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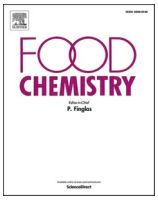

Upgrading hazelnut skins: Green extraction of polyphenols from lab to semi-industrial scale

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ABSTRACT

Hazelnut skins (HS) are usually managed as waste; however, this by-product is a source of bioactive compounds, with potential applications in feed and food sectors. Phenolic compounds can be extracted using green protocols combining enabling technologies and green solvents. This work investigates subcritical water extraction (SWE) of bioactive compounds from HS. A laboratory-scale study was performed on four different batches, with significant batch-to-batch heterogeneity. The evaluation of polyphenolic profiles and antioxidant activities afforded promising results compared to the benchmark of reflux maceration. To evaluate process effectiveness, the extraction protocol was replicated on a semi-industrial plant that processed 8 kg of matrix. Downstream processes have been optimized for scale-up, demonstrating the effectiveness of SWE in retaining product concentration and bioactivity avoiding excipients in spray-drying phase. Hazelnut extracts exhibited antibacterial properties against animal- and food-borne pathogens, supporting their potential use as sustainable feed ingredients for improved hazelnut production and animal farming practices.

1. Introduction

As the world moves toward a greener future, it is crucial to pursue and optimize innovative industrial strategies that prioritize environmental sustainability and minimize the ecological footprint of economic activity. In this context, the reduction of food waste and the recovery of processing waste for re-use in production processes have assumed considerable importance worldwide thanks to raised awareness in administrators and citizens (Perino & Chemat, 2019).

The production of hazelnuts (*Corylus avellana* L.) has increased significantly in recent years with over one million tonnes produced annually. Turkey is currently the leading producer of hazelnuts,

Abbreviation list: CE, Conventional Extraction; CFU, Colony Forming Unit; CTs, Condensed tannins; DE, Dry Extract; DM, Dry Matrix; FD, Freeze Drying; GAE, Gallic Acid Equivalents; HAT, Hydrogen Atom Transfer; HS, Hazelnut Skin; HSE, Hazelnut Skins Extract; HTs, Hydrolysable Tannins; MAE, Microwave-assisted extraction; MASWE, Microwave-Assisted Subcritical Water Extraction; MBC, Minimum Bactericidal Concentration; MHB, Mueller Hinton Broth; MIC, Minimum Inhibitory Concentration; ORACFL, Oxygen Radical Absorbance Capacity; PEF, Pulsed Electric Field; ROS, Reactive Oxygen Species; S/L, Solid-Liquid Ratio; SD, Spray Drying; SET, Single Electron Transfer; SFE, Supercritical-Fluid Extraction; SI-SWE, Semi-Industrial Scale Extraction; TEq, Trolox equivalents; TPC, Total phenolic content; UAE, Ultrasound-Assisted Extraction; UHPLC-Q-Orbitrap HRMS, Ultra High Performance Liquid Chromatography coupled with quadrupole-orbitrap high-resolution mass spectrometry.

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contributing 65 % of global production, followed by Italy, the United States and Azerbaijan, with 8 %, 6.5 %, and 6.3 %, respectively (FAOSTAT, 2023).

The economic interest in this sector focuses exclusively on the kernel, (Milošević & Milošević, 2017). The residual components are made up of the skin, which is separated during the roasting process and constitutes 2.5 % of the total weight, and the shell. While these parts currently lack economic value, and are usually managed as waste, several works have already explored their potential valorization as part of a circular economy model (Allegrini et al., 2022; D'Ambra et al., 2023). Indeed, hazelnut skins (HS) are particularly rich in dietary fibers, phenolic compounds, polyunsaturated fatty acids, tocopherols and significant levels of essential minerals, making them a promising source of bioactive compounds at low cost (Ivanović et al., 2020; Özdemir et al., 2014). Phenolic compounds are the most abundant of the bioactive compounds in HS (Fernández-Agulló et al., 2013; Zhang et al., 2021) and have demonstrated higher antimicrobial activity against Gram-positive bacteria, with lesser efficacy against Gram-negative bacteria (Di Michele et al., 2021).

Nowadays, industries involved in the extraction of natural products must pursue process intensification for environmentally friendly procedures and safer extraction protocols to remain competitive. Green extraction has emerged as a promising strategy with which to reduce the environmental impact, energy consumption and health hazards associated with the use of traditional organic solvents (Chemat et al., 2019). Several enabling technologies have been developed and tested for this purpose, including ultrasonic-assisted extraction (UAE) (Verdini et al., 2021), pulsed electric field (PEF) (Naliyadhara et al., 2022), supercritical-fluid extraction (SFE) (Herrero et al., 2015), microwave-assisted extraction (MAE) (Grillo et al., 2023; Pogorzelska-Nowicka et al., 2024) and microwave-assisted subcritical water extraction (MASWE) (Salgado Ramos et al., 2023). The use of green extraction technologies for agri-food waste valorization paves the way for the development of a more resilient and sustainable food industry (Voss et al., 2023).

MAE is widely known to significantly reduce extraction times compared to conventional techniques (Alexandru et al., 2014). The application of this advanced method to HS has already been optimized to increase phenolic yields, using ethanol as the extraction solvent (Odabaş & Koca, 2016). However, the use of water in subcritical conditions for extraction enables the isolation of organic compounds with preserved molecular functionalities. Under subcritical conditions, the solvent properties of water are like those of organic solvents, and it becomes able to dissolve several components of medium and low polarity due to changes in water properties at increasing temperature and pressure (Zhang et al., 2020). Subcritical water can therefore behave similarly to hydro-alcoholic mixtures, enabling the extraction of a wide range of chemical species, due to systematic decreases in the permittivity, viscosity and surface tension of water, coupled with an increase in the diffusion coefficient (Haghighi & Khajenoori, 2013).

Even though the industrial development of subcritical solvent extraction is still challenging, some works on pilot-scale processes have been recently described. For example, subcritical conditions were recently exploited in a pilot-scale semi-continuous process for the extraction of polyphenols from spent black tea (Rajapaksha & Shimizu, 2022). However, the presence of ethanol was still necessary, as ethanol–water (50 % *w*/w) was used as solvent in subcritical conditions, instead of only water. A pilot-scale subcritical water extraction of flavonoids from satsuma mandarin peel was also investigated (Ko et al., 2016), by using a 8 L scale plant, and a solute/solvent ratio of 1/34. The scale was enhanced to 25 L in a work described by Alonso-Riaño et al., 2023or the subcritical water extraction of brewer's spent grain valorization.

The application of subcritical water extraction (SWE) to hazelnut waste has recently been described, with higher yields generally being obtained, compared to solvent extraction (Büyükkileci & Sürek, 2018). However, HS extractions are currently performed on the lab-scale, and a proof of concept for industrial-scale processes is still lacking.

When SWE is performed under microwave irradiation, the dielectric heating favors the attainment of subcritical conditions, with temperatures ranging between 100 and 374 °C being achieved under pressure.

Although some studies reported the use of non-conventional technologies for the extraction of polyphenols from agri-food residues (Panzella et al., 2020), there is a lack of knowledge concerning the combination of these methods with subcritical water.

The aim of this work is to exploit SWE both at laboratory (using MASWE) and semi-industrial scale for the production of a HS extract as a greener alternative active against animal and foodborne pathogens, in the perspective of a circular economy approach for feeding.

With this purpose, the SWE of bioactive compounds from HS with the aim of maximizing extraction yields and phenolic content. The effectiveness of MASWE has been examined on four different HS samples and compared to a conventional maceration protocol with a hydroalcoholic solution. The bioactive profiles and the antioxidant and antimicrobial activities of the hazelnut skins extracts (HSE) were then evaluated.

Even though the challenges related to the industrial development of intensified processes are still manifold, advancements in the design of innovative industrial reactors present promising opportunities for the scale-up of extraction processes (Gallina et al., 2022). The potential application of the SWE technology on a larger scale was therefore evaluated in a customized semi-industrial extraction plant (Cravotto et al., 2022). The optimization of valorization protocols for agricultural by-products can create new commercial opportunities for farmers and hazelnut processors, boosting economic growth and innovation in the agri-food sector.

2. Materials and methods

2.1. Samples and chemicals

Four batches of HS (B1, B2, B3 and B4) were supplied by four different companies located in Italy, after quality check. All the analyses have been performed according to EU standards following Commission Implementing Regulation (EU) 2024/771 of 29 February 2024 amending Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for the official control.

The batches were characterized by different origins, in terms of cultivar and industrial processing. B1 and B2 originate from the same industrial plant but belong to two different Italian hazelnut cultivars (B1 from Campania region, Southern Italy, B2 from Piedmont region, Northern Italy). B3 is an HS from an industrial plant located in the Umbria region (Central Italy) in which both Italian and foreign hazelnuts are processed (many of Turkish origin). B4 derives from a feed company that collects different HS from different sources, with these then being subjected to quality controls to obtain uniformity and consistency in the batches for the formulation of feed components for livestock and pets, and to ensure safety in terms of contaminants and microbiology related risks. The samples were stored at room temperature in a dry environment to avoid metabolite degradation and used without undergoing pre-treatment processes. All chemicals used in this study were purchased from Sigma-Aldrich (Steinheim, Germany) and used without further purification.

2.2. Extraction protocols

2.2.1. Microwave-assisted subcritical water extraction (MASWE)

MASWE was performed according to Cravotto et al., 2022. HS were suspended in distilled water in a 1:30 solid/liquid ratio. The vessels were placed into a multimodal microwave reactor (SynthWAVE™, Milestone Srl, Bergamo, Italy) capable of exploiting an external supply of inert gas (N_2). For each test, appropriate purging with N_2 was performed three times to remove oxygen from the system, thus reducing oxidative stress

on the biomass. The reaction chamber was then pressurized with N_2 (0.5 MPa). The samples were heated at 150 °C with a maximum irradiation power of 1500 W for 30 min under magnetic stirring (329 x g). The resulting solution was filtered under vacuum, and the biomass was thoroughly washed with water (3 × 10 mL). The liquid fraction was then lyophilized (LyoQuest-85, Telstar, Madrid, Spain) and the dry extracts were stored at 4 °C. Three separate samples of hazelnut skins from each batch were treated with the extraction process under the same conditions. Each extraction was performed three times to validate the reproducibility of the experimental results.

2.2.2. Conventional extraction (CE)

Conventional extraction was performed using a hydroalcoholic reflux maceration protocol. HS (3.4 g) was suspended in a hydroalcoholic solution (EtOH/H_2O 70:30, 100 mL), resulting in solid/liquid ratio of 1:30, and heated under magnetic stirring at 100 °C for 1 h. Subsequently, the solution was filtered under vacuum, and the solvent was evaporated under vacuum. The liquid fraction was then lyophilized (LyoQuest-85, Telstar, Madrid, Spain) and the dry extracts were stored at 4 °C. Three separate samples of hazelnut skins from each batch were treated with the extraction process under the same conditions. Each extraction was performed three times to validate the reproducibility of the experimental results.

2.2.3. Semi-industrial-scale subcritical water extraction (SI-SWE)

Experiments were carried out using a pilot-scale customized prototype (Tropical Food Machinery Srl, Busseto, Italy, Fig. 1).

The system employs a recirculating flow of pressurized hot water and includes a 40 L recirculation tank (2), as well as two stainless steel cylindrical extractors that can be used separately (3). Each extractor is equipped with five perforated baskets (4) with a 0.5 mm mesh for holding the biomass. The system is heated via steam conduction through the heat exchanger. The pressurized hot water flows both radially and vertically through the perforated baskets to facilitate the extraction process. The final volume of water used for a single extraction, using only a single 100- L tank at a time, was 150 L, including the pipes. The reactor uses a conventional steam heating system and is pressurized through the steam pressure auto generated by water at high temperature in the sealed environment. Therefore, the pressure in the pilot plant does not need adjustment as it reaches equilibrium according to the thermal state. The final pressure was around 0.8 ± 0.02 MPa, and the temperature was set at 150 °C for 30 min.

HS extraction scale-up was performed with 8 kg of biomass suspended in water (150 L) for each experiment. In total, 25 extractions were carried out, treating 200 kg of HS.

The extracts were then pumped into an expansion tank where they were subjected to a pressure of 0.02 MPa. This pressure drop leads to flash evaporation, where some of the liquid rapidly vaporizes, resulting in an immediate reduction in the liquid volume by 25–30 %. Additionally, this evaporative cooling phenomenon lowers the temperature of the remaining liquid to 70 °C. Following the flash evaporation, the concentration process continues under vacuum conditions. This vacuum helps to further reduce the liquid's volume by an additional 50 %.

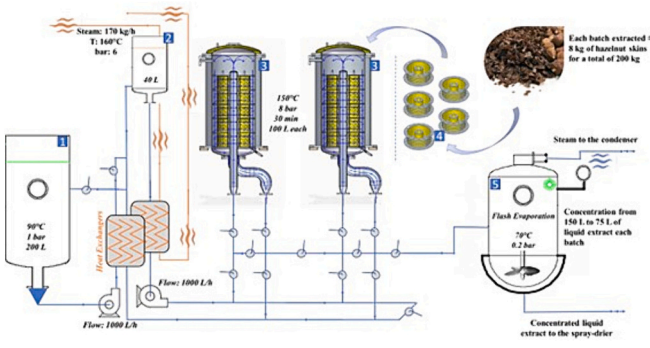

Fig. 1. Semi-industrial subcritical water extraction process-flow diagram. 1: Pre-heating tank; 2: Recirculation tank; 3: Two extractors; 4: Perforated baskets; 5: Evaporation unit.

The concentrated liquid fraction was then dried using an industrial spray dryer (Agroindustry Tecco Srl, Cuneo, Italy). An aliquot was subjected to laboratory-scale drying, for the sake of comparison, using two different tools: a small-scale spray dryer (KD-SD-2000, Zhengzhou Keda Machinery and Instrument Equipment Co., Ltd., Zhengzhou, China) operating at three different temperatures (120, 160 and 200 °C), and a freeze dryer (LyoQuest-85, Telstar, Madrid, Spain).

2.3. Characterization of hazelnut skin extracts (HSE)

2.3.1. Total phenolic content (TPC)

Total phenolic content was determined according to the Folin–Ciocalteu protocol (Aimone et al., 2023). After sample preparation, the solution was allowed to stand for 25 min before readings were taken at 725 nm on a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Gallic acid solutions (0.01 to 0.45 mg/mL) were used to prepare the calibration curve. The concentration of total phenolic compounds was expressed as mg of gallic acid equivalents (GAE)/gram of dry matrix (DM), and mg GAE/g of extract. Analyses were repeated three times on each sample replicate obtained for each batch.

2.3.2. Tannin determination

Tannin determination was performed according to Peri and Pompei (1971), with some adjustments (Peri & Pompei, 1971). In the total tannin analysis, a solution of hemisulfate cinchonine (0.5 % *w/v*, 600 μL) was mixed with hydroalcoholic solutions (EtOH/H_2O 50:50 *v*/v) of the extracts (20 mg/mL, 600 μL) in an Eppendorf tube. The obtained solutions were shaken and then left at 4 °C for 14 h to promote the precipitation of the tannate cinchonine. The samples were then centrifuged at 18,135 x*g* for 2 min at 10 °C (Allegra 64R, Beckman Coulter Srl, Cassina De' Pecchi, Italy). The supernatants were separated and analyzed using the Folin–Ciocalteu test (as described in section 2.3.1). Total semi-quantitative tannin content was calculated as a difference and expressed as GAE.

The tannic fraction was also analyzed in terms of hydrolysable (HTs) and condensed tannins (CTs) (Scalbert et al., 1989). The precipitates obtained after centrifugation were washed twice with distilled water to remove hemisulfate cinchonine and were resuspended in 600 μL of a hydroalcoholic mixture (EtOH/H_2O 50:50, (*v*/v)). Afterwards, this suspension (500 μL) was mixed with HCl 36 % (5:2 v/v, 250 μL) and an aqueous solution of formaldehyde 4.8 % (250 μL). The mixture was shaken and incubated for 14 h, then centrifuged at 18,135 x*g* for 5 min at 10 °C. The supernatant, containing the HT fraction, was analyzed using the Folin–Ciocalteu method. The precipitate, however, contained the CTs, and the quantity of this fraction was determined by calculating the difference between the total and the HTs. Analyses were repeated three times on each sample replicate obtained for each batch.

2.3.3. Phenolic compound identification and quantification

The identification of phenolic compounds in both HS and HSEs was performed as reported in Martini et al. (2017). Briefly, 1.25 g of sample was added to 6.25 mL of extraction solution (methanol:water:formic acid solution; 70:28:2, v/v), and the solution was then homogenized and incubated for 60 min at 37 °C under stirring. After extraction, the mixture was centrifuged at 4185 x*g* for 20 min (4 °C) and the supernatant was withdrawn. The pellet resulting from the centrifugation was further extracted by adding the same amount of extraction solution as reported above, and the same protocol was repeated. The supernatants resulting from the three cycles of extraction were mixed and, after appropriate dilution, analyzed using UHPLC-MS. The system consisted

of a UHPLC Ultimate 3000 separation module coupled to a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). The separation of phenolic compounds was accomplished using a C18 column (Acquity UPLC HSS C18 Reversed phase, 2.1 × 100 mm, 1.8 µm particle size, Waters, Milan, Italy). The chromatographic separation and mass spectrometry settings are fully described in Martini et al. (2020). Quantification was carried out by building external calibration curves with the available standard compounds, as reported in Table S2.

2.3.4. Antioxidant activity

2.3.4.1. DPPH radical scavenging activity. DPPH• radical scavenging activity was measured as reported in Boffa et al. (2016). Various dry-extract concentrations were prepared via sequential dilution, and absorbance was measured at 515 nm using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The obtained absorbance data were processed using Bobo Least Squares software (version 0.9.1.), with probit regression analysis. The EC_{50} value represents the extract concentration required to inhibit 50 % of DPPH· radicals and is expressed as µg dry extract (DE) per mL of solution. These values were compared with the EC_{50} values obtained using a reference standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), expressed as Trolox equivalents (TEq.) per gram of extract (mmol g^{-1}). Analyses were repeated three times on each sample replicate obtained for each batch.

2.3.4.2. Oxygen radical absorbance capacity (ORACFL). To determine ORACFL, one gram of each extract was separately mixed with a buffer 75 mM, pH 7.2 containing 13.19 g K_2HPO_4 and 10.26 g KH_2PO_4 in 900 mL deionized water, homogenized in a Ultra-Turrax homogenizer (Ultra Turrax T25 Basic, IKA Labortechnik Janke & Kunkel GmbH, Stavfen, Germany) for 1 min, and then vortexed for 2 min. The homogenates were centrifuged at 4185 x*g* for 20 min at 4 °C and the supernatant was used to determine antioxidant capacity via ORACFL method, which is based on the fluorescence decay rate of a probe in the presence of a radical oxygen species, compared with that of Trolox, as reported by Zulueta et al. (2009). The ORACFL assays were carried out on a FLUOstar OPTIMA microplate fluorescence reader (BMG LABTECH, Offenburg, Germany) at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The procedure was based on the method reported by Branciari et al. (2015). All reaction mixtures were prepared in duplicate and data are expressed as micromoles of TEq. per gram of sample (mmol g^{-1}).

2.3.4.3. Fe-chelating activity. The ability of extracts to chelate Fe^{2+} was assessed using the colorimetric method proposed by Santas et al. (2010), with minor modifications. Initially, various dry extract concentrations were prepared via sequential dilution, and a final volume of 0.5 mL was achieved for each dilution. Then, 1.6 mL of an acetate buffer (4.10 g/L sodium acetate, pH 5.5) and 0.2 mL of a $FeSO_4$ solution (45.5 mg/L in acetate buffer) were added to each sample, and allowed to react for 5 min. Following the incubation period, a ferrozine solution (393.9 mg/L in acetate buffer) was added, and sample absorbance was read at 562 nm using a spectrophotometer after 15 min of reaction (Agilent Technologies, Santa Clara, CA, USA). Distilled water was used as the blank. A control sample with distilled water instead of ferrozine was used to correct for unequal color in the sample solutions, and a reference sample without extracts was used to establish the standard chelating activity of the ferrozine. The percentage of Fe^{2+}-ferrozine complex formation was calculated as follows (Eq. 1):

$$Fe^{2+}\ \text{chelating ability}\ (\%) = \frac{(Abs_{sample} - Abs_{control}) - Abs_{reference}}{Abs_{reference}} \times 100 \qquad (1)$$

The absorbance data obtained were processed using Bobo Least Squares software (version 0.9.1.) to define proper Probit regression and determine the EC_{50} concentration for the Fe-chelating activity of the extracts (Locatelli et al., 2009). The same protocol was applied for a standard chelator, EDTA, and the results were subsequently expressed in terms of EDTA equivalents.

2.3.5. Antimicrobial activity

The antimicrobial activity of HSEs was assayed against the most relevant animal and food-borne pathogens. The strains were bought from Microbiologics (St. Cloud, MN, USA) and the growth conditions of each microorganism are reported in Table 1. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were measured using the broth microdilution technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022), European Committee on Antimicrobial Susceptibility Testing (The European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2018) and to the protocol provided in Wiegand et al., 2008.

For each microorganism, colonies were resuspended in sterile saline solution and suspension turbidity was measured spectrophotometrically at an optical density of 600 nm (OD600). The bacterial suspension used for the assay was prepared by adjusting the number of bacteria to 5×10^5 CFU/mL, in either Mueller Hinton Broth (MHB) or MHB supplemented with 5 % lysed horse blood and 20 mg/L β-NAD (*L. monocytogenes* and *C. jejuni*), and by vigorous vortexing.

An aliquot of each suspension (100 µL) was added to a 96-well microplate containing the same volumes of two-fold serial dilution of the extracts, ranging from 0.156 to 40 mg/mL. Two controls were set up: a positive control using broth medium and bacterial suspension; and a negative control using broth medium and the extract at the same concentration tested. The microplate was then incubated according to the specific bacterial growth conditions reported in Table 1. The MIC was defined as the lowest concentration of extract that produced no bacterial growth. The MBC was determined by sub-culturing the media used for MIC determination. An amount of 10 µL from the wells, corresponding to the MIC and to the higher MIC concentrations, was plated on either Muller Hinton Agar or Blood Agar and then incubated according to the specific bacterial growth conditions. The MBC was represented as the lowest HSE concentration that was able to kill 99.9 % of the bacterial inoculum after 24 h of incubation, as demonstrated by the total absence of growth. The standard reading was established after 24 h and all experiments were performed in triplicate ($n = 3$) on a homogeneously mixed sample for each batch.

3. Results and discussion

3.1. Lab-scale extractions

The extraction of phenolic compounds is strongly influenced by their chemical nature, the technology employed and the presence of interfering substances (Alara, Abdurahman and Ukaegbu, 2021). Moreover, the solubility of phenolic substances is strictly dependent on their degree of polymerization and the polarity of the solvent used for the extraction.

Table 1
Strains used for assay and growth conditions.

Bacterial strains	Growth conditions
Gram-positive	
Staphylococcus aureus (ATCC 29213)	37 °C for 24 ± 2 h, aerobiosis
Bacillus cereus (ATCC 11778)	30 °C for 24 ± 2 h, aerobiosis
Listeria monocytogenes (ATCC 13932)	37 °C for 24 ± 2 h, aerobiosis
Gram-negative	
Salmonella enteritidis (ATCC 13076)	37 °C for 24 ± 2 h, aerobiosis
Escherichia coli (ATCC 25922)	37 °C for 24 ± 2 h, aerobiosis
Pseudomonas aeruginosa (ATCC 27853)	25 °C for 24 ± 2 h, aerobiosis
Campylobacter jejuni (ATCC 33291)	41.5 °C for 48 ± 2 h, microaerophilia

In the present work, the efficacy of the MASWE protocol was assessed and compared with CE. Both extraction methods were initially evaluated in terms of dry extract yield and TPC (Table 2).

The extraction yields for MASWE were similar for the B1E, B2E and B3E extracts (21–23 %). However, a higher yield (nearly 25 %) was achieved for B4E, indicating that the presence of extractable compounds was slightly higher here than for the other three samples (Table 2). The comparative analysis of the HS extraction efficiency of MASWE and CE demonstrated small differences, with a slightly higher yield being observed for the CE of B3E and B4E, although the extraction time is twice as high.

The TPC of HSE is reported as mg GAE/g ext. (thus indicating the selectivity of the extraction process for polyphenols), and mg GAE/g of dry matrix (DM), which represents the overall amount of phenolic compounds extracted per gram of biomass (Table 2).

Selectivity expresses how many mg of total polyphenols are present per g of dry extract; therefore, it can be inferred from the table that the majority fraction of the extract is polyphenols, for almost all samples, except for B1E from MASWE. To justify the TPC results for the B3E-CE and B4E-CE samples, which are above 1000 mg GAE per gram of extract, two standard phenolic compounds, epicatechin and tannic acid, were compared to gallic acid for their activity in a Folin-Ciocalteu assay. The results showed that these two compounds give higher signal response than gallic acid in the TPC assay (Fig. S1). Consequently, when present in high amounts in an extract, the contribution of these compounds can result in an overexpression of the TPC value if expressed as gallic acid equivalent units. This is the case for B3E-CE and B4E-CE, which are much richer in tannins than the other samples (Fig. S2).

The differences in TPC in the analyzed batches may be caused by environmental conditions, geographical origin, agronomic applications and sample collection time, which are closely linked to the biosynthetic pathways of the secondary metabolites (Lucchetti et al., 2018). Furthermore, the chemical composition of HS is strongly modified during industrial hazelnut processing, as the phenolic compounds may degrade during these procedures. This occurs especially in roasting processes, which, besides physical changes, cause significant chemical modifications in bioactive substances (Binello et al., 2018).

The comparison of the results achieved using CE and MASWE shows that water, despite being in its subcritical phase, is still slightly less effective at extracting polyphenols from HS than the hydroalcoholic solution (Table 2). However, the fact that the extraction time is halved for MASWE should be taken into consideration.

Nonetheless, MASWE technology avoids the use of organic solvents, and, consequently, reduces costs, environmental concerns and the number of operational units required. This approach reduces the risks posed by solvent-related product contamination, while also minimizing environmental pollution from solvent manufacturing and potential leakage during the process. Additionally, the removal of flammable solvents enhances operational safety for personnel, and reduces costs as there is no need for specialized production lines that conform to the Equipment for Potentially Explosive Atmospheres (ATEX) directive in European Union member states (European Parliament & Council of the European Union, 2014).

Table 2
Extraction yield (% amount of toal extract over treated biomass) and TPC of HSEs ($n = 3$).

	MASWE[a]			CE[b]		
	Yield (%)	TPC Selectivity (mg GAE/g of dry extract)	TPC Yield (mg GAE/g DM)	Yield (%)	TPC Selectivity (mg GAE/g of dry extract)	TPC Yield (mg GAE/g DM)
B1E	21.9 ± 0.7	489.5 ± 8.0	103.7 ± 5.0	22.5 ± 0.9	781.4 ± 20.0	176.3 ± 10.0
B2E	22.9 ± 0.8	551.3 ± 5.0	126.1 ± 4.0	22.0 ± 0.1	866.0 ± 22.0	191.0 ± 4.0
B3E	22.5 ± 0.1	885.8 ± 19.0	199.0 ± 3.0	26.3 ± 0.4	1158.2 ± 16.0	305.2 ± 5.0
B4E	24.9 ± 0.2	778.4 ± 16.0	194.2 ± 5.0	27.8 ± 0.3	1172.1 ± 60.0	325.7 ± 14.0

[a] MASWE: Microwave-assisted subcritical water extraction, solid/liquid ratio 1:30, 150 °C, 30 min;
[b] CE: Conventional extraction, solid/liquid ratio 1:30, 100 °C, 1 h.

In summary, the HSE Folin–Ciocalteu assay revealed notable selectivity toward polyphenols, particularly for B3E and B4E. MASWE showed promising results as it provides a polyphenol-rich extract, while avoiding the need for organic solvents.

However, while MASWE can yield higher concentrations of desired compounds in shorter times, the total energy required (heat and maintenance of specific pressures) can impact the overall sustainability of the process. Therefore, the greatest potential for improving the environmental performance of microwaves is related to the expected decarbonization of the electricity mix, which would reduce the impacts by 6 %–24 % (Gallego-Schmid et al., 2018). Furthermore, the new generation microwave systems would include better insulation and microwave waveguide technologies that minimize energy loss, meanwhile maximizing the specific absorption rate of microwaves in water and plant materials to decrease heating times and energy requirements. The utilization of combined heat and power systems through a closed-loop system could also reduce the waste heat generation.

By focusing on these measures, the environmental footprint of microwave-assisted subcritical water extraction can be significantly reduced, making it a more sustainable option for extracting valuable compounds from natural materials.

3.1.1. *Polyphenolic fraction composition*

Further studies were conducted to more comprehensively characterize the phenolic compounds recovered using the different extraction techniques. Tannins, phenolic acids, flavan-3-ols and flavonols have been recognized in the literature as the principal polyphenols present in hazelnuts (Pycia et al., 2020).

Quantitative analysis revealed that tannins were the predominant class of polyphenols in HSEs, particularly in the CE samples. These compounds can be broadly classified into two groups: CTs, flavonoids without a sugar core, and HTs, composed of ellagic and gallic acid with a sugar core, mainly glucose (Kumar Das et al., 2020). The distribution of CTs and HTs in the HSE tannin fraction was investigated (Fig. S2).

The B3E and B4E MASWE extracts displayed higher percentages of total tannins in their phenolic fraction (53 % and 50 %, respectively) than their B1E and B2E analogs (45 % and 32 %, respectively). All of the CE extracts contain higher proportions of tannins in their phenolic fraction than the MASWE extracts, with the highest value being obtained for B4E (79 %). By contrast, the HSEs recovered using MASWE present larger fractions of non-tannin phenolic compounds. These results are consistent with a study by Aimone et al. (2023) on the MASWE extraction of tannins, in which it was revealed that the yield of monomeric phenols is positively correlated to extraction temperature.

The principal fraction of tannins in all the HSEs is composed of CTs. Notably, the MASWE B3E and B4E contain almost double the percentages of HTs than the CE extracts, even if less abundant than CTs. However, the TPC measured with the Folin-Ciocalteu method showed a higher value for the CE extracts. This is because the higher presence of total HTs concentration in MASWE compared to CE extracts did not offset the contribution from the CTs (Table S1).

UHPLC-MS analyses were then exploited for the quantification of phenolic acids, flavan-3-ols and flavonols in HSEs (Table 3). A more detailed quantification of the phenolic compounds detected in HS and HSEs is reported in the Supplementary Material. (Tables S3, S4 and S5).

MASWE samples contained approximately double the quantity of phenolic acids than CE extracts, and up to 3.5 times the amount of flavan-3-ols, in all four batches. The considerable amount of phenolic acids, such as gallic and ellagic acid (Table S4-S5), in the MASWE

Table 3
UHPLC-MS quantification of phenolic acids, flavan-3-ols and flavonols in HSEs.

		Phenolic acids	Flavan-3-ols	Flavonols
		mg/g of extract		
MASWE	*B1E*	1.11 ± 0.012	11.43 ± 0.11	1.04 ± 0.003
	B2E	1.23 ± 0.016	12.79 ± 0.08	0.63 ± 0.002
	B3E	1.19 ± 0.023	12.12 ± 0.03	0.51 ± 0.002
	B4E	1.36 ± 0.031	14.84 ± 0.06	0.51 ± 0.003
CE	*B1E*	0.39 ± 0.001	2.99 ± 0.013	1.52 ± 0.006
	B2E	0.57 ± 0.001	5.57 ± 0.011	2.00 ± 0.003
	B3E	0.56 ± 0.007	3.54 ± 0.011	1.85 ± 0.028
	B4E	0.75 ± 0.002	6.22 ± 0.017	3.60 ± 0.017

MASWE: microwave-assisted subcritical water extraction; CE: conventional extraction.

samples can be attributed to the partial depolymerization of the HTs when exposed to higher temperatures and pressures, which is further facilitated by the slightly acidic nature of water in its subcritical state (Smeriglio, Barreca, Bellocco, & Trombetta, 2017). By contrast, hydro-alcoholic maceration at 100 °C may have promoted hydrolysis to a lesser degree. The high amounts of flavan-3-ols, especially catechin and epicatechin (Tables S4 and S5), in the MASWE extracts may be related to the products of CT hydrolysis, as caused by the favorable depolymerization conditions present during MASWE. Furthermore, the flavonols fraction was higher in the conventional extracts, with quercetin and its glycosylated form being the main compounds identified.

The higher abundance of monomeric phenolic compounds in the MASWE samples may have beneficial implications in terms of nutraceutical value, as polyphenols with higher polymerization degrees tend to have lower intestinal absorption rates (Smeriglio, Barreca, Bellocco, & Trombetta, 2017).

3.1.2. Antioxidant activity

Interest in the use of natural antioxidants, from both consumers and industry, has rapidly grown in recent decades. Antioxidant compounds can be used in human and animal diets to prevent the overproduction of reactive oxygen species (ROS), which lead to oxidative stress, a condition implicated in several diseases, such as cancer, diabetes, arthrosclerosis, arthritis, neurodegenerative diseases and premature ageing (Lourenço et al., 2019; Xu et al., 2021). In addition, they also find applications as food preservatives, reducing oxidative damage in products and consequently improving their quality and shelf life (Bensid et al., 2022).

The assessment of antioxidant activity in natural extracts can be conducted with different approaches and methodologies. The scavenging capacity of radical species can be evaluated using two different mechanisms: hydrogen atom transfer (HAT) and single electron transfer (SET). Alternatively, chelation capacity toward transition metals, which are responsible for ROS production through Fenton-type oxidation, can serve as an indicator of antioxidant capacity, since the chelation of these metals prevents the subsequent formation of radical species (Gulcin, 2020).

In the present study, the antioxidant properties of HSEs were investigated using three different assays: DPPH (HAT+SET mechanism), ORAC (HAT) and Fe-chelating activity (metal-chelating mechanism). These methods were selected because of their ability to target different, distinct mechanisms, enabling a comprehensive evaluation of the antioxidant potential of HSEs to be carried out (Fig. 2).

The antioxidant activity of the MASWE samples, measured using the DPPH method, was highest for B3E, followed by B4E (higher in this sample than in the same extract obtained using CE), B2E and B1E in that order (7.41, 4.51, 2.66 and 1.68 mmol TE eq./g extract, respectively). The same trend is observed in the batches when the ORAC test was used, but the differences between the samples was smaller (7.24, 6.86, 6.60, and 6.548 mmol TE/g extract for B3, B4, B2 and B1, respectively). Quite interestingly, when using the ORAC assay, the extracts recovered after

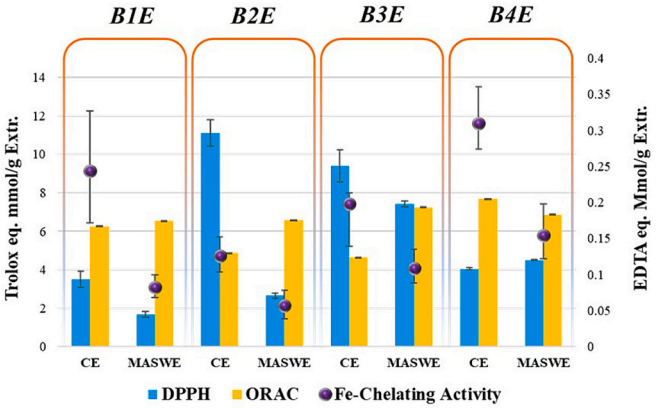

Fig. 2. Antioxidant activity of HSEs measured using DPPH, ORAC and Fe-chelating activity assays. MASWE: microwave-assisted subcritical water extraction; CE: conventional extraction. Confidence intervals are reported as error bars.

MASWE show higher antioxidant properties than the conventional ones (except for B4E). By contrast, Fe-chelating activity was highest in the CE samples; in the order B4E > B1E > B3E > B2E. The antioxidant-activity trend observed in HSEs, as determined by DPPH and ORAC assays, showed a positive correlation with the amount of polyphenols in the extract. This observation suggests that these are the compounds in the extracts that exert the majority of the antioxidant effect. By contrast, the Fe-chelating activity did not show a direct correlation with polyphenol content, implying that this specific property is influenced by other compounds. In general, all of the extracts showed significant antioxidant activity, with some differences being observed among the batches. B3E and B4E, due to their significant antioxidant power, were confirmed to be the samples with the highest content of bioactive compounds.

3.1.3. Antibacterial effect of hazelnut extracts

The antimicrobial properties of HSEs against animal- and food-borne pathogens were evaluated, and the results are summarized in Table 4, where activity is indicated with colors ranging from dark green (high activity) to red (low activity). All of the extracts displayed antimicrobial activity against the tested strains, showing higher efficacy against Gram-positive than Gram-negative bacteria. Notably, it was observed that the MIC and MBC values were almost identical for each sample and bacterial strain tested. This indicates that the concentration required to inhibit bacterial growth is also sufficient to kill the bacteria.

B. cereus was the bacteria most susceptible to all of the HSEs, whether obtained via CE, active at the concentration of 0.156 mg/mL, or MASWE, active at concentrations ranging between 0.312 and 0.625 mg/mL. *S. aureus* also showed to be susceptibility to all the HSEs, showing a MIC of 0.312 mg/mL with all of the HSEs obtained via CE, while the extracts from MASWE were active at concentrations of 0.312 and 0.625 mg/mL. Similarly, *L. monocytogenes* was characterized by a MIC and MBC of 0.312 mg/mL for all conventional extracts, while concentrations ranging from 0.312 to 0.625 mg/mL were registered for MASWE.

On the other hand, the conventional extracts showed low antimicrobial activity against Gram-negative bacteria. Remarkably, the HSEs obtained using MASWE displayed significantly higher antimicrobial activity toward Gram-negative bacteria than the extracts obtained using the conventional method. *S. enteritidis* growth was inhibited by concentrations of 5 and 1.25 mg/mL of the HSEs obtained via the CE and MASWE methods, respectively. For *P. aeruginosa,* the conventional HEs are active at the concentrations of 5 and 10 mg/mL, while the green extracts are active at concentrations of 2.5 and 5 mg/mL. *E. coli* appears as the least sensitive strain, with MIC values ranging from 10 to 40 mg/mL for the conventional extracts and 2.5–5 mg/mL for the green ones. The MIC/MBC values for *C. jejuni* ranged from 2.5 to 5 mg/mL for the

Table 4
MIC and MBC values for all the hazelnut skins extracts assayed with different bacterial strains. The values are expressed as mg/mL.[a]

Bacterial strain	Test	*B1E*		*B2E*		*B3E*		*B4E*	
		CE	MASWE	CE	MASWE	CE	MASWE	CE	MASWE
Gram-positive									
S. aureus	MIC	0.312	0.625	0.312	0.625	0.312	0.625	0.312	0.312
	MBC	0.312	0.625	0.312	0.625	0.312	0.625	0.312	0.625
B. cereus	MIC	0.156	0.625	0.156	0.625	0.156	0.312	0.156	0.312
	MBC	0.156	0.625	0.156	0.625	0.156	0.312	0.156	0.312
L. monocytogenes	MIC	0.312	0.625	0.312	0.312	0.312	0.625	0.312	0.312
	MBC	0.312	0.625	0.312	0.625	0.312	0.625	0.312	0.312
Gram-negative									
S. enteritidis	MIC	5	1.25	5	1.25	5	1.25	5	1.25
	MBC	5	1.25	5	1.25	5	1.25	5	2.5
E. coli	MIC	10	5	10	2.5	20	2.5	40	2.5
	MBC	10	5	10	2.5	20	2.5	40	2.5
P. aeruginosa	MIC	5	2.5	10	2.5	10	2.5	10	5
	MBC	5	2.5	10	2.5	10	2.5	10	5
C. jejuni	MIC	2.5	1.25	2.5	1.25	2.5	1.25	2.5	1.25
	MBC	2.5	1.25	2.5	1.25	2.5	1.25	5	1.25

MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; MASWE: microwave-assisted subcritical water extraction; CE: conventional extraction.
[a] Colors range from dark green (high activity) to red (low activity). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

extracts processed under conventional techniques, while lower values were registered for MASWE samples.

Interestingly, MIC and MBC were the same for all the bacterial strains tested, except for B4E, which showed an MBC of one-fold dilution more than its MIC (0.625 mg/mL and 2.5 mg/mL for *S. aureus* and *S. enteritidis*, respectively). Furthermore, despite the different chemical profiles and antioxidant activities registered, no significant differences in the antimicrobial activity of the HSEs from the four HS batches were observed.

In general, the differences in antimicrobial activity could be related to the higher amount of Flavan-3-ols in MASWE compared to CE. These are a group of flavonoids that appear to have greater activity against Gram-positive than Gram-negative bacteria (Cushnie & Lamb, 2005).

Variations in antimicrobial activities against bacteria may reflect the differences in the cell-surface structures of Gram-negative and Gram-positive species. The synergistic association of the antimicrobial and health-promoting effects of phenolic compounds can provide a basis for the formulation of natural preservatives with supplementary nutritional benefits, intended for incorporation into human and animal diets (Cueva et al., 2010). However, further research is necessary to determine the optimal dosage, toxicity and efficacy of these compounds before they can be widely adopted to reduce the use of synthetic antibiotics (Santas et al., 2010).

3.2. Semi-industrial scale extraction (SI-SWE)

The laboratory-scale extraction strategy used on HS has provided promising results, as evidenced by the high TPC and antioxidant properties observed in the HSEs. However, in order to meet the current market demand for nutraceutical products, it is imperative that extraction protocols suitable for industrial-scale production are developed. The direct implementation of new technologies at an industrial scale is still a challenge and does not guarantee immediate feasibility. In order to assess the suitability of this extraction strategy for the industrial market, a scale-up experiment was performed on a SI-SWE system (Fig. 1) at Tropical Food Machinery, in Busseto (PR, Italy).

The protocol used in the laboratory-scale (MASWE) experiments was reproduced as closely as possible during SI-SWE. However, SI-SWE was carried out at full load using the entire reactor volume to maximize time and water-consumption efficiency. This resulted in an average of 8 kg of HS per extraction, suspended in 150 L water, which translates into a lowering of the solid-liquid ratio from 1:30, on the lab-scale, to 1:18.75 for SI-SWE.

On the base of the previous results, batch B4 was selected for the scale-up experiment (and the main polyphenols were also identified in the starting material, as reported in Table S3).

Furthermore, the product from the feed mill has greater consistency of quality, and it is available in larger quantities, a fundamental condition to ensure reproducible scale-up protocols given the variable nature of industrial waste.

An extraction yield of approximately 18.19 %, based on the total weight of the extracted matrix, was achieved on the semi-industrial scale; this value is lower than that achieved on the laboratory scale (24.94 %). On other hand, the scale-up process successfully preserved extract quality in terms of TPC selectivity, as evidenced in Fig. S3. Indeed, an increase in the extraction selectivity toward polyphenolic compounds was observed, (from 778 to 875 mg GAE/g extr.). The decrease in extraction yield can be explained by some challenges and losses that occurred when adapting the extraction process to an industrial setting. In particular, the halved liquid/solid ratio may have negatively affected the overall extraction efficiency by reducing the driving force for mass transfer. However, the reduction in the solid-liquid ratio (s/l) contributed to a decrease in water consumption, and, simultaneously, allowed twice as much matrix to be extracted at the same time with only a relatively modest decrease in yield, of around 25 %. Furthermore, a conventional heating technique (steam conduction) was used in the industrial setting, rather than microwaves, and this may have contributed to the difference in product yield. Nevertheless, the preservation of extract qualities represents a significant preliminary achievement.

From the perspective of a zero-waste approach, after extraction and drying, the biomass waste from SI-SWE could be reused in livestock to increase circularity. Hazelnut by-products have been previously tested to be used as poultry litter (Sarica & Cam, 2000) with interesting results. As an alternative, the wastes post SI-SWE processes could be used as substrate for biogas fermentations. HS have already been tested for their potentials in biogas production; their composition in fibers, indeed, could improve the metabolism of methane producing bacteria,

increasing biogas plant performances. (Şenol & Zenk, 2020).

3.2.1. Comparison of drying methods

The liquid extract obtained from SI-SWE was used to evaluate the most suitable drying method. This study focuses on the impact of two different drying technologies (freeze drying (FD) and spray drying (SD)) on extract quality, and the TPC and antioxidant activities of the dried extracts were used to evaluate this impact.

FD is a well-established technology that offers advantages in preserving the integrity of bioactive compounds by avoiding thermal degradation. However, it is associated with high energy and time consumption, which may limit its suitability for large-scale production.

SD is a continuous process that enables rapid and efficient water removal from liquid samples. However, one of the major drawbacks of this method is the potential degradation of thermosensitive molecules due to the elevated temperatures employed. Additionally, optimizing the spray drying process often requires the use of additives, such as maltodextrins, to decrease the moisture content and water activity of the particles, to avoid stickiness (Sobulska & Zbicinski, 2021). This addition can increase the cost of the process and reduce the concentration of key bioactive compounds in the final dried extract.

In addition to showing remarkable environmental benefits, SWE allows spray-drying to be carried out without the need for excipients (Aimone et al., 2023).

Therefore, the SD protocol was investigated on the laboratory scale at three different temperatures, 120 °C, 160 °C and 200 °C, while industrial SD worked at 180 °C. The concentration of polyphenols in the dried samples was found to be unaffected by the drying methodologies. However, there was a noticeable difference in the antioxidant activities of the FD sample and those dried using SD, on both the laboratory and industrial scales (Fig. S4). The major activity detected in the FD sample can be attributed to the preservation of the most thermolabile bioactive compounds present in the HSEs. No significant differences in results, in terms of TPC and antioxidant activity, were observed in the various SD conditions tested on the laboratory scale. Consequently, the optimized protocol was established as operating at a drying temperature of 120 °C to reduce process energy consumption. This drying technology was also found to be scalable when applied to HSEs, as the laboratory- and industrial-scale SD experiments displayed no significant differences in terms of TPC and bioactivity.

The choice of drying method should consider process requirements, extract quality and energy consumption. In conclusion, both FD and SD have advantages and limitations in the drying of HSEs. FD preserves bioactive compounds and maintains high antioxidant activity but requires more energy and time. By contrast, SD is a fast and efficient drying technology, but may result in the degradation of thermosensitive molecules, lowering the antioxidant activity of the extracts. The SD process demonstrated scalability when applied to HSEs, making it a viable option for large-scale production.

3.2.2. Characterization of semi-industrial scale extracts

The antioxidant capacity, in terms of DPPH, ORAC and Fe-chelating activity, of the B4E produced using MASWE (dried with FD) and SI-WE (dried with industrial SD) have been compared, together with the class of phenolic compounds extracted, and the results can be seen in Fig. 3.

The main differences in antioxidant activity between MASWE and SI-SWE are observed in the DPPH test. As has already been reported in section 5.3.1., industrial SD influences the antioxidant activity measured in the DPPH assay, reducing efficacy from 4.51 to 2.57 mmol TE/g dry extract respect FD, and the Fe-chelating activity results are also lower for the extract obtained on the semi-industrial scale (0.15 vs 0.09 mmol EDTA eq/g dry extract for the MASWE and SI-SWE, respectively).

However, the antioxidant activity measured using the ORAC assay did not reveal significant differences in the two samples, suggesting that HAT scavenger compounds are sufficiently maintained (6.86 and 7.11 mmol TE/g extr. For the MASWE and SI-SWE, respectively).

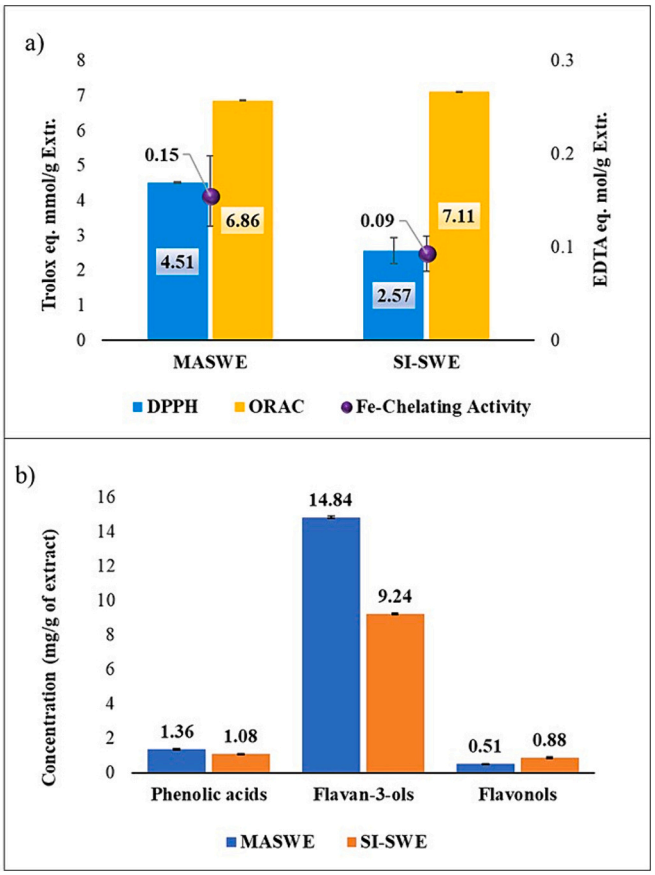

Fig. 3. Comparison of microwave-assisted subcritical water extraction and semi-industrial scale subcritical water extraction. Confidence intervals are reported as error bars. a) Antioxidant activity. b) class of phenolic compounds.

In interpreting this outcome, the different mechanisms of action of the assays must be considered (Dudonné et al., 2009). Although the content of phenolic compounds and flavan-3-ols is lower in the semi-industrial extract, flavonols are present in higher quantities. The differences are not particularly striking, however, meaning that the two extracts can be considered similar in terms of polyphenol content (Table S3).

3.2.3. Comparison of antimicrobial activity

The antimicrobial activity of the B4E produced with MASWE (dried with FD) and SI-WE (dried with industrial SD) were compared, and the results are reported in Table 5.

The antimicrobial activity results of SI-SWE were comparable to those observed for MASWE (Table 5), for all strains, except against the Gram-positive bacteria *Listeria monocytogenes,* and the Gram-negative bacteria *Campylobacter jejuni,* where the semi-industrial extract displayed lower antimicrobial effects.

4. Conclusion

The extraction of total phenolic compounds from HS using SWE has been investigated on both the laboratory and semi-industrial scales. The use of water in its subcritical conditions allowed the avoidance of ethanol, but providing extracts with comparable antioxidant activity. While all extracts display antimicrobial activity against the tested strains, it is worth noting that the green extracts have higher activity against Gram-negative bacteria than the conventional extracts, thus setting the stage for further studies on the application of green extracts from agri-food waste against animal- and food-borne pathogens.

Table 5
Comparison of antimicrobial activity of lab and semi-industrial B4E[a]

Gram-positive

Bacterial strain	*S. aureus*		*B. cereus*		*L. monocytogenes*	
Test (*mg/mL*)	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
MASWE	0.312	0.625	0.312	0.312	0.312	0.312
SI-SWE	0.312	0.625	0.312	0.312	1.25	1.25

Gram-negative

Bacterial strain	*S. enteritidis*		*E. coli*		*P. aeruginosa*		*C. jejuni*	
Test (*mg/mL*)	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
MASWE	1.25	2.5	2.5	2.5	5	5	1.25	1.25
SI-SWE	1.25	2.5	2.5	2.5	5	5	2.5	2.5

[a] Colors range from dark green (high activity) to red (low activity). MASWE: microwave-assisted subcritical water extraction; semi-industrial scale subcritical water extraction; MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; MASWE: microwave-assisted subcritical water extraction; CE: conventional extraction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Furthermore, MASWE presents advantages in terms of cost-effectiveness, process safety and reduced environmental impact. The SI-SWE system, coupled with industrial SD, successfully reproduced the lab-scale process in terms of product concentration, extract quality and bioactivity. This consistency in the chemical profiles and bioactivity of the laboratory- and semi-industrial scale products paves the way for the use of HS extracts as a natural low-cost bioactive product for feeds, thus reducing reliance on synthetic antibiotics. In addition, SWE meets the principles of green chemistry for waste valorization, according to a circular economy approach.

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CRediT authorship contribution statement

Giorgio Capaldi: Writing – original draft, Investigation, Formal analysis, Data curation. **Monica Voss:** Writing – original draft, Investigation, Formal analysis, Data curation. **Silvia Tabasso:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Valentina Stefanetti:** Investigation, Formal analysis, Data curation. **Raffaella Branciari:** Methodology, Conceptualization. **Salah Chaji:** Investigation, Formal analysis. **Giorgio Grillo:** Investigation, Formal analysis, Data curation. **Christian Cravotto:** Methodology, Investigation. **Davide Tagliazucchi:** Formal analysis, Data curation. **Domenico Pietro Lo Fiego:** Methodology, Investigation, Formal analysis, Data curation. **Massimo Trabalza Marinucci:** Supervision, Methodology, Conceptualization. **Rossana Roila:** Formal analysis, Data curation. **Antonio Natalello:** Methodology, Conceptualization. **Davide Pravettoni:** Methodology, Conceptualization. **Giancarlo Cravotto:** Writing – review & editing, Supervision. **Claudio Forte:** Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140999.

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