Non-targeted NMR approach to unveil and promote the biodiversity of globe artichoke in the Mediterranean area

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Abstract

The globe artichoke is an important constituent of the Mediterranean diet, being rich in bioactive compounds. Artichoke is widely cultivated in Italy, especially in the Apulia region, with a multitude of local varieties. Most of this genetic material is endangered and its metabolic profile is not yet characterized. In this work, we aimed at dissecting landrace biodiversity by characterizing the metabolic profiles of edible hearts, i.e. the edible inner part of the flower heads, and external bracts of artichoke flower heads, using a simple, fast, and affordable analytical methodology. A non-targeted spectroscopic approach combining NMR experiments and multivariate data analysis provided a comprehensive picture of the chemical composition of 16 artichoke landraces, some of which are at risk of extinction. A special focus was on hydrosoluble compounds, contributing to the functional food value of the artichoke. Moreover, a possible correlation between the metabolic composition and the head color was established.

Our analyses highlight the nutraceutical diversity and value of newly studied artichoke landraces. Specifically, the hearts of the deep purple-colored "Nero del Salento" are rich in both mono- and dicaffeoylquinic acids along with inulin, while the hearts of the green "Bianco di Taranto" and "Centofoglie di Rutigliano" are characterized by a relatively higher content of dicaffeoylquinic acids. The results can help promote endangered local varieties for production and commercialization, against the ongoing genetic erosion and loss of crop diversity.

Keywords:

Cynara cardunculus var. *scolymus*, NMR, Nutraceuticals, Caffeoylquinic acids, Inulin, Flower head color, Mediterranean biodiversity, Multivariate data analysis, Fingerprinting, Class discrimination

Abbreviations

No keyword abbreviations are available

1 Introduction

Nowadays, plants are considered as a source of natural healthy food able to provide, through the diet, not only nutrients but also functional elements with positive effects on human wellness.

The globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] is a typical vegetable crop cultivated in the Mediterranean countries, where its wild progenitor and other species of the genus *Cynara* also grow (Sonnante et al., 2003). This crop can be found with a wide range of local varieties (landraces), especially in Italy (Gatto et al., 2013; Pagnotta et al., 2017; Pavan et al., 2018) and it contributes considerably to the agricultural economy of the Mediterranean area. Italy is the top world producer with about 379,000 t, followed by Egypt and Spain (FAOSTAT-Food and Agriculture Organization of the United Nations Statistics Division. Crops production domain data, 2016).

The globe artichoke is widely used in the Mediterranean diet. Its immature flower head is consumed as a fresh or cooked vegetable and is recognized as a valuable nutrient food. Indeed, artichoke plants are an excellent source of bioactive compounds and nutraceuticals (phytochemicals providing health benefits). Nearly all parts of the plant are rich in polyphenols (flavonoids, phenolic acids, hydroxycinnamic acid derivatives, anthocyanins), lignans, dietary fibers (inulin and pectin), terpenoids (sesquiterpene lactones), as well as saturated and unsaturated fatty acids (Lattanzio et al., 2009; Negro et al., 2012; Pandino et al., 2011). Caffeic acid, chlorogenic acids, and dicaffeoylquinic acids, especially cynarin (1,3-di-*O*-caffeoylquinic acid) are well represented in artichoke. Plants use polyphenols, and hydroxycinnamic acids in particular, for pigmentation, growth, reproduction, resistance to pathogens, and response to environmental stress (Lattanzio et al., 2006). The content and distribution of polyphenols vary according to the genotype/variety and the plant tissue (De Paolis et al., 2008; Lombardo et al., 2010; Negro et al., 2012; Schütz et al., 2004; Sonnante et al., 2010). In particular, it has been observed that both inner bracts and receptacles are extremely rich in polyphenols compared to the external bracts, which contain a lower amount of them (Negro et al., 2012). In addition, the artichoke immature flower heads are rich in inulin, fibers, and minerals. Therefore, the globe artichoke hearts, i.e. the edible inner part of the flower heads, are considered a functional food. The leaves of artichoke plants show a similar chemical composition as the flower head and their extracts have been used for a long time as choleretics, hepatoprotective agents, with antioxidative, diuretic, anticarcinogenic, anti-HIV, as well as antifungal and antibacterial activity (Gebhardt, 2001; Thompson Coon and Ernst, 2003; Zayed et al., 2020). Moreover, polyphenols extracted from the globe artichoke have been proven to possess anti-proliferative activity on different human cancer cells, including hepatoma and breast cancer cells (Miccadei et al., 2008; Mileo et al., 2016). Importantly, only a small part of the globe artichoke flower head is suitable for consumption, and over 60% of the biomass (leaves, stems, external bracts, roots, and seeds) is discarded. The residues deriving from the globe artichoke cultivation and home or industrial processing can be used as animal feed (Martinez Teruel et al., 1998) or for fuel and fiber production (Femenia et al., 1998). In addition, these bio-wastes possess health-promoting effects as they have high phytochemical contents. Thus in recent years, possible exploitation of the artichoke residues for the extraction of beneficial molecules, e.g. antioxidants to be used as food additives or in cosmetics, has been proposed (D'Antuono et al., 2018; Ruiz-Cano et al., 2014; Sánchez-Rabaneda et al., 2003; Zayed and Farag, 2020).

While the usages of artichoke plants are well explored (Bekheet and Sota, 2019), still few ~~relevant~~ analytical approaches are reported as useful for exploring the biodiversity of the metabolites contained in ~~are reported to exploit the metabolite biodiversity of~~ this plant. Plant metabolomics, combining high-throughput analytical chemistry and multivariate data analysis, represents a reliable and powerful tool to study the complexity of phytochemistry, since it allows to measure and compare simultaneously a pool of metabolites from crude natural extracts (Salem et al., 2020; Valentino et al., 2020). Many examples of targeted methods are reported for the identification and quantification of the most common classes of metabolites in artichokes (Fratianni et al., 2007; Pandino et al., 2011; Petropoulos et al., 2018). The data derived from these studies are crucial to define the metabolic composition of metabolites of this plant and to understand the distribution of metabolites in the different plant parts. Nevertheless, metabolomics offers an alternative approach consisting of a non-targeted method, which allows evaluating simultaneously a large number of variables affecting the intrinsic and extrinsic features of the samples under investigation (Gao et al., 2019; Horn et al., 2019; Medina et al., 2019; Shao et al., 2019). An increasing number of~~The~~ applications of non-targeted NMR spectroscopic methods to the analysis of food matrices ~~has been increasingly~~are reported in the ~~recent~~ literature (Ballin and Laursen, 2019; Gallo et al., 2015; Sundekilde et al., 2019; Valentino et al., 2020). Recently, the application of the non-targeted NMR method to the development of food classifiers was reported, unveiling the potential of this analytical approach, even when highly variable spectrometers were employed (Gallo et al., 2020; Musio et al., 2020; Ragone et al., 2020). NMR-based profiling of plant metabolomics is now emerging as a new strategy to characterize different genotypes and plant parts (de Falco et al., 2016; Farag et al., 2018), and great efforts are paid to reach a standardized protocol.

This study aimed at analyzing and valorizing artichoke germplasm, including some newly recovered landraces, for its content in nutraceutical compounds, in order to promote selected varieties for cultivation, sustainable food production and marketing. To this end, a non-targeted NMR method was used to reveal the small variations in the metabolic composition of both the artichoke edible hearts and the discarded outer bracts. Through the identification of a pool of water-soluble metabolites characteristic for each landrace, it was possible to obtain valuable information on the high biodiversity of the genetic resources of the artichoke, correlated to factors of quality, nutritional value, and health

benefits. Moreover, a possible correlation was attempted between the bioactive compounds content and the flower head color.

2 Materials and methods

2.1 Plant ~~Material~~material

Globe artichoke plants were grown at the *ex-situ* field collection held at the Institute of Biosciences and Bioresources (IBBR-CNR, Bari, Italy). Standard agronomic procedures ~~following~~according to local practices were used for irrigation, plant protection, and weed control ~~as described by~~ (Negro et al., 2012). Sixteen local varieties, arranged in rows (one to three for each variety) in the field collection, were considered for this study: ten landraces are traditionally grown in different areas of the Apulia region in southern Italy; the remaining six landraces/varieties of other Italian or European origins were selected to compare Apulian local material with varieties from other provenances. Immature artichoke flower heads were harvested at the commercial stage (see pictures in Table 1) in April-May 2017 and collected in triplicates from each available row (e.g. Moretto_1, Moretto_2, Moretto_3, see results section) of the 16 varieties. Each flower head was separated into external bracts and heart, the latter ~~being constituted by~~consisting of the edible portion, which is the lowest part of internal bracts and the receptacle. Plant material was immediately sliced, ground to a fine powder in liquid nitrogen, stored at – 80 °C, and then freeze-dried. Each lyophilized organ was stored at 4 °C in a sealed plastic bag protected from light and under vacuum until used for analyses. Triplicates of the same sample and organ were combined, for a total of 60 samples, 30 per organ (external bracts and hearts).

alt-text: Table 1

Table 1

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List of the artichoke local varieties sampled for NMR analyses.[Instruction: Table 1: We noted that artichoke images are of different size, could you please enlarge/reduce it to make each picture of the same size?]

~~Landrace~~Landrace	~~Color of outer bracts~~Color of outer bracts	~~Morpho-agronomical classification~~Morpho-agronomical classification ~~a~~	~~Origin~~Origin	~~Number of rows in the collection field~~Number of rows in the collection field	~~Flower head~~Flower head
Bianco di Taranto	Green	out	Italy_Apulia	1	
Verde di Putignano	Green	out	Italy_Apulia	3	
Verde di Castellana	Green	out	Italy_Apulia	1	
Centofoglie di Rutigliano	Green	out	Italy_Apulia	1	
Scapoli-Isernia	Green	Romanesco	Italy_Molise	3	
Bianco di Pertosa	Green	Romanesco	Italy_Campania	1	
Violetto di Putignano	Purple	out	Italy_Apulia	1	
Locale di Calimera	Purple	out	Italy_Apulia	2	
Nero del Salento	Purple	out	Italy_Apulia	3	
Locale di Supersano	Purple	out	Italy_Apulia	2	
Nero di Ostuni	Purple	out	Italy_Apulia	2	
Moretto	Purple	Violetto	Italy_Emilia Romagna	3	

					
Violetto di Toscana	Purple	Violetto	Italy_Toscana	2	
Brindisino	Intermediate	Catanese	Italy_Apulia	1	
Catanese	Intermediate	Catanese	Italy_Sicilia	1	
Salanquet	Intermediate	Romanesco	France	3	

Table Footnotes

[a] Sonnante et al. (2002); Out: outgroup.

2.2 Chemicals

3-(Trimethylsilyl)− 2,2,3,3-tetradeutero-propionic acid sodium salt (TSP-d_4, CAS N. 24493−21−8, 99%D, Armar Chemicals, Döttingen, Switzerland), sodium azide (NaN_3, CAS N. 26628−22−8; ≥99.5%, Sigma-Aldrich, Milan, Italy), deuterium oxide (D_2O, CAS. N. 7789−20−0, 99.86%D, Eurisotop, Saclay, France), and dimethyl sulfoxide-d_6 (DMSO-d_6, CAS. N. CAS Number 2206−27−1, 99.9%D, Eurisotop) were used for the preparation of NMR samples. NMR tubes (Norell 509-UP 7) were provided by Norell, Landisville NJ, US.

Hydrochloric acid (HCl, 37%, CAS Number ~~7647-01-0,~~7647−01−0, Sigma-Aldrich), sodium oxalate (NaOOCCOONa, ≥99.5%, CAS Number ~~62-76-0,~~62−76−0, Sigma-Aldrich). Water (H_2O, CAS Number ~~7732-18-5,~~7732−18−5, Sigma-Aldrich) was doubly deionized (resistivity: ~~18 MΩ·cm)~~ 18 MΩ·cm) by using a Milli-Q water purification system (Merck Millipore, Darmstadt, Germany).

2.3 NMR measurements

From each sample, ~~25~~25 mg of artichoke lyophilized powder was placed into a test tube. The sample solution was prepared by adding ~~1.5~~1.5 mL of oxalate buffer at pH 4.2 (pH value was reached after addition of 37% HCl to ~~100~~100 mL of an aqueous solution containing ~~0.25~~0.25 M of $Na_2C_2O_4$ and $2.5 \cdot 10^{-3}$ M of NaN_3), then submitted to sonication at ~~40~~40 kHz for ~~5~~5 min, shaken for ~~1~~1 min in a VORTEX at ~~2500~~2500 rpm, and centrifuged (Ettich Rotofix ~~32~~32 A, ~~A, 4700~~ 4700 g, ~~g, 15~~ 15 min). Using an automated system for liquid handling (SamplePro Tube, Bruker BioSpin) the NMR tubes were filled in with ~~630~~630 µL of the supernatant solution and ~~70~~70 µL of 0.20% of a TSP solution in D_2O. The stability of the sample prepared according to this procedure was checked over ~~24~~24 h to exclude any possible changes in metabolic composition during the duration of the analysis (see Supplementary Material for further details, Fig. S1). A Bruker Avance ~~400~~400 MHz spectrometer equipped with a ~~5~~5 mm inverse probe and a BACS autosampler was employed to perform the 1D ^{1}H NOESY NMR experiments implemented with a selective pre-saturation step to remove the residual water signal. The following acquisition parameters were used: pulse program (noesygppr1d); the size of FID (TD, ~~64~~64 K); spectral width (SW, ~~8013~~8013 Hz); transmitter offset (ca. ~~4.70 ppm;~~ 4.70 ppm, exact chemical shift value was set on the residual water signal); 90° hard pulse (p1, optimized by automatic procedure keeping the pulse length as short as possible (< ~~10 µs);~~ 10 µs); dummy scans (ds, 4); the number of scans (ns, 32); loop count for 'td0' (TD0, 4); mixing time (d8, ~~10 ms);~~ 10 ms); recycle delay (d1, ~~3~~3 s); presaturation (pl9, calculated by command "pulse ~~25~~25 Hz" after optimization of p1). The repeatability of the NMR analysis performed upon application of the described acquisition parameters was checked through the statistical analysis of ten replicated experiments. For two selected signals at ~~2.96 ppm~~ 2.96 ppm for asparagine and ~~3.19 ppm~~ 3.19 ppm for choline the value of Coefficient of Variation (CV%) was calculated as an index of repeatability. The following formula was used: CV% ~~= (σ/µ) × 100,~~ = (σ/µ) × 100, where σ and µ stand for standard deviation and mean, respectively (see Supplementary Material for further details, Fig. S2).

Each spectrum was acquired using TOPSPIN 2.1 software (Bruker BioSpin GmbH, Rheinstetten, Germany) under an automatic procedure that lasts around ~~22~~22 min and encompasses sample loading, temperature stabilization for ~~5~~5 min, tuning, matching, and shimming. Free induction decays (FIDs) were Fourier transformed by using MestreNova; the phase and the baseline were automatically corrected, and the spectra were referenced to the ~~TSP~~TSP-d_4 singlet ~~(0.00 ppm).~~(0.00 ppm).

2.4 Pre-treatment of raw data for the statistical analysis

The raw data (FIDs) relative to the 1D ^{1}H NOESY NMR experiments were processed by a single operator using Mestrelab and segmented into regular-sized (0.04 ppm) intervals (buckets) in the range of [9.50, 0.50] ppm. The underlying area of each bucket was calculated and normalized to the total intensity. The areas of the buckets in the

region [5.10, 4.15] ppm, corresponding to the residual water signal, were set to 0. The data matrices were imported into MetaboAnalyst 5.0, and buckets were subjected to mean-centering and divided by the standard deviation of each variable (Unit Variance scaling). Multivariate statistical analyses were performed: Principal Component Analysis (PCA), Hierarchical Clustering Dendrogram (HCD), Partial Least Square-Discriminant Analysis (PLS-DA). PCA and HCD were used to have an overview of the data. PLS-DA was used as a supervised method that uses multivariate regression techniques to extract via a linear combination of original variables (X) the information that can predict the class membership (Y). The PLS regression is performed using the *plsr* function provided by R *pls* package (Wehrens, 2007). The classification and cross-validation are performed using the corresponding wrapper function offered by the caret package. To assess the significance of class discrimination, a permutation test is performed. In each permutation, a PLS-DA model is built between the data (X) and the permuted class labels (Y) using the optimal number of components determined by cross-validation for the model based on the original class assignment. MetaboAnalyst supports two types of test statistics for measuring class discrimination. The first one is based on prediction accuracy during training. The second one is separation distance based on the ratio of the between the group sum of the squares and the within the group sum of squares (B/W-ratio). If the observed test statistic is part of the distribution based on the permuted class assignments, the class discrimination cannot be considered significant from a statistical point of view (Bijlsma et al., 2006).

3 Results

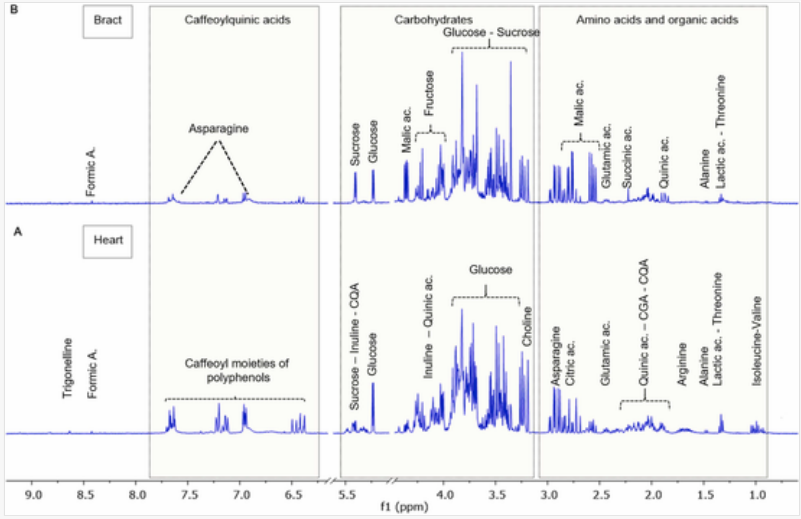
3.1 Metabolite ~~profiling~~identification and statistical analysis

The set of artichoke samples under investigation was opportunely selected in a way to explore the metabolic diversity of local varieties mainly cultivated in the Apulia region, a major area for artichoke production not only in Italy but also at the international level. As listed in Table 1, ten different Apulian landraces were investigated: "Bianco di Taranto", "Verde di Putignano", "Verde di Castellana", "Centofoglie di Rutigliano", "Violetto di Putignano", "Locale di Calimera", "Nero del Salento", "Locale di Supersano", "Nero di Ostuni", and "Brindisino". Additionally, five varieties from other Italian regions were considered, namely "Scapoli Isernia", "Bianco di Pertosa", "Violetto di Toscana", "Moretto", and "Catanese". Also, one variety of French origin, namely "Salanquet", was analyzed. In general, artichokes are classified into the following four main varietal groups according to morpho-agronomical traits, such as harvest time and flower head structure: "Spinosi", "Violetti", "Catanesi", and "Romaneschi", although much local germplasm does not fall in any of these categories (Sonnante et al., 2002). All investigated varieties have diverse morpho-agronomical characteristics, including a different color of the head outer bracts (Table 1), which can be considered an important commercial trait.

The spectroscopic analysis showed that both the external bracts and the hearts are rich in hydrosoluble metabolites, though they distribute differently within the two parts of the plant (Fig. 1) and according to the landrace (see Supplementary Material for further details, Fig. S3). The identification of the metabolites~~e profile~~ based on the chemical shifts and the multiplicity of the signals in the 1D ^{1}H NOESY NMR spectra, along with 2D COSY experiments (see Supplementary Material for further details, Fig. S4), was established by comparison with spectra of reference compounds. In the case of polyphenolic metabolites, the assignments of the signals were accomplished by comparison with data reported in the HMDB Database (Wishart et al., 2018) and the literature (D'Amelio et al., 2015; de Falco et al., 2016).

alt-text: Fig. 1

Fig. 1



Typical 1D ^{1}H NOESY NMR spectra normalized to the total intensity of the aqueous extracts of the inner parts, i.e. hearts (A) and the external bracts (B) of the artichoke head. The rectangles indicate the typical spectral regions containing the signals assigned to the main classes of metabolites found in hearts (A) and external bracts (B). CQA:caffeoylquinc acid; CGA: chlorogenic acid.

The aqueous extracts contained a pool of free amino acids, like isoleucine, valine, threonine, alanine, arginine, glutamic acid, and asparagine, the majority being classified as essential amino acids. The composition in terms of organic acids was also noticeable, as demonstrated by the presence of lactic, quinic, succinic, malic, citric, fumaric, and formic acids. The main sugars detected in the NMR spectra were glucose, sucrose, and inulin as ascertained by the doublets at 5.23, 5.40, and ~~5.43 ppm~~ 5.43 ppm attributed to α-glucose, sucrose, and inulin, respectively (see Supplemental material for further details, Table S1). Among these energy sources for human health, taking into account the intensity of the abovementioned signals and the molar weights (glucose < sucrose < inulin), it can be stated that glucose was the most representative sugar, independently of the tested varieties.

All the analyzed samples contained a range of hydroxycinnamic derivatives, the major chemical components of polyphenolic compounds in artichoke (Lattanzio et al., 2009). Among them, chlorogenic acids (3-caffeoylquinic and 5-caffeoylquinic acids), and dicaffeoylquinic acids (1,3-dicaffeoylquinic, 1,5-dicaffeoylquinic and 3,5-dicaffeoylquinic acids) were identified.

Also, the sampled artichokes contained a variety of other metabolites such as choline, betaine, uridine, and trigonelline, which were distributed in both parts of the flower head.

The data derived from sixty 1D ^{1}H NOESY NMR spectra (30 for each sample considered, including hearts and external bract samples) were investigated through multivariate data analysis (MVDA) to detect the main general differences in metabolic composition between the external bracts and the hearts, without taking into account the ~~origin~~landrace and the origin of each sample. The unsupervised Principal Component Analysis (PCA) (see Supplementary Materials for further details, Fig. S5) and the supervised Partial Least Squares Discriminant Analysis (PLS-DA) revealed two main clusterings along component 1 (Fig. 2~~A).~~ A). These two groups correspond to the two parts of the plant, namely the external bracts and the hearts. The variables ~~(0.04 ppm~~ (0.04 ppm sized buckets of the spectra), and the metabolites which exerted the highest contribution to the two groups of samples could be defined by measuring the Variable Importance in Projection (VIP) (Fig. 2B). Specifically, the heart samples were characterized by high levels of carbohydrates, and, in particular, glucose and sucrose. Moreover, among all the amino acids found, the bucket containing the signal of isoleucine was found to be a variable with a relevant VIP. Among the caffeoylquinic acid derivatives, 5-caffeoylquinic acid (5-CQA) was more abundant in the inner part of the artichoke, while 4,5-caffeoylquinic acid content was relatively higher in the external bracts (Fig. 2B).

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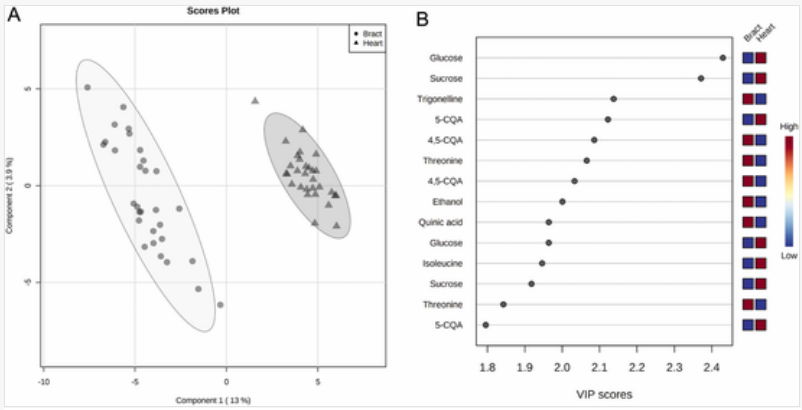
Fig. 2



PLS-DA applied to the 30 spectra of artichoke external bracts and the 30 spectra of hearts by using UV-scaled 0.04 ppm-sized bucketing. A) Scores plot Component 1 ~~vs~~vs Component 2 where the observations are indicated as follows: external bracts as circles; hearts as triangles. The ellipse represents the 95% confidence region. The explained variances are shown in brackets. B) VIP scores containing the variables which contributed significantly to the distribution of the external bracts and the heart samples according to the PLS-DA model along the PC1. The colored boxes on the right indicate the relative concentration of the corresponding metabolite in each group under study.

~~Indeed~~On the other hand, the distribution of the ~~observed distribution of the~~external bracts ~~samples related to external bract~~ ~~was~~ was associated with the spectral regions containing the signals assigned to quinic acid, trigonelline, threonine, and ethanol.

3.2 Analysis of external bracts

A PCA and a Hierarchical Clustering Dendrogram (HCD) were carried out on the aqueous extracts of the external bracts to extrapolate information ~~regarding~~on the metabolic composition of the ~~studied~~ varieties under investigation. PCA was performed on the 30 regular-sized spectra ~~(0.04 ppm)~~ (0.04 ppm) relative to the external bracts. As a result, the samples were distributed mainly along PC1 (Fig. 3A).

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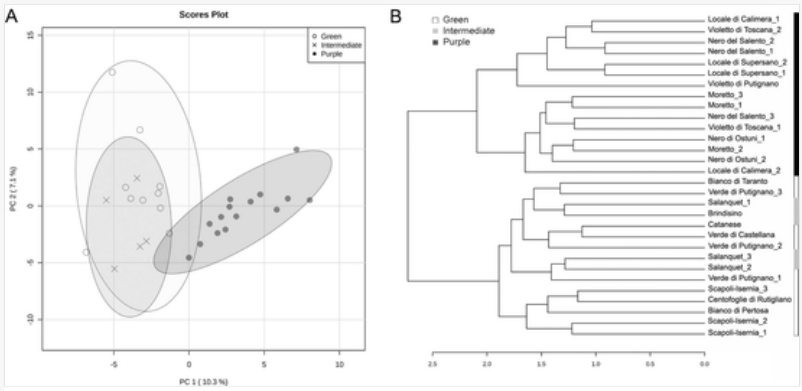
Fig. 3



PCA was applied to the 30 spectra of external bracts by using UV-scaled 0.04 ppm-sized bucketing. A) Scores plot PC1 ~~vs~~vs PC2 where the scores are indicated as follows: circles, crosses, black circles for green, intermediate and purple colored external bracts, respectively. B) Hierarchical Clustering Dendrogram (HCD) applied on the 30 regular sized spectra of external bracts, using the following parameters: distance measure, Spearman; clustering algorithm, Average.

The observed clustering is in perfect accordance with the color of the bracts, as described in Table 1. Also, HCD revealed two main groups: the first cluster contained samples characterized by purple external bracts, and the second one consisted of the samples with green or intermediate colored bracts (Fig. 3B).

PLS-DA was ~~carried out~~performed to facilitate the identification of ~~the~~ metabolites that contribute most ~~contributing~~ to the distribution of ~~the~~ samples~~, enabling the discrimination between the three color-based groups of samples~~ based on the color of the external bracts. The model was validated by applying the 10-fold cross-validation method to exclude any overfitting. As depicted in the scores plot (Fig. 4~~A),~~ A), the samples were mainly distributed along component 1.

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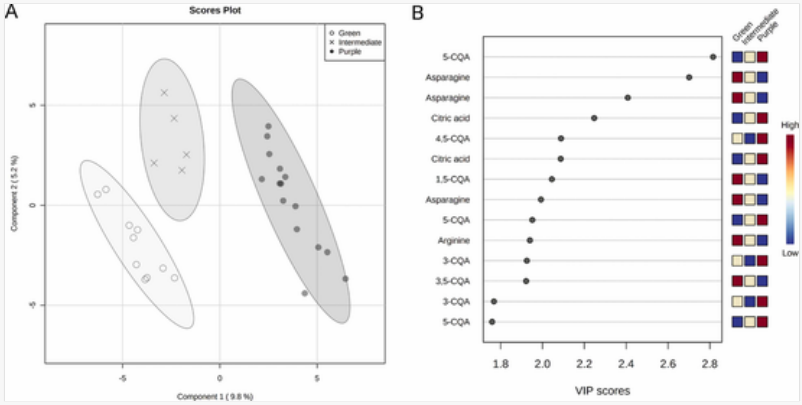
Fig. 4



PLS-DA was applied to the 30 spectra of external bracts by using UV-scaled 0.04 ppm-sized bucketing. A) Score plots Component 1 ~~vs~~vs Component 2, where the observations are indicated as follows: circles, crosses and black circles for green, intermediate and purple colored external bracts, respectively. Ellipse indicates the confidence region (95%). B) VIP scores plot containing the variables which contributed significantly to the distribution of the bracts according to the PLS-DA model along the PC1. The colored boxes on the right indicate the relative concentration of the corresponding metabolite in each group under study.

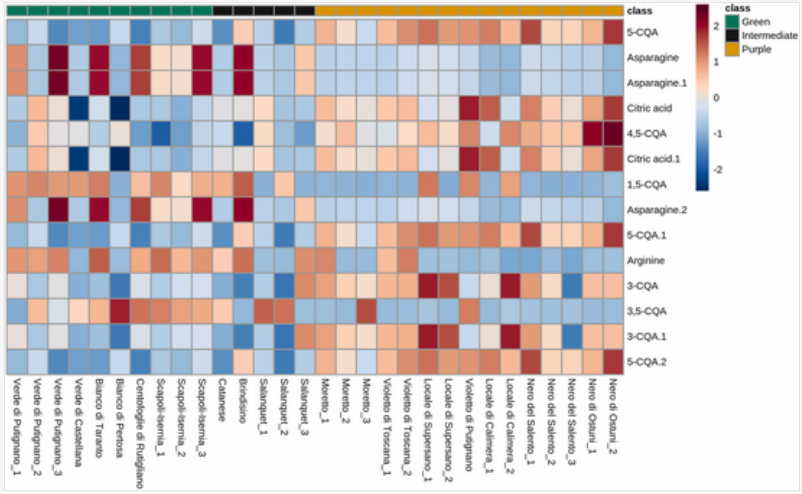
The identification of the most influential metabolites in the observed PLS-DA scores distribution was obtained by combining the information coming from the corresponding loadings (see Supplementary Material for further details, Fig. S6B) and the VIP scores (see Supplementary Material for the full list of variables with VIP >1, Table S2). Fourteen buckets, corresponding to eight metabolites, were ~~detected~~ idendified among the variables ~~which~~that contributed ~~more relevantly~~most significantly to the ~~pattern~~distribution of the ~~PLS-DA scores plot~~samples along component 1. As summarized in Fig. 4B, hydroxycinnammic acids significantly influenced the grouping of samples related to bract color. Specifically, for purple samples, the buckets containing the signals of the following metabolites showed a VIP value higher than 1.76: 5-caffeoylquinic, 4,5-dicaffeoylquinic, and 3-caffeoylquinic acids. On the other hand, samples deriving from the green outer bracts were characterized by a high level of 1,5-dicaffeoylquinic, and 3,5-dicaffeoylquinic acids.

Further metabolites with VIP ~~>1.80~~≥ 1.80 were asparagine, citric acid, and arginine. Asparagine and arginine were found predominantly in the green outer bracts, while citric acid was mainly present in the purple ones. The analysis of the hierarchical clustering heatmap shows the correlations between the most contributing variables to the PLS-DA scores pattern according to the VIP values and the samples (Fig. 5). This heatmap helped detect the most evident differences in the metabolic composition among the studied varieties. As a result, among the landraces with purple external bracts, "Locale di Supersano" and "Locale di Calimera" were particularly rich in 3-caffeoylquinc acid. "Nero del Salento" and "Nero di Ostuni" contained high levels of citric acid and 5-caffeoylquinic acid. Among the green samples, "Verde di Putignano", "Bianco di Taranto" and "Centofoglie di Rutigliano" were the local varieties richest in asparagine. Among the varieties characterized by intermediate-colored bracts, "Brindisino" was rich in both arginine and asparagine, and had a high level of 5-caffeoylquinc and 1,5-dicaffeoylquinic acids. "Salanquet", the artichoke variety of French origin, contained high levels of 3,5-dicaffeoylquinic acid.

Images are optimised for fast web viewing. Click on the image to view the original version.

alt-text: Fig. 5

Fig. 5



Hierarchical Clustering Heatmap on the normalized data related to external bracts of artichoke flower heads. Distance measure: Pearson; clustering algorithm: Ward. Each colored cell on the map corresponds to a concentration value in the data table, with samples in columns and metabolites in rows.

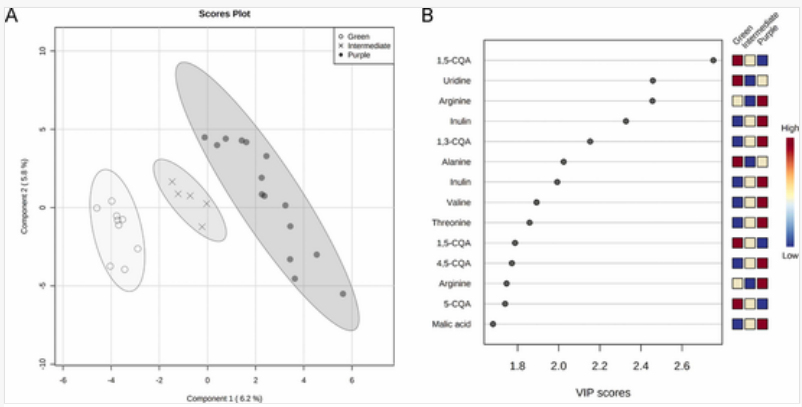
3.3 Analysis of hearts

The high biodiversity of the globe artichoke plants was confirmed also when the unsupervised PCA (see Supplementary Materials for further details, Fig. S7) and the supervised PLS-DA (Fig. 6~~A)~~ A) were performed on the 30 samples of the artichoke hearts. Upon removal of one sample which behaved as a strong outlier according to the Hotelling's test ("Verde di Castellana"), the remaining samples were distributed predominantly along component 1.

Images are optimised for fast web viewing. Click on the image to view the original version.

alt-text: Fig. 6

Fig. 6



PLS-DA was applied to the 0.04 ppm regular-sized buckets obtained upon UV-scaling from the 1D ^{1}H NOESY NMR spectra of 29 heart samples belonging to 15 different varieties. Scores plot Component 1 ~~vs~~vs Component 2. A) Scores plot Component 1 ~~vs~~vs Component 2, where the observations are indicated as follows: circles, crosses and black circles for heart samples obtained from green, intermediate and purple colored heads, respectively. Ellipse indicates the confidence region (95%). The explained variances are shown in brackets. B) VIP scores plot containing the variables which contributed significantly to the distribution of the hearts

according to the PLS-DA model along the PC1. The colored boxes on the right indicate the relative concentration of the corresponding metabolite in each group under study.

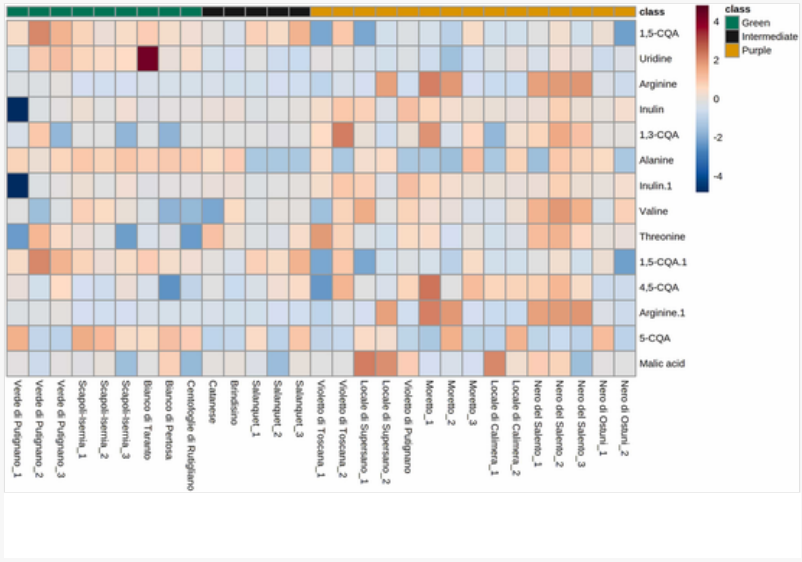
The analysis of the loadings (see Supplementary Material for further details, Fig. S8B) along with the VIP scores (Fig. 6B; Table S3) demonstrated that the heart samples collected from artichokes with green external bracts were particularly rich in 1,5-dicaffeoylquinic and 5-caffeoylquinic acids, ~~along with~~ uridine, and alanine. Among the caffeoyl derivatives, 1,3-dicaffeoylquinic and 4,5-dicaffeoylquinic acids were well represented in the heart samples with purple outer bracts. Also, amino acids such as arginine, valine, and threonine were contained at high levels in this cluster of samples. Importantly, the heart samples from purple artichokes were particularly rich in inulin.

The analysis of the hierarchical clustering heatmap (Fig. 7) helped to unveil some slight variations in the metabolic composition of the different local varieties under investigation, confirming the biodiversity of such landraces. It was found that the hearts of green artichoke varieties were richer in 5-caffeoylquinic acid, 1,5-dicaffeoylquinic acid, and uridine. Among this group of samples, "Bianco di Taranto" was the variety with the highest content of uridine. ~~Within~~Among the heart samples of purple artichokes~~,~~ "Nero del Salento", "Moretto", and "Locale di Supersano" had the highest levels of malic acid and amino acids, including ~~like~~ arginine, valine, and threonine~~, and malic acid~~. ~~Besides~~Furthermore, 1,3-dicaffeoylquinic acid was well represented in the local variet~~y~~ies of "Violetto di Toscana" and "Nero del Salento".

Images are optimised for fast web viewing. Click on the image to view the original version.

alt-text: Fig. 7

Fig. 7



Hierarchical Clustering Heatmap on the normalized NMR data related to the hearts of artichoke flower heads. Distance measure: Pearson; clustering algorithm: Ward. Each colored cell on the map corresponds to a concentration value in the data table, with samples in columns and metabolites in rows.

Also, the statistical results of the hierarchical clustering heatmap highlighted that inulin (VIP > 2.3, Fig. 6B) was distributed in all the heart samples studied, though a higher level could be detected in the artichoke heads with purple external bracts.

4 Discussion

The globe artichoke biodiversity in the Mediterranean area, and in particular in Italy, is broadly recognized, but it suffers from a high risk of erosion, due to a limited number of varieties vegetatively propagated and grown. In southern Italy and especially in Apulia, where artichoke local varieties are still cultivated and coexist with modern seed cultivars, many artichoke ecotypes are neglected and at risk of extinction (~~Accogli Rita et al., 2018~~Accogli et al., 2018). The valorization of local varieties for their peculiar nutritional quality aims at preserving their cultivation for sustainable healthy food production (Conversa et al., 2020). Therefore, ~~F~~efficient and reliable analytical methods are needed~~therefore necessary~~, such as the non-targeted NMR ~~non-targeted~~ approach, ~~the~~whose extraordinary potential ~~of which re~~lies ~~i~~on the rapidity of the analysis and reproducibility of the results (Gallo et al., 2015).

We used a non-targeted NMR approach to define the metabolic ~~profile~~composition and nutritional value of 16 local varieties, some of which are at a high risk of extinction, and promote their sustainable production and

commercialization. ~~The a~~Analysis of ~~the~~ NMR spectra revealed a different distribution of ~~hydrosoluble~~water-soluble metabolites in artichoke hearts and external bracts. The aqueous extracts contained primary metabolites (amino acids, organic acids, and sugars) along~~side~~ with ~~the~~ secondary metabolites, mainly caffeoylquinic acids, as typically found in artichoke natural extracts (de Falco et al., 2016; Negro et al., 2012; Pandino et al., 2011). ~~Mostly e~~Essential amino acids were found, among which arginine is considered a conditionally essential amino acid, as the human body ~~cannot~~is unable to sufficiently synthesize it during pregnancy, adolescent growth, or recovery from trauma (De Koning, 2013).

The characteristic organic acids of plant foods, such as lactic, succinic, citric and malic acids, were also detected, which are known to be produced by the plant during carbohydrate degradation. ~~Notably~~In particular, malic and citric acids contribute to confer a bitter taste, thus a~~ffecting~~ltering the flavor of artichoke heads, mainly determined by cynaropicrin (Cravotto et al., 2005). Moreover, citric acid was shown to have both antioxidant and anti-inflammatory effects (Abdel-Salam et al., 2014).

Other metabolites such as choline, betaine, uridine, and trigonelline were also found in ~~our~~the samples studied. These compounds ~~contribute to enhancing~~help improve the nutraceutical quality of artichoke heads. Trigonelline is a bioactive alkaloid, a source of vitamin B3, ~~that~~which was also found in artichoke leaves through NMR analysi~~e~~s (Farag et al., 2018). It has hypoglycemic, neuroprotective, antibacterial, antiviral, and anti-tumor activities, and has been shown to reduce diabetic auditory neuropathy and platelet aggregation (Zhou et al., 2012). Choline is the precursor of the osmoprotectant glycine betaine and is itself an essential nutrient for humans (McNeil et al., 2001).

In our study, caffeoylquinic acids and sugars exerted the highest contribution to the clustering of the two organ-specific groups. 5-Caffeoylquinic acid was more abundant in hearts than in external bracts, while 4,5-caffeoylquinic acid content was higher in external bracts. In previous studies it was found ~~A~~a different distribution of caffeoylquinic acids and, in general, of polyphenols ~~among~~ in the ~~head~~ tissues ~~was also found in previous studies~~of the flower head, confirming that polyphenols follow various physiological/biochemical changes during plant growth and organ specificity, ensuring protection from biotic and abiotic stressors (Blanco et al., 2018; Lattanzio et al., 2009; Lombardo et al., 2018; Pandino et al., 2011). Hydroxycinnamic acids are indeed involved in the biosynthesis of the lignin (Faulds and Williams, 1999) and act against UV-light damage (Moglia et al., 2009), conferring physical resistance to external bracts, hence protecting the inner parts of the flower head. Moreover, the different distribution of hydroxycinnamates may be attributed to the various genotypes tested (Lombardo et al., 2010). In addition, our results show that quinic acid, which is involved in the biosynthesis of phenolic acids through the shikimic acid pathway (Ghasemzadeh and Ghasemzadeh, 2011), is more abundant in external bracts, exposed to environmental factors.

Sugars such as glucose and sucrose were detected at higher levels in hearts with respect to external bracts. Indeed, head tissues are considered as preferential sink organs for organic compounds (Fadda et al., 2018), where carbohydrates and amino acids accumulate to provide energy and precursor molecules for the development of embryos and seeds, being flower organs developed from the receptacle (Borghi and Fernie, 2017). Carbon is drawn into flower head tissues in the form of sucrose or, after hydrolysis, fructose and glucose. Moreover, reserve polysaccharides, like inulin, are accumulated gradually during petal development (Borghi and Fernie, 2017).

The multivariate statistical analys~~e~~is, performed on both artichoke hearts and external bracts, led to the identification of the differenti~~al~~ly abundant metabolites and clustering of the tested varieties in three color-based groups, mainly due to amino acid and mono- and dicaffeoylquinic acid distribution. As for amino acids, green and intermediate-colored external bracts showed a higher content of arginine and asparagine compared to bracts of purple varieties. Purple hearts showed instead more arginine, valine and threonine with respect to hearts of green and intermediate-colored varieties. Notably, arginine and asparagine have a high N:C ratio and arginine is an ideal storage compound and an N-mobilizer in plants, accumulating during seed germination and nitrogen transport (Lea et al., 2007).

Our NMR approach allowed the detection and organ distribution of the abundant 5-caffeoylquinc acid and the differentiation of the dicaffeoylquinic isomers in the three color-based groups. It was previously established that the main compounds found to be responsible for the antioxidative properties of extracts from artichoke heads are 5-caffeoylquinic acid and 3,5-dicaffeoylquinic acid, and to a lesser extent 1,5-dicaffeoylquinic acids (Garbetta et al., 2014). We ~~could~~were able to detect these compounds and ~~differentially trace~~track them in the two organs and ~~the~~ three color-based groups. These three compounds were all previously found in artichoke receptacles of different cultivars (Albergamo et al., 2017; Pandino et al., 2011; Petropoulos et al., 2018), even though comparison with previous findings is not possible due to different varieties, plant growth and analytical condition used (de Falco et al., 2016; Lombardo et al., 2010). Consistency with previous studies can still be found for the presence of dicaffeoylquinic acids in external bracts (Albergamo et al., 2017; de Falco et al., 2016; Dosi et al., 2013).

The edible part of the globe artichoke is characterized by a high content of reducing sugars, with inulin representing up to 75% of the total glucosidic content (Lattanzio et al., 2002). In our study, the artichoke hearts, in particular the purple ones, showed a higher content of inulin compared to external bracts. Such evidence is in agreement with the data reported in the literature (Okey and Williams, 1920; Zeaiter et al., 2019). However, external bracts, representing a by-product of artichoke processing, could be still used for the extraction of this beneficial compound (López-Molina et al.,

2005; Ruiz-Cano et al., 2014), thus contributing to circular economy development. Several species belonging to the Asteraceae family are rich in water-soluble polysaccharide inulin, especially in their roots or rhizomes. It has been found that the degree of inulin polymerization (DP) can vary considerably among species, with globe artichoke having a higher DP compared to other Asteraceae crops (López-Molina et al., 2005). A higher DP can have a positive effect on inulin beneficial properties, for instance, prebiotic activity, fat absorption, water binding capacity, and digestibility (Azorín-Ortuño et al., 2009).

5 ~~CONCLUSION~~Conclusion

The need for safe and healthy food leads to the valorization of local varieties and to the promotion of local food products characterized by a high ~~variation~~diversity in bioactive compounds. The application of a non-targeted NMR approach and chemometrics allowed to unveil the diversity of metabolite content in traditional landraces from the Apulia region, where hotspots of biodiversity have been found for several crop species, including the globe artichoke itself (Curci et al., 2016; Pavan et al., 2018), cowpeas (Lioi et al., 2019a; Zuluaga et al., 2021), common bean (Lioi et al., 2019b), chickpea (Pavan et al., 2017), etc.

Our study may help select the artichoke landraces to be promoted for both human consumption and the extraction of beneficial compounds (even from biowastes as external bracts), and breeding purposes. The varieties richer in mono- and dicaffeoylquinic acids could be used in future studies for the identification of the late metabolic steps leading to the synthesis of ~~such~~these bioactive compounds, ~~still~~ not yet elucidated.

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CRediT authorship contribution statement

Emanuela Blanco: Conceptualization, ~~Methodology; Investigation; Validation;~~Methodology, Investigation, Validation, Writing ~~– Original Draft;~~– original draft, Writing ~~– Review~~– review & ~~Editing;~~editing, Visualization. **Biagia Musio:** Conceptualization, ~~Methodology; Investigation; Validation;~~Methodology, Investigation, Validation, Formal ~~analysis;~~analysis, Data ~~Curation;~~Curation, Writing ~~– Original Draft;~~– original draft; Writing ~~– Review~~– review & ~~Editing;~~editing, **Stefano Todisco:** ~~Investigation and formal~~ Investigation, Formal analysis. **Piero Mastrorilli:** ~~Resources and~~ Resources, Supervision. **Vito Gallo:** ~~Methodology;~~Methodology, Formal ~~analysis; Validation;~~analysis, Validation, Data ~~Curation; Resources;~~curation, Resources, Writing ~~– Review~~– review & ~~Editing;~~editing, Supervision. **Gabriella Sonnante:** ~~Conceptualization; Methodology; Validation; Resources;~~Conceptualization, Methodology, Validation, Resources, Writing ~~– Review~~– review & ~~Editing; Supervision;~~editing, Supervision, Project ~~administration and~~ administration, Funding acquisition.

Declaration of Competing Interest

The authors disclose no conflicts of interest.

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Appendix A Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2022.104539.

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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Highlights

- Metabolic profile of globe artichoke edible part and external bracts established.
- Non-targeted ^{1}~~H-NMR-~~H NMR metabolomics of hydrosoluble nutraceutical ~~compounds~~compounds.
- Relation between artichoke flower head color and metabolite composition revealed.
- Biodiversity of the Mediterranean globe artichoke landraces investigated.

Appendix A Supplementary material

Multimedia Component 1

Supplementary material

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Queries and Answers

Q1

Query: Please confirm that given names and surnames have been identified correctly and are presented in the desired order, and please carefully verify the spelling of all authors.

Answer: We confirm that given names and surnames are correct and in the right order.

Q2

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact j.yesujoshwa@elsevier.com immediately prior to returning your corrections.

Answer: We confirm that our article is a regular item, to be included in a regular issue of the journal.

Q3

Query: Please approve that the affiliations link the authors with their correct departments, institutions, and locations.

Answer: We approve affiliations and related links to authors.

Q4

Query: If you wish to acknowledge a funding source, please type the full funder name, country and grant IDs in the text, if available: Correctly acknowledging the primary funders and grant IDs of your research is important to ensure compliance with funder policies. We could not find any acknowledgement of funding sources in your text. Is this correct?

Answer: We listed our funding sources under Funding section. If necessary, add them also in the Acknowledgements section: "This work was supported by: Regione Puglia Administration under Rural Development Program 2014–2020, Projects ‘Biodiversity of vegetable crops in Puglia (BiodiverSO)’ and ‘Biodiversity of Apulian vegetable species (BiodiverSO Veg) n. 2’, Measure 10, Sub measure 10.2; CNR project ‘Bio-Memory‘ (Delibera CDA CNR n.157, 17/06/2020; delibera n. 239 7/10/2020); Italian Ministry MUR project ‘ALIFUN - Sviluppo di alimenti funzionali per l’innovazione dei prodotti alimentari di tradizione italiana‘ (ARS01_00783_ALIFUN, Decreto concessione n.0002852, 30/11/2021)."