Applied Food Research 5 (2025) 101083

Contents lists available at ScienceDirect

Applied Food Research

journal homepage: www.elsevier.com/locate/afres

^{1}H-NMR metabolomic fingerprinting for differentiation of organic and conventional Italian brown and white rice

Federico Brigante [a,b], Zoe Giannioti [a], Pavel Solovyev [a], Luana Bontempo [a,b,*]

[a] Traceability Unit, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1 38098, San Michele all'Adige, TN, Italy
[b] Fondazione OnFoods, via Università n. 12 43121, Parma, Italy

ARTICLE INFO

Keywords:
Cultivation methods
Organic food
Brown and white rice
1h NMR
Metabolomic fingerprinting

ABSTRACT

Conventional farming is the most common agricultural system in Italy, but the demand for organic alternatives is steadily increasing, given the environmental and health concerns of the consumers. Nuclear Magnetic Resonance (NMR) offers fast, non-destructive, and reproducible analysis for such purposes. In this work, the selected NMR metabolomics approach led to the development of supervised and unsupervised multivariate statistical models which classified Italian brown and white rice samples according to cultivation practice. Amino acids (aspartate, tryptophan, lysine, leucine), sugars (maltose, sucrose, glucose), and purines (adenosine, guanosine, oxypurinol) were the main metabolites proposed as markers for rice authentication. Quantification results showed that organic samples were higher in amino acids and nitrogen compounds and conventional brown rice being the only with high purine content.

1. Introduction

Nearly half of the European rice production is concentrated in Italy, specifically in the northern rural areas, where significant degradation of water quality is noted as a result of high-input monoculture farming (Vaglia et al., 2022). Conventional cultivation is the most common agricultural system in the country, however, organic alternatives are becoming increasingly prominent (Bacenetti et al., 2016). Contributing to this trend is the "Farm to Fork" strategy implemented by the European Commission (EC), which aims to achieve the Green Deal target of 25 % EU agricultural land under organic farming by 2030 (EC, 2021). Moreover, notably high demand has been observed for organic products, with studies mentioning both environmental and health concerns as drivers behind the consumer willingness to pay premium prices for these products (Katt & Meixner, 2020).

Rice is a significant source of dietary nutrients, such as carbohydrates, vitamins and minerals, and exhibits differences both in the nutritional and sensory properties, according to the milling process applied (Gondal et al., 2021). In brown rice production, only the outermost layer of the kernel is removed, and so the rice is significantly richer in the nutrients contained in the bran layer (including essential fatty acids, fiber, thiamine, B vitamins, and important minerals) compared to white rice, which loses the bran layers and germ removed during polishing (Dinesh Babu et al., 2009).

Metabolomic approaches have emerged in recent years as a powerful tool in rice traceability and quality assessments, due to the effect of genetic and environmental factors on the metabolic profile (Uawisetwathana & Karoonuthaisiri, 2019). Examples of analytical techniques widely used in rice metabolomics for the identification of product variety, quality and origin, include Nuclear Magnetic Resonance (NMR) (Song et al., 2016), Liquid Chromatography-Mass Spectrometry (LC-MS) (Cheng et al., 2023) and Gas Chromatography-Mass Spectrometry (GC–MS) (Ch et al., 2021), respectively. The discrimination between organic and conventional cultivation systems has been achieved through the combination of different analytical techniques with multivariate data analysis. Examples include ^{1}H NMR and isotope ratio mass spectrometry (IRMS) (among others) combined with principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and linear discriminant analysis (LDA) for tomatoes (Hohmann et al., 2015) and milk (Erich et al., 2015). More recently, an untargeted UHPLC - MS metabolomics approach distinguished organic from conventional Chinese rice samples (Xiao et al., 2018), while untargeted ^{1}H NMR-based metabolomics have differentiated organic from conventional pomegranate (Villa-Ruano et al., 2020), honey

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* Corresponding author at: Traceability Unit, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1 38098, San Michele all'Adige, TN, Italy.

E-mail address: luana.bontempo@fmach.it (L. Bontempo).

https://doi.org/10.1016/j.afres.2025.101083
Received 28 May 2025; Received in revised form 6 June 2025; Accepted 13 June 2025

(Consonni et al., 2019) and coffee (Consonni et al., 2018).

NMR has often been preferred over other techniques due to its reliability, sensitivity, reproducibility and specificity in food metabolite detection, as well as its non-destructive nature and relatively simple sample preparation (Nagana Gowda & Raftery, 2021) . Untargeted approaches overcome the limitation of targeted analysis, which requires a predetermined knowledge of compound availability, while the use of multivariate methodologies at the final stage of data analysis significantly reduced the complexity of NMR data (Sundekilde et al., 2019).

No previous studies have focused on the NMR characterization of rice and the use of multivariate statistics to propose new markers for the classification of rice samples. The objective of this study was to classify Italian brown and white rice according to the cultivation systems by ^{1}H NMR untargeted metabolomics and propose a set of metabolites for validation and future authentication tests.

2. Materials and methods

2.1. Sample collection

A set of authentic organic brown (OBR, $n = 5$), and white rice (OWR, $n = 11$) samples of different varieties were collected directly from producers in Northern Italy (Lombardy and Piedmont provinces). Conventional brown (CWR, $n = 4$) and white (CWR, $n = 14$) and organic brown ($n = 2$) rice samples of different varieties were purchased in local supermarkets in the Trentino-Alto Adige region in northern Italy. The organic samples from the supermarket exhibited the corresponding organic logo certification from the European Union (See supplementary material).

2.2. Sample preparation

Rice samples were introduced into a laboratory grinder (Retsch Grindomix GM200) and milled for 30 s at 8000 rpm until a fine powder was obtained. Then, 100 mg of each sample was weighed in an Eppendorf tube and 900 μL of Milli-Q water was added (18.2 MΩ.cm^{-1} at 25 °C, 3 ppb TOC) (Q-POD®, Millipore Advantage A10). Next, 100 μL of D_2O containing the standard 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSMP-d_4) for internal reference was added. Samples were sonicated for 30 min and centrifuged for 15 min at 14,000 x g (Eppendorf *minispin plus*). Finally, 600 μL of the supernatant was transferred to an NMR tube (509-UP, Norell Inc.) for analysis.

2.3. NMR spectroscopy

NMR spectra were acquired using a Bruker Avance Neo 600 spectrometer, operating at a base frequency of 600.13 MHz for ^{1}H nuclei. The spectrometer featured a broadband Z-gradient probe for 5 mm sample tubes and a 24-position refrigerated SampleCase autosampler (Bruker BioSpin GmbH, Rheinstetten, Germany). The acquisition and processing of all spectra were automated using Topspin 4.3.9 in conjunction with Icon NMR 6.1.0. The deuterium lock signal was optimized for a 9:1 H_2O/D_2O (v/v) mixture. Proton NMR spectra were recorded using the following specific settings: the noesygppr1d pulse sequence was employed with a power level of 51.16 dB (25 Hz suppression window). The sweep width (SW) was 20.83 ppm, with a time domain (TD) of 131,072 (128 K) data points and an acquisition time of 5.2428 s. Each spectrum was obtained with 64 scans (NS) and 4 dummy scans (DS). The relaxation delay (D1) was set to 10 s, and the receiver gain (RG) was consistently fixed at 16. The baseopt digitization mode was utilized. Before each spectrum acquisition, automatic probe tuning (ATMA routine) and automatic shimming (TOPSHIM) were performed. Spectra were then automatically processed using the apk0.noe phase correction program in TopSpin. Each rice sample constituted a single biological replicate in the NMR analysis.

2.3.1. Quality assurance

A system suitability test (SST) was run at the beginning of each batch of samples. For the ^{1}H linewidth test for calibration quality, a standard sample of 1 % chloroform in acetone-d_6 was used. The linewidths at 0.55 % and 0.11 % were measured. The SSTs included a water signal suppression experiment. The analyzed solution was a 2.0 mM sucrose sample in 10 % D_2O/90 % H_2O and 2 % NaN_3 standard according to the ASTM International (ASTM International, 2015). The linewidths of DSS at 0.55 % and 0.10 % were measured. For internal temperature control, a solution of 99.8 % MeOD was used. The registered internal temperature was 300.00±0.05 K. All SSTs passed all the specifications for each experiment (See Supplementary Material for a summary of specifications). The temperature of the NMR facility was measured every 30 min, and it was 20.8 ± 0.2 °C during the whole study. The full width at half maximum (FWHM) of the TSMP-d_4 peak was the criterion used for spectra reacquisition, all the classes presented an average FWHM in the acceptable range around or 1.0 Hz (Bliziotis et al., 2020; Deborde et al., 2019). For reproducibility, the signal-to-noise ratio of the TSMP-d_4 peak was calculated and the class-CV % calculated for each class (See Supplementary material).

2.4. Spectra pre-processing

In metabolomics, spectral editing before data analysis is necessary to enhance the signal-to- noise ratio (SNR), eliminate instrumental artifacts, and improve system interpretation (Martin et al., 2018). This process involves modifying both the free induction decay (FID) and the spectrum prior to statistical analysis. FID operations include solvent suppression, apodization, and Fourier transform. Post-Fourier transform operations involve phase correction (0th and 1st order), baseline correction, referencing, alignment, window selection, bucketing (or binning), region removal, and normalization. For this study, except for apodization, 0th and 1st order phase correction, and baseline correction, all steps were applied to the spectra using the PepsNMR package (Martin et al., 2018) in R Studio (R Core Team, 2024). PepsNMR includes algorithms that enhance classification performance through improved alignment techniques (warping), minimizing human manipulation during alignment. Proper alignment is crucial as signal misalignment can lead to incorrect results and assumptions during statistical modeling. Solvent suppression was achieved using the noesygppr1d pulse sequence from Bruker. Spectra were manually referenced to the TMSP-d4 internal standard at 0.00 ppm. Window selection spanned from 10.00 to 0.2 ppm. Binning, which partitions the spectrum into segments of predetermined duration (measured in ppm), was performed with precision to avoid data oversimplification and critical variable loss. Four binning values were tested, with the final binning window set at 0.03 ppm. The aligned spectra were then normalized to the mean using the PepsNMR package's normalization function. The normalized, aligned spectra were exported as a bucket table in csv format (Final dimension of data matrices: 18×326).

2.5. Identification of variables

Identification of the individual compounds was performed by comparison with the Human Metabolome Database (HMDB) (Wishart et al., 2022), the BBIOREFCODE database of NMR metabolites (v.2.01, Bruker BioSpin GmbH, Rheinstetten, Germany) or manually by comparison with the literature data (Almoselhy et al., 2014; Ben Youssef et al., 2016; Davoren & Mason, 2023; Fathi et al., 2017; Huo et al., 2017; Ingallina et al., 2023; Lalwani et al., 2020; Roslund et al., 2008). For each compound, the assignment, the multiplicity, and coupling constant (J) when possible, were reported.

2.6. Quantification of compounds

Quantification of compounds in rice was performed with the same software, concentrations, and protocols as in Brigante et al. (2025). Sucrose was used as external standard for quantification. Metabolites with a signal-to-noise ratio higher than 10 were selected. Only identified metabolites with minimal or no overlap and were quantified.

2.7. Statistical analyses

The csv files (one for the white rice samples and one for the brown rice samples) were imported into MetaboAnalyst 6.0 (Pang et al., 2024) for supervised and unsupervised statistical analyses. Prior to statistical analyses, the data were filtered using the incorporated variance and abundance filters with median absolute deviation and median intensity value, respectively, set to a 10 % according to the size of the data matrix. Next, scaling was performed by Pareto scaling. Principal component analysis was performed in the dataset to find preliminary clustering between the samples with 95 % confidence ellipses for outlier detection. A hierarchical cluster analysis (HCA) with average distance measure and ward linkage and a heatmap visualization was performed in both datasets. This unsupervised technique allowed a visualization of the preliminary clustering including the relative intensity of the bins in a color- coded scale to make inferences about the groups of bins that contribute to the clusterings. For supervised analysis, orthogonal partial least squares discriminant analysis (OPLS-DA) was applied. This analysis divides the variation into two components, the predictive or correlated to the response matrix Y and the orthogonal or uncorrelated to the response matrix. Internal validation of the models was performed by k-fold cross-validation ($k = 5$) and the parameters accuracy, R2 (goodness of fit), and Q2 (predictability) were obtained. A permutation test ($n = 100$) was conducted to determine whether a random permutation of the Y-block would produce a better model than the original one. The variable selection method used was the Variable Importance in Projection (VIP) score, since variables (bins) having a VIP score greater than 1 were almost half of the original variables, those with VIP score making the top 15 and top 14 were selected for brown and white rice, respectively (all with VIP scores greater than 1.3). For quantification results, ANOVA tests with Tukey's post-hoc test was performed between the different classes to search for significant differences between metabolites.

3. Results

3.1. ^{1}H NMR spectroscopy

The results from the NMR analysis of brown and white rice are presented in Fig. 1 and Table 1. Each signal was assigned a number or a number with a letter if they belonged to the same compound or family of compounds. Specific panels in the figure show zoomed regions for better observation of the signals. The signals with the highest intensity were those of carbohydrates, including mono and disaccharides. The group of monosaccharides included both anomers of glucose, and *scyllo*-inositol while the disaccharides in rice included sucrose and maltose (two glucose molecules with an α(1→4) bond). Other saccharide signals belonged to oligosaccharides, particularly to the raffinose family oligosaccharides (RFOs). Their characteristic broad signals and high molecular weight makes their exact identification a challenge for NMR, that requires the help of separation or 2D NMR techniques with authentic standards.

Signals belonging to fatty acids were also present in brown and white

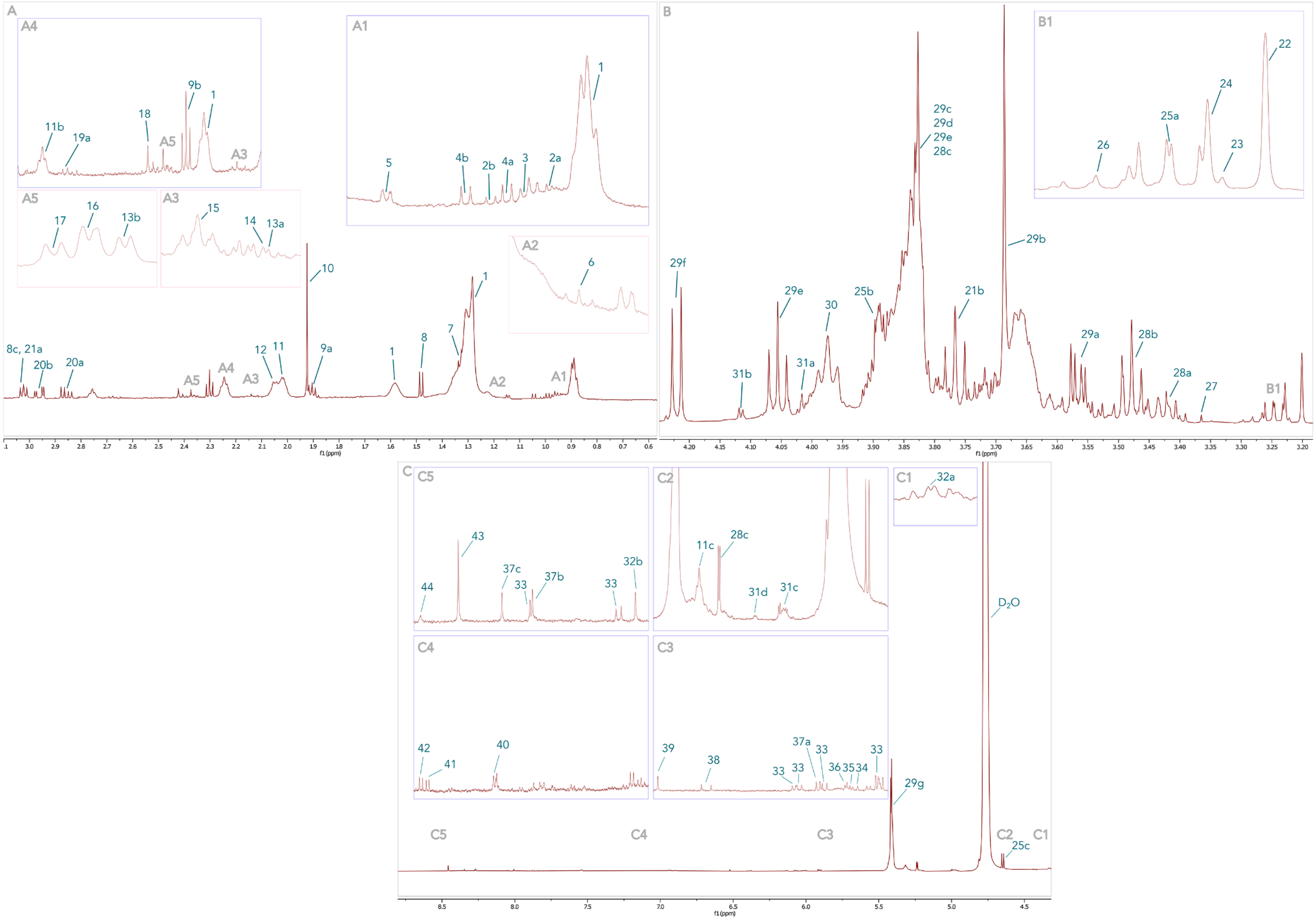

Fig. 1. General ^{1}H—NMR spectrum of rice. (A): Region from 0 to 3.1 ppm. (B): Region from 3.2 to 4.2 ppm. (C): Region from 4.5 to 9 ppm.

Table 1
Identification of compounds in rice by ^{1}H NMR[a].

Number	Compound	Assignment	Multiplicity[a]	Chemical shift (ppm)	Coupling constant, J [Hz]
1	Fatty acids	ω-CH_3	m	0.88–0.92	–
2a	Isoleucine	δ-CH_3	t	0.94	-[b]
3	Leucine	δ-CH_3	d	0.96	6.4
4a	Valine	γ-CH_3	d	0.99	7.0
2b	Isoleucine	γ-CH_3	d	1.01	7.1
4b	Valine	γ'-CH_3	d	1.04	7.0
5	Isobutyrate	CH_3	d	1.14	6.1
6	Ethanol	CH_3	t	1.18	
1	Fatty acids	Aliphatic CH_2 chain	m	1.28–1.31	–
7	Lactate	β-CH_3	d	1.33	-[b]
8	Alanine	β-CH_3	d	1.48	7.2
1	Fatty acids	β-CH_2	m	1.59	–
9a	GABA	β-CH_2	qt	1.91	-[b]
10	Acetate	α-CH_3	s	1.93	–
11a	Unsaturated Fatty Acids (UFAs and PUFAs[e])	Allylic CH_2	m	2.03	–
12	N-acetyl carbohydrates	$\mathbf{CH_3}$-CO-NH	s	2.04–2.07	–
13a	Glutamate	β-CH_2	q	2.13	–
14	Glutamine	β-CH_2	q	2.15	–
15	Glutarate	α -CH_2	t	2.17	7.2
1	Fatty acids	α-CH_2	m	2.23	–
9b	GABA	α -CH_2	t	2.30	7.3
13b	Glutamate	γ-CH_2	m	2.35	–
16	3-Hydroxybutyrate	α-CH_2	d	2.36	-[b]
17	Isobutyrate	α-CH	m	2.37	–
18	Succinate	α, β-CH_2	s	2.42	–
19a	Aspartate	β-CH	dd	2.67	(17.5, 9.0)
11b	Unsaturated Fatty Acids (PUFAs[e])	Bis-allylic CH	m	2.75	–
19b	Aspartate	β'-CH	dd	2.80	(17.7, 3.4)
20a	Asparagine	β-CH	dd	2.86	-[b]
20b	Asparagine	β'-CH	dd	2.96	(16.9, 4.2)
21a	Lysine	ε-CH_2	t	3.02	-[b]
8c	GABA[e]	γ-CH_2	t	3.03	-[b]
22	Choline	N-$(CH_3)_3$	s	3.20	–
23	Phosphocholine	N-$(CH_3)_3$	s	3.21	–
24	Glycerophosphocholine	N-$(CH_3)_3$	s	3.22	–
25a	β-D-Glucose	H-2	dd	3.23	(9.4, 8.0)
26	β-Maltose	H-2	dd	3.28	(9.6, 8.0)
27	*Scyllo*-Inositol	CH-1,2,3,4,5,6	s	3.36	–
28a	α-Glucose	CH-4	m	3.42	–
28b	α-Glucose	H-2	dd	3.50	-[b]
29a	Sucrose	H-2g[c]	dd	3.54	(9.8, 3.8)
29b	Sucrose	CH-1f	s	3.68	–
21b	Lysine	α-CH	t	3.75	-[b]
29c	Sucrose	CH-6 g	m	3.82	–
29d	Sucrose	CH-6f	m	3.82	–
29e	Sucrose	CH-5 g	m	3.83	–
28c	α-Glucose	CH-5	m	3.84	–
25b	α-Glucose	CH_2–6	m	3.90, 3.75	–
30	α-Maltose	CH-3	m	3.97	-[b]
31a	RFOs	CH	m	4.01	–
29e	Sucrose	CH-4f	t	4.06	8.6
31b	RFOs	CH	m	4.11	–
29f	Sucrose	CH-3f	d	4.22	8.8
32a	Guanosine	CH-13	dd	4.41	-[b]
25c	α-Glucose	CH-1	d	4.64	7.9
31c	RFOs	CH-1Gal''c	m	4.98–5.00	–
31d	RFOs	CH-3Gal'c	m	5.10	–
28c	α-Glucose	CH-1	d	5.23	3.7
11c	Unsaturated Fatty acids (UFAs and PUFAs[e])	Olefinic CH	m	5.30–5.31	–
29g	Sucrose[d]	CH-1 g	d	5.40–5.42	4.0
33	Purines	CH	d	5.90	-[b]
33	Purines	CH	d	5.91	-[b]
34	UMP[e]	CH	d	5.98	-[b]
35	CMP[e]	CH	d	5.99	-[b]
36	CTP[e]	CH	d	6.00	-[b]
33	Purines	CH	d	6.05	6.2
37a	Adenosine	CH-2	d	6.08	6.2
33	Phosphorylated purines	CH	d	6.12	-[b]
33	Phosphorylated purines	CH	d	6.13	-[b]
38	Sinapic acid	Vinylic CH	d	6.39	15.9
39	Fumarate	α, β-HC=CH	s	6.53	–
40	Tryptophan	CH-7	d	7.55	-[b]
41	2-Deoxyuridine	CH-4	d	7.85	7.5
42	Uridine	CH-4	d	7.87	8.1

(*continued on next page*)

Table 1 (*continued*)

Number	Compound	Assignment	Multiplicity[a]	Chemical shift (ppm)	Coupling constant, J [Hz]
32b	Guanosine	CH-2	s	8.00	–
33	Purines	CH	d	8.05	7.8
37b	Adenosine	CH-11	s	8.26	–
33	Purines	CH	s	8.27	–
37c	Adenosine	CH-15	s	8.34	–
43	Formate	HCOO-	s	8.47	–
44	IMP[e]	CH-2	s	8.55	–

[a] s:singlet, d:doublet, t:triplet, q:quartet, qt: quintuplet, dd:doublet of doublets, st:sextet.

[b] the coupling constant could not be calculated due to low intensity or overlapping of signals.

[c] f:fructose, g:glucose, Gal:Galactose.

[d] The anomeric protons of other disaccharides are overlapped in this region.

[e] UMP: Uracil monophosphate; CMP: Cytidin monophosphate; CTP: Cytidine triphosphate; IMP: Inosine monophosphate; GABA: gamma aminobutyiric acid. UFAs: Monounsaturated fatty acids; PUFAs: Polyunsaturated fatty acids.

rice spectra. The corresponding subtype of fatty acid signal (monounsaturated – UFA or polyunsaturated – PUFA) was written in parenthesis in Table 1 for clarification. Their structural similarity and interactions with the solvent causing the broadening of the signals did not allow their individual identification but the diagnostic signals of saturated, monounsaturated and polyunsaturated fatty acids were identified. Polyunsaturated fatty acids were distinguished by the resonance of bis-allylic protons that are absent in monounsaturated and saturated fatty acids. The rest of their resonances: alpha and beta CH_2 groups, the remaining CH_2 groups of the aliphatic chain, and hydrogen atoms in allylic double bonds were also identified but could not be discriminated against for each subgroup of fatty acids.

Then, the region between 1.1 and 3.15 ppm was dominated by signals from organic acids, along with some resonances between 6.5 and 8.5 ppm for those with vinylic hydrogen atoms or directly attached to carboxylic groups like the characteristic resonance of formic acid. The characteristic methyl groups of some amino acids were found in the zone between 0.8 and 1.1 ppm; some CH_2 and CH aliphatic resonances between 2.4 and 3.0 ppm; and the CH aromatic resonances in the low-field region beyond 7.5 ppm.

The group of purines showed a large number of signals in the zone between 5.8 and 8.4 ppm, belonging to the characteristic CH between the two nitrogen atoms from the indole group. However, some signals could not be assigned to a specific molecule after finding various matches against the databases and bibliography for the same signal. In the case of substituted and phosphorylated purines, the resonances were shifted towards the low-field region compared to their corresponding aglycones and in some cases did not allow the assignment of the signal to a single compound.

3.2. *Unsupervised analysis*

3.2.1. *Principal component analysis (PCA)*

The scores plot of the PCA analysis for brown and white Italian rice is presented in Fig. 2. In the case of brown rice, the total explained variance between the first two components was 93.2 %, a clear preliminary clustering of all the samples was observed and no outliers were detected since all the samples of both groups were inside the corresponding confidence interval. For white rice, 70.7 % of the total variance was explained by the first two principal components, with no detected outliers. For this dataset, an overlapping zone was observed in the first quadrant of the plot (upper-left corner, Fig. 2B). A series of factors could be responsible for the observed overlap in white rice samples. Firstly, this overlap could be due to the different milling processes that remove the bran layer, turning brown rice into white rice. This process results in the loss of metabolites contained in the bran that could contribute to differentiation. Consequently, the remaining endosperm, which is mainly composed of carbohydrates, fats, and proteins, could accentuate the similarity between some of the varieties. So, the ideal proposed markers should be independent of the milling process. Secondly, the

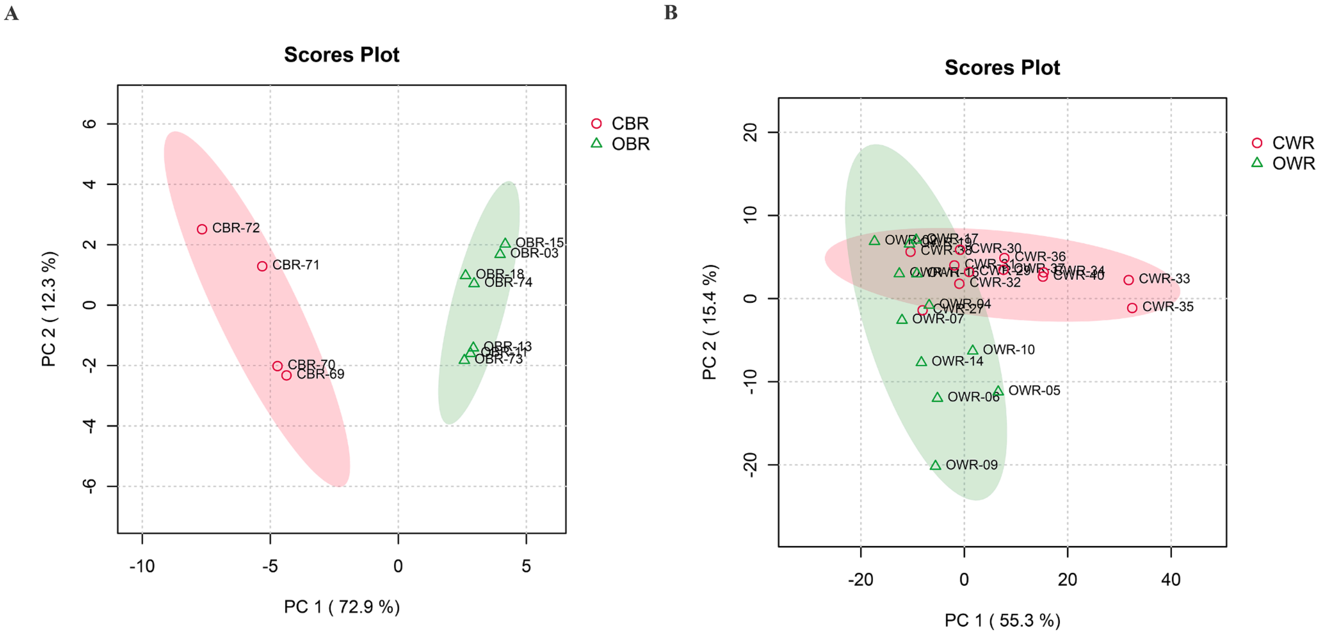

Fig. 2. PCA scores plot of rice samples. (A) Brown rice. (B) White rice.

specific environmental conditions including soil status, irrigation, and climate could have an impact for samples with the same geographic origin regardless of the cultivation system applied. This is also important for future authentication of rice samples since the proposed markers should ideally be resistant to different environmental conditions and be independent of the geographic origin of the samples.

3.2.2. Hierarchical cluster analysis (HCA)

The results of the HCA analysis are presented in Fig. 3. The organic samples were correctly grouped in two clusters and the heatmap visualization showed that CBR gave lower relative intensity in the bins that created the classification, indicated by a brighter green color (Fig. 3A, upper-left corner). The relative intensity of bins from carbohydrates, purines, and organic acids were higher for this class. On the other hand, OBR samples showed mostly a higher intensity in bins from regions from 0.8–3.0 ppm belonging to aminoacids and organic acids and from carbohydrates in the zone from 4.6 to 5.6 ppm with less contribution from purines.

In the case of white rice, the cluster of CWR contained 3 of the OBR samples, in agreement with the preliminary overlap observed in the PCA analysis. Further exploration of these 3 samples revealed that one of them, OSMR-8, was subjected to a semi-milling process (and it was labeled after this to keep track of this information). During this particular milling process, only part of the bran layer is removed, this contrasts with fully milled (white) rice, where the bran and germ are completely removed. A previous study highlighted that the distribution of nutrients such as proteins, fiber, and polyphenolic compounds, varied significantly with the degree of milling, with semi-milled rice retaining more of these beneficial components and keeping less resistant starch (Sirisoontaralak et al., 2020; Wang et al., 2021). Therefore, the metabolite composition of this type of rice placed it closer to CWR, as shown by the HCA results. This sample, even if it was not pointed out as an outlier neither by visual nor statistical results, was removed from subsequent classification models since a higher number of biological replicates is necessary to propose markers that are independent of the milling process.

The other 2 samples in this group were the only ones of the Rosa Marchetti variety which is part of the Italian tradition, particularly known in the Piedmont region and owing its name to the discoverer, Marchetti, and its rose-colored (rosa) hue after cooking. Possible reasons for the observed grouping of these samples could be a distinctive metabolite composition and/or the degree of milling as in the previous case. Regarding the variables causing the clustering, a set of variables between 1.2–3.2 ppm were predominant for the clustering of the rest of OWR (Fig. 3B, upper-left corner), while for CWR a particular zone of higher relative intensity was observed from variables between 3.2–5.5 ppm that belonged mostly to carbohydrates (Fig. 3B, lower-right corner).

3.3. Classification of Italian white and brown rice

No additional samples could be obtained within the experimental timeframe to construct an independent external validation set, which is necessary for the validation of markers in food samples. As a result, the discriminant markers identified in this study should be considered potential at this stage.

The results of the OPLS-DA models for the classification of Italian white and brown rice are presented in Fig. 4. The classification models consisted of one predictive and one orthogonal component in both cases. The cross-validation parameters of each model were for brown rice: R2X=0.599; R2Y=0.998; Q2=0.983 and for white rice: R2X=0.432; R2Y=0.805; Q2=0.583. The values of Q2 and the permutation tests of both models with p-values lower than 0.05 and 0.01, respectively, indicated the absence of overfitting in the models (Supplementary Figure 1).

For brown rice, an excellent separation was achieved by the predictive and orthogonal components (Fig. 4A) in agreement with the results from the unsupervised models. For the white rice samples, only a slight overlap was observed between the confidence intervals of the classes but most importantly the samples were not overlapped (Fig. 4B). This result demonstrated the statistical power of the technique of maximizing the separation between classes, resolving the situation from the unsupervised analysis where an overlapping of samples was presented.

The variable selection method gave the top discriminating variables of each model, the results are presented in Fig. 5 and a table with the

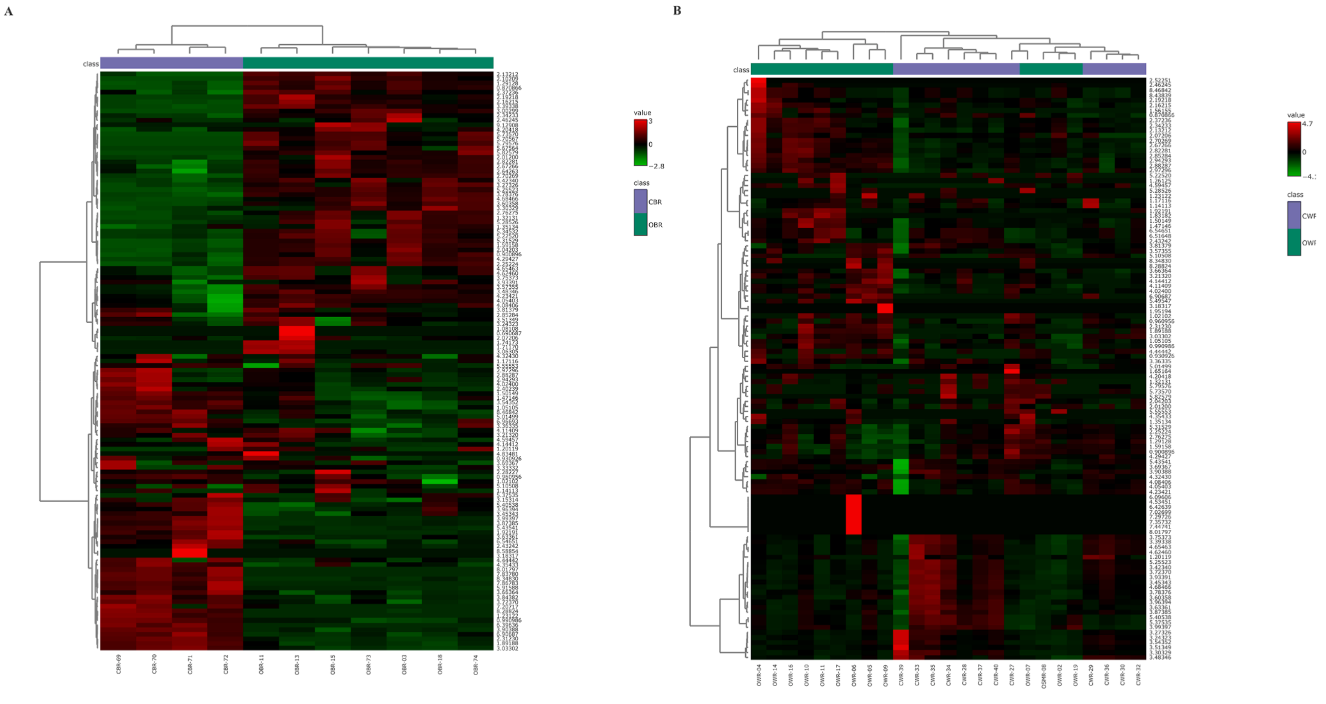

Fig. 3. HCA and heatmap of rice samples. (A) Brown rice (B) White rice.

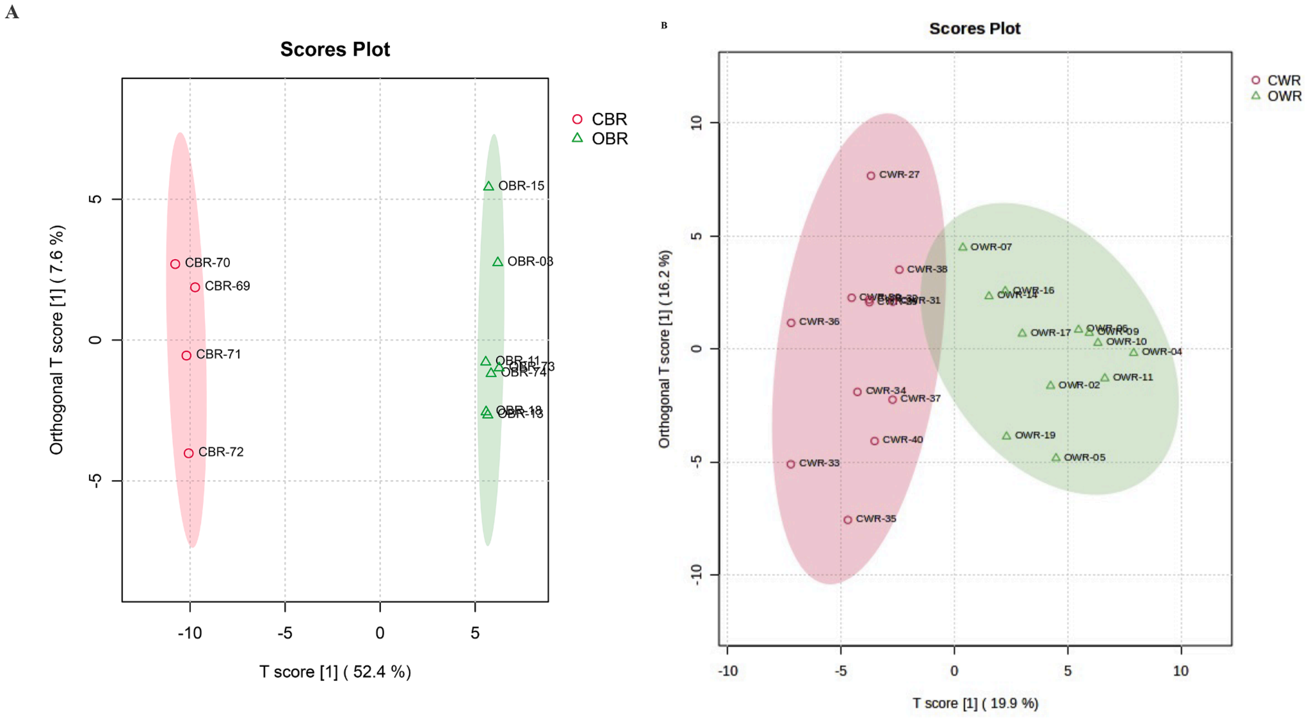

Fig. 4. OPLS-DA score plot of rice samples. (A) Brown rice (B) White rice.

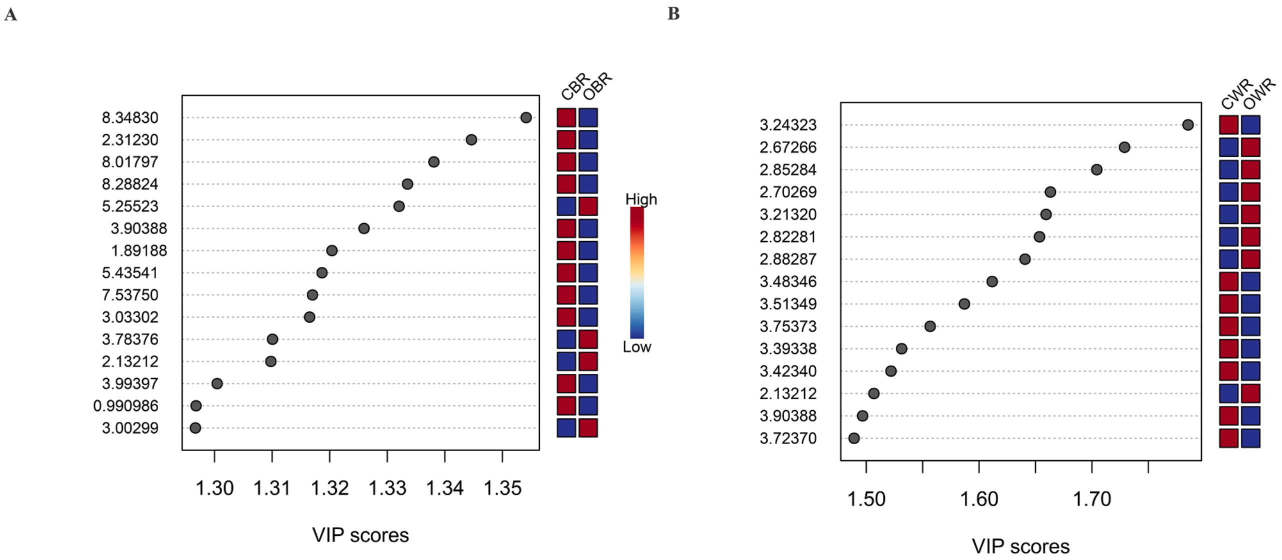

Fig. 5. Variable selection results from OPLS-DA models. (A) Brown rice (B) White rice.

association between discriminating bins and the identified metabolites is shown in Supplementary Table 1. Quantification results are presented in Table 2. A group of metabolites were selected as discriminant for both types of italian rice. This group of metabolites was composed of β-Glucose, α-Glucose, asparagine, and glutamate/glutamine (Fig. 5A). In the latter case, the selection pointed to a CH_2 group that showed the same chemical shift in both molecules. The quantification of glutamate and glutamine was not possible due to structural similarity between the metabolites that caused the final identification to be inconclusive. Regarding fatty acids, the β-CH_2 signal from fatty acids was higher in OBR. Unfortunately, identifying fatty acids individually, hence neither their quantification, is not possible in the NMR analysis of food matrices due to the structural similarity, the hydrophobic interaction of the non-polar metabolites causing aggregation in a polar solvent, as well as the dipole-dipole interactions with the solvent creating inhomogeneous broadening of the signals in the spectrum of this family of metabolites. Given this lack of specificity, this signal could be considered as a general indicator acting in conjunction with the rest of the selected metabolites.

Asparagine and glutamate/glutamine showed higher relative intensity in organic rice samples, while β-Glucose for conventional rice samples. Asparagine showed higher concentration in organic samples but only significant differences for white rice samples, while the overall concentration was higher in both types of brown rice, indicating that the consumption of brown rice causes a greater contribution of this amino acid to the diet. The quantification of β-Glucose was not possible due to great overlapping with the one anomeric proton of maltose at 4.67 ppm, so the analysis was done based on the total intensity of the doublet, since it is also proportional to the concentration. Interestingly, α-Glucose was

Table 2
Quantification of compounds in rice in mg/100 g[a].

Compound/Class	CBR (*n* = 4)	OBR (*n* = 7)	CWR (*n* = 14)	OWR (*n* = 12)
Isoleucine	1.7 ± 0.1^{a}	6.4 ± 0.7^{b}	2.1 ± 0.2^{c}	2.4 ± 0.7^{d}
Valine	1.7 ± 0.1^{b}	4.2 ± 0.5^{a}	1.5 ± 0.2^{b}	1.7 ± 0.5^{b}
Alanine	4.1 ± 0.5^{b}	12.8 ± 0.8^{a}	3.0 ± 0.4^{c}	$4 \pm 1^{b,c}$
Acetate	7 ± 1^{a}	2.9 ± 0.5^{b}	2.5 ± 0.8^{b}	2.2 ± 0.8^{b}
GABA	8.6 ± 0.8^{b}	13 ± 2^{a}	3.5 ± 0.5^{c}	4 ± 1^{c}
Succinate	2.0 ± 0.3^{b}	2.5 ± 0.3^{a}	1.3 ± 0.2^{c}	1.5 ± 0.4^{c}
Asparagine	14 ± 4^{a}	16 ± 2^{a}	7.2 ± 0.7^{c}	9 ± 2^{b}
Choline	4.6 ± 0.1^{b}	5.7 ± 0.8^{a}	2.0 ± 0.5^{d}	2.6 ± 0.8^{c}
Phosphorylcholine	0.8 ± 0.1^{a}	0.62 ± 0.07^{b}	0.33 ± 0.05^{c}	ND[c]
Glycerophosphocholine	4.9 ± 0.6^{b}	7 ± 1^{a}	3.9 ± 0.6^{b}	2.8 ± 0.9^{c}
Scyllo-inositol	0.016 ± 0.003^{a}	0.012 ± 0.001^{b}	0.006 ± 0.001^{d}	0.008 ± 0.003^{c}
α-Glucose	33 ± 3^{a}	96 ± 8^{a}	$(8 \pm 2)\mathrm{x}10^{1b}$	$(5 \pm 1)\mathrm{x}10^{1b}$
Sucrose	$(106 \pm 1)\mathrm{x}10^{1a}$	$(110 \pm 1)\mathrm{x}10^{1a}$	$(33 \pm 5)\mathrm{x}10^{1b}$	$(32 \pm 9)\mathrm{x}10^{1b}$
β-Maltose	NQ[c]	$(17 \pm 3)\mathrm{x}10^{1a}$	$(15 \pm 4)\mathrm{x}10^{1a}$	$(9 \pm 3)\mathrm{x}10^{1b}$
Adenosine	5 ± 1	NQ[c]	ND[c]	ND[c]
Sinapic acid	3.2 ± 0.2	NQ[c]	ND[c]	ND[c]
Fumarate	1.2 ± 0.2^{b}	1.8 ± 0.4^{a}	0.9 ± 0.1^{b}	1.2 ± 0.3^{b}
Deoxyuridine	3.2 ± 0.7	NQ[c]	ND[c]	ND[c]
Uridine	5 ± 1	NQ[c]	ND[c]	NQ[c]
Guanosine	6 ± 1	NQ[c]	ND[c]	NQ[c]
Formate	2.7 ± 0.4^{a}	2.0 ± 0.4^{b}	0.5 ± 0.1^{d}	0.8 ± 0.2^{c}

[a] Different italicized letters indicate significant differences.
[b] gamma aminobutyiric acid.
[c] ND: Not detected, NQ: Not quantified.

the only metabolite that showed higher relative intensity in OBR and CWR samples. In agreement with this finding, the glucose concentration showed significant differences between both types of rice but no significant differences were found for the cultivation method. This also support previous findings where the consumption of white rice has led to higher glycemic index in blood compared to brown rice (Foster-Powell et al., 2002). This could be connected to ~~(~~degree of milling, cultivation system, and also to intrinsic factors like the distribution of glucose anomers in each rice variety. Sucrose and α-Maltose were also selected as discriminating molecules for brown rice also with higher concentration in CBR but no statistical differences between cultivation systems for sucrose. However, the concentration of maltose showed significant differences being higher in conventional white rice, this accompanied the trend of glucose, showing that conventional system caused an increase in carbohydrate concentrations. *Scyllo*-inositol could discriminate both types of rice varieties and cultivation systems, also following the previously found trend for glucose and maltose. Maltose, along with glucose, and fructose, constitute the free sugars fraction in rice (Lee et al., 2019). Our results are in agreement with previous studies where higher levels of sugars for other matrices like fruits and vegetables were found in conventional farming systems. This system relies heavily on the use of pesticides for pest control. So, this alleviates stress in the plants and allows the allocation of their energy to the synthesis of more sugars (Rahman et al., 2021).

Adenosine, guanosine, and oxypurinol were selected for the discrimination of the cultivation systems of brown rice with higher concentrations in CBR. Surpringsingly, adenosine and other purines were not identified (ND label in Table 2) or detected in very low signal intensity that did not allow quantification (NQ label in Table 2) in the rest of the varieties. Brown rice has previously shown a higher content of purines and nucleotides than other white and colored varieties of rice (Zhao et al., 2024). Moreover, purines and pyrimidines have been intimately connected to the germination process of brown rice showing differential metabolic pathways, making them good candidates for brown rice authenticity. These metabolites are essential building blocks for nucleic acid synthesis that intervene in vital processes such as organogenesis, storage organ development, and leaf senescence (Chuan-ying et al., 2022). Regarding amino acids, gamma-aminobutyric acid (GABA) was selected for the discrimination of brown rice, with statistically significant and higher concentrations in OBR. The natural concentration of this free amino acid in brown rice is low, but its high nutritional value has motivated the seek for alternatives to augment its concentration. Previous research demonstrated that the genes and metabolites involved in the GABA shunt and polyamine degradation pathway were altered to increase the levels of GABA in brown rice after treatment with pulsed light (Zhang et al., 2024). The consumption of germinated brown rice has shown to be a very good alternative to increase the intake of this amino acid. The levels of GABA in brown rice were studied in different varieties, in varying soaking and germination times where the GABA content was increased in rice varieties Jyothi and Chitteni and Njavara from 0 h to 72 h of soaking and germination (Thomas et al., 2023). Another study found that the highest concentration of GABA and glutamic acid was reached after 48 h of germination in white and colored rice, with colored rice showing higher values for both metabolites (Kamjijam et al., 2020).

Tryptophan, leucine, and lysine were the other amino acids in the model for the discrimination of rice with lysine being the only one selected also for white rice, with a higher relative intensity in OBR and CWR. Lysine is a limiting amino acid in rice and previous studies have found that a direct proportion exists between the nitrogen (N) rate in the cultivation system and the lysine content, with no significant differences between brown and white rice (Chang et al., 2008; Ning et al., 2010). On this basis, a possible reason for the observed results could be the varying types, proportion and quantity of N input from both cultivation systems used across the different farms for the samples of our study.

Lastly, choline and aspartate were selected for the white rice discrimination, with higher concentrations in OWR compared to CWR. Particularly for choline, the concentration was higher in both organic classes of rice (OWR and OBR) than the respective conventional counterparts, but it was selected as a discriminating molecule only for OWR. Previous studies have found higher contents of choline connected to organic farming systems in vegetables (Lucarini et al., 2020), and organic soy sauce (Kamal et al., 2022) in agreement with our results. The univariate analysis demonstrated statistically significant differences among all rice classes, confirming the discriminatory power of the proposed markers. Notably, these differences were detected rapidly and efficiently using NMR spectroscopy, highlighting the technique's potential for high-throughput screening and routine quality control applications in rice authentication. Importantly, these results carry analytical robustness within the context of this study, as they do not require external validation to confirm statistical significance, and thus support the multivariate preliminary results.

4. Conclusion

^{1}H NMR metabolomics achieved the complete characterization of Italian brown and white rice, giving a comprehensive view of the nutritional profile, and classifying rice cultivation systems fast, efficiently, and with excellent statistical parameters. Due to the limited sample size, this study did not permit the construction of a external validation model. Consequently, emphasis was placed on monovariate analysis, where quantitative NMR data demonstrated statistically significant differentiation among rice groups. While multivariate analyses were impacted by group size constraints, they nonetheless revealed discernible clustering patterns, indicating underlying compositional differences. These findings, although preliminary, underscore the potential of NMR-based metabolomic approaches for varietal discrimination and a fast tool for rice production methods analysis. Importantly, our findings lay the groundwork for more comprehensive future studies involving larger, more diverse sample sets from additional providers and even different geographic regions across Italy. Such efforts will be critical for the development of a robust rice authentication framework, with applications in quality control and the detection of food fraud, thereby supporting consumer protection and regulatory oversight. Cultivation systems showed an impact on rice nutritional profile, as demonstrated by the variety of molecules proposed as markers. Purines were a chemical family selected exclusively for conventional brown rice while choline was selected for organic white rice. Carbohydrates and amino acids were selected for both types of rice, with conventional cultivation practices showing a higher carbohydrate content in both types. However, as always, the use of sugars as markers is intended only in conjuction with the rest of metabolties from different chemical families, since sugars are ubiquitous to cereals in both cultivation practices.

Ethical statement - studies in humans and animals

This study did not involve any human participants, human data, human tissue, or animals. Therefore, ethical approval and informed consent were not required.

Funding

This research was funded by the European Union’s Horizon 2020 Research and Innovation Programme under the Marie Sklodowska-Curie grant, agreement no 956,265. FoodTraNet. The work was supported by the Ministero dell’Università e della Ricerca [PE00000003 ON FOODS]; Provincia Autonoma di Trento [FRUITOMICS, FESR 2014–2020].

Disclaimer

This study was carried out within the ON Foods – Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods” project and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – Mission 4 Component 2 Investment 1.3 – D.D. 1550 11/10/2022,

PE0000003, CUP B43C22000770006). This manuscript reflects only the authors’ views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

CRediT authorship contribution statement

Federico Brigante: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Zoe Giannioti:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Pavel Solovyev:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Luana Bontempo:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Acknowledgements

The authors acknowledge the support of “ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods” to Fondazione Edmund Mach, funded by the Italian Ministry of University and Research, PNRR, Mission 4 Component 2 Investment 1.3 - Project code PE00000003, CUP B43C22000770006. This study has also received funding from the Autonomous Province of Trento, with EU co-financing (FRUITOMICS, FESR 2014–2020 Program of the Autonomous Province of Trento, Italy)

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101083.

Data availability

Data will be made available on request.

References

Almoselhy, R. I. M., Allam, M. H., El-Kalyoubi, M. H., & El-Sharkawy, A. A. (2014). 1H NMR spectral analysis as a new aspect to evaluate the stability of some edible oils. *Annals of Agricultural Sciences, 59*(2), 201–206. https://doi.org/10.1016/j.aoas.2014.11.006

ASTM International. (2015). Standard practice for measuring andreporting performance of Fourier-Transform nuclear magnetic resonance (FT-NMR) spectrometers for liquid samples. *ASTME2977-15* (p. 30). ASTM International.

Bacenetti, J., Fusi, A., Negri, M., Bocchi, S., & Fiala, M. (2016). Organic production systems: Sustainability assessment of rice in Italy. *Agriculture, Ecosystems & Environment, 225*, 33–44. https://doi.org/10.1016/j.agee.2016.03

Ben Youssef, S., Fakhfakh, J., Tchoumtchoua, J., Halabalaki, M., & Allouche, N. (2016). sucrose, efficient purification and complete NMR characterization of galactinol, raffinose, and stachyose isolated from *Pinus halepensis* (Aleppo pine) seeds using acetylation procedure. *Journal of Carbohydrate Chemistry, 35*(4), 224–237. https://doi.org/10.1080/07328303.2016.1207781

Bliziotis, N. G., Engelke, U. F. H., Aspers, R. L. E. G., Engel, J., Deinum, J., Timmers, H. J. L. M., Wevers, R. A., & Kluijtmans, L. A. J. (2020). A comparison of high-throughput plasma NMR protocols for comparative untargeted metabolomics. *Metabolomics : Official Journal of the Metabolomic Society, 16*(64). https://doi.org/10.1007/s11306-020-01686-y

Brigante, F. I., Solovyev, P., Fabroni, S., Campanelli, G., Montemurro, F., & Bontempo, L. (2025). Classification of farming systems by NMR widely targeted metabolomics: A cauliflower case study. *Journal of Food Composition and Analysis, 138*, Article 107018. https://doi.org/10.1016/j.jfca.2024.107018

Chang, E. H., Zhang, S. F., Wang, Z. Q., Wang, X. M., & Yang, J. C. (2008). Effect of nitrogen and phosphorus on the amino acids in root exudates and grains of rice during grain filling. *Acta Agronomica Sinica, 34*(4), 612–618. https://doi.org/10.1016/S1875-2780(08)60024-3

Cheng, B., Shi, S., Pan, K., Nie, J., Xing, J., Wang, X., Li, L., Tang, J., Liu, J., Cao, C., & Jiang, Y. (2023). Untargeted metabolomics based on UHPLC-Q-exactive-ms reveals metabolite and taste quality differences between Koshihikari Rice from China and Japan. *International Journal of Gastronomy and Food Science, 31*, Article 100680. https://doi.org/10.1016/j.ijgfs.2023.100680

Chuan-ying, R., Shu-wen, L., Li-jun, G., Bin, H., Ying-lei, Z., Wen-gong, H., Bo, L., Wei, L., & Wei-hong, L. (2022). The metabolomics variations among rice, brown rice, wet germinated brown rice, and processed wet germinated brown rice. *Journal of Integrative Agriculture, 21*(9), 2767–2776. https://doi.org/10.1016/j.jia.2022.07.025

Ch, R., Chevallier, O., McCarron, P., McGrath, T. F., Wu, D., Nguyen Doan Duy, L., Kapil, A. P., McBride, M., & Elliott, C. T. (2021). Metabolomic fingerprinting of volatile organic compounds for the geographical discrimination of rice samples from China, Vietnam and India. *Food Chemistry, 334*, Article 127553. https://doi.org/10.1016/j.foodchem.2020.127553.046

Consonni, R., Polla, D., & Cagliani, L. R. (2018). Organic and conventional coffee differentiation by NMR spectroscopy. *Food Control, 94*, 284–288. https://doi.org/10.1016/j.foodcont.2018.07.013

Consonni, R., Bernareggi, F., & Cagliani, L. R. (2019). NMR-based METABOLOMIC approach to differentiate organic and conventional Italian honey. *Food Control, 98*, 133–140. https://doi.org/10.1016/j.foodcont.2018.11.007

Davoren, E., & Mason, S. (2023). 1H-NMR protocol for rapid diagnosis of purine and pyrimidine metabolic disorders in urine. *STAR Protocols, 4*(2), Article 102181. https://doi.org/10.1016/j.xpro.2023.102181

Deborde, C., Fontaine, J. X., Jacob, D., Botana, A., Nicaise, V., Richard-Forget, F., Lecomte, S., Decourtil, C., Hamade, K., Mesnard, F., Moing, A., & Moliné, R. (2019). Optimizing 1D 1H-NMR profiling of plant samples for high throughput analysis: Extract preparation, standardization, automation and spectra processing. *Metabolomics : Official Journal of the Metabolomic Society, 15*(28). https://doi.org/10.1007/s11306-019-1488-3

Dinesh Babu, P., Subhasree, R. S., Bhakyaraj, R., & Vidhyalakshmi, R. (2009). Brown Rice-beyond the color reviving a lost health food - A review. *American-Eurasian Journal of Agronomy, 2*(2), 67–72.

Erich, S., Schill, S., Annweiler, E., Waiblinger, H. U., Kuballa, T., Lachenmeier, D. W., & Monakhova, Y. B. (2015). Combined chemometric analysis of 1H NMR, 13C NMR and stable isotope data to differentiate organic and conventional milk. *Food Chemistry, 188*, 1–7. https://doi.org/10.1016/j.foodchem.2015.04.118

European Commission. (2021). Communication from the commission to the european parliament, the council, the European economic and social committee and the committee of the regions empty on an action plan for the development of organic production. *COM*, 141 final, 25 March 2021 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52021DC0141R%2801%29 [accessed 29 July 2024].

Fathi, F., Brun, A., Rott, K. H., Falco Cobra, P., Tonelli, M., Eghbalnia, H. R., Caviedes-Vidal, of Metabolites E, Karasov, W. H., & Markley, J. L. (2017). NMR-based identification in polar and non-polar extracts of avian liver. *Metabolites, 7*(4), 61. https://doi.org/10.3390/metabo7040061

Foster-Powell, K., Holt, S. H., & Brand-Miller, J. C. (2002). International table of glycemic index and glycemic load values: 2002. *The American Journal of Clinical Nutrition, 76*(1), 5–56. https://doi.org/10.1093/ajcn/76.1.5

Gondal, T. A., Keast, R. S., Shellie, R. A., Jadhav, S. R., Gamlath, S., Mohebbi, M., & Liem, D. G. (2021). Consumer acceptance of brown and white rice varieties. *Foods, 10*(8), 1950. https://doi.org/10.3390/foods10081950

Hohmann, M., Monakhova, Y., Erich, S., Christoph, N., Wachter, H., & Holzgrabe, U. (2015). Differentiation of organically and conventionally grown tomatoes by chemometric analysis of combined data from Proton Nuclear magnetic resonance and mid-infrared spectroscopy and stable isotope analysis. *Journal of Agricultural and Food Chemistry, 63*(43), 9666–9675. https://doi.org/10.1021/acs.jafc.5b03853

Huo, Y., Kamal, G. M., Wang, J., Liu, H., Zhang, G., Hu, Z., Anwar, F., & Du, H. (2017). 1H NMR-based metabolomics for discrimination of rice from different geographical origins of China. *Journal of Cereal Science, 76*, 243–252. https://doi.org/10.1016/j.jcs.2017.07.002

Ingallina, C., Di Matteo, G., Spano, M., Acciaro, E., Campiglia, E., Mannina, L., & Sobolev, A. P. (2023). Byproducts of globe artichoke and cauliflower production as a new source of bioactive compounds in the green economy perspective: An NMR study. *Molecules, 28*, 1363. https://doi.org/10.3390/molecules28031363

Kamal, G. M., Uddin, J., Bin Muhsinah, A., Wang, X., Noreen, A., Sabir, A., & Musharraf, S. G. (2022). 11H NMR-based metabolomics and 1313C isotopic ratio evaluation to differentiate conventional and organic soy sauce. *Arabian Journal of Chemistry, 15*(1), Article 103516. https://doi.org/10.1016/j.arabjc.2021.103516

Kamjijam, B., Bednarz, H., Suwannaporn, P., Na Jom, K., & Niehaus, K. (2020). Localization of amino acids in germinated rice grain: Gamma-aminobutyric acid and essential amino acids production approach. *Journal of Cereal Science, 93*. https://doi.org/10.1016/j.jcs.2020.102958. Article 102958.

Katt, F., & Meixner, O. (2020). A systematic review of drivers influencing consumer willingness to pay for organic food. *Trends in Food Science & Technology, 100*, 374–388. https://doi.org/10.1016/j.tifs.2020.04.029

Lalwani, A. M., Yilmaz, A., Bisgin, H., Ugur, Z., Akyol, S., & Graham, S. F. (2020). The biochemical profile of post-mortem brain from people who suffered from epilepsy reveals novel insights into the etiopathogenesis of the disease. *Metabolites, 10*, 261. https://doi.org/10.3390/metabo10060261

Lee, J. H., Woo, K. S., Jo, C., Jeong, H. S., Lee, S. K., Lee, B. W., Lee, Y. Y., Lee, B., & Kim, H. J. (2019). Quality evaluation of rice treated by high hydrostatic pressure and atmospheric pressure plasma. *Journal of Food Quality, 2019*. https://doi.org/10.1155/2019/4253701. Article 4253701.

Lucarini, M., Di Cocco, M. E., Raguso, V., Milanetti, F., Durazzo, A., Lombardi-Boccia, G., Santini, A., Delfini, M., & Sciubba, F. (2020). NMR-based metabolomic comparison of brassica oleracea (Var. italica): Organic and conventional farming. *Foods, 9*, 945. https://doi.org/10.3390/foods9070945

Martin, M., Legat, B., Leenders, J., Vanwinsberghe, J., Rousseau, R., Boulanger, B., Eilers, P. H. C., De Tullio, P., & Govaerts, B. (2018). PepsNMR for ˆ1H NMR metabolomic data pre-processing. *Analytica Chimica Acta, 1019*, 1–13. https://doi.org/10.1016/j.aca.2018.02.067

Nagana Gowda, & Raftery, D. (2021). NMR-Based metabolomics. *Advances in Experimental Medicine and Biology, 1280*, 19–37. https://doi.org/10.1007/978-3-030-51652-9_2

Ning, H., Qiao, J., Liu, Z., Lin, Z., Li, G., Wang, Q., Wang, S., & Ding, Y. (2010). Distribution of proteins and amino acids in milled and brown rice as affected by nitrogen fertilization and genotype. *Journal of Cereal Science, 52*(1), 90–95. https://doi.org/10.1016/j.jcs.2010.03.009

R Core Team. (2024). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rahman, S. M. E., Mele, M. A., Lee, Y. T., & Islam, M. Z. (2021). Consumer preference, quality, and safety of organic and conventional fresh fruits, vegetables, and cereals. *Foods, 10*(1), 105. https://doi.org/10.3390/foods10010105

Roslund, M. U., Tähtinen, P., Niemitz, M., & Sjöholm, R. (2008). Complete assignments of the 1H and 13C chemical shifts and *J*H,H coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopyranosides. *Carbohydrate Research, 343*(1), 101–112. https://doi.org/10.1016/j.carres.2007.10.008

Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A., MacDonald, P., Wishart, D., Li, S., & Xia, J. (2024). MetaboAnalyst 6.0: Towards a unified platform for metabolomics data processing, analysis and interpretation. *Nucleic Acids Research, 52*(W1), W352–W361. https://doi.org/10.1093/nar/gkae253

Sirisoontaralak, P., Keatikasemchai, S., Mancharoen, C., & Nakornpanom, N. (2020). Development of lightly milled black rice with easy cooking and retaining health benefits. *Journal of Food Science and Technology, 57*, 3762–3771. https://doi.org/10.1007/s13197-020-04408-3

Song, E. H., Kim, H. J., Jeong, J., Chung, H. J., Kim, H. Y., Bang, E., & Hong, Y. S. (2016). A 1H HR-MAS NMR-based metabolomic study for metabolic characterization of rice grain from various oryza sativa L. cultivars. *Journal of Agricultural and Food Chemistry, 64*(15), 3009–3016. https://doi.org/10.1021/acs.jafc.5b05667

Sundekilde, U. K., Eggers, N., & Bertram, H. C (2019). NMR-based metabolomics of food. *NMR-Based Metabolomics*, 335–344. https://doi.org/10.1007/978-1-4939-9690-2_18

Thomas, S., Aneena, E. R., Pathrose, B., Thomachan Panjikkaran, S., Sharon, C. L., Prameela, P., Ajisha, K. H., & Athira, K. A. (2023). Augmentation of gamma amino butyric acid (GABA), antioxidant potential and nutrient qualities in germinated brown rice. *Food and Humanity, 1*, 349–357. https://doi.org/10.1016/j.foohum.2023.06.010

Uawisetwathana, U., & Karoonuthaisiri, N. (2019). Metabolomics for rice quality and traceability: Feasibility and future aspects. *Current Opinion in Food Science, 28*, 58–66. https://doi.org/10.1016/j.cofs.2019.08.008

Vaglia, V., Bacenetti, J., Orlando, F., Alali, S., Bosso, E., & Bocchi, S. (2022). The environmental impacts of different organic rice management in Italy considering different productive scenarios. *Science of The Total Environment, 853*, Article 158365. https://doi.org/10.1016/j.scitotenv.2022.158365

Villa-Ruano, N., Rosas-Bautista, A., Rico-Arzate, E., Cruz-Narvaez, Y., Zepeda-Vallejo, L. G., Lalaleo, L., Hidalgo-Martínez, D., & Becerra-Martínez, E. (2020). Study of nutritional quality of pomegranate (punica granatum L.) juice using 1H NMR-based METABOLOMIC approach: A comparison between conventionally and organically grown fruits. *LWT, 134*, Article 110222. https://doi.org/10.1016/j.lwt.2020.110222

Wang, Z., Zhang, M., Liu, G., Deng, Y., Zhang, Y., Tang, X., Li, P., & Wei, Z. (2021). Effect of the degree of milling on the physicochemical properties, pasting properties and in vitro digestibility of Simiao rice. *Grain & Oil Science and Technology, 4*(2), 45–53.

Wishart, D. S., Guo, A., Oler, E., Wang, F., Anjum, A., Peters, H., … Gautam, V. (2022). HMDB 5.0: The human metabolome database for 2022. *Nucleic Acids Research, 50* (D1), D622–D631. https://doi.org/10.1093/nar/gkab1062

Xiao, R., Ma, Y., Zhang, D., & Qian, L. (2018). Discrimination of conventional and organic rice using untargeted LC-MS-based metabolomics. *Journal of Cereal Science, 82*, 73–81. https://doi.org/10.1016/j.jcs.2018.05.012

Zhang, G., Xu, J., Wang, Y., Hua, D., Zhang, H., He, Y., … Sun, J. (2024). A multi-omics study revealed the effect of pulsed light treatment on germinated brown rice: Promotion of sprouting efficiency and gamma-aminobutyric acid enrichment. *Food Bioscience, 61*, 104196. https://doi.org/10.1016/j.fbio.2024.104196

Zhao, M., Zhai, L., Tang, Q., Ren, J., Zhou, S., Wang, H., Yun, Y., Yang, Q., Yan, X., Xing, F., et al. (2024). Comparative metabolic profiling of different colored rice grains reveals the distribution of major active compounds and key secondary metabolites in green rice. *Foods, 13*, 1899. https://doi.org/10.3390/foods13121899