

Green optimisation of microwave-assisted extraction of phenolic compounds from rice straw lignocellulosic residues and LC-HRMS-based cultivar profiling

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ABSTRACT

Rice straw was valorised as a source of antioxidant phenolic compounds. Microwave-assisted extraction (MAE) was optimised using a Box–Behnken design to assess the influence of temperature, ethanol concentration, solvent volume and extraction time on yield, total phenolic content (TPC) and antioxidant capacity. Temperature and time were the key factors. Optimal conditions (80 °C, 60% ethanol, 50 mL, 40 min) produced an 18.9% yield and 796 mg GAE/100 g DW, with high antioxidant activity (FRAP 32.3; ABTS 43.2 μmol TE/g DW). LC-ESI/LTQ-Orbitrap-HRMS/MS enabled detailed profiling of phenolic compounds, with 4-hydroxybenzoic acid and quercetin-3-glucoside as major contributors. The optimised workflow was applied to eleven cultivars harvested in 2019–2020, revealing cultivar- and year-dependent differences in phenolic fingerprints and antioxidant performance. Overall, the integrated MAE–Orbitrap approach efficiently produced phenolic-rich extracts and supported comparative cultivar profiling, although further evidence on stability, safety and regulatory aspects is needed for food-related applications.

1. Introduction

Rice (*Oryza sativa* L.) is a cereal of high nutritional value and one of the world's main crops, being rich in carbohydrates, proteins, lipids, fibre and ash. However, some variations may exist between different varieties (Rodríguez, 2007). In the Valencian Community, rice cultivation covers a significant area and contributes to the sustainability of the local ecosystem. The main native varieties grown in this region are *Bomba*, *J Sendra*, *Italia Onice* and *Sirio*. These varieties differ in grain size and shape, disease resistance, and irrigation requirements. Among them, *Bomba* rice has received particular attention due to its ability to remain loose and whole after cooking (Maroto Luján, 2016).

The main residue generated by rice cultivation is rice straw (Ribó, Albiach, Pomares, & Canet, 2017). This waste consists of plant stems and leaves, which are produced in large quantities. It is a low-value material. It typically contains 35 wt% cellulose, 18 wt% hemicellulose, 20 wt%

lignin and 15 wt% ash (Ribó et al., 2017). Traditionally, straw was burned. This practice has negative environmental consequences, including greenhouse gas emissions and air pollution. Recent studies continue to report that rice straw burning releases harmful pollutants and is associated with respiratory health concerns and air-quality deterioration (Le, Khoi, & Mallick, 2022). Combustion smoke, together with unfavourable meteorological conditions, increases the risk of acute respiratory diseases among people living near rice-growing areas (Domínguez-Escribà & Porcar, 2010).

Moreover, these emissions contribute large amounts of highly contaminating atmospheric particles and volatile organic compounds (Phuong et al., 2021), which adversely affect air quality, climate regulation, the hydrological cycle, and atmospheric visibility. Consequently, the Spanish Regional Ministry of Agriculture, Rural Development, Climate Emergency and Ecological Transition, in alignment with the European Union's Common Agricultural Policy (CAP) requirements,

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implemented regulatory measures (Order 7/2016, April 18). These measures progressively restricted open-field burning and fully prohibited it from the 2022 harvest onwards (Conselleria d'Agricultura, 2016). This regulation increased the need for alternative management strategies for the approximately 100,000 t of rice straw left on the land after each harvest (Çapanoğlu, Nemli, & Tomas-Barberan, 2022; Torregrosa, Miguel Giner, & Velázquez-Martí, 2021).

The global shift towards a circular economy has intensified the search for sustainable and environmentally friendly strategies to manage agricultural and food residues. Owing to its recalcitrant composition and low biodegradability. It is therefore a promising feedstock for the production of value-added products (Gummert, Van Hung, Chivenge, & Douthwaite, 2019; Kaur, Kaur, Kaur, & Kaur, 2024; Martínez-Guillén, Álvarez-Martínez, Micol, & Barrajón-Catalán, 2025). Rice straw is commonly explored as a source of cellulose or lignin. However, it can also provide phenolic compounds with potential applications in the food, cosmetics, and pharmaceutical industries (Ramos et al., 2023). Phenolic compounds constitute a wide family of plant-derived metabolites formed through secondary metabolic pathways. They are characterised by the presence of at least one aromatic ring bearing one or more hydroxyl groups. In plants, they play multiple roles, including the prevention or delay of cellular oxidation through their involvement in pigmentation processes and their participation in defence mechanisms against pathogen attack (Apak, Özyürek, Güçlü, & Çapanoğlu, 2016). Within plant tissues, these compounds are distributed either in soluble form within vacuoles or bound to insoluble components of the cell wall (Rajbhar, Dawda, & Mukundan, 2015). From a nutritional perspective, epidemiological evidence indicates that dietary intake of phenolics contributes to the prevention of degenerative diseases, largely attributed to their antioxidant properties (Apak et al., 2016). In rice straw, phenolics are mainly classified as phenolic acids, stilbenes, and flavonoids (Ramos et al., 2023). These compounds show substantial structural diversity. This diversity affects physicochemical properties and extraction behaviour. Most rice-straw phenolics are covalently bound to insoluble polymers. Efficient extraction is therefore needed to release them while limiting degradation (Martínez, 2010).

Solid-liquid extraction is the conventional approach for releasing bioactive compounds, with key parameters including solvent composition, particle size, extraction time, temperature, solid-to-liquid ratio, and pH (Rajbhar et al., 2015). Karimi et al. (2014) extracted phenolic and flavonoid compounds from the straw and seed husks of several rice varieties using Soxhlet extraction with aqueous-organic solvents at high temperatures. Higher recovery was reported when acid hydrolysis was applied. This treatment stabilised phenolics at acidic pH and increased their solubility in organic solvents (Rajbhar et al., 2015).

In recent years, extraction methods aligned with green-processing principles have been developed to obtain natural bioactives more efficiently. These techniques often reduce solvent consumption and processing time, while maintaining high yields and good reproducibility (Ramos et al., 2023). Green extraction methods, such as subcritical water extraction (SWE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), or their combination, are effective alternatives to conventional extraction methods and meet sustainability requirements (Ramos et al., 2023). Recent reviews highlight that MAE is often described as a time- and solvent-efficient technique, offering reduced solvent consumption and lower environmental impact compared to conventional methods (López-Salazar, Camacho-Díaz, Arenas Ocampo, & Jiménez-Aparicio, 2023), and its efficacy in extracting phenolics from agro-food residues, including in combination with NaDESs, has been increasingly documented (Bezerra & Koblit, 2025; Zhu, Ma, Zhang, Zhang, & Sun, 2025). However, quantitative sustainability metrics (e.g., energy demand and solvent recovery) are not always reported. These metrics were not assessed in the present study.

Several studies illustrate the potential of these techniques in rice by-products. Wataniyakul, Pavasant, Goto, and Shotipruk (2012) reported

enhanced recovery of total phenolics from defatted Thai rice bran using SWE after microwave pretreatment. Freitas, González-Martínez, and Chiralt (2020) obtained extracts with high antioxidant and antimicrobial activity by combining ultrasound pretreatment with reflux heating from rice straw wastes from *J. Sendra* var. from *L'Albufera Natural Park* rice fields (Valencia, Spain). Leonarski et al. (2025) used MAE techniques to recover anthocyanins from black rice bran. To the best of our knowledge, MAE has not been reported for the extraction of phenolic compounds from Bomba rice straw or other Mediterranean cultivars intended for the production of antioxidant-rich extracts. In addition, most studies focus on single batches or cultivars. They often lack statistically validated optimisation and high-resolution compositional profiling. This limits the transferability of extraction protocols across genotypes and harvest conditions.

Among the analytical techniques available for investigating phenolic metabolites, liquid chromatography coupled to high-resolution tandem mass spectrometry in negative ion mode, employing an electrospray source and a hybrid high-resolution mass analyser (LC-ESI/LTQ-Orbitrap-HRMS) is a powerful and versatile option (Kodikara, Netticadan, Bandara, Wijekoon, & Sura, 2024; Laveriano-Santos et al., 2022; Vallverdú-Queralt et al., 2017). Its high mass accuracy and resolution enable confident compound identification in complex plant matrices, including agro-industrial by-products.

These advances, combined with modern chemometrics, have accelerated HRMS-based metabolomics applications in food authenticity, cultivar discrimination and quality assessment, where multivariate models (e.g., PCA/OPLS) are routinely used to extract robust markers from complex datasets (García-Pérez, Becchi, Zhang, Rocchetti, & Lucini, 2024). Moreover, the combination of a linear ion trap (LTQ) with the Orbitrap analyser enables multistage fragmentation (MS^n), facilitating structural elucidation of isomeric phenolics and the detection of low-abundance metabolites. These features, together with the sensitivity provided by electrospray ionisation (ESI), make this platform a reference standard for metabolomic profiling, cultivar authenticity, and the characterisation of antioxidant extracts derived from lignocellulosic residues or rice cultivars (Pinto et al., 2023).

Microwave-assisted extraction (MAE) optimised by response surface methodology (RSM) is increasingly used to improve global recovery of antioxidant extracts from agro-residues (Elzaawely, Maswada, El-Sayed, & Ahmed, 2017; Holic et al., 2018; Islam et al., 2026; Menzel, González-Martínez, Vilaplana, Diretto, & Chiralt, 2020). In rice straw, however, optimisation studies often focus on single batches or cultivars and rarely provide compound-resolved evidence to interpret the observed functional responses. LC-HRMS/MS (e.g., Orbitrap) enables high-confidence compound annotation and supports robust phenolic fingerprinting (Chen et al., 2025). Its application to rice straw extracts remains limited. To date, Box-Behnken-optimised MAE has not been integrated with LC-ESI/LTQ-Orbitrap-HRMS/MS profiling and evaluated across multiple cultivars and harvest years. The novelty of this work lies in combining these approaches under harmonised protocols across eleven cultivars over two years, enabling cultivar-dependent compositional interpretation under comparable extraction conditions. Therefore, this study aimed to optimise the MAE of phenolic compounds from Bomba rice straw (BRS) using a RSM approach to obtain a global phenolic-rich antioxidant extract. These were further characterised by LC-ESI/LTQ-Orbitrap-HRMS/MS to enable compound-level interpretation. The optimised protocol was then applied to rice straw from 11 cultivars harvested in 2019–2020 in the L'Albufera Natural Park area (Valencia, Spain), including Bomba, J. Sendra, Sirio, Albufera, Fonsa, Arpa, Onice, Italia, and a pigmented cultivar (black rice). This design allowed evaluation of protocol robustness and assessment of cultivar-dependent differences in phenolic composition and antioxidant performance.

2. Materials and methods

2.1. Materials and chemicals

Rice straws from different rice cultivars were collected from fields located in the L'Albufera Natural Park area (Valencia, Spain) after the 2019 and 2020 harvest seasons. Rice straw from eleven cultivars was evaluated. These included *Bomba*, *J. Sendra*, *Sirio*, *Albufera*, *Fonsa*, *Arpa*, *Onice*, and *Italia*, and a pigmented cultivar (black rice). Full details of the plant material (cultivar, origin/harvest information, and sample coding) are provided in the Supplementary Material (Table S1). Rice straw was washed with water to remove impurities and dried for 20 h in a climatic chamber (Dycometal CM/081, Barcelona, Spain) at 40 °C and 50% relative humidity. Dried rice straw was ground in a high-speed rotatory mill (ZM 200, Restech, Hann, Germany) using a 0.5 mm pore size sieve and stored in a desiccator.

Flavonoids ((-)-epicatechin, hesperidin, quercetin-3-glucoside, myricetin, apigenin, genistein, kaempferol and rutin, taxifolin), phenolic acids (4-hydroxybenzoic acid, 3,4-dihydroxyhydrocinnamic acid, *p*-coumaric acid, sinapic acid, 3,5-dihydroxybenzoic acid, ferulic acid, vanillic acid, syringic acid, protocatechuic acid, gallic acid and caffeic acid), and other phenolics (vanillin, catechol and phthalic acid) were purchased from Sigma-Aldrich (Madrid, Spain). Standards were stored protected from light.

Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) were purchased from Sigma-Aldrich (Madrid, Spain). Other reagents were analytical grade and were supplied by Sigma-Aldrich (Madrid, Spain).

2.2. Characterisation of Bomba rice straw (BRS)

The chemical composition of BRS was determined according to the Association of Official Analytical Chemists (AOAC) methods (AOAC, 2023). All determinations were carried out in triplicate, and the results were reported on a dry weight (DW) basis (g/100 g DW). Moisture content was measured by drying samples in an oven at 105 ± 5 °C until constant weight. Crude protein content was determined by the Kjeldahl method (N x 6.25, AOAC 978.04) using a Pro-Nitro-M steam distillation unit (JP Selecta, Barcelona). Ash content was determined after calcination in an oven at 600 ± 15 °C for 2 h (AOAC 923.03), and crude fat was calculated by Soxtec extraction with petroleum ether for 1 h (AOAC 920.85). Total carbohydrate content was then calculated by difference, according to Eq. (1):

$$\begin{aligned} \text{Total carbohydrates} &= \left(\frac{\text{g}}{100 \text{ g rice straw}} \right) \\ &= 100 - (\text{moisture} + \text{fat} + \text{ash} + \text{protein}) \end{aligned} \quad (1)$$

2.3. Microwave-assisted extraction and characterisation from BRS

MAE was carried out using a FlexiWAVE microwave oven (Milestone, Bergamo, Italy). A 2 g dry sample was placed in a round-bottom flask, and ethanol/water was added at 40, 50, or 60% (v/v). The solvent volume was set to 50, 65, or 80 mL to adjust the solid-to-liquid ratio. Samples were extracted at 500 W for the selected time (5.0, 22.5, or 40 min) and temperature (40, 60, or 80 °C). After the extraction step, the ethanolic solutions were vacuum-filtered, and the supernatant was removed using a rotary evaporator, followed by freeze-drying (Telstar, LYOQUEST-55 PLUS, Terrassa, Spain). The dried extracts were stored at room temperature, protected from light, until analysis.

Four extraction variables (temperature, ethanol concentration, solvent volume and extraction time) were selected based on previous MAE literature for phenolic recovery from lignocellulosic plant matrices (Aquino et al., 2023; Khalfi, Garrigós, Ramos, & Jiménez, 2024;

Melikoglu, 2025). The selected MAE factors were chosen because they directly govern mass transfer and microwave-assisted solubilisation of bound phenolics from lignocellulosic matrices. Temperature enhances diffusion and matrix softening, promoting the release of phenolics, while extraction time controls the extent of desorption/solubilisation and the approach to equilibrium. Ethanol concentration (ethanol/water ratio) modulates solvent polarity and microwave absorption, balancing the extraction of phenolic acids and flavonoids, whereas solvent volume (solid-to-liquid ratio) influences the concentration gradient and mixing efficiency, affecting the driving force for mass transfer. For these reasons, the effects of these variables were evaluated on three responses (extraction yield, total phenolic content (TPC) and antioxidant activity by ferric reducing antioxidant power assay (FRAP) using a three-level Box–Behnken design (BBD) (Table 1).

A total of 29 experiments were carried out randomly, including five central points. Each experimental run was performed once, and each response (TPC, FRAP) was analysed in triplicate from the same extract; results are reported as the mean of the three measurements. RSM was used for model fitting and optimisation of MAE parameters based on BBD results. Experimental data were fitted to a second-order (quadratic) polynomial model including linear, interaction and quadratic terms.

2.3.1. Extraction yield

The extraction yield (%) was calculated using Eq. (2):

$$\text{Extraction yield (\%)} = \frac{W_{\text{ext}} (\text{g})}{W_0 (\text{g})} \cdot 100 \quad (2)$$

where W_0 is the initial weight of dried rice straw (before extraction), and W_{ext} is the weight of dried extract obtained after extraction.

2.3.2. Determination of TPC

TPC was determined by the Folin-Ciocalteu colourimetric assay, following the method described by Singleton and Rossi with minor modifications (Singleton & Rossi, 1965). An aliquot (0.25 mL) of the extract was mixed with 1.25 mL of Folin-Ciocalteu reagent (1:10, v/v, in deionised water) and 1.0 mL of 7.5% (w/v) aqueous sodium carbonate solution. The mixture was then kept in the dark at room temperature for 30 min, after which the absorbance was measured at 765 nm using a UV–Vis spectrophotometer (BioMate-3, Thermo Scientific, MA, USA). Calibration curves were prepared using gallic acid as a standard (10–500 mg/kg, coefficient of determination (R^2) = 0.998). Results were expressed as mg gallic acid equivalents per 100 g of dry matter (mg GAE/100 g DW).

2.3.3. Determination of antioxidant activity

2.3.3.1. FRAP assay. Antioxidant activity was assessed by FRAP assay, following the procedure described by Apak et al. (2016). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM ferric chloride solution in a 10:1:1 (v/v/v) ratio. An aliquot (100 µL) of extract was added to 3.0 mL of FRAP reagent. The mixture was incubated for 30 min, after which the absorbance was measured at 593 nm using a UV–Vis spectrophotometer. Calibration curves were prepared using Trolox as a standard (six concentration levels: 10–500 mg/kg, and external calibration). Linearity was confirmed in the working range (R^2 = 0.995). Results were expressed as µmol Trolox equivalents per gram of dry matter (µmol TE/g DW).

2.4. Analysis of extracts at optimal conditions

Extracts obtained under the selected optimal MAE conditions were performed in triplicate as independent extractions ($n = 3$), and each extract was analysed in triplicate using the same analytical procedures applied to the BBD responses to ensure comparability. Accordingly, extraction yield, TPC and FRAP were determined as described in Section

Table 1

Box-Behnken experimental design (BBD) matrix and response values obtained from BRS by MAE.

Run	Independent variables				Responses		
	Temperature (°C)	Ethanol (% v/v)	Solvent volume (mL)	Extraction time (min)	Yield (%)	TPC (mg GAE/100 g DW)	FRAP (μmol TE/g DW)
1	60	60	50	5.0	8.67	338.51	15.62
2*	60	60	65	22.5	9.72	368.00	20.22
3	60	80	50	22.5	9.22	379.19	16.39
4	40	60	50	22.5	7.77	273.49	16.18
5	80	60	65	5.0	12.25	494.12	24.79
6	60	60	80	40.0	10.98	419.09	21.59
7	60	40	50	22.5	9.12	371.46	17.87
8	40	60	65	5.0	7.64	213.88	10.78
9	60	40	80	22.5	6.97	283.80	13.72
10*	60	60	65	22.5	11.04	438.54	25.68
11	60	80	65	5.0	9.34	390.58	18.37
12	80	80	65	22.5	12.27	561.05	29.03
13	40	80	65	22.5	9.61	282.04	14.67
14	60	80	65	40.0	11.25	441.30	23.33
15	40	40	65	22.5	8.51	235.30	11.97
16	60	80	80	22.5	10.36	456.85	20.56
17	40	60	80	22.5	9.36	353.58	17.87
18	60	60	80	5.0	10.18	425.67	18.61
19*	60	60	65	22.5	10.09	370.92	19.07
20	60	40	65	40.0	9.13	367.24	17.21
21	80	60	65	40.0	15.86	727.67	39.08
22	40	60	65	40.0	9.24	408.53	16.07
23	80	60	50	22.5	15.14	734.13	36.49
24*	60	60	65	22.5	10.89	483.58	21.50
25*	60	60	65	22.5	10.70	466.54	19.82
26	80	40	65	22.5	16.10	600.64	27.60
27	60	60	50	40.0	9.57	437.40	19.59
28	80	60	80	22.5	14.31	623.21	33.87
29	60	40	65	5.0	9.55	356.30	16.29

* Central points.

2.3. In addition, antioxidant capacity under optimum conditions was further assessed by the 2,2-azino-bis-ethylbenzothiazoline-6-sulphonic acid assay (ABTS) and 1,1-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH).

2.4.1. ABTS assay

The ABTS assay was performed according to the procedure described by Rejeb, Dhen, Gargouri, and Boulila (2020). The ABTS radical cation was generated by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate, and the mixture was kept in the dark at room temperature for 16 h. This solution was then diluted with ethanol to obtain a working solution with an absorbance of 0.70 ± 0.01 at 734 nm.

Subsequently, 150 μL of extract was combined with 2.85 mL of ABTS working solution, and after 30 min of incubation at room temperature in the dark, the absorbance was recorded at 734 nm. Calibration curves were prepared using Trolox as a standard (six concentration levels and external calibration). Linearity was confirmed in the working range ($R^2 = 0.992$). Results were expressed as μmol Trolox equivalents per gram of DW (μmol TE/g DW). All measurements were performed in triplicate.

2.4.2. DPPH assay

The DPPH radical-scavenging activity was determined according to the method described by Rejeb et al. (2020), with minor modifications. 150 μL of the extract was added to 2.85 mL of the DPPH solution (0.1 mM in methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm. Radical scavenging activity was expressed as the percentage of inhibition (I%). All measurements were performed in triplicate.

2.4.3. Metabolomic analysis by LC-ESI/LTQ-Orbitrap-HRMS

Targeted metabolomic profiling of the BRS extract was performed as described in a previous study (Rejeb et al., 2020), with minor modifications. The analysis was performed using an LC-ESI/LTQ-Orbitrap-HRMS system comprising an Accela chromatograph (Thermo

Scientific, Hemel Hempstead, UK) equipped with a photodiode array detector, a quaternary pump and a thermostatted autosampler. This chromatographic system was seamlessly coupled to an LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, Hemel Hempstead, UK) operated in negative electrospray ionisation mode. The main instrumental settings were configured as follows: source voltage, 3.5 kV; sheath gas, 50 a.u. (arbitrary units); auxiliary gas, 20 a.u.; sweep gas, 2 a.u.; and capillary temperature, 375 °C (Automatic gain control (AGC) target 5×10^5 for MS mode and 5×10^4 for MSⁿ mode).

Chromatographic separation was performed on an Atlantis T3 Column (2.1 × 100 mm, 3 μm, Waters Corporation, Milford, MA, USA), maintained at 50 °C. The mobile phase consisted of solvent A (water with 0.05% formic acid) and solvent B (methanol with 0.05% formic acid). The elution programme for solvent B (t (min), %B) was as follows: (0,0); (2,0); (10,100); (11,100); (11.1, 0); (15, 0). The flow rate was 400 μL/min with an injection volume of 5 μL. Fourier transform mass spectrometry (FTMS) scan mode was employed to analyse extracts from BRS, maintaining a resolving power of 30,000 with a scan mass range from m/z 100–700.

Phthalic acid was identified based on retention time, chemical characteristics, and MS/MS fragmentation behaviour, and subsequently confirmed using the online database FoodDB (fooddb.ca). The most intense ions in the FTMS spectrum were selected for product-ion (MS²) scans. MS² data were acquired at a resolving power of 15,000 at m/z 600, allowing the determination of both precursor and fragment ions. High-energy collisional dissociation was applied with a collision energy of 35% and an activation time of 10 ms to induce fragmentation of the selected precursor ions.

Quantification was performed using authentic commercial standards (Sigma-Aldrich; compounds listed in Section 2.1) by external calibration. Calibration curves were prepared in methanol containing 0.05% formic acid over the range 0.005–5 μg/mL. Linearity was acceptable for all analytes ($R^2 \geq 0.98$). Quantification was based on three independent extracts ($n = 3$) and triplicate injections per extract, and results are

reported as mean $\pm$ SD. Instrument control and subsequent data processing were carried out using Xcalibur v3.0 software (ThermoFisher Scientific, Hemel Hempstead, UK).

2.5. Application of the optimal MAE conditions using different rice straw cultivars

Eleven rice straw cultivars were extracted under the optimal MAE conditions to obtain phenolic-rich extracts (Table S1). The extracts were analysed based on their polyphenolic profiles, TPC, and antioxidant capacity. Under optimal conditions, extraction was performed in triplicate as independent extractions ($n = 3$), and each extract was analysed in triplicate.

The selection included both traditional and modern genotypes cultivated in 2019 and 2020. This design captured variability across genotypes and harvest years, and evaluated interannual variation in metabolite accumulation.

All samples were collected post-harvest from field-grown rice in the Valencian Community (Spain), under standard agronomic practices. Straw was air-dried, milled, and stored in dry, dark conditions until extraction. The selected cultivars represent the germplasm diversity grown in eastern Spain. *Bomba*, *J Sendra*, and *Sirio* are traditional Spanish varieties commonly cultivated in the *L'Albufera Natural Park*, valued for their culinary quality and local adaptation. *Albufera*, *Fonsa*, *Arpa*, and *Onice Italia* represent modern genotypes introduced to improve agronomic performance, including higher yields and resistance to lodging or disease. The pigmented variety (*N_20*) was included for its distinctive anthocyanin-rich profile, which has been associated with elevated antioxidant potential. Including two harvest years (2019 and 2020) supported the evaluation of genotype and environmental influences on the accumulation of bioactive phenolics in rice straw.

The polyphenolic composition of the rice straw extracts was analysed by high-resolution mass spectrometry (LC-ESI/LTQ-Orbitrap-HRMS) using accurate mass (FTMS) and diagnostic MS² fragmentation patterns following standard criteria, enabling the identification and relative quantification of phenolic acids and flavonoids. In parallel, the TPC and the antioxidant potential of each extract were evaluated using three complementary assays: ABTS, FRAP, and DPPH radical scavenging activity. These methods provide complementary insights into the reducing capacity and free-radical-neutralisation potential of the phenolic extracts.

2.5.1. Chemometric analysis (PCA and Pearson correlations)

In order to explore relationships between the main phenolic constituents and antioxidant capacity across cultivars, chemometric analyses were performed using a subset of the most representative quantified phenolics (i.e., those consistently detected and/or occurring at the highest concentrations across samples). Specifically, quercetin-3-glucoside, 3,4-dihydroxyhydrocinnamic acid, ferulic acid, 4-hydroxybenzoic acid, protocatechuic acid, *p*-coumaric acid, syringic acid, sinapic acid, vanillic acid, vanillin, and taxifolin (as a cultivar-specific marker for *N_20*) were considered together with TPC, FRAP, ABTS and DPPH results. Prior to analysis, variables were autoscaled (z-scores) to account for different measurement units and ranges. Pearson product-moment correlations were computed and visualised as a heatmap. Principal component analysis (PCA) was performed on the correlation matrix, and the first two components were used for interpretation and graphical representation (biplot) because they accounted for the majority of the overall variance.

2.6. Statistical analysis

Data are presented as mean $\pm$ SD. The number of independent extractions (n) and analytical replicates is indicated for each dataset.

The BBD and related analysis were performed using Statgraphics Centurion XVI (Statistical Graphics, Rockville, MD., USA). Graphical

evaluation of the main effects and factor interactions was performed. Analysis of variance (ANOVA) was applied at a 95% confidence level ($p < 0.05$). R^2 was used to assess a model's predictive ability. In addition, ANOVA was applied to assess lack of fit, ensuring that the regression model was adequate for maximising the studied responses. To improve interpretation, interactions and influence of the variables were also analysed using RSM plots and a Pareto diagram.

Principal component analysis (PCA) was performed using Statgraphics Centurion XVI (Statistical Graphics, Rockville, MD, USA) to reduce dataset dimensionality and identify the main components explaining the variance among the tested rice straw cultivars. The number of components was selected according to the scree plot.

3. Results and discussion

3.1. Characterisation of Bomba rice straw (BRS) waste

The chemical composition of BRS was determined to assess its potential as a renewable resource for applications in agriculture, bioenergy and environmental management. BRS was composed of $12.4 \pm 0.2\%$ ash, $7.2 \pm 0.4\%$ moisture, $3.2 \pm 0.5\%$ proteins, $7.5 \pm 0.9\%$ fat and $69.6 \pm 0.6\%$ carbohydrates. These results are in line with those reported in the literature (Ramos et al., 2023), with slight differences depending on the rice straw composition, which varies across crop varieties analysed. Carbohydrates represented the major fraction and may serve as feedstock for ethanol or biogas production. Understanding this fraction is essential for optimising conversion processes and improving bioenergy yields (Sharma, Singh, & Arya, 2020). Ash content, indicative of the mineral residue after complete combustion, is crucial for assessing rice straw's inorganic composition and its potential for nutrient recycling. A high ash content can affect soil fertility and the efficiency of combustion processes in bioenergy production. A low protein content was obtained, indicating limited nitrogenous material, but it is still relevant for evaluating suitability for animal feed and composting. Fat content can still influence its energy content and suitability for specific industrial processes, such as biofuel production. However, this may also impact the digestibility and nutritional value of rice straw-based feeds. Overall, the presence of proteins and fat could enhance the nutritive value of rice straw-based feeds and support sustainable agricultural practices (Ramos et al., 2023).

3.2. MAE optimisation

The effects of extraction time, ethanol concentration, solvent volume and extraction temperature on the MAE of phenolic compounds from BRS were evaluated in terms of extraction yield, TPC and FRAP responses using a BBD. The design matrix and the corresponding results for all experiments are presented in Table 1. Yield, TPC, and FRAP values obtained from BRS under MAE conditions ranged from 6.97 to 16.10%, 213.88 to 734.13 mg GAE/100 g DW, and 10.78 to 39.08 μ mol TE/g DW, respectively.

Combinations of the lower and higher levels (-1 , 0 , $+1$) provided by the BBD increased extraction yield but did not always enhance TPC or FRAP. This occurs because yield reflects global extract mass, where compounds other than phenolics, with or without antioxidant activity, may also be recovered.

The central-point experiments (runs 2, 10, 19, 24, and 25) were included as independent replicates to estimate pure error and assess the process's repeatability within the experimental domain. These runs yielded mean values of $10.5 \pm 0.6\%$ (extraction yield), 425.5 ± 53.7 mg GAE/100 g DW (TPC), and 21.6 ± 2.5 μ mol TE/g DW (FRAP). The dispersion observed (especially for TPC and FRAP) may be related to the intrinsic heterogeneity of the plant matrix and to minor run-to-run variability during post-extraction handling (solid-liquid separation of finely milled straw, solvent removal, and freeze-drying). Overall, the central-point replicates indicated adequate precision and agreed with

the fitted model.

ANOVA results are presented in Table 2. The models displayed high R^2 values for yield, TPC, and FRAP (0.9118, 0.9057, and 0.9199, respectively), indicating strong agreement between predicted and experimental data. The lack-of-fit of all models was not significant ($p > 0.05$), with high p -values of 0.10, 0.49, and 0.48 for yield, TPC, and FRAP, respectively, confirming appropriate model performance.

A second-order polynomial equation was used to obtain a mathematical model for each response variable as a function of the independent factors (Eqs. (3)–(5)), with A, B, C and D representing temperature, ethanol concentration, solvent volume and extraction time, respectively, within the tested domain.

$$\text{Yield} = -7.4200 - 0.0246A - 0.0946D + 0.0037A^2 - 0.0031AB + 0.0027BC \quad (3)$$

$$\text{TPC} = -520.6050 + 5.6618A + 7.2781D \quad (4)$$

$$\text{FRAP} = 3.5461 - 0.4649A - 0.1685D + 0.0086A^2 - 0.0075B^2 \quad (5)$$

Fig. 1 shows the Pareto diagrams for yield, TPC, and the FRAP at a 95% confidence level. Although all variables had a positive effect, not all showed significant linear main effects.

Despite this, ethanol concentration and solvent volume were included as independent variables because they are key mechanistic factors in solid-liquid extraction (solvent polarity and solvent conditions relative to the solid). They are necessary to define an operating window for the process. Their inclusion also allows for the possible effects of curvature and interaction effects in the response surface model (Gupta, Dubey, & Tripathy, 2025). The Pareto chart (Fig. 1) indicates that temperature and time were the most influential factors for yield, with temperature showing the strongest positive effect.

Under MAE, temperature and time often dominate because microwave energy couples with polar components (hydroalcoholic mixtures and residual moisture in the biomass) and promotes rapid volumetric dielectric heating, which enhances diffusion and mass transfer. This contrasts with conventional heating, where heat transfer is slower and more surface-limited (Mao, Robinson, & Binner, 2021). In rice straw, this effect can be reinforced by matrix features that hinder conventional extraction, such as a silica-rich, waxy surface layer. Microwave-assisted treatments have been reported to disrupt this silicified/waxy structure and increase accessibility of the lignocellulosic matrix (Lü & Zhou, 2011; Ma, Liu, Chen, Wu, & Yu, 2009).

In addition, a substantial fraction of straw phenolic compounds (e.g.,

ferulic and p-coumaric acids) occurs in insoluble-bound form linked to cell-wall polymers, so higher temperature and sufficient residence time favour their release from the matrix (Linh, Fujita, & Sakoda, 2017; Shahidi & Yeo, 2016). Consequently, yields, TPC, and FRAP increased as the temperature rose within the tested range. Temperature typically enhances extraction by increasing solute solubility and mass-transfer coefficients while reducing solvent viscosity (de Hoyos-Martínez, Merle, Labidi, & Charrier – El Bouhtoury, 2019). However, very high temperatures may reduce selectivity and promote phenolic degradation. The maximum temperature is constrained by solvent properties and the open MAE configuration used (Tomasi, Machado, Boaventura, Botelho, & Santos, 2022).

For yield, significant factors ($p < 0.05$) were temperature (A), the quadratic term of temperature (AA), the temperature-ethanol interaction (AB), extraction time (D) and the ethanol-concentration-volume interaction (BC). For TPC, temperature (A) and extraction time (D) were the only significant positive factors. Finally, for FRAP, the most influential factors were temperature (A), extraction time (D), the quadratic term of temperature (AA) and the quadratic term of ethanol percentage (BB).

Regarding TPC and antioxidant capacity by FRAP, a significant positive effect was observed for the linear term of temperature and extraction time, considering the factors set as extremes (+1; −1) for time (40; 5 min) and temperature (80, 40 °C). This agrees with other studies on the optimisation of phenolic compounds extracted from agri-food waste. For example, in the study by Wang et al. (2013), in which the extraction of phenolic compounds from pomegranate leaves was favoured by the positive interaction between temperature and time, these factors were set to the maximum (+1) in the design optimum. Another example is the study by Quiles-Carrillo, Mellinas, Garrigos, Balart, and Torres-Giner (2019), which reported positive interactions between temperature and time and established these factors at maximum (+1) levels for optimising the extraction of phenolic compounds with antioxidant activity from carob pods. Tomasi, Santos, Boaventura, and Botelho (2023) obtained the highest TPC in chestnut shell extracts after 5 min of extraction at 93 °C, indicating a positive effect of temperature.

On the other hand, within the investigated experimental domain, solvent volume (solid-to-liquid ratio) and ethanol concentration did not show a statistically significant linear main effect on the three responses. However, they were retained to define a practical operating window and to capture potential quadratic and interaction effects. This does not imply that solvent selection is irrelevant. However, under temperature-controlled MAE, the extraction was governed by thermal and time-

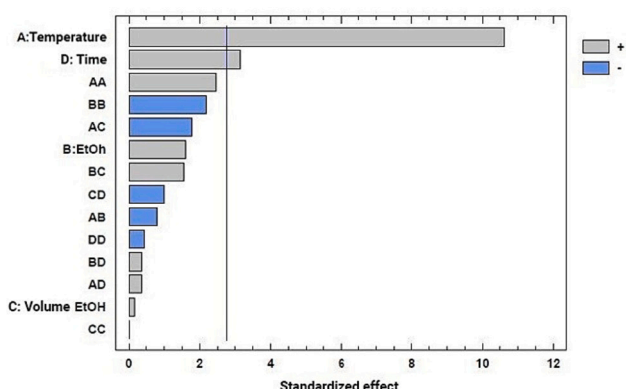
Table 2

ANOVA results for response surface quadratic models of BRS extraction (A (temperature), B (ethanol concentration), C (solvent volume) and D (extraction time)).

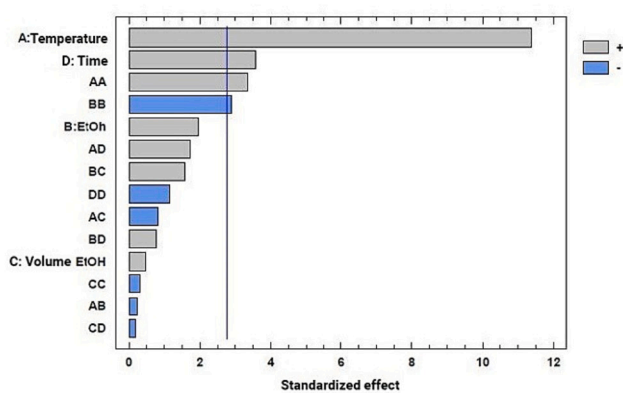
Source	Yield			TPC			FRAP		
	Sum of Squares	F-Value	P-Value	Sum of Squares	F-Value	P-Value	Sum of Squares	F-Value	P-Value
A	95.09	304.94	0.00*	324,724.00	112.81	0.00*	889.89	129.09	0.00*
B	0.59	1.91	0.24	7313.93	2.54	0.18	26.12	3.79	0.12
C	0.60	1.93	0.24	65.42	0.02	0.88	1.39	0.20	0.68
D	5.90	18.91	0.01*	28,243.70	9.81	0.03*	87.53	12.70	0.02*
AA	14.52	46.56	0.01*	17,362.70	6.03	0.07	76.76	11.13	0.03*
AB	6.05	19.40	0.01*	1862.90	0.65	0.46	0.41	0.06	0.82
AC	1.47	4.70	0.09	9120.55	3.17	0.14	4.64	0.67	0.46
AD	1.01	3.23	0.15	378.30	0.13	0.73	20.25	2.94	0.16
BB	2.20	7.06	0.06	13,724.60	4.77	0.09	57.76	8.38	0.04*
BC	2.70	8.66	0.04*	6832.77	2.37	0.19	17.26	2.50	0.19
BD	1.36	4.37	0.10	395.73	0.14	0.72	4.09	0.59	0.48
CC	1.99	6.40	0.06	0.70	0.00	0.98	0.66	0.10	0.77
CD	0.01	0.01	0.93	2780.89	0.97	0.38	0.24	0.04	0.86
DD	0.60	1.93	0.24	546.78	0.19	0.68	9.24	1.34	0.31
Lack-of-fit	12.15	3.90	0.10	32,268.60	1.12	0.49	79.30	1.15	0.48
Pure error	1.25			11,513.70			27.58		
Cor. Total	151.99			464,508.00			1335.74		
R^2	0.9118			0.9057			0.9199		
Adj. R^2	0.8237			0.8115			0.8399		

* Significant model terms, $p < 0.05$.

(a) TPC



(b) FRAP



(c) Yield

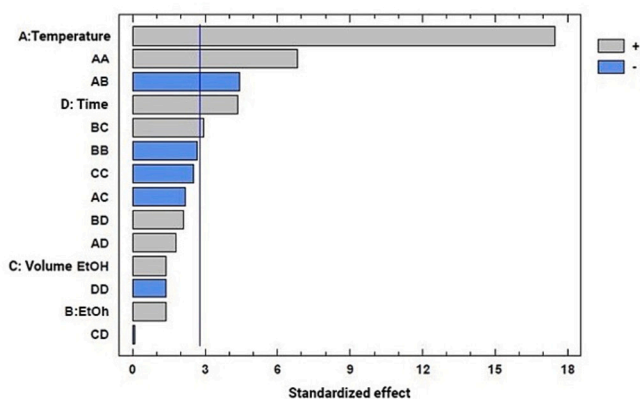


Fig. 1. Pareto charts obtained for the extraction yield, TPC and FRAP of *Bomba* rice straw.

driven mechanisms enhanced by microwave irradiation. In MAE, microwaves interact primarily with polar components (ethanol/water mixtures and residual water in the biomass) via dielectric heating, enabling rapid volumetric heating, promoting matrix swelling/softening, and facilitating efficient mass transfer (Alara, Abdurahman, & Ukaegbu, 2021; Melikoglu, 2025). Thus, temperature and extraction time dominated the release of bound phenolic compounds from lignocellulosic structures. In contrast, changes in solvent composition or volume may contribute more weakly as main effects and/or emerge through non-linear (quadratic) or interaction terms. Similar behaviour has been reported in other MAE-RSM studies, where solvent-related factors (ethanol proportion and/or solvent-to-solid conditions) may

become non-significant as linear terms within a given domain, while temperature/time dominate the responses under MAE (Ameer et al., 2017; Gupta et al., 2025).

Solvent composition can still influence extraction by modifying solute solubility and solvent penetration into the matrix (Roselló-Soto et al., 2019). Extraction yield may also increase with solvent polarity (Kaderides, Papaioikonomou, Serafim, & Goula, 2019). Several studies have reported a parabolic relationship between phenolic recovery and ethanol proportion, with intermediate mixtures yielding higher recoveries. This supports the quadratic behaviour observed in this work (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015).

Nonetheless, other authors have indicated that higher ethanol concentrations can disrupt hydrogen and hydrophobic interactions between phenolics and proteins, or between phenolics and cellulose (Karaogul & Nedjip, 2025; X. Zheng et al., 2013). From a microwave perspective, solvent composition affects the dielectric properties of the medium and can therefore modify heating behaviour and mass-transfer kinetics (Kaderides et al., 2019). In contrast, in conventional solid-liquid extraction (e.g., maceration or Soxhlet), heating is slower and non-volumetric. Extraction is also more equilibrium-controlled, making solvent polarity and solid-to-liquid ratio more influential than under MAE (Melikoglu, 2025).

Significant interactions between extraction variables on yield were further examined using 3D response surface plots (Fig. 2). The temperature–ethanol surface (Fig. 2a) confirmed the strong positive effect of temperature on yield, while ethanol concentration had a moderate influence. In contrast, the ethanol (% v/v) vs solvent volume (mL) surface (Fig. 2b) was comparatively flat, indicating a limited effect of solvent volume and only minor changes from ethanol concentration. Overall, these surfaces visually support the Pareto and ANOVA outcomes and highlight that yield is primarily temperature-driven, with solvent-related factors exerting secondary effects mainly through interactions within the tested ranges.

3.3. Extraction optimisation

The optimal conditions for BRS were determined independently for each response, aiming to maximise extraction yield, TPC and FRAP (Table 3) to identify the factor settings that maximise each parameter within the studied range.

A multi-response optimisation was used to identify conditions that balanced extraction yield, TPC and FRAP. Yield was included as a process response because it reflects overall mass recovery and practical extract productivity (important for downstream use and scale-up). TPC was included as a practical screening index of phenolic-enriched extracts, while acknowledging that the Folin-Ciocalteu assay is non-specific and can be affected by other reducing constituents. Therefore, yield and TPC were interpreted only as global indicators, and antioxidant capacity (FRAP) was used as a functional response to prioritise extracts with higher redox activity. This multi-response strategy (global indices plus antioxidant assays, followed by compound-resolved interpretation) is consistent with recent MAE-RSM studies using TPC and antioxidant assays as optimisation targets (Domínguez-Rodríguez, Castro-Puyana, & Marina, 2025; Ramírez-Brewer, Quintana, & García-Zapateiro, 2024).

The optimal MAE conditions for obtaining phenolic-rich extracts with high antioxidant capacity from BRS were 40.0 min, 50.0 mL of 60.0% (v/v) aqueous ethanol, and 80 °C.

Validation experiments were performed in triplicate under the selected optimum conditions, yielding $18.9 \pm 0.9\%$ (yield), 796.1 ± 77.6 mg GAE/100 g DW (TPC), and 32.3 ± 1.4 $\mu\text{mol TE/g DW}$ (FRAP). At the optimum, predicted values were 15.94% (yield), 766.56 mg GAE/100 g DW (TPC) and 37.28 $\mu\text{mol TE/g DW}$ (FRAP). The corresponding 95% confidence intervals (CI) for the mean response were 14.13–17.74, 594.03–939.09 and 28.84–45.71, while the 95% prediction intervals (PI) were 13.56–18.32, 538.63–994.49 and 26.13–48.42, respectively

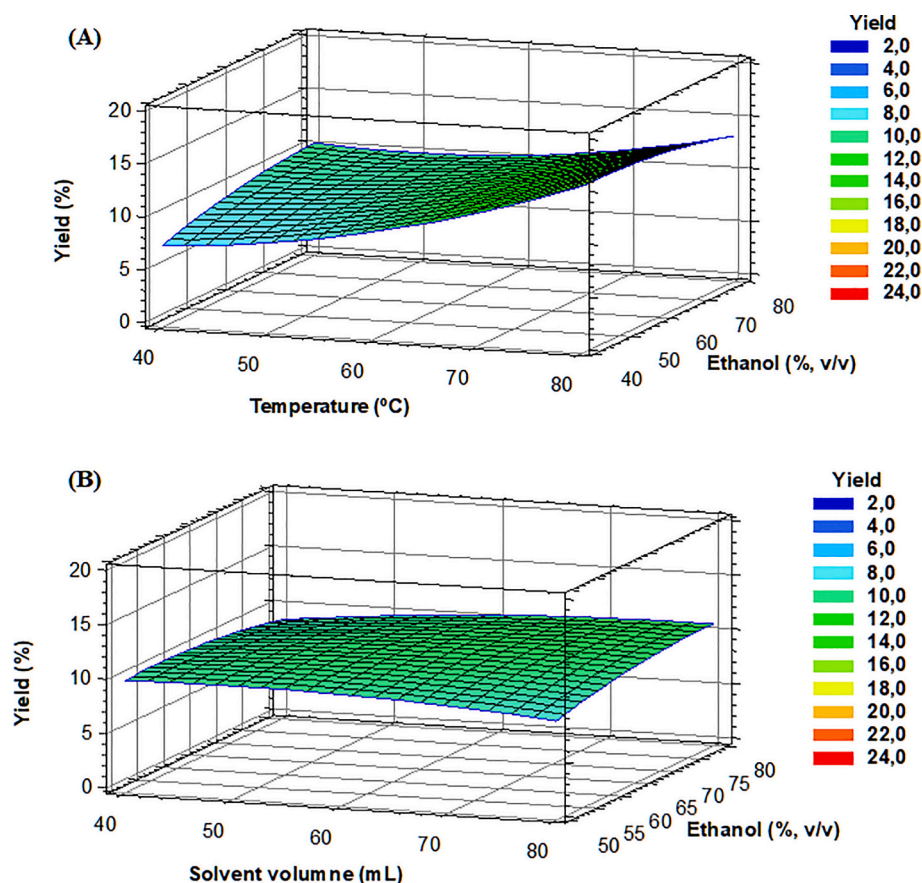


Fig. 2. Response surface plots for extraction yield (%). (a) Effect of temperature (°C) and ethanol concentration (% v/v) on yield at fixed extraction time (22.5 min) and solvent volume (65 mL). (b) Effect of solvent volume (mL) and ethanol concentration (% v/v) on yield at fixed extraction time (22.5 min) and temperature (60 °C).

Table 3

MAE optimal conditions and predicted responses for phenolic compounds from BRS.

Responses	Yield	TPC	FRAP	Multi-response optimisation
	(%)	(mg GAE/100 g DW)	(μmol TE/g DW)	Yield/TPC/FRAP (%)/(mg GAE/100 g DW)/(μmol TE/g DW)
Predicted values	16.2	765.9	37.7	15.9 / 766.6 / 37.2
Desirability				0.972504
<i>Optimal factors</i>				
Extraction time (min)	37.6	40.0	40.0	40.0
Ethanol volume (mL)	50.0	50.0	52.1	50.1
Ethanol (% v/v)	40.5	57.1	61.7	60.0
Temperature (°C)	80.0	80.0	80.0	80.0

(Table S2). Relative prediction errors have been reported and considered acceptable for process optimisation of lignocellulosic matrices. The Box-Behnken model showed good predictive performance, achieving an overall desirability of 0.972504. The antioxidant capacity was also evaluated using the ABTS assay under optimal extraction conditions, yielding a value of 43.2 ± 1.8 μmol TE/g DW. To benchmark MAE against a conventional reference method, Soxhlet extraction was performed as described by Karimi et al. (2014) with minor modifications. Briefly, rice straw was extracted with 60% (v/v) ethanol under reflux at 80 °C for 2 h. Under these specific conditions, MAE provided a higher extraction yield ($18.9 \pm 0.9\%$) than Soxhlet ($10.3 \pm 0.4\%$), while reducing extraction time (40 min vs 2 h). In addition, Soxhlet produced lower phenolic-related responses (TPC: 342.26 ± 45.6 mg GAE/100 g DW; FRAP: 14.7 ± 0.4 μmol TE/g DW; ABTS: 28.3 ± 2.3 μmol TE/g DW) compared with the MAE optimum conditions. This comparison is intended as a conventional benchmark within the tested conditions,

following common practice in MAE studies that evaluate performance using global responses (yield, TPC and antioxidant assays), and, when available, complementary compound-level profiling.

This MAE-Soxhlet benchmarking approach is consistent with recent literature. For instance, a recent study compared MAE with conventional extraction (Soxhlet) and maceration using global performance metrics (e.g., yield, TPC and antioxidant assays). The results demonstrated that MAE can deliver phenolic-rich extracts with higher antioxidant responses than conventional approaches under the evaluated conditions. That extraction method influences the qualitative composition of the extracts when assessed by LC-MS/MS (Ramírez-Brewer et al., 2024).

Studies in the field emphasise that conventional solid-liquid extraction methods (including Soxhlet/maceration) typically involve longer processing times and higher resource demand, whereas MAE is often reported as a time-efficient alternative, although outcomes remain matrix- and condition-dependent (Dominguez-Rodríguez et al., 2025).

Accordingly, direct cross-study comparisons should be interpreted with caution, as they are influenced by variety, solvent system, and process severity. Karimi et al. (2014), using a conventional extraction procedure across several rice straw varieties, reported TPC values ranging from 211 ± 20 to 318 ± 14 mg GAE/100 g DW.

In addition, Menzel et al. (2020) evaluated aqueous and hydro-alcoholic extractions of dry-milled rice straw and reported TPC values of 225 mg GAE/100 g DW for the first aqueous extraction, with lower values for subsequent extractions and for ethanol- and methanol-based systems. Varietal effects have also been reported, with straw phenol concentrations ranging from 291 to 417 mg gallic acid/100 g DW across different rice varieties and growing locations (Jumrus et al., 2022).

Freitas et al. (2020) reported a TPC of 486 ± 4 mg GAE/100 g DW using UAE combined with heating. In this context, the TPC achieved under MAE optimum conditions (796 mg GAE/100 g DW) supports the effectiveness of MAE for the recovery of phenolics from rice straw under the studied conditions.

Beyond rice straw, the suitability of MAE for lignocellulosic by-products has also been demonstrated in other agro-industrial residues (e.g., wheat and sugarcane bagasse), frequently in combination with response surface designs and targeted phenolic profiling, reinforcing MAE as a practical option for agro-waste valorisation.

For instance, Kirusnaruban, Gasparre, Nandasiri, Eskin, and Rosell (2025) demonstrated that water-based MAE can effectively extract phenolic acids from wheat, achieving considerable yields with a green solvent system. Similarly, Ovatlarnporn et al. (2024) optimised the MAE of sugarcane bagasse phenolics using a BBD, achieving significant antioxidant and antidiabetic activities in the resulting extracts. Moreover, Álvarez-Romero et al. (2023) showed that MAE outperformed UAE for recovering phenolics from barley, employing RSM and BBD optimisation. These examples indicate that, although matrix composition and structure can markedly influence extraction performance, MAE can be successfully applied across diverse lignocellulosic by-products when

operating conditions are appropriately optimised.

3.4. Main phenolic compounds profile by LC-ESI/LTQ-Orbitrap-HRMS analysis

The selected operating point represents the optimum for overall extract performance. The phenolic compound profile of the extract was characterised using LC-ESI/LTQ-Orbitrap-HRMS to use for compound-level interpretation across cultivars. Phenolic compounds were annotated by generating molecular formulas from accurate-mass data and matching isotopic patterns. Candidate formulas were then searched in the Dictionary of Natural Products (Chapman & Hall/CRC). Analytes were confirmed using MS/MS (MS^2) fragmentation and comparison with literature and spectral databases, in line with recent Orbitrap-HRMS/MS phytochemical profiling approaches (Bouzabata et al., 2022; Crescenzi et al., 2023; Horai et al., 2010; Kim & Kim, 2025; Kodikara et al., 2024; Neveu et al., 2010). The main flavonoids and phenolic acids identified and quantified are summarised in Table 4.

A total of 18 compounds were quantified: 5 flavonoids (apigenin, 0.01 ± 0.00 mg/100 g; genistein, trace; kaempferol, trace; quercetin-3-glucoside, 2.33 ± 0.66 mg/100 g; rutin, trace); 11 phenolic acids (caffeic acid, trace; 3,5-dihydroxybenzoic acid, 0.06 ± 0.01 mg/100 g; 3,4-dihydroxyhydrocinnamic acid, 10.25 ± 0.01 mg/100 g; ferulic acid, 0.53 ± 0.05 mg/100 g; gallic acid, 0.003 ± 0.00 mg/100 g; 4-hydroxybenzoic acid, 52.36 ± 6.67 mg/100 g; protocatechuic acid, 0.06 ± 0.01 mg/100 g; *p*-coumaric acid, 4.40 ± 0.01 mg/100 g; syringic acid, 0.15 ± 0.01 mg/100 g; sinapic acid, 1.36 ± 0.001 mg/100 g; vanillic acid, 0.34 ± 0.02 mg/100 g); and 2 other phenolics (catechol, 0.05 ± 0.01 mg/100 g; vanillin, 0.43 ± 0.03 mg/100 g). In addition, phthalic acid was also identified by MS^2 fragmentation, showing a theoretical ion mass $[M-H]^-$ of 165.0193 and an experimental value of 165.0184 (Table 4). Quantitatively, the most abundant compounds were 4-hydroxybenzoic acid (4-HBA) (52.36 ± 6.67 mg/100 g DW), 3,4-

Table 4

Identification and quantification of major phenolic compounds present in BRS by LC-ESI/LTQ-Orbitrap-HRMS and by multi-stage mass analysis MS^2 fragmentation using LC/ESI/LTQ-Orbitrap-HRMS.

Peak No.	Compound	Molecular formula	RT (min)	Ion mass [M-H] ^{−*}		mDa error ^{**}	mg/100 g ^{***}
				Theoretical	Experimental		
Flavonoids							
1	Quercetin-3-glucoside	C ₂₁ H ₂₀ O ₁₂	6.75	463.0882	463.0864	1.80	2.33 ± 0.66
2	Apigenin	C ₁₅ H ₁₀ O ₅	8.09	269.0456	269.0447	0.90	0.01 ± 0.00
4	Genistein	C ₁₅ H ₁₀ O ₅	8.10	269.0456	269.0448	0.80	Tr
6	Kaempferol	C ₁₅ H ₁₀ O ₆	8.10	285.0405	285.0392	1.30	Tr
10	Rutin	C ₂₇ H ₃₀ O ₁₆	6.72	609.1461	609.1434	2.70	Tr
Phenolic acids							
12	Caffeic acid	C ₉ H ₈ O ₄	5.17	179.035	179.0343	0.70	Tr
14	3,5-Dihydroxybenzoic acid	C ₇ H ₆ O ₄	1.93	153.0193	153.0188	0.50	0.06 ± 0.01
15	3,4-Dihydroxyhydrocinnamic acid	C ₉ H ₁₀ O ₄	5.03	181.0506	181.0504	0.20	10.25 ± 0.01
16	Ferulic acid	C ₁₀ H ₁₀ O ₄	6.24	193.0506	193.0499	0.70	0.53 ± 0.05
17	Gallic acid	C ₇ H ₆ O ₅	0.93	169.0142	169.0133	0.90	0.01 ± 0.00
18	4-Hydroxybenzoic acid	C ₇ H ₆ O ₃	3.70	137.0244	137.0239	0.50	52.36 ± 6.67
19	Protocatechuic acid	C ₇ H ₆ O ₄	1.71	153.0193	153.0186	0.70	0.06 ± 0.01
20	<i>p</i> -coumaric acid	C ₉ H ₈ O ₃	5.93	163.0401	163.0396	0.50	4.40 ± 0.01
22	Syringic acid	C ₉ H ₁₀ O ₅	5.72	197.0455	197.0446	0.90	0.15 ± 0.01
23	Sinapic acid	C ₁₁ H ₁₂ O ₅	6.35	223.0612	223.0607	0.50	1.36 ± 0.001
24	Vanillic acid	C ₈ H ₈ O ₄	5.20	167.035	167.0343	0.70	0.34 ± 0.02
Other							
25	Catechol	C ₆ H ₆ O ₂	1.93	109.029	109.0289	0.10	0.05 ± 0.01
26	Vanillin	C ₈ H ₈ O ₃	5.74	151.0401	151.0395	0.60	0.43 ± 0.03
Phenolic acids	Identified by MS ² fragmentation using LC/ESI/LTQ-Orbitrap-HRMS						MS ² fragment ion [M-H] [−]
27	Phthalic acid	C ₈ H ₆ O ₄	5.56	165.0193	165.0184	0.90	121.0287

* Annotation was supported by accurate mass (formula constraints and isotopic pattern matching), database searching (Dictionary of Natural Products), and MS/MS (MS^2) confirmation using literature and spectral resources (e.g., Phenol-Explorer and MassBank) (Horai et al., 2010; Neveu et al., 2010).

** mDa error: absolute mass error (mDa) between measured and theoretical m/z (Horai et al., 2010).

*** Quantification was performed by external calibration with commercial standards. Results are reported as mean $\pm$ SD ($n = 3$); nd: not detected ($<LOD$); Tr: traces ($<LOQ$).

dihydroxyhydrocinnamic acid (10.25 ± 0.01 mg/100 g DW), and quercetin-3-glucoside (2.33 ± 0.66 mg/100 g DW).

Phenolic compounds in rice straws are typically bound to insoluble polymers through covalent bonds and are recognised for their antioxidant and antimicrobial properties (Borrega et al., 2022). Recent research has highlighted that the main phenolic compounds in rice straw include non-flavonoids, such as hydroxybenzoic acids and hydroxycinnamic acids, as well as flavonoids, including flavanols, flavones, isoflavones, and flavonols. However, the efficiency of phenolic compounds extraction can vary significantly depending on the method used. Studies highlight variability in phenolic compounds across extraction methods and rice straw varieties, underscoring the importance of optimising extraction techniques to maximise the yield and bioactivity of these valuable compounds. These findings support efficient phenolic extraction from rice straw, an abundant and underutilised resource.

Comparison with previous studies supports the efficacy of the MAE conditions. For instance, the extraction yield of quercetin-3-glucoside at 2.33 mg/100 g DW is consistent with reported values for the extraction of rice straw phenolic compounds using various methods, as this is the main flavonoid identified. The high concentration of 3,4-dihydroxyhydrocinnamic acid (10.25 ± 0.01 mg/100 g DW) indicates efficient recovery of hydroxycinnamic acids under MAE. This type of compound has been previously reported during alkaline pretreatment processes designed to remove lignin and produce reactive cellulosic fibres (Borrega et al., 2022). Li, Qi, Luo, Khan, and Wan (2015) utilised nanofiltration membranes in related research to effectively isolate hydroxycinnamic acids from alkaline lignocellulosic hydrolysates derived from Chinese rice straw. Similarly, Zheng, Zheng, Xue, Hu, and Gao (2017) analysed phenolic acids from rice straw extracted with 0.5 M NaOH. The main phenolic compounds detected in their study included gallic acid, epigallocatechin gallate, phthalic acid, vanillic acid, syringic acid, vanillin, coumaric acid, and ferulic acid, with coumaric and ferulic acids being the predominant phenolic acids at 0.7 wt% and 0.4 wt%, respectively.

Karimi et al. (2014) used solid-liquid extraction with 80% (v/v) methanol under reflux for 2 h at 90 °C to extract phenolic and flavonoid compounds from different rice straw varieties, obtaining extracts with high antioxidant activity. Kaempferol (1.107 mg/100 g DW) was the main flavonoid compound found in *Ali Kazemi* variety, followed by apigenin (0.153 mg/100 g DW). In contrast, the *Hashemi* variety was rich in caffeic acid (1.020 mg/100 g DW), whereas the *Khazar* variety showed apigenin as the main flavonoid (0.124 mg/100 g DW). In another study, Menzel et al. (2020) identified ferulic, *p*-coumaric and protocatechuic acids as major phenolic acids present in rice straw from the *L'Albufera Natural Park* of Valencia by aqueous extraction. Although individual concentrations differed, the overall phenolic-acid profile was consistent with our results. Nurika, Suhartini, Azizah, and Barker (2020) also investigated the use of rice straw inoculated with the fungus *Serpula lacrymans* for the production of bio-based compounds, obtaining a vanillin concentration of 2.596 µg/g, which is markedly lower than the value reported in the present study.

MAE has been highlighted for its extraction efficiency, showing high yields of phenolic compounds with preserved bioactivity and antioxidant properties from agricultural by-products, such as rice straw, compared to traditional methods (Ramos et al., 2023).

This approach reduces extraction time and can be operationally more efficient, while enhancing the recovery of valuable compounds, such as quercetin-3-glucoside and 4-hydroxybenzoic acid, which are known for their significant antioxidant and antimicrobial activities (Ciulu et al., 2018; Elez Garofulić et al., 2024). The identified phenolic compounds in BRS have significant health benefits, underscoring their potential applications in the food, pharmaceutical and cosmetic industries. Quercetin-3-glucoside possesses potent antioxidant and anti-inflammatory properties, supports cardiovascular health, and exhibits potential anticancer activities (Nile, Nile, Shin, Park, & Oh, 2021). 4-HBA stands out for its strong antimicrobial and antioxidant activities,

making it particularly beneficial for skin health and protection against oxidative stress. Similarly, 3,4-dihydroxyhydrocinnamic acid has demonstrated antioxidant properties that may help reduce inflammation and oxidative damage (Park et al., 2019). Ferulic acid, another powerful antioxidant present in BRS extracts, is known for its role in preventing chronic diseases, such as heart disease and cancer (Ciulu et al., 2018). Sinapic acid, with its combined antioxidant, anti-inflammatory and antimicrobial properties, supports overall health and aids in disease prevention (Lopez-Corona et al., 2022). Lastly, vanillin provides notable antioxidant and anti-inflammatory benefits beyond its use as a flavouring agent, potentially reducing oxidative stress and the risks of chronic diseases (Iannuzzi, Liccario, & Sirangelo, 2023).

The extraction of phenolic compounds from rice straw using MAE aligns with global trends towards sustainable and circular economies. Rice straw, typically considered an agricultural waste, can be transformed into a valuable source of bioactive compounds. This mitigates environmental issues associated with rice straw disposal and provides a sustainable source of natural antioxidants for various industrial applications, including the development of functional foods, nutraceuticals, and cosmeceuticals, which promote health and well-being. This approach supports the sustainable valorisation of rice straw by reducing the need for waste disposal, thereby minimising the ecological footprint of agricultural processes (Illankoon, Milanese, Collivignarelli, & Sorlini, 2023).

3.5. Application of optimal MAE: comparative phenolic compounds profiles across cultivars and harvests

To assess genotype effects on phenolic composition, 11 cultivar-harvest combinations were analysed using the same LC-ESI/LTQ-Orbitrap-HRMS workflow (representing eight cultivars (e.g., *Bomba*, *Sirio*, *J Sendra*) harvested in 2019 and 2020, Table S1) were analysed using the same LC-ESI/LTQ-Orbitrap-HRMS workflow previously applied to *Bomba* from 2020. These samples encompassed a representative range of genotypes cultivated in the Valencian Community, covering both japonica and indica subspecies, as well as a pigmented variety (N20).

A total of 26 phenolic compounds were confidently identified, with full details including molecular formulas, accurate masses, mass errors (ppm), and characteristic fragments presented in Tables S3 and S4 (Supporting Information).

Overall, the results revealed substantial variability in phenolic compounds profiles across cultivars and harvest years, confirming that genotype is a major determinant of phenolic composition. These findings are in good agreement with previous research on phenolic diversity in rice straw and other cereal by-products (Devraj, Panoth, Kashampur, Kumar, & Natarajan, 2020; Ma, Zhang, Rong, Wu, & Sun, 2022; Pang et al., 2018).

The chromatographic analysis (Table S5) revealed that 4-HBA was the most abundant compound in most cultivars, with notably higher levels in AL20 and S20 (93.9 mg/100 g and 90.9 mg/100 g), well above the 52.4 mg/100 g observed in the *Bomba* 2020 reference. In contrast, the lowest 4-HBA content was found in S19 (26.4 mg/100 g DW), suggesting strong genotypic influence and potential seasonal effects. Cultivars such as OJ19 (86.9 mg/100 g DW) and S20 (90.9 mg/100 g DW) also exhibited high levels, indicating that 4-HBA biosynthesis is highly genotype-dependent but also stable across harvest years. This compound plays a structural role in lignin and acts as a key antioxidant, making these cultivars attractive candidates for extractive valorisation (Joshi, Chandrakar, & Wasewar, 2021).

The second most prevalent phenolic acid, 3,4-dihydroxyhydrocinnamic acid, showed high inter-cultivar variability, peaking in F20 (359.9 mg/100 g DW), AR20 (308.6 mg/100 g DW), and A20 (291.4 mg/100 g DW). These values obtained were close to three times those found in the *Bomba* and *J Sendra* cultivars, which presented lower concentrations (102–120 mg/100 g), suggesting a strong genotypic

effect that could reflect differences in phenylpropanoid pathway flux or cell wall composition. Comparable cultivar-dependent variations in hydroxycinnamic acid composition have also been described for maize and wheat straws (Tramontina et al., 2024).

Among flavonoids, quercetin-3-glucoside was the most abundant and variable, reaching up to 62.7 mg/100 g DW in A_20 and 60.3 mg/100 g DW in AR_20. Other cultivars such as S_19 and N_20 also showed relatively high values (47.1 and 28.2 mg/100 g, respectively), whereas Bomba samples (B_19 and B_20) exhibited moderate levels (<25 mg/100 g DW). The elevated quercetin-3-glucoside in A_20 and AR_20 suggests upregulation of flavonol biosynthesis, potentially linked to environmental triggers or varietal traits. Glucosylated flavonoids, such as quercetin-3-glucoside, offer increased stability and bioavailability, thereby enhancing their functional potential for nutraceutical and antioxidant applications (Hu, Luo, Yang, & Cheng, 2025).

Taxifolin was detected only in N_20, the pigmented cultivar. The pigmented pericarps are associated with enhanced flavonoid diversity and concentration, as shown in black rice and red sorghum brans (Seong et al., 2022).

Minor flavonoids, such as apigenin and kaempferol, were detected at trace levels across all samples. Apigenin was slightly elevated in S_19 and F_20, suggesting possible cultivar-specific activation of flavone biosynthetic pathways.

The distribution of additional minor phenolics, such as vanillin and catechol, also showed cultivar-dependent trends. Vanillin was most abundant in F_20 (121.4 mg/100 g DW) and AL_20 (96.2 mg/100 g DW), possibly reflecting enhanced oxidative cleavage of ferulic acid residues. While catechol, a catecholamine-related compound with antioxidant and antimicrobial properties, reached its maximum in N_20 (68 mg/100 g DW), which may relate to the unique phenolic biosynthesis profile of pigmented rice varieties. As a bioactive compound with dual antioxidant and sensory relevance, its presence may signal oxidative stress markers or specific enzymatic activity in the straw (Yan et al., 2022).

Ferulic and *p*-coumaric acids, although present at lower levels, displayed genotype-linked variation, particularly higher ferulic content in pigmented N20 and starchy F20.

3.6. Application of optimal MAE conditions: comparative TPC and antioxidant capacity across rice cultivars, rice straw cultivar–harvest combinations

To complement the metabolomic profiling of phenolic compounds, the antioxidant capacity of the rice straw extracts was evaluated using four standard spectrophotometric assays: TPC, FRAP, ABTS, and DPPH (Table 5). The results revealed substantial variability among cultivars, reflecting genotype-dependent differences in antioxidant accumulation. These assays also provided complementary information to the phenolic profiles obtained by high-resolution mass spectrometry.

The TPC ranged from 486.7 ± 13.3 mg GAE/100 g (JS_19) to 1101.8

± 15.9 mg GAE/100 g (S_19). The highest values were observed for S_19 and A_20 (1065.7 ± 165.9 mg GAE/100 g) and S_20 (981.9 ± 29.3 mg GAE/100 g), all exceeding 980 mg GAE/100 g. These results are consistent with the elevated concentrations of hydroxybenzoic and hydroxycinnamic acids observed by LC-ESI/LTQ-Orbitrap-HRMS, particularly 4-HBA and DHHCA, which are known to contribute significantly to antioxidant activity (Colombo, Moretto, Barberis, Frosi, & Papetti, 2024; Martínez-Guillén et al., 2025; Ramos et al., 2023).

A_20 showed the highest ABTS radical scavenging capacity (54.6 ± 8.8 μ mol TE/g DW). A_20, a lesser-studied japonica variety traditionally cultivated in southern Valencia, showed the highest antioxidant activity in the ABTS assay, emphasising its potential as a source of bioactive compounds for extractive applications. Following for B_20 (46.1 ± 2.2 μ mol TE/g DW), S_19 (38.6 ± 3.0 μ mol TE/g DW), and F_20 (39.3 ± 4.0 μ mol TE/g DW). The ANOVA confirmed that these differences were statistically significant ($p < 0.05$). Similar trends were observed in FRAP, with S_19 (37.5 ± 6.0 μ mol TE/g DW) and B_20 (39.0 ± 2.2 μ mol TE/g DW) showing the highest reducing power. These cultivars also contained notable amounts of quercetin-3-glucoside, a flavonol with potent antioxidant properties (Jumrus et al., 2022). Although the assays differ in radical species, the consistent trends and ANOVA results support the reliability of the findings.

Likewise, Sirio 2019 (S_19) and Bomba 2020 (B_20) exhibited strong FRAP and ABTS values, in agreement with their complex phenolic composition. These findings align with previous reports showing that rice straw from japonica cultivars tends to accumulate higher levels of phenolic acids and flavonols than indica types, particularly under Mediterranean growing conditions (Elzaawely et al., 2017).

N_20 contained taxifolin and catechol (compounds with reported antioxidant activity (Seong et al., 2022)) but showed modest TPC and ABTS values. According to the ANOVA, these differences were significant ($p < 0.05$), suggesting either matrix effects or limited solubility of these compounds under the extraction conditions used.

J Sendra 2019 and 2020 (JS_19 and JS_20) showed the lowest TPC and antioxidant activity, in agreement with their poor phenolic profiles as determined by LC-ESI/LTQ-Orbitrap-HRMS.

These observations highlight cultivar differences relevant for rice-straw valorisation, from an analytical perspective. The agreement between LC-MS data and antioxidant assays indicates internal consistency of the extraction and profiling protocols. From a valorisation perspective, A_20, S_19, F_20, and B_20 are promising candidates for the development of bio-based antioxidant systems suitable for incorporation into active packaging materials, biostimulants, or nutraceutical ingredients. These results also underline the importance of integrating chemical profiling with functional bioassays to identify optimal cultivar-harvest combinations for circular bioeconomy applications (Martínez-Guillén et al., 2025).

An important aspect of this study was the comparison of the same cultivar harvested in different years: Bomba (B_19 vs B_20), Sirio (S_19

Table 5

TPC and antioxidant capacity of the different extracts obtained using the MAE technique from different rice straw plant cultivars ($m \pm SD$, $n = 3$; values in the same column followed by the same letter are not significantly different ($p < 0.05$)).

Cultivar–harvest combinations	TPC		FRAP		ABTS		DPPH	
	mg GAE/100 g DW		μ mol TE/g DW		μ mol TE/g DW		Inhibition (%)	
B_19	796.1 ^{ab}	± 77.6	52.1 ^b	± 3.0	43.2 ^{bc}	± 1.8	71.0 ^b	± 0.4
OI_19	860.7 ^{ab}	± 90.9	34.8 ^b	± 2.9	39.7 ^b	± 5.3	76.5 ^b	± 0.9
JS_19	486.7 ^a	± 13.3	17.0 ^a	± 3.5	19.8 ^a	± 2.4	37.5 ^a	± 1.1
S_19	1101.8 ^b	± 15.9	37.5 ^b	± 6.0	38.6 ^b	± 3.0	82.6 ^{bc}	± 1.8
N_20	880.7 ^{ab}	± 73.5	35.9 ^b	± 2.8	40.3 ^b	± 5.0	79.0 ^b	± 0.8
AL_20	954.7 ^b	± 54.0	32.6 ^b	± 2.6	44.1 ^{bc}	± 1.7	71.7 ^b	± 0.8
F_20	710.4 ^{ab}	± 78.7	27.5 ^{ab}	± 2.5	39.3 ^b	± 4.0	60.6 ^b	± 0.8
S_20	981.9 ^b	± 29.3	40.1 ^b	± 10.3	38.8 ^b	± 6.7	88.1 ^c	± 3.1
B_20	918.1 ^b	± 84.1	39.0 ^b	± 2.2	46.1 ^{bc}	± 2.2	85.9 ^{bc}	± 0.7
JS_20	818.1 ^{ab}	± 23.4	32.3 ^b	± 7.6	36.3 ^b	± 4.1	71.1 ^b	± 2.3
A_20	1065.7 ^b	± 165.9	42.2 ^b	± 6.8	54.6 ^c	± 8.8	92.8 ^c	± 2.0

vs S_20), and J Sendra (JS_19 vs JS_20). This comparison provides insights into the environmental and agronomic factors that influence phenolic accumulation and antioxidant activity, independent of genotype. For *Sirio*, S_19 showed significantly higher TPC and FRAP than S_20, although both years had comparable concentrations of major phenolics, such as 4-HBA and 3,4-dihydroxyhydrocinnamic acid. This result suggests that extrinsic factors, such as climate, soil moisture, or harvest timing, may have affected extraction efficiency or metabolite stability. Conversely, B_20 showed higher ABTS and DPPH values than B_19, indicating possible changes in antioxidant availability or synergistic interactions among phenolic compounds in the 2020 harvest. *J Sendra* samples (JS_19 vs JS_20) showed lower antioxidant values in 2019, followed by higher values in 2020. This pattern is consistent with year-to-year variability in phenolic composition and antioxidant response.

These patterns align with recent findings in other crops. Harvest timing and environmental conditions can influence phenolic accumulation and extraction yield. For instance, Dai et al. (2024) reported that flavonoid and phenolic content in *Rheum officinale* leaves varied across growth stages. These results highlight the value of timing-specific harvesting to optimise bioactive yield. A study on coloured rice straws also showed wide variation in phenolic and antioxidant content across locations, even within the same variety, suggesting a strong genotype-by-environment interaction (Jumrus et al., 2022).

3.7. Correlation analysis and principal component analysis (PCA)

Pearson's correlation analysis revealed strong agreement among antioxidant metrics. FRAP and DPPH were almost perfectly correlated ($r = 1.00$). Both were strongly correlated with TPC ($r = 0.92$). ABTS also correlated positively with FRAP and DPPH ($r = 0.83$ for both) and with TPC ($r = 0.78$). These results indicate that the four assays consistently captured antioxidant-related variability across rice straw extracts (Fig. 3).

Among individual phenolic compounds, quercetin-3-glucoside showed the strongest positive association with antioxidant

performance. It correlated with TPC ($r = 0.56$), FRAP ($r = 0.63$), ABTS ($r = 0.65$), and DPPH ($r = 0.63$). Protocatechuic acid exhibited moderate positive correlations with TPC ($r = 0.39$), FRAP ($r = 0.38$) and DPPH ($r = 0.38$). It also correlated strongly with taxifolin ($r = 0.79$).

In contrast, several hydroxycinnamic- and hydroxybenzoic-related compounds showed high mutual correlations. Ferulic acid correlated with *p*-coumaric acid ($r = 0.98$). Vanillic acid correlated with vanillin ($r = 0.87$). 3,4-Dihydroxyhydrocinnamic acid with vanillin ($r = 0.86$). This pattern suggests a shared accumulation trend across cultivars. However, these compounds showed weak or negative associations with TPC, FRAP, and DPPH (typically -0.10 to -0.26). They showed modest positive correlations with ABTS (up to $r = 0.41$). This result indicates assay-dependent sensitivity to compositional differences (Fig. 3).

Differences between FRAP, ABTS and DPPH are expected because these assays probe related but non-identical antioxidant mechanisms and may show different sensitivity to extract composition (Huynh, Wongmaneeprutip, & Vangnai, 2024). Accordingly, correlations were therefore interpreted as compositional associations rather than direct causality (Shahidi & Samarasinghe, 2025). From a structure-activity perspective, flavonols (e.g., quercetin derivatives) often show strong electron-transfer and radical-scavenging efficiency due to their hydroxylation pattern. Hydroxycinnamic acids (e.g., ferulic and *p*-coumaric derivatives) can also contribute to antioxidant capacity. However, their response can be more assay- and matrix-dependent. In rice straw, phenolic acids are a predominant phenolic family. Their occurrence in different extractable forms can influence functional responses across cultivars and harvest years (Maia, Pessoa-Junior, & Roberto, 2023; Ramos et al., 2023).

PCA summarised these relationships and highlighted the variables driving sample differentiation (Fig. 4). The first two principal components explained 67.26% of total variance (PC1 = 39.89%, eigenvalue of 5.982; PC2 = 27.36%, eigenvalue of 4.106). PC1 represented a compositional gradient. It showed positive loadings for vanillin (0.366), vanillic acid (0.365), 3,4-dihydroxyhydrocinnamic acid (0.330), ferulic acid (0.314), *p*-coumaric acid (0.305) and syringic acid (0.300). PC1 showed negative loadings for protocatechuic acid (-0.295), TPC

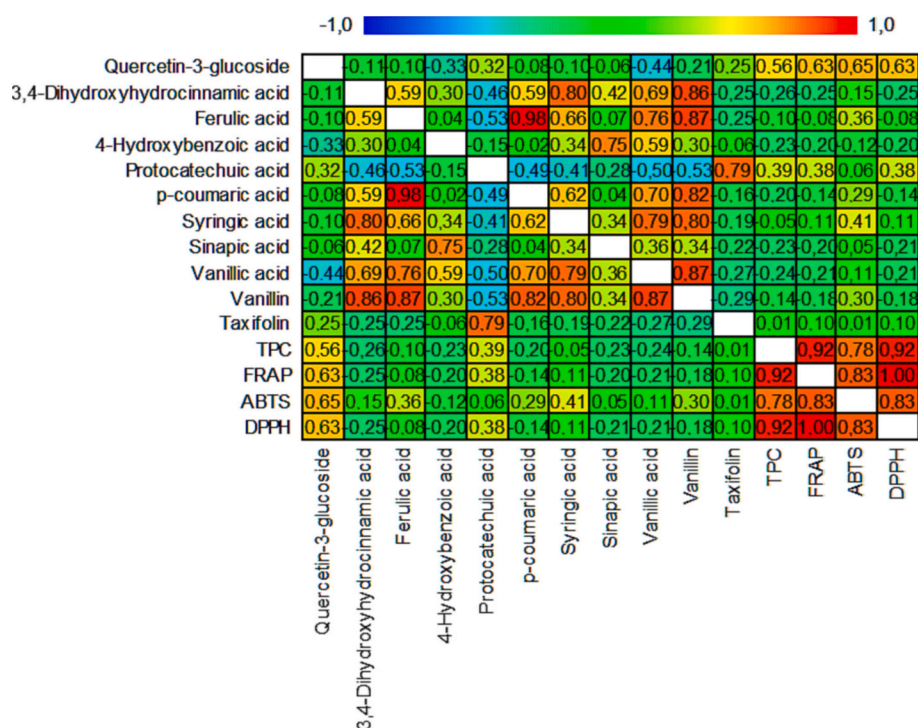


Fig. 3. Pearson correlation matrix among the selected phenolic compounds and antioxidant parameters (TPC, FRAP, ABTS and DPPH) in rice straw extracts (colours indicate correlation strength and direction (-1 to $+1$)).

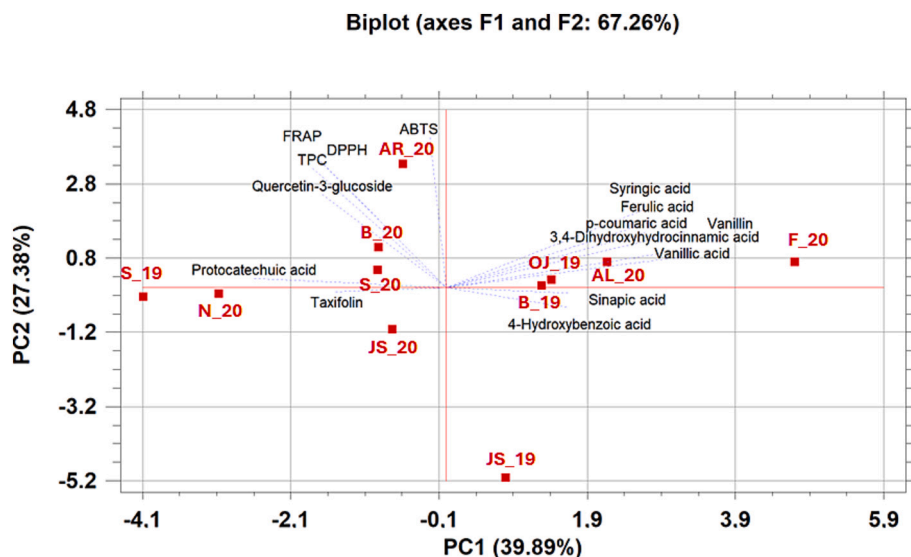


Fig. 4. PCA biplot (PC1 vs PC2) data of the selected phenolics and antioxidant parameters (TPC, FRAP, ABTS and DPPH). Vectors represent variable loadings, and points correspond to rice straw samples (cultivar-harvest year).

(−0.206), FRAP (−0.198), DPPH (−0.198) and quercetin-3-glucoside (−0.191). Therefore, samples with positive PC1 scores were characterised by higher contributions of hydroxycinnamic- and benzoic-related compounds. Samples with negative PC1 scores were associated with higher antioxidant indices and quercetin-3-glucoside or protocatechuic acid.

PC2 was mainly driven by antioxidant capacity variables, showing the highest positive loadings for ABTS (0.479), FRAP (0.413), DPPH (0.413), and TPC (0.382). Quercetin-3-glucoside (0.297) and syringic acid (0.247) also contributed.

Taxifolin was included due to its specificity in N_20. It showed low contributions (PC1 = −0.171; PC2 = −0.013). This result supports its use as a marker compound rather than a main driver of antioxidant variation.

In the score plot, cultivar separation matched the loading patterns (Fig. 4). Samples with positive PC1 scores (particularly F_20, and to a lesser extent AL_20 and OJ_19) were associated with higher contributions of vanillin, vanillic acid and hydroxycinnamic acids (ferulic, *p*-coumaric, syringic and 3,4-dihydroxyhydrocinnamic acids). This pattern indicates a phenolic-acid-driven profile.

Samples with negative PC1 scores (e.g., S_19, N_20, B_20 and S_20) aligned more closely with quercetin-3-glucoside and protocatechuic acid. Depending on their PC2 position, they also aligned with overall antioxidant indices. Along PC2, A_20 and B_20 showed the strongest association with antioxidant capacity (the upper region of the biplot). JS_19 and, to a lesser extent, JS_20 appeared clearly separated in the lower region, indicating a weaker antioxidant response. N_20 aligned with the taxifolin vector, supporting taxifolin as a cultivar-specific marker.

Regarding the TPC, FRAP, ABTS and DPPH, Figs. 3 and 4 indicate two main patterns. First, TPC and the antioxidant assays (FRAP, DPPH, ABTS) form a coherent block. These results support a shared antioxidant-related dimension across extracts. Second, a phenolic-acid cluster (e.g., ferulic, *p*-coumaric and vanillic, vanillin-related compounds) showed strong mutual correlations but weaker associations with FRAP and DPPH. This pattern is consistent with separation along PC1. PC1, therefore, discriminated phenolic-acid-rich profiles (positive PC1) from samples associated with higher antioxidant indices and flavonoid markers (negative PC1). PC2 represents an overall gradient in antioxidant capacity. This interpretation strengthens the clustering conclusions by linking sample positioning to the dominant variables driving separation.

PCA also showed a harvest-year effect within cultivars, as reflected by the consistent displacement of 2019 vs 2020 samples in the score space (Fig. 4). B_19 and B_20 are separated on opposite sides of PC1. B_19 had positive PC1 scores, whereas B_20 had negative PC1 and higher PC2 scores. This pattern indicates a shift in phenolic composition and its contribution to antioxidant capacity between years. A similar trend was observed for S_19 vs S_20. S_19 was located further towards negative PC1, whereas S_20 clustered closer to the origin. This result suggests lower compositional contrast in 2020. JS_19 showed an outlier pattern with strongly negative PC2 scores, whereas JS_20 was less extreme. These results support a strong year-to-year modulation of phenolic composition and antioxidant response.

Cultivar- and harvest-year differences are expected in cereal matrices due to genotype and genotype-by-environment effects on phenolic metabolism and antioxidant traits (Jumrus et al., 2022; Pott et al., 2023; Shim et al., 2025). Seasonal conditions (e.g., temperature regime, water availability/drought, radiation/UV exposure, and biotic pressure) can modulate the phenylpropanoid pathway and thereby shift the accumulation of phenolic acids and flavonoids, leading to measurable changes in antioxidant-related traits (Jumrus et al., 2022; Ninkuu et al., 2025). In rice, drought- and climate-related responses have been linked to the regulation of phenylpropanoid biosynthesis (e.g., via PAL-related control points), supporting that year-to-year climatic variability can alter phenolic profiles and functional antioxidant readouts (Shim et al., 2025).

4. Conclusions

In this study, MAE was optimised as a resource-efficient and time-saving strategy for the release of naturally bound phenolic antioxidants from the lignocellulosic matrix (cellulose, hemicellulose, lignin) of BRS. Optimal MAE conditions were 40 min, 50 mL of 60% (v/v) ethanol and 80 °C. Under these conditions, validation experiments (triplicate) yielded $18.9 \pm 0.9\%$ extraction yield, 796.1 ± 77.6 mg GAE/100 g DW TPC, and 32.3 ± 1.4 $\mu\text{mol TE/g DW}$ FRAP; ABTS antioxidant capacity was 43.2 ± 1.8 $\mu\text{mol TE/g DW}$. The innovation of this work lies not only in the optimisation of MAE, but also in the integration of high-resolution LC-ESI/LTQ-Orbitrap-HRMS to provide a robust, compound-level chemical fingerprint and quantification of key phenolics, including flavonols and phenolic acids, and in the validation of the optimised protocol across a broad genetic background (multiple cultivar-harvest combinations). 4-HBA was a dominant marker across most cultivars,

whereas other compounds such as 3,4-dihydroxyhydrocinnamic acid and quercetin-3-glucoside showed strong inter-cultivar variability. The pigmented straw also exhibited distinctive compounds (e.g., taxifolin and catechol), supporting the value of varietal selection to tailor extracts towards targeted applications.

Overall, the proposed MAE-Orbitrap workflow proved reproducible across multiple cultivar–harvest combinations in this study and enabled the identification of cultivar–harvest combinations that produce antioxidant-rich extracts. These results support the valorisation of rice straw into well-characterised extracts with potential functional value.

By integrating experimental design with high-resolution profiling, the approach provides an operational basis for scale-up and circular bioeconomy-oriented valorisation. Potential applications in food preservation, cosmetics and active/intelligent packaging remain exploratory and will require additional evidence on extract stability, sensory impact, safety/toxicology and regulatory compliance. In addition, protocol transferability beyond the present laboratory will require inter-laboratory validation using harmonised operating and analytical procedures. To facilitate these real-world applications, future work will assess the scale-up of the optimised MAE to pilot-scale microwave systems (larger batch or continuous-flow) with efficient solvent recovery/recycling. As highlighted in the literature, industrial scale-up of microwave-based extraction may require design and process-control adaptations to ensure uniform energy distribution and reproducible temperature profiles. Finally, techno-economic and quantitative sustainability metrics (e.g., energy demand, solvent recovery efficiency and comparative indicators), in line with the Principles of Green Analytical Chemistry, will be required to benchmark MAE against conventional solid–liquid extraction and other emerging extraction technologies, supporting the sustainable valorisation of this residue into a high-value product.

CRedit authorship contribution statement

Laura San Sebastián: Writing – original draft, Investigation. **Emily P. Laveriano-Santos:** Writing – review & editing, Investigation, Formal analysis. **Rosa María Lamuela-Raventos:** Methodology, Conceptualization. **Alfonso Jiménez:** Writing – review & editing, Project administration, Funding acquisition. **Anna Vallverdú-Queralt:** Writing – review & editing, Methodology, Data curation, Conceptualization. **María Carmen Garrigós:** Writing – review & editing, Project administration, Funding acquisition. **Marina Ramos:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2026.148723>.

Data availability

Data will be made available on request.

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