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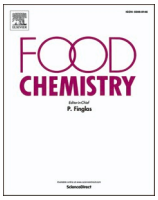

Unraveling genetic, compositional, and organoleptic traits of elephant garlic of different geographical origins

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ABSTRACT

The ancient native variety of elephant garlic, known as "Aglione della Valdichiana" and cultivated in the Valdichiana area of Tuscany, Italy, has gained recent recognition in the National Catalog of Local Varieties. The renewed interest in traditional products has led to a focus on identifying local varieties of elephant garlic, driven by their distinctive organoleptic and nutritional characteristics. However, other types of elephant garlic nowadays available on the market appear similar, but challenges exist in discerning their origin and composition. This study focused on characterizing elephant garlic from Lazio, Italy, and the Val di Chiana region through genetic, chemical, and aromatic analyses to understand genetic and geographic influences. ISSR markers differentiated elephant garlic from common varieties and highlighted regional genetic diversity. Chemical analysis revealed higher polyphenol content and antioxidant activity in elephant garlic compared to common garlic. Moreover, analysis highlights the variability in the concentrations of sulfur-containing compounds between common and elephant garlic. Aromatic and sensory assessments underscored distinctions between garlic types and regions, emphasizing the significant impact of geographic origin and genetic background on metabolite profiles in *Allium* genotypes.

1. Introduction

Allium represents one of the largest genera within the monocotyledonous plants, comprising over 900 bulbous species renowned for their extensive diversity in both morphological and physiological features (Han et al., 2020). The genus originated in eastern Asia during the late Eocene, with nearly all species primarily distributed across the northern hemisphere, spanning from the arid sub-tropical regions to the boreal zone (Li et al., 2016). In addition to commercially significant species such as common garlic (*A. sativum*), onion (*A. cepa*), chives (*A. schoenoprasum*), shallot (*A. cepa* var. *aggregatum*), and leek (*A. ampeloprasum* var. *porrum*), numerous others are utilized as ornamental plants and in traditional medicinal practices (Fritsch & Friesen, 2002).

The interest in *Allium* species lies in their bioactive compounds, which have been recognized for their potential health benefits, including anticancer, anti-atherosclerotic, anti-diabetic, antiplatelet, anti-asthmatic, and lipid-lowering activities (Fritsch & Keusgen, 2006; Singh and Singh, 2008). These bioactive compounds can be categorized into two primary groups. The first group comprises sulfur-containing compounds, such as allin and its derivatives, which contribute to the characteristic sensorial attributes of the bulbs and primarily exhibit antimicrobial activity (Corzo-Martínez et al., 2007; Hanen et al., 2012). The second group includes sulfur-free polyphenolic compounds. Both groups of bioactive compounds function as natural antioxidants, preventing oxidative damage to cellular components and potentially reducing the risk of chronic diseases (Liu et al., 2006; Ma et al., 2011).

Allium ampeloprasum L. represents a "species-complex" characterized by various cytotypes spread throughout the regions bordering the Mediterranean Sea and extending from North Africa and Southwest Asia to Southern England (Guenaoui et al., 2013). The *A. ampeloprasum* species-complex is commonly acknowledged to encompass four distinct gene-pools: wild leek, European leek cultivars, Egyptian kurrat, and great-headed garlic (Guenaoui et al., 2013). Wild leek, *A. ampeloprasum*,

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constitutes the primary gene pool, exhibiting considerable genetic variation concerning flower colour, bulb size, and modes of reproduction, which may include cloves, bulblets, or seeds (Figliuolo & Di Stefano, 2007). Wild *A. ampeloprasum* is widely valued in Tunisia for its medicinal properties and as a culinary ingredient in local cuisine, and it is widespread across the whole country landscape. *A. ampeloprasum* var. *porrum* J. Gay is the first most important domesticated vegetable selected from the primary wild gene-pool and comprises leek cultivars, normally tetraploids, predominantly allogamous with 20% of self-fertilization (De Clercq et al., 2003). These cultivars are worldwide growth due to their adaptation and tolerance to low temperatures. *A. ampeloprasum* var. *kurrat* Schweinf, commonly referred to as kurrat, is cultivated in the Middle and Near East, especially in Egypt (De Clercq et al., 2003).

A. ampeloprasum var. *holmense*, commonly known as "great headed garlic", "elephant garlic", "big tex garlic", or "Tahiti garlic", represents the third cultivated member within the *A. ampeloprasum* species complex. This vegetable is recognized as a cultigen, propagated vegetatively worldwide, featuring large, typically seedless, flower heads, with its primary geographic origin traced to the Mediterranean region. The substantial size of *A. ampeloprasum* var. *holmense* bulbs (exceeding 500 g when fresh), the vigorous structure of the whole plant, and its sterility are apparently associated with high ploidy levels (4×, 6× or 8×) (Fritsch & Friesen, 2002; Khedim, Aïnouche, & Amirouche, 2020).

Elephant garlic (*A. ampeloprasum* var. *holmense*) has emerged as a potential alternative to common garlic (*A. sativum*) in culinary applications, whether consumed fresh or processed, owing to its closely resembling flavor profile, coupled with a milder impact on breath odor and enhanced digestibility compared to common garlic (Block, 2011). Consequently, elephant garlic has garnered appellations such as "garlic for people who do not like garlic", "kissing garlic", and "garlic-like" (Lu et al., 2011). Its high digestibility compared to common garlic can be attributed in part to lower levels of fibers and sulfur-containing compounds, such as alliin and N-gamma-glutamyl-S-allyl cysteine (Ceccanti et al., 2021). Although both alliin and allicin, two organosulfur compounds responsible for the characteristic pungent aroma, are also present in elephant garlic, they occur in lower concentrations than in common garlic. For instance, Kim et al., 2017 reports that Alliin and allicin, were present at concentrations of 23.63 and 5.58 mg/g dw, respectively while Elephant garlic contained just 2.61 mg/g dw of alliin and 0.43 mg/g dw of allicin. Notably, elephant garlic bulbs from Poland have been reported to exhibit higher concentrations of polyphenols and greater antioxidant activity than common garlic bulbs (Najda et al., 2016). Moreover, Ceccanti et al. (2021) report that total phenolic content was 2-fold higher in elephant garlic than in common garlic. Conversely, Lu et al. (2011) observed opposite results in common and elephant garlic bulbs from the U.S.A., a discrepancy that might depend on the geographic origin of the bulbs analyzed and on their harvesting period.

In Italy, numerous historical records document the utilization of *A. ampeloprasum* var. *holmense* since the early 16th century, commonly referred to as "Aglione" (where the suffix "one" in Italian denotes a big size, whereas "aglio" means garlic) (Terzaroli et al., 2022). One notable cultivation area is Val di Chiana, a valley located in Central Italy within the Tuscany region, where a local landrace known as "Aglione della Val di Chiana" is predominantly cultivated, particularly in the provinces of Arezzo and Siena. This landrace has garnered recognition as a historically significant and nutritionally valuable vegetable, which has recently been rediscovered and acknowledged by its inclusion in the list of Traditional Agronomic and Edible Products of Italy (MiPAAF, 2017). Moreover, it has been listed in the National Registry of Biodiversity for agricultural and food crops (MIPAAF, 2021), as well as in the Regional Catalog of local varieties in Tuscany.

The resurgence of interest in traditional products has prompted a notable focus on identifying and/or rediscovering local varieties of elephant garlic cultivated across various regions of Central Italy, particularly in the Lazio and the Umbria regions. This renewed attention is motivated by the high market value of these local varieties, attributed to their distinctive organoleptic and nutritional characteristics determined by both the genotype and the cultivation area. This trend is particularly evident in productions limited to specific and defined regions, where efforts are made to recognize and preserve the uniqueness of these products to prevent eventual commercial fraud. However, in the case of *A. ampeloprasum* var. *holmense*, the bulbs available in the local and national markets exhibit similar morphological characteristics, in terms of size and shape, yet their precise origin remains obscure. To address this challenge, our study aimed to conduct comprehensive analytical tests on bulbs of an accession of *A. ampeloprasum* sourced from Val di Chiana, comparing them with those of elephant garlic genotypes cultivated in the northern part of the Lazio region, as well as from those of commercial varieties of common garlic grown in the same area (Lazio). These analytical assessments encompassed examinations of genetic, chemical, and aroma profiles, alongside investigations into phenolic compositions and antioxidant properties. Through these analyses, we aimed to discern and authenticate the unique characteristics of each genetic material, thus facilitating their proper characterization and safeguarding their authenticity within the marketplace.

2. Material and methods

2.1. Plant material

The fresh bulbs of *Allium ampeloprasum* var. *holmense* were purchased at a local market in Tarquinia, a small town in the province of Viterbo located on the northern coast of the Lazio region. Based on the size of the bulbs and their respective cloves, four different putative genotypes were identified, named very large (from here onwards XL_Eleph), large (L_Eleph), medium (M_Eleph), and small (S_Eleph) elephant garlic, as detailed in Table S1. The seller stated that local farmers, who have been cultivating this vegetable for several years in this geographical area, supplied these "Aglione" bulbs. The different elephant garlic bulbs are shown in fig. S1. Concurrently, two commercial varieties of *A. sativum* widely cultivated in that area and referred to as white and Spanish red garlic (WG and SR, respectively) were also procured from the same local market. The four elephant garlic genotypes and the two commercial garlic varieties were field grown at the ARSIAL experimental station located in Tarquinia (Lazio Region, Province of Viterbo: 42.226036, 11.736633). On 25 October 2022, cloves were sown directly in the field, without any pretreatment, adopting the local traditional agricultural practices. Cloves were planted in rows separated by 0.5 m, with a distance of 0.3 m between plants. The plant material was arranged in a randomized block design with three replications and each experimental unit (plot) consisted of 30 plants per accession. All genetic entries were harvested on June 28, 2023. Six bulbs for each entry (two bulbs for each replicate) were randomly selected in each plot of the experimental field. In addition, six bulbs of an accession of "Aglione della Val di Chiana" (VC_Eleph) were randomly collected from a farm located in the typical cultivation area of this landrace (Tuscany Region, province of Siena).

2.2. Molecular analysis

Total genomic DNA was isolated from 100 mg of bulb of a single individual plant for each genetic entry, using the "NucleoSpin® Plant II kit" (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. The quality and concentration of the extracted DNA were determined through 1.5% agarose gel electrophoresis stained with ethidium bromide, using known concentrations of λ phage DNA as a reference. All DNA samples were then diluted to 20 ng/μL and stored at −20 °C until PCR amplification. DNA samples were amplified with ten primers from the University of British Columbia (UBC) collection (Table S2), previously used in the genetic characterization of different *Platanus* species (Ciaffi et al., 2018) and for studying inter- and intra-

varietal genetic variability in Italian artichoke germplasm collection (Alicandri et al., 2023). PCR reactions were performed in a final volume of 25 μL containing approximately 20 ng of genomic DNA, 0.5 μM of each primer, and 12.5 μL of Go Taq Hot Start Colorless Master Mix, 2× (Promega, USA). All reactions were carried out using an Eppendorf Thermal Cycler (Mastercycler Gradient) with the following thermal cycling conditions: 4 min initial denaturation step at 94 °C, followed by 35 cycles of amplification, each 1 min at 94 °C, 1 min at 52–55 °C, 2 min at 72 °C, and a final extension at 72 °C for 7 min. Each Inter-Simple Sequence Repeat (ISSR) PCR reaction was performed independently two times to confirm the accuracy of amplification. ISSR fragments were separated on 1.5% (*w/V*) agarose gels, stained with ethidium bromide (0.001%), and visualized under UV light with the UVITEC Essential V6 Gel Imaging and Documentation System (Cleaver Scientific, Rugby, United Kingdom). The size of amplification products was estimated using a DNA marker with sizes ranging from 100 to 3000 bp (SMOBIO Technology, Hsinchu City, Taiwan).

2.3. Morphological characterization

Morphological analyses were conducted on three different bulbs for each genetic entry. Linear dimensions were obtained using a digital caliper (Digital Caliper, Hylka Tools, UK) with an accuracy of ±0.1 mm. Weight was determined using a bench scale (PFB-2000-2, Kern & Sohn GmbH, Ballingen, Germany) with an accuracy of ±0.01 g. For morphological characterization the following parameters were analyzed: Equatorial diameter (De); Polar diameter (Dp); Thickness (T); Diameter geometric mean ($Dgm = (De*Dp*T)*0{,}333\ mm$); Diameter arithmetic mean ($Dam = (De + Dp + T)/3\ mm$); Surface area ($SA = 0{,}785*De*Dp\ mm^2$); Cross-sectional area ($CSA = 0{,}785*(De + Dp + T)2/3\ mm^2$); Sphericity index ($SI = De/\sqrt{Dp*T}$) (Bahnasawy, 2007). For dry matter measurement, fresh garlic bulbs were collected and weighed to establish their initial weight. The garlic samples were then subjected to a controlled drying process using an oven set to 70 °C until constant weight was reached.

After drying, the garlic was re-weighed to determine its dry weight. The dry matter percentage is calculated using the formula: Dry Matter % = (dry weight/initial weight)*100.

2.4. Chemical characterization

For chemical analysis, four fresh cloves (for each genotype) were peeled, immediately grounded using liquid nitrogen and analytical mill (A 11 basic Analytical mill. IKA-Werke GmbH & Co. KG, Staufen Germany) and stored at −18 °C until analysis.

2.4.1. Polyphenols and flavonoids

Polyphenol quantification was carried out according to the method described by Henríquez et al. (2010). Briefly, 1 g of grounded sample was homogenized in 10 mL of 70% acetone solution and centrifuged at 11200*g* for 5 min. 500 μL of extract was mixed with 750 μL of saturated Na_2CO_3 solution, 950 μL of distilled water, and 125 μL of Folin-Denis' reagent. The mixture was then incubated at 36 °C for 30 min and read at 765 nm with a UV–Vis spectrometer (Perkin Elmer Lambda 850+). Phenolic concentration was calculated by interpolation with a calibration curve (30–300 μg/mL) of gallic acid (R^2 0.9996), and the results were expressed as mg gallic acid equivalents on a fresh weight basis (mg GAE/g fw). Analyses were performed in triplicate by considering samples from three different bulbs for each genetic entry.

The total flavonoid content was quantified according to Dewanto et al. (2002) slightly modified. Briefly, 500 mg of grounded sample were homogenized with 2 mL of 80% ethyl alcohol solution. The mixture was centrifugated for 5 min at 11200 g, and 500 μL of the supernatant was collected and mixed with 45 μL of 5% $NaNO_2$ solution and 300 μL of distilled water. After 6 min at room temperature, 45 μL of 10% $AlCl_3$ was added to the mix and incubated for additional 5 min. Then, after the addition of 300 μL of 1 M NaOH and 300 μL of distilled water, the mixture was incubated at room temperature for 5 min and then read at 510 nm using a UV–Vis spectrometer (Perkin Elmer Lambda 850+). Total flavonoid content, expressed as mg of quercetin equivalents/g fw, was calculated using a quercetin calibration curve ranging between 10 and 500 μg/mL (R^2 0.9992). Analyses were carried out in triplicate as previously described.

2.4.2. Antioxidant activity by DPPH method

Analyses were carried out according to the protocol described by Blois (1958) slightly adapted. Two stock solutions were prepared, one of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), used as a positive control, and a 0.2 mM DPPH solution, used as a negative control. Grounded tissues (1 g) was homogenized in 10 mL of 80% ethanol solution and then centrifuged at 11200 rpm for 10 min. The supernatant (100 μL) was added to 1 mL of a DPPH solution (3.9 mg DPPH dissolved in 100 mL of methanol/water 80:20 *v*/v). The mixture was incubated for 1 h in the dark at room temperature. Afterward, the absorbance was read at 517 nm with a spectrophotometer (Perkin Elmer Lambda 850+). DPPH scavenging capacity was calculated according to the following formula: scavenging capacity (%) $= \frac{\text{Abs Blank–(Abs Sample–Abs Control)}}{\text{Abs Blank}} \times 100$. Samples were analyzed in triplicate as previously described.

2.4.3. Antioxidant activity by ABTS method

The ABTS radical scavenging activity was evaluated according to Re et al. (1999), starting from a stock solution of ABTS (7 mM in water), activated (2.45 mM final concentration) with potassium persulfate solution. Before use, ABTS + solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm. Twenty microliters of the sample was added to 980 μL of ABTS + solution and left to react for 5 min. After this time, the solution's absorbance was read at 734 nm. ABTS scavenging capacity was calculated according to the following formula: scavenging capacity (%) $\frac{(\text{Abs ABTS–Abs Sample})}{\text{Abs ABTS}} \times 100$. Samples were analyzed in triplicate as previously described.

2.5. Aromatic profile

The analysis of the aromatic profile of elephant and common garlic samples was carried out on 5 g of cut material, in triplicate as previously described. A PerkinElmer Clarus® SQ 8 GC/MS system with a PerkinElmer Turbomatrix™ HS-40 was used for the analysis. Standard 20 mL headspace vials and aluminum crimped caps with PTFE-lined silicon septa were used (PerkinElmer Inc. Italy). The experimental conditions are those reported by Kathryn Lawson-Wood (2016) with slight modifications, summarized in Table S3. Volatiles identification was achieved using the Amdis (© 2003 Tobias Kind) and National Institute of Standards and Technology (NIST) Mass Spectral Database (NIST 98, Version 2.0, USA) data bank. Only compounds with an 80% match or more were selected. Selected peaks were then quantified using TurboMass software (TurboMass R, Version 5.4.2 PerkinElmer Inc., USA, 2008), by integration of the peaks area. The area of each peak was then normalized to the sum of the areas (Modesti et al., 2021).

2.6. Sensory analysis

For sensory evaluation, samples were prepared as follows: the external tunics were removed, and three cloves were taken from three different bulbs for each genetic entry, cut into cubes of approximately 2 cm on each side, combined, and mixed. Each panelist received five units of the seven different garlic samples, placed in covered Styrofoam cups and labeled with 3-digit codes.

The descriptive sensory test was conducted at the Sensory Analysis Laboratory of the University of Tuscia. To ensure the reliability of the study, a panel of eight evaluators underwent rigorous training following

official regulations, including UNI EN ISO 8586-1, 2012 and UNI EN ISO 13299, 2010. These evaluators had prior experience in sensory evaluation of common garlic. The profile sheet was developed according to ISO 11035:1994 standards. However, modifications were made to reduce the M value and to adapt it specifically for assessing elephant garlic samples (Table S4). Before the official tests, the panelists underwent two specialized training sessions, despite their previous experience in evaluating common garlic samples. Each session, lasting 1.5 h, focused on familiarizing the panelists with the sensory characteristics of elephant and common garlic samples, as well as instructing them on the proper methodology for evaluating aroma solely through olfactory senses. During these training sessions, the panelists practiced identifying the sensory attributes necessary to describe the aroma comprehensively, ensuring they could assign intensity values consistently on the 5-point scale adopted for the study. This thorough training aimed to standardize the evaluation process and enhance the reliability and accuracy of the sensory assessments conducted.

After the training sessions, the eight participants carried out the sensory evaluation in a dedicated sensory evaluation laboratory, adhering to the standards outlined in UNI EN ISO 8589, 2014. The participants evaluated seven samples (five from the elephant garlic genotypes and two from the common garlic varieties), which were presented to them in a randomized monadic sequential order. For each sample, the participants assigned intensity values (5-point scale) to each descriptor listed in the profile sheet.

2.7. Non-destructive evaluation

A mass-based (MS) *E*-nose was used to further investigate the aromatic fingerprint. The E-nose is based on an array of 12 quartz microbalances (QMBs) and was designed, developed, and assembled at the University of Rome Tor Vergata (Capuano et al., 2020). The sensors are functionalized by Mg, Co, Cu, Zn, Fe, Mn, Sn, free base (H2) of 5, 10, 15, 20-tetrakis-(4-butyloxyphenyl) porphyrin (TBPP), free base (H3), copper, phosphorus and manganese complexes of 5,10,15-triphenylcorrole (TPC); the control of the instrument and data collection are managed by custom software in MATLAB (Capuano et al., 2020). The sampling protocol for measurement includes the following steps: 5 g of cut garlic samples were incubated in closed vials at 30 ± 1 °C for 20 min. The headspace was extracted for 60 s using a stream of filtered air and delivered into the electronic nose sensor cell. After each measurement, the E-nose system was cleaned for 180 s. Sensor signals were calculated as the resonant frequency shift between the sensors exposed to pure air and to the sample. Three different vials were analyzed for each sample.

2.8. Data analysis

In the ISSR molecular markers analysis, only distinct, reproducible, and well-resolved fragments ranging from 300 to 2500 bp were considered. DNA fragments (bands) across all genotypes were scored as present (1) or absent (0) for each of the ISSR markers, and the raw data were imported into a Microsoft EXCEL spreadsheet to construct a binary matrix. The genetic diversity for each ISSR locus was evaluated by the following parameters: total number of bands obtained for each ISSR primer (TB), number of polymorphic bands (PB), percentage of polymorphism (Pol %), the allele with the highest frequency (MAF), expected heterozygosity (He), and PIC (Polymorphic Information Content), with the latter three parameters determined using Power Marker 3.25 software (Liu et al., 2006). The phylogenetic relationships among the genotypes were assessed by estimating the genetic distances using Nei's coefficient (Nei, 1973). The resulting distances matrix was employed to generate a phylogenetic tree using the UPGMA algorithm (Unweighted Pair-Group Method with Arithmetic Averages) in MEGAX software (Kumar et al., 2018).

Morphological and compositional data were statistically analyzed through the Shapiro–Wilk and Bartlett test to verify normality and homogeneity of variances. Once these prerequisites were established, data were compared by one-way ANOVA and Tukey's HSD post hoc test with $p \leq 0.05$. The statistical tests were performed using GraphPad p Prism 7.01. Sensory data were processed with XL-Stat (Addinsoft, Paris, France) software through principal component analysis (PCA) and Hierarchical Clustering analysis with the Ward method (Euclidean distance). GC–MS data were autoscaled and used for a PCA with Venetian-blind as validation method (with blind thickness = 1). Organosulfur compounds were selected, normalized and then used to build a heatmap. The correlation between volatile compounds and sensory attributes was analyzed by Pearson correlation. *E*-nose numerical data were autoscaled and then analyzed with partial least square discriminant analysis (PLSDA) using Venetian-blind as validation method (with a blind thickness = 1). Finally, the E-nose measurement was also used to build a linear regression model using a Principal Components Regression (PCR) with the QMBs response as predictor variables and GC–MS data as response variables. Multivariate analyses were performed by using Matlab R2013a (MathWorks®, Natick, MA, USA) and PLS Toolbox (Eigenvector Research, Inc., Manson, WA, USA).

3. Results and discussion

3.1. Genetic relationships among garlic genotypes

For all analyzed genotypes, the 10 ISSR primers produced a total of 136 distinguishable bands ranging in size from 300 bp to 2.5 Kb (Fig. S2), with an average of 13.6 bands per primer (Table 1). The number of bands varied from eight (UBC 850 and UBC 834) to 18 (UBC 832), with the same primers showing the lowest (7) and the highest (17) number of polymorphic bands, respectively. In total, 131 polymorphic bands were detected with an average of 13.1 polymorphic bands per primer and 96% polymorphism. He and PIC values had relatively uniform values for the ten ISSR markers, with an average of 0.329 and 0.268, respectively (Table 1). In particular, He values ranged from 0.296 (UBC850) to 0.354 (UBC 881), while PIC values were comprised between 0.243 (UBC850) and 0.287 (UBC 881). The UPGMA dendrogram based on genetic distances between the seven garlic genotypes, estimated using the Nei coefficient (1973) using ISSR molecular markers, is reported in Fig. 1.

The dendrogram clearly showed the presence of two main clusters that distinguish the two *A. sativum* commercial varieties from the five *Allium ampeloprasum* var. *holmense* genotypes. Furthermore, based on the Nei's genetic distances, the upper main cluster containing the five elephant garlic genotypes could be further divided into two sub-clusters.

Table 1
Genetic diversity parameters of seven garlic specimens genotyped using 10 ISSR markers: white garlic, Spanish red garlic, elephant garlic from Val Di Chiana, and four sizes of elephant garlic from the Lazio region (very large, large, medium, small).

ISSR marker	TB	PB	Pol %	MAF	He	PIC
UBC 832	18	17	94%	0.762	0.331	0.268
UBC 842	12	12	100%	0.774	0.327	0.268
UBC 850	8	7	88%	0.804	0.296	0.243
UBC 840	13	12	92%	0.747	0.339	0.271
UBC 851	17	16	94%	0.782	0.317	0.259
UBC 848	17	16	94%	0.765	0.327	0.265
UBC 834	8	8	100%	0.750	0.347	0.281
UBC 860	16	16	100%	0.786	0.327	0.270
UBC 881	15	15	100%	0.752	0.354	0.287
UBC 857	12	12	100%	0.786	0.327	0.270
MEAN	13.6	13.1	96%	0.771	0.329	0.268
ST. DEV.				0.019	0.016	0.012
TOTAL	136	131				

TB: total number of bands; PB: number of polymorphic bands; Pol %: polymorphism percentage; MAF: Major Allele Frequency; He: Expected Heterozygosity; PIC: Polymophism Information Content

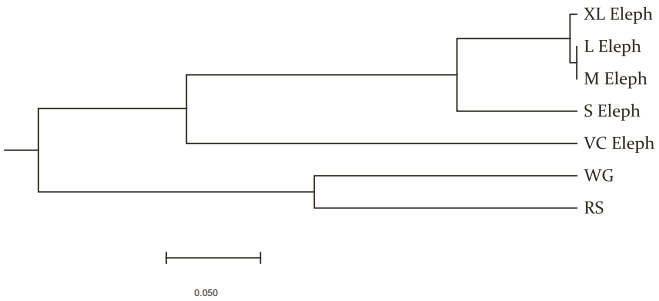

Fig. 1. UPGMA dendrogram of the genetic relationships among the seven garlic genotypes (WG: white garlic; SR: Spanish red garlic; VC_Eleph: elephant garlic from Val Di Chiana; XL_Eleph, L_Eleph, M_Eleph and S_Eleph: very large, large, medium and small elephant garlic from Lazio region), generated by the Nei coefficient (Nei, 1973) using ten ISSR molecular markers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The first sub-cluster included the four putative genotypes cultivated in the Lazio region (namely L_Eleph, M_Eleph, XL_Eleph, and S_Eleph) and the second comprised the "Val di Chiana" accession (VC_Eleph). These results imply a probable shared genetic origin among the four Lazio putative genotypes identified only based on bulb size, while also highlighting their genetic distinctiveness from the "Val di Chiana" genotype. Finally, a clear genetic differentiation was also found for the two common garlic commercial varieties WG and RS.

3.2. Physical and chemical characterization

The morphological measurements are detailed in Table 2. Remarkably, for all the parameters under analysis, the two common garlic varieties WG and SR exhibited a high level of dissimilarity, setting them distinctly apart from elephant garlic genotypes. In the latter case, all acquired data consistently aligned with the predetermined size criteria of classification of the different genotypes: XL_Eleph displayed the highest values, while S_Eleph exhibited the lowest. High similarity in morphological parameters was observed between VC_Eleph and L_Eleph. Although the elephant garlic bulbs of different geographic origins were extremely similar in shape and aspect, they displayed different sensory and metabolomic profiles as shown below.

The dry matter content resulted similar in all the genotypes analyzed, while the total polyphenol content varied significantly between common garlic and elephant garlic genotypes, ranging from 2.6 mg/g fw in WG to 10.23 mg/g fw in XL_eleph (Table 3). Slight differences were also observed in the content of flavonoids, although a definitive trend between common garlic and elephant garlic genotypes was not readily discernible. To assess the antioxidant capacity, two different approaches were employed (namely DPPH and ABTS assays). While the radical scavenging capacity was generally higher when measured using DPPH, the observed antioxidant capacity in the different samples exhibited a consistent trend with both methods. XL_Eleph and VC showed the highest antioxidant capacity followed by L_Eleph and M_Eleph. On the other hand, S_Eleph, SR, and WG had the lowest scavenging capacity. Previous research has demonstrated that the antioxidant activity of elephant garlic, as measured by DPPH and ABTS assays, is higher than that of normal garlic (Kim et al., 2019). It is well known that the health-promoting properties of garlic are largely attributed to its polyphenol and organosulfur compounds (Phan et al., 2019). Some studies reports that polyphenols are the main compounds responsible for garlic's antioxidant properties as well as health benefits (Nazzaro et al., 2020; Caputo et al., 2020). As known, the amount of secondary metabolites is affected by different factors, among others, the genotype plays a crucial role (Pellegrini and Ponce, 2020). As observed, elephant garlic seems to have a higher amount of polyphenols than common garlic, in accordance with previous findings (Ceccanti et al., 2020; Caputo et al., 2020; Loppi et al., 2021), thus suggesting that certain allium species, such as *A. ampeloprasum*, may offer greater health benefits than others.

As known, the antioxidant activity of garlic, is also due to its sulfur-containing compounds (Phan et al., 2019). The heatmap in Fig. 2 illustrates the relative concentrations of various sulfur-containing compounds across the different garlic samples. The concentrations of S-allyl mercapto-L-cysteine and dimethyl sulfide show significant variability among the samples. Notably, Elephant garlics exhibited a higher concentration of S-allyl mercapto-L-cysteine compared to the common garlics. This compound is known for its strong antioxidant properties (Rais et al., 2023), which could confirm the higher antioxidant activity

Table 2
Differences in morphological traits (means ± sd of three measurements) of the seven common and elephant garlic genotypes (WG: white garlic; SR: Spanish red garlic; VC_Eleph: elephant garlic from Val Di Chiana; XL_Eleph, L_Eleph, M_Eleph and S_Eleph: very large, large, medium and small elephant garlic from Lazio region). Different letters indicate statistically significant differences among genotypes at $p \leq 0.05$ (ANOVA analysis, Tukey test).

	Weight (g)	Diameter (cm)	Surface area (cm^2)	Cross-sectional area	Shape index
WG	23.02 ± 18.60 d	43.31 ± 2.81 d	1082 ± 159 d	3168 ± 437 e	1.31 ± 0.12 c
SR	24.30 ± 10.32 d	37.42 ± 6.12 d	1049 ± 328 d	3298 ± 1107 e	1.14 ± 0.01 d
VC_Eleph	305.91 ± 11.20 b	84.34 ± 2.14 ab	4950 ± 227 b	16,890 ± 792 b	1.47 ± 0.04 b
XL_Eleph	414.92 ± 32.31 a	94.73 ± 3.82 a	7259 ± 613 a	21,157 ± 1709 a	1.71 ± 0.08 a
L_Eleph	293.82 ± 55.01 b	83.71 ± 4.84 ab	5319 ± 681 b	16,562 ± 1919 b	1.23 ± 0.06 b
M_Eleph	232.84 ± 39.92 b	74.32 ± 4.72 b	4662 ± 533 b	13,054 ± 1666 c	1.33 ± 0.06 c
S_Eleph	119.31 ± 47.52 c	58.61 ± 8.18 c	2584 ± 688 c	8206 ± 2323 d	1.15 ± 0.01 d

Table 3
Differences in dry matter, total polyphenols and flavonoids, and antioxidant activity (DPPH and ABTS methods) (means ± sd of three measurements) of the seven common and elephant garlic genotypes (WG: white garlic; SR: Spanish red garlic; VC_Eleph: elephant garlic from Val Di Chiana; XL_Eleph, L_Eleph, M_Eleph and S_Eleph: very large, large, medium and small elephant garlic from Lazio region). Different letters indicate statistically significant differences among genotypes at $p \leq 0.05$ (ANOVA analysis, Tukey test).

	Dry matter (%)	Total polyphenols (mg/g fw)	Total Flavonoids (μg/g fw)	DPPH radical scavenging activity (%)	ABTS radical scavenging assay (%)
WG	40.11 ± 1.91 a	2.62 ± 0.51 b	1.32 ± 0.31 b	19.10 ± 3.21 b	15.30 ± 0.38 e
SR	40.08 ± 2.52 a	4.36 ± 0.12 b	1.42 ± 0.25 ab	18.52 ± 2.43 b	19.19 ± 0.38 de
VC_Eleph	38.42 ± 3.84 a	9.81 ± 0.34 a	1.54 ± 0.34 ab	35.36 ± 8.85 ab	29.08 ± 0.15 ab
XL_Eleph	39.61 ± 0.32 a	10.23 ± 0.51 a	1.22 ± 0.54 b	42.91 ± 9.06 a	31.41 ± 1.91 a
L_Eleph	36.02 ± 1.24 a	10.40 ± 0.72 a	1.39 ± 0.13 ab	34.24 ± 8.83 ab	25.30 ± 0.91 bc
M_Eleph	35.42 ± 0.34 a	9.56 ± 0.29 a	1.76 ± 0.22 a	37.30 ± 6.80 ab	24.49 ± 2.22 bc
S_Eleph	35.73 ± 0.91 a	10.07 ± 0.44 a	1.19 ± 0.42 b	20.85 ± 2.91 b	23.35 ± 1.38 cd

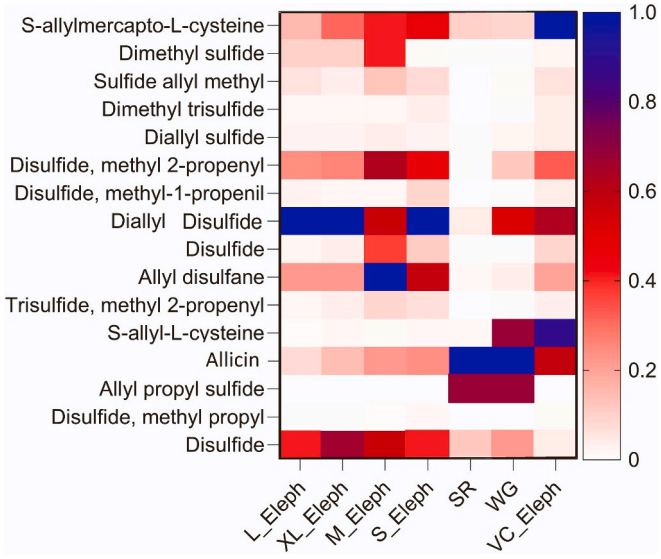

Fig. 2. Heatmap performed on volatile organosulfur compounds measured in seven garlic genotypes (WG: white garlic; SR: Spanish red garlic; VC_Eleph: elephant garlic from Val Di Chiana; XL_Eleph, L_Eleph, M_Eleph and S_Eleph: very large, large, medium and small elephant garlic from Lazio region). Each cell represents the normalized relative abundance (mean oh three replicates) of each compound in each of the seven genotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

observed in the Elephant garlics genotypes. The concentration of diallyl disulfide was slightly higher in elephant garlic, while was almost negligible in WG and SR. Similarly, allyl disulfane and trisulfide, methyl 2-propenyl, known for their antimicrobial and antioxidant properties (Kim et al., 2017), shows moderate levels across most elephant garlic samples but is significantly lower in WG and SR. On the contrary, allicin, allyl propil sulfide and S-allyl-L cysteine are relatively higher in WG and SR. Garlic is rich of bioactive compounds, with allicin and S-allyl cysteine being two of the most prominent and well-studied components (Thakur et al., 2024). Allicin is extremely unstable and rapidly breaks down into other volatiles chemicals, such as diallyl disulfide, diallyl trisulfide, diallyl sulfide and trisulfides (Thakur et al., 2024). However, this compound, the "essence of garlic," is one of garlic's biologically active components and it is the main responsible for its distinctive odor (Reyes et al., 2017) therefore its prevalence in WG and SR genotype was expected. The analysis highlights the variability in the concentrations of sulfur-containing compounds between common and elephant garlic. Elephant garlic shows higher levels of S-allyl mercapto-L-cysteine, contributing to its strong antioxidant properties and overall higher antioxidant activity. On the other hand, common garlic genotypes like WG and SR have higher concentrations of volatile sulfur compounds especially allicin, which are responsible for garlic's distinctive odor. This variability underscores the complex interplay between polyphenols and organosulfur compounds in determining the health-promoting properties of garlic. However, organosulfur compounds are highly unstable and challenging to quantify precisely because they undergo numerous reactions that can be influenced by a wide range of factors (Rais et al., 2023). Specifically, when garlic bulb is crushed, alliin is converted to allylsulfenate through the action of the enzyme alliinase and the cofactor pyridoxal phosphate. This allylsulfenate then dimerizes to form diallyl thiosulfinate (allicin), alongside other volatile compounds such as diallyl tetrasulfide, diallyl trisulfide, diallyl disulfide, diallyl sulfide, allyl methyl tetrasulfide, allyl methyl trisulfide, allyl methyl disulfide, allyl methyl sulfide, allyl mercaptan, dimethyl trisulfide, methyl allyl thiosulfinate, (Bastaki et al., 2021). The findings suggest that while both polyphenols and organosulfur compounds contribute to garlic's bioactivity, the stability and abundance of polyphenols make them key contributors to its antioxidant effects.

3.3. Sensory evaluation

Sensory evaluations of the seven elephant and common garlic genotypes were carried out through direct sniffing and aromatic profile mapping.

Sensory data (intensity scores of the 11 discriminant descriptors) were used to build a PCA model (Fig. 3). As indicated in Table S5, the eigenvalue estimates revealed that the first component exhibited substantial discriminating power, accounting for 60.70% of the variance. When combined with the second component, the explained variance increased to 80.07%. The remaining components (PC3–6) demonstrated negligible contributions, with eigenvalues <1. PC1 showed a positive correlation with descriptors related to oniony and cooked vegetables while exhibiting a negative correlation with descriptors indicating pungency, aromatic intensity, persistence, and a distinctly garlicky aroma with sulfur notes. On the other hand, PC2 demonstrated positive correlations with fresh and dried vegetal scents, particularly those resembling heart and mushroom, while showing negative correlations with cooked notes. The results of the descriptive analysis underscored the high discriminating power of the selected descriptors. Through exclusive olfactory evaluation, the panel successfully differentiated not only between common and elephant garlic samples but also between elephant garlic samples of different geographic origins (Fig. 3). Indeed, the elephant garlic from Val di Chiana (VC_Eleph) displayed a more delicate aroma compared to the genotypes from the Lazio region. Its vegetal note carried strong hints of onion and cooked vegetables, while the other elephant garlic samples exhibited less pronounced onion and cooked vegetable notes, with XL_Eleph displaying strong undergrowth hints. On the contrary, common garlic samples (namely WG and SR) were located in the opposite quadrant of the graph, being characterized by strong attributes of pungency, garlicky aroma, and sulfur notes. Sensory data were also utilized for Hierarchical Clustering analysis (HCA). As depicted in Fig. S3, common and elephant garlic samples were effectively discriminated based on the sensory descriptors evaluated. Additionally, albeit to a lesser extent, the elephant garlic sample from Val di Chiana exhibited discernible differences from those of the Lazio region. Sensory data confirmed that elephant garlic is characterized by a more delicate aroma compared to common garlic, consistently with previous findings (Caputo et al., 2020). The preference for elephant garlic among individuals sensitive to the intense flavor and aroma of common garlic may be attributed to its milder taste profile. The distinct aromatic profile of elephant garlic, characterized by different sulfur compounds and higher levels of alcohols and esters, contributes to its unique and delicate flavor profile. Despite its milder flavor, elephant garlic still offers great health benefits associated with garlic consumption, including higher polyphenols content as previously described and as already stated by other authors (Ceccanti et al., 2020; Caputo et al., 2020; Loppi et al., 2021).

3.4. Aromatic characterization

Using HS GC–MS approach a total of 37 volatile compounds were identified in the different samples. Volatiles can be broadly categorized into distinct chemical classes: aldehydes, alcohols, amines, esters, mercaptans and sulfides (Table S6; S7). This comprehensive categorization not only enhances the understanding of the garlic aroma profile but also serves as a foundation for further exploration into the distinctive chemical composition of different common and elephant garlic genotypes. Hence, GC–MS data were used for the construction of a PCA model (Fig. 4). Four PCs were selected with a cumulative explained variance of 84.22% (PC1 42.29%, PC2 18.06%, PC3 16.17%, and PC4 8.06%). The entire HS of common garlic varieties (WG and SR) revealed volatile acids, esters, thiols, and mercaptans as the most abundant

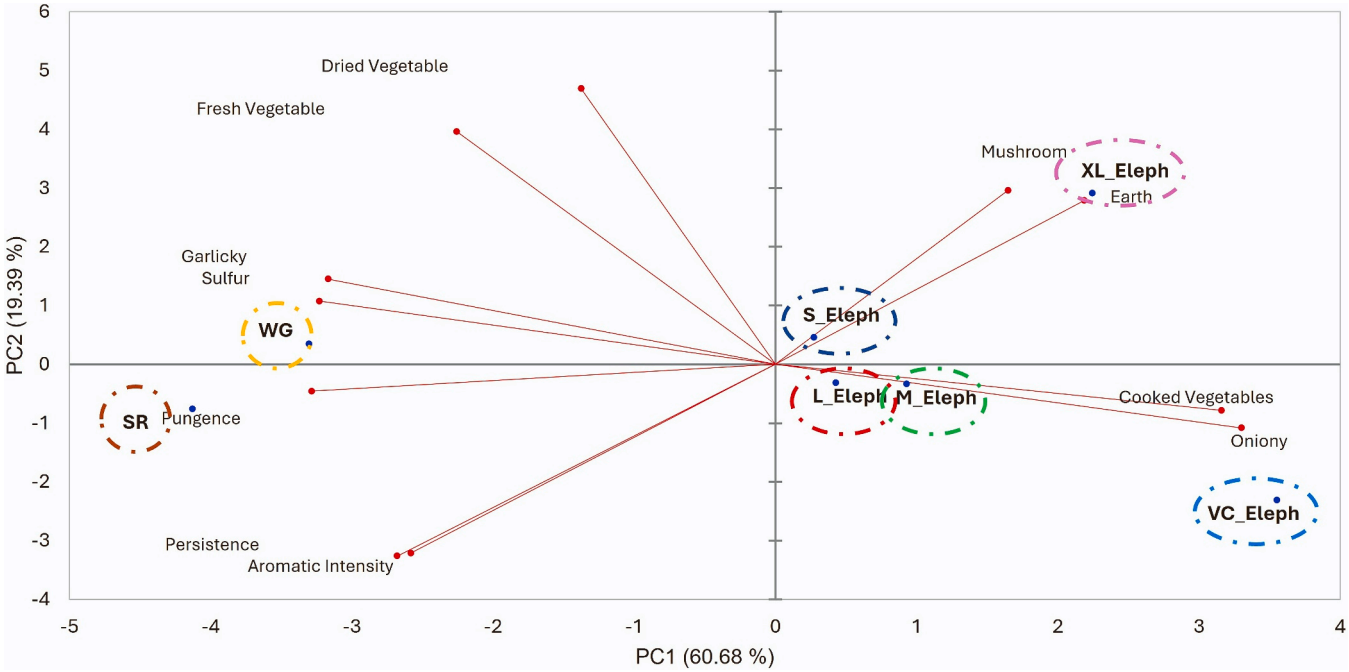

Fig. 3. Biplot (PC1 vs PC2) of the PCA performed sensory data. The intensity scores of the 11 discriminant descriptors were used as predictor variables while the different garlic genotypes (WG: white garlic; SR: Spanish red garlic; VC_Eleph: elephant garlic from Val Di Chiana; XL_Eleph, L_Eleph, M_Eleph and S_Eleph: very large, large, medium and small elephant garlic from Lazio region) were used as response variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

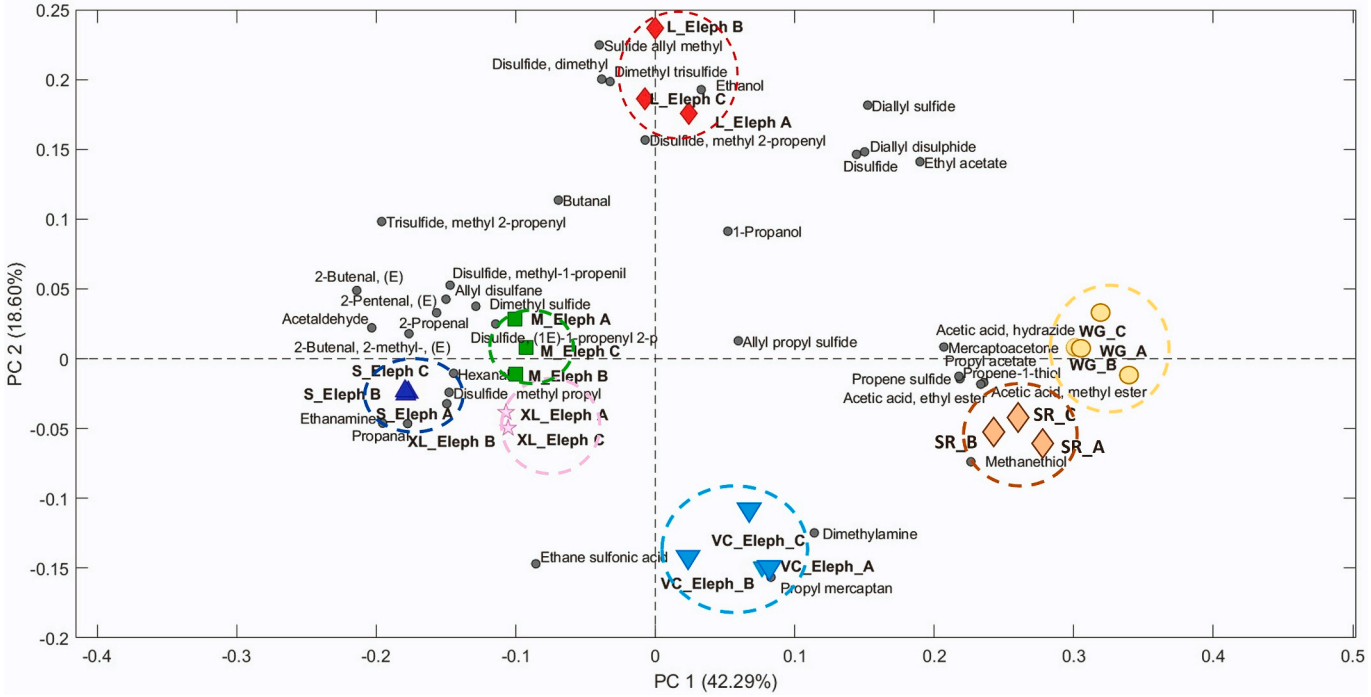

Fig. 4. Biplot (PC1 vs PC2) of the PCA performed on identified free VOCs in garlic samples. The detected compounds were used as predictor variables while the different garlic genotypes (WG: white garlic; SR: Spanish red garlic; VC_Eleph: elephant garlic from Val Di Chiana; XL_Eleph, L_Eleph, M_Eleph and S_Eleph: very large, large, medium and small elephant garlic from Lazio region) were used as response variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds, highlighting important differences compared to elephant garlic samples, where their relative concentration nearly halved. Sulfides emerged as the predominant chemical class across elephant garlic samples from the Lazio region, particularly in the S_Eleph, M_Eleph, and XL_Eleph genotypes. This finding is in accordance with several published studies (Ascrizzi & Flamini, 2020; Block et al., 1992, 1993; Block et al., 1993). Compared to all the other samples, elephant garlic from Val Di Chiana (VC_Eleph) exhibited a decidedly less complex aromatic profile, which was characterized by the presence of three specific compounds, namely propyl mercaptan, ethane sulfonic acid, and

dimethylamine. These findings align perfectly with the sensory classification results, where VC_Eleph exhibited a more delicate aroma compared to other elephant garlic genotypes. Additionally, the common garlic varieties WG and SR consistently displayed a completely different aromatic profile. The concurrence observed between sensory evaluation and GC–MS analysis suggests that, despite utilizing distinct methodologies, they offer valuable and complementary insights.

To elucidate further the correlation between sensory perception and chemical composition, a Pearson correlation analysis was conducted between sensory descriptors and individual volatile compounds (Fig. S4). Specifically, descriptors such as garlic, dried vegetable, sulfur, pungency, persistence, and aromatic intensity exhibited positive correlations with compounds like disulfide, acetic acid, propyl acetate, mercaptoacetone, propene sulfide, diallyl sulfide, ethyl acetate, propen 1 thiol, and dimethyl sulfide. These descriptors consistently characterized the common garlic samples. Conversely, descriptors associated with elephant garlic samples from the Lazio region, such as earth and mushroom, showed positive correlations with compounds like ethane sulfonic acid, acetaldehyde, dimethyl sulfide, 1-propanol, butanal, 2, butanal (E), disulfide 2-propenal, disulfide dimethyl, hexanal and allyl propyl sulfide. Onion and cooked vegetables, which characterize the sensory profile of the VC_Eleph sample, exhibited positive correlations with compounds like propyl mercaptan, 2-propenal, methyl trisulfide, allyl disulfane, ethan sulfonic acid. The correlation between sensory perception and chemical composition, as revealed through Pearson correlation analysis, underscores the effectiveness of sensory evaluation in discriminating the diverse aromatic profiles of different garlic samples. The positive correlations between specific sensory descriptors and corresponding volatile compounds provide valuable insights into the distinct characteristics of each *Allium* genotype, affirming the complementary nature of sensory evaluation and GC–MS analysis in enhancing our understanding of garlic flavor profiles.

3.5. Non-destructive characterization

The efficacy of HS-GC–MS is centered on the measurement of specific volatile compounds. In contrast, the *E*-nose, functioning as a detection instrument, can identify simple and complex aromas through features such as an array of non-specific sensors and a well-suited pattern recognition system. In the case of the garlic production chain, *E*-nose applications included early detection of fungal infection (Makarichian et al., 2022), monitoring processes (Makarichian et al., 2022), and cultivars classification (Trirongjitmoah et al., 2015; Eyupoglu, 2019; Abbey et al., 2001). As such, the aromatic fingerprint was also evaluated using QMBs e-nose. The PLS-DA model describes about 93% of the variability on the first two latent variables (LV1 56.40 and LV2 35.94%) (Fig. 5). The interpretation of PLS-DA score plots typically relies on the premise that the distance between points reflects the similarity between samples. Consequently, clusters observed in the score plots are construed as groups of comparable samples. The plot revealed the presence of three distinct clusters. The first cluster included S, L, and XL elephant garlic genotypes, the second the two common garlic varieties (WG and SR), while the third, well separated from the others, comprised the VC_Eleph genotype. The M_Eleph genotype stands between the common garlic varieties and the remaining three elephant garlic genotypes from the Lazio region. These results are in line with what was observed in the analytical and sensory analysis. Hence, the formed clusters not only closely resemble the clusters identified in the PCA of HS-GC–MS but also exhibit distinctions based on various sensors. This observation implies significant aromatic variations among different garlic samples. Specifically, the QMB 1, 2, 6, 7, 10, and 11, functionalized by Co, Mn, Sn, copper, and phosphorus, respectively, showed proximity with S_Eleph, L_Eleph, XL_Eleph and to a lesser extent with M_Elph and the common garlic varieties. On the other hand, VC_Eleph was related to sensors 3, 4, 5, 8, and 12 (Cu, Zn, Fe, TBPP and TPC). When molecules adhere to the coated layer above the *E*-Nose QMBs' surface, there is a resulting shift in their oscillation frequency. This shift is directly proportional to the absorbed mass, and the subsequent alteration in the electrical signal is

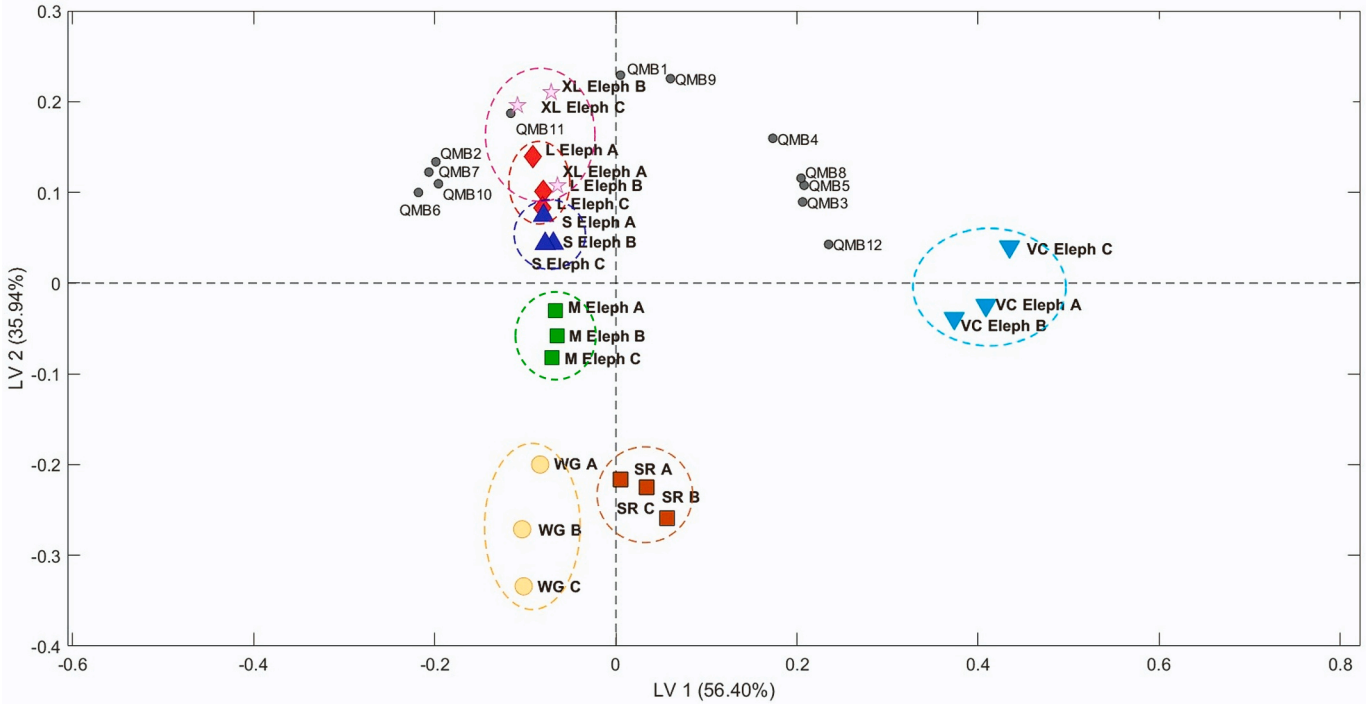

Fig. 5. Biplot (LV1 vs LV2) of the PLSDA performed on autoscaled *E*-nose measurement. The QMBs signals were used as predictor variables while the different garlic genotypes (WG: white garlic; SR: Spanish red garlic; VC_Eleph: elephant garlic from Val Di Chiana; XL_Eleph, L_Eleph, M_Eleph and S_Eleph: very large, large, medium and small elephant garlic from Lazio region) were used as response variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

measured. QMBs are constructed from thin quartz discs, typically coated with various materials. Consequently, each sensor exhibits an individual and distinctive response based on its sensitivity and selectivity, influenced by specific functionalization (Capuano et al., 2020; Muñoz-Castells et al., 2023). The various sensor functionalization play a crucial role in offering initial insights into the volatile profile of garlic. This is attributed to the strategic use of molecules on the sensor surface, displaying varying affinities toward specific classes of aromatic compounds (Capuano et al., 2020; Di Natale et al., 2014). The inherent response of each sensor is influenced by the chemical structure and concentration of volatile compounds (VanDeventer, 2001). Consequently, the effectiveness of an *E*-nose system in discrimination relies on its capacity to generate a measurable response to aroma components and distinguish between aromas with diverse constituents. It is important to note that the complexity of an aroma, characterized by the diversity and quantity of its constituent substances, directly correlates with the sensor response. Essentially, the greater the complexity of the aroma, in terms of both the quality and quantity of compounds, more pronounced the sensors' response. This heightened sensitivity significantly enhances the electronic nose's capability to discern and differentiate among various aromas. The statistical response of the PLS-DA model is represented by the confusion matrix (Table S8). The different classes were identified with a variable accuracy between 80 and 100% in calibration and between 30 and 100% in cross-validation. The results indicate that the model did not perfectly discriminate the different samples. However, upon reviewing the misclassifications, it can be asserted that they align with the previously mentioned observations. For instance, the common garlic varieties WG and SR have been assigned in the respective classes in cross-validation, suggesting high similarity between these two samples. The model consistently misclassifies the elephant garlic samples from the Lazio region, indicating notable aromatic resemblance. On the other hand, the Val di Chiana elephant garlic genotype was correctly assigned to the respective class highlighting a distinct and unique aromatic bouquet.

PCR is a statistical technique employed for modeling relationships between dependent variables (E-nose data) and independent variables (volatiles) when strong correlations and multicollinearity issues exist among them. This analysis aids in comprehending the interrelationships between variables and is particularly valuable in assessing the E-nose's predictive capability for individual-identified volatiles (Muñoz-Castells et al., 2023). In constructing the PCR model, two principal components were selected, resulting in a cumulative explained variability of about 85% (PC1 77.12, PC2 8.20). The model's prediction correlation coefficients (R^2) and Errors in calibration and cross-validation (RMSEC and RMSECV) are presented in Table S9. The highest correlation coefficients with R^2 above 0.70 in calibration and 0.60 in cross-validation (highlighted in green) were observed for 2-Butanal (E), acetaldehyde, acetic acid, hydrazide, dimethylamine, mercaptoacetone, propene sulfide, propene-1-thiol and propyl acetate. Satisfactory prediction abilities, with R^2 between 0.69 and 0.50 in calibration and 0.60 and 0.40 in cross-validation (highlighted in yellow) were also achieved for 2-Propenal, acetic acid ethyl ester, acetic acid methyl ester, allyl propyl sulfide, butanal, diallyl disulfide, diallyl sulfide, dimethyl sulfide, disulfide, ethane sulfonic acid, ethanamine, methanethiol, trisulfide, methyl 2-propenyl. Conversely, the model exhibited poor performance (R^2 below 0.5 in calibration) in predicting the other compounds. Overall, the model struggled to predict quantitatively concentrations of 16 of the identified volatiles while correctly identifying (with different accuracy ranging between 0.51 and 0.75) the concentration of 21 compounds. Despite the clear limitations, the model demonstrated good predictive capabilities for crucial volatiles such as sulfide compounds. It is important to highlight that the model, developed with a somewhat limited sample size, could see enhancements through the analysis of more samples in comparable conditions. This would contribute to bolstering data robustness and improving predictive accuracy.

4. Conclusions

The results obtained in this study indicated that molecular, metabolic and sensory analyses could be used as suitable and integrated tools to discriminate between and within genotypes belonging to different *Allium* species. Molecular analysis using ISSR markers allowed to clearly distinguish the elephant garlic genotypes from commercial common garlic varieties, and the genetic distinctiveness of the elephant garlic genotypes of different geographic origin. Detailed morphological measurements and antioxidant profiles highlighted differences between garlic and elephant garlic genotypes, with the latter exhibiting higher polyphenols and antioxidant activity. Moreover, analysis highlights the variability in the concentrations of sulfur-containing compounds between common and elephant garlic. The PCA model based on HS GC–MS as well as sensory data illustrated the aromatic variations among genotypes. These differences were particularly marked not only by comparing common garlic and elephant garlic genotypes, but also between elephant garlic genotypes from different geographic areas. Consistently, *E*-nose analysis revealed distinct clusters and indicated aromatic similarities between certain garlic genotypes as observed in aromatic analytical measurement. PCR analysis was employed to model relationships between E-nose data and identified volatiles. The model exhibited satisfactory predictive abilities for sulfur compounds, with varying degrees of accuracy. However, limitations were noted in predicting the concentrations of other volatile classes, emphasizing the need for further sample analysis to enhance model robustness. In conclusion, the extensive analyses encompassing molecular, chemical, and non-destructive characterizations have yielded insights into the genetic diversity, metabolomic profiles, and aromatic variations among the different allium genotypes analyzed. Notably, the pronounced differences observed in the different elephant garlic genotypes highlight the significant impact of their geographical origin and genetic background on the overall formation of secondary metabolites, even when the morphological characteristics of their bulbs appear highly similar.

Ethic statements

Ethical approval to conduct the sensory study was not required. All sensory analyses were conducted with the explicit consent of participants. Participants were provided with a detailed explanation of the study's purpose, procedures, and use of the data, and they voluntarily agreed to participate via the statement “I am aware that my responses are confidential, and I agree to participate in this survey ”. An affirmative reply was required to enter the survey. The questionnaires used in the study were provided to participants ensuring clarity and comprehensibility. The study was conducted with the utmost respect for participants' rights and confidentiality. Any identifiable information collected from participants was anonymized to protect their privacy. The methodology employed in this study was designed to minimize potential harm or discomfort to participants while maximizing the validity and reliability of the results. Sensory panelists were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

CRediT authorship contribution statement

Margherita Modesti: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Serena Ferri:** Writing – review & editing, Formal analysis. **Enrica Alicandri:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Mariateresa Cardarelli:** Writing – review & editing, Supervision. **Mario Ciaffi:** Writing – review & editing, Supervision, Conceptualization. **Diana De Santis:** Writing – review & editing, Supervision, Data curation, 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.

Data availability

Data are available in the paper and in supplementary material

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140643.

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