6630-201803004
110. Zhong, L., Lin, Y., Wang, C., Niu, B., Xu, Y., Zhao, G., Zhao, J. (2022). Chemical profile, antimicrobial and antioxidant activity assessment of the crude extract and its main flavonoids from tartary buckwheat sprouts. *Molecules*, *27*(2), art. no. 374. https://doi.org/10.3390/molecules27020374
111. Zhou, M., Wieslander, G., Tang, Y., Tang, Y., Shao, J., Wu, Y. (2016). Chapter 11 – Bioactive compounds in buckwheat sprouts. In M. Zhou, I. Kreft, S.H. Woo, N. Chrungoo, G. Wieslander (Eds.), *Molecular Breeding and Nutritional Aspects of Buckwheat*, Academic Press, Cambridge, Massachusetts, United States pp. 151-159. https://doi.org/10.1016/B978-0-12-803692-1.00011-0
112. Zhou, X., Cheng, Sh., Yang, Y., Zhou, Y., Tang, W., Zhang, X., Wang, Q., Li, Z. (2011a). Toward a novel understanding of buckwheat self-defensive strategies during seed germination and preliminary investigation on the potential pharmacological application of its malting products. *Journal of Medicinal Plants Research*, *5*(32), 6946-6954. https://doi.org/10.5897/JMPR11.555
113. Zhou, X., Wang, Q., Yang, Y., Zhou, Y., Tang, W., Li, Z. (2011b). Anti-infection effects of buckwheat flavonoid extracts (BWFEs) from germinated sprouts. *Journal of Medicinal Plants Research*, *6*(1), 24-29. https://doi.org/10.5897/JMPR11.535
114. Zhou, X.L., Chen, Z.D., Zhou, Y.M., Shi, R.H., Li, Z.J. (2019). The effect of tartary buckwheat flavonoids in inhibiting the proliferation of MGC80-3 cells during seed germination. *Molecules*, *24*(17), art. no. 3092. https://doi.org/10.3390/molecules24173092
115. Zhou, Y., Wang, H., Cui, L., Zhou, X., Tang, W., Song, X. (2015). Evolution of nutrient ingredients in tartary buckwheat seeds during germination. *Food Chemistry*, *186*, 244-248. https://doi.org/10.1016/j.foodchem.2015.03.115
116. Zhu, F. (2021). Buckwheat proteins and peptides: biological functions and food applications. *Trends in Food Science & Technology*, *110*, 155–167. https://doi.org/10.1016/j.tifs.2021.01.081
117. Zielińska, D., Szawara-Nowak, D., Zieliński, H. (2013). Antioxidative and anti-glycation activity of buckwheat hull tea infusion. *International Journal of Food Properties*, *16*(1), 228-239. https://doi.org/10.1080/10942912.2010.551308
118. Zieliński, H., Honke, J., Bączek, N., Majkowska, A., Wronkowska, M. (2019). Bioaccessibility of D-chiro inositol from water biscuits formulated from buckwheat flours fermented by lactic acid bacteria and fungi. *LWT – Food Science & Technology*, *106*, 37-43. https://doi.org/10.1016/j.lwt.2019.02.065
119. Znamirowska, A., Sajnar, K., Kowalczyk, M., Kluz, M., Buniowska, M. (2020). Effect of addition of spelt and buckwheat hull on selected properties of yoghurt. *Journal of Microbiology, Biotechnology and Food Sciences*, *10*(2), 296-300. https://doi.org/10.15414/jmbfs.2020.10.2.296-300
120. Zych-Wężyk, I., Krzepiłko, A. (2012). Determination of total phenolic compound content and antioxidant properties of edible buckwheat sprouts. *Ecological Chemistry and Engineering A*, *19*(4-5), 441-449. https://doi.org/10.2428/ecea.2012.19(04)046

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