Volatile Profile, Antioxidant Capacity and Phenolic Content of Istrian Garlic Ecotypes

Iva BAŽON[1,2] (✉)
Dean BAN[1,2]
Igor LUKIĆ[1,2]
Nikola MAJOR[1]
Mario FRANIĆ[1,2]
Bernard PREKALJ[1,2]
Smiljana GORETA BAN[1,2]

Summary

Garlic (*Allium sativum* L.) is among the most important vegetable crops used in diet because of its sensory properties and positive health effects. Local producers in Istria (Croatia) have preserved old garlic ecotypes primarily for domestic consumption. Such ecotypes are adapted to specific agro-ecological conditions of Istria and their biochemical properties have not been investigated yet. The aim of this study is to determine the diversity of Istrian garlic ecotypes based on their biochemical profile for further selection of ecotypes with higher biological value in breeding programs. Garlic bulbs were collected from nine locations in Istria and analyzed after two months by chromatographic and spectrometric techniques. Volatiles diallyl sulfide, methyl allyl disulfide, diallyl disulfide, methyl allyl trisulfide, and diallyltrisulfide showed less variability among ecotypes, while total phenolics ranged from 28.5 to 69.5 mg gallic acid equivalents/100 g fresh weight. Two ecotypes and a cultivar abundant in phenolics also showed high antioxidant capacity measured by FRAP or DPPH assays. Several ecotypes stood out with higher dry matter content indicating their potential for longer storage. It was assumed that the determined variability in composition could be partly attributed to different genotypes and growing conditions. This research can contribute to the preservation of garlic biodiversity in Istria as well as to the establishment of a garlic breeding program.

Key words

bioactive compounds, breeding, chromatography, DPPH, FRAP, organosulfur compounds

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia

[2] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, 10000 Zagreb, Croatia

✉ Corresponding author: iva@iptpo.hr

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Introduction

Garlic (*Allium sativum* L.) is the world second most cultivated *Allium* species and is used as a food condiment for its beneficial health effects (Kamenetsky et al., 2005; Lanzotti, 2006). Fritsch and Friesen (2002) have classified *Allium sativum* L. into the *Longicuspis*, *Subtropical* and *Ophioscorodon* groups which mainly differ in bolting habit and topset size. However, *Allium longicuspis* is considered as the closest garlic relative based on recent molecular studies (Pooler and Simon, 1993; Volk et al., 2004; Ipek et al., 2015). Garlic originated in Central Asia from where it has spread worldwide and today is mostly cultivated in subtropical and temperate climates (Fritsch and Friesen, 2002). Garlic production in Croatia amounts around 26 thousand tons in 2019 (Croatian Buerau of Statistics, 2021).

Allium species are rich in organosulfur compounds and contain four cysteine sulfoxides, among which alliin is the most abundant in garlic and gives to it its characteristic flavor (Block, 2005). After the disruption of garlic bulb tissue, the enzyme alliinase transforms alliin into allicin which quickly decomposes into diallyl disulfide and other volatile compounds (Block, 1985; Block, 2005). Organosulfur compounds are usually associated with antibiotic and anticarcinogenic properties in humans and with insecticidal and repellent activity within plant defense mechanisms (Block, 2005). Phenolics are powerful antioxidants that protect against oxidative stress by scavenging free radicals (Lachowicz et al., 2016) and play an important role in sensory attributes of plant-derived food (Tomás-Barberán and Espin, 2001). Sulfur volatiles and phenolics are garlic secondary metabolites and their biosynthesis is usually associated with ecotype or genotype in conjunction with growth conditions (Isah et al., 2019).

Despite garlic vegetative propagation, its content of phytochemicals was found to be a variable parameter (Bravo, 2009; Martins et al., 2016). Garlic landraces and ecotypes are an important source of diversity (Baghalian et al., 2006; Shaaf et al., 2014). The assessment of available garlic diversity is important for breeding programs (Barboza et al., 2020).

Various garlic ecotypes are grown on small farms on the Istrian peninsula and are cultivated using traditional cultivation practices in specific agro-ecological conditions. Garlic ecotypes from Istria were recently characterized by Prekalj et al. (2019) and variability in bulb color, shape and other investigated morphological traits were found. However, Istrian garlic ecotypes have not yet been investigated for their phytochemical content. The aim of this research is to assess the diversity of Istrian garlic ecotypes based on specific sulfur volatile compounds, total phenol content (TPC), antioxidative capacity (AOC) and dry matter content (DM). This research also aims to contribute to the selection of ecotypes with superior nutritional value.

Material and Methods

Plant Material and Sampling

Eleven garlic ecotypes and two cultivars were collected from nine locations on the Istrian peninsula and were given accession numbers: Lindar (IPT 337, IPT 338), Rovinj (IPT 340), Pićan (IPT 341), Tinjan (IPT 342), Gračišće (IPT 343, IPT 344), Beram (IPT 345), Kaligarići (IPT 346, IPT 347) and Vižintini (IPT 348, IPT 349). The local names are listed in Table 1. 'Istarski crveni' cv. is listed in the List of varieties of the Republic of Croatia and has been maintained at the Institute of Agriculture and Tourism, Poreč, Croatia (N 45°13'20.30", E 13°36'6.49"). Cultivar 'Music' has been introduced and grown for several years at Lindar location. The accession IPT 337 was the initial material for cv. 'Istarski crveni' and is considered the most similar to cv. 'Istarski crveni'. In total, ten cloves, each from a different bulb, were sampled for TPC and AOC analyses, and cloves from two additional bulbs were sampled for the analysis of garlic volatiles two months after harvest.

Table 1. Local names of investigated garlic accessions

Accession	Local name
IPT337	Istarski crveni
IPT338	Istarski bijeli
IPT340	Istarski crveni
IPT341	Istarski crveni
IPT342	Mješavina
IPT343	Istarski crveni
IPT344	Istarski bijeli
IPT345	Istarski crveni
IPT346	Istarski crveni without scape
IPT347	Istarski crveni with scape
IPT348	Istarski crveni without scape
IPT349	Istarski crveni with scape
Cultivars	
cv. 'Istarski crveni'	cv. 'Istarski crveni'
cv. 'Music'	cv. 'Music'

Determination of Organosulfur Volatile Compounds

Extraction, analysis and identification of volatile compounds were carried out according to the method from Bažon et al. (2020). Garlic volatile compounds were isolated using headspace solid-phase microextraction (HS-SPME). Representative cloves of uniform garlic bulbs were peeled, crushed and homogenized and 0.3 grams were placed into a 10 mL glass vial containing a stirring bar. Four mL of distilled water were added before sealing with a PTFE/silicone septum. The headspace in the vial was equilibrated at 40°C for 5 minutes, and the extraction was carried out at 40°C for 5 minutes with stirring at 800 rpm. The headspace was sampled using a 1 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μmHS-SPME fibre, purchased from Supelco (Bellefonte, PA, USA).

Analysis of volatile compounds was carried out using a gas chromatograph with a flame ionization detector (GC-FID;

model 3350, Varian Inc., Harbour City, CA, USA). The GC oven temperature started from 40°C to 245°C at 6°C/min and was kept for 10 min at this temperature. Volatile compounds were desorbed by inserting the SPME fibre into the GC injection port for 10 min in splitless mode. The column Rtx-WAX 60 m × 0.25 mm i.d. × 0.25 μmdf (Restek, Belafonte, PA, USA) was used. Injector and detector temperatures were 245°C and 248°C, respectively. Helium was used as carrier gas at 17.5 psi at the column head, while detector gases were hydrogen and air. Blank runs were injected before sample analysis.

The identification of volatile aroma compounds was performed by comparing the retention times with those of the standards allyl mercaptane (≥90%), allyl sulfide (≥97%), allyl disulfide (80%), and by comparing their linear retention indices relative to n-alkanes with those reported in literature. Volatile compounds were additionally identified by comparison of their mass spectra to those from the NIST05 mass spectral library after GC-MS analysis using a Varian 3900 GC coupled to a Varian Saturn 2100T mass spectrometer (Varian Inc., Harbour City, CA, USA), with the same column and analysis parameters as previously described. The amounts were presented as peak area counts obtained by GC-FID analysis.

Extraction of Soluble Bioactive Compounds

The extraction, measurement of total phenolic content and determination of antioxidant capacity was carried out according to Major et al. (2018). Bioactive compounds were extracted by ultrasound-assisted extraction (UAE) in 80% methanol. The samples were sonicated, left to extract at room temperature and then centrifuged. The solution was filtered with a nylon filter (0.45 μm) prior to analysis.

Measurement of Total Phenolic Content

Folin-Ciocalteu assay (Singleton and Rossi, 1965) was used to evaluate the total phenolic content (TPC). Samples were mixed with Folin–Ciocalteu reagent and 6% sodium carbonate. The absorbance was measured after 60 minutes at 750 nm on a UV/Vis spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan). TPC was expressed as mg of gallic acid equivalents per gram sample in fresh weight (mg GAEQ/g FW).

Determination of Total Antioxidant Capacity

Total antioxidant capacity was evaluated by Ferric Reducing Antioxidant Power (FRAP) (Benzie and Strain, 1996) and by the scavenging ability of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Brand-Williams, Cuvelier, and Berset, 1995) using a spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan). FRAP antioxidant capacity was obtained by mixing the sample with FRAP reagent. The absorbance was measured at 593 nm after 4 minutes of reaction time and the results were expressed as mmol of Fe^{2+} equivalents per g sample in fresh weight (mmol Fe^{2+}EQ g^{-1} FW). DPPH radical scavenging activity was determined by mixing the sample with 0.1 mM DPPH radical. After 30 minutes, the absorbance was measured at 517 nm. The results were expressed as mmol of Trolox equivalents per g sample in fresh weight (mmol TEQ/FW).

Dry Matter Content

For the determination of dry matter content (DM) five grams of peeled garlic were dried in a forced oven (Memmert UF 160, Büchenbach Germany) at 105°C until constant weight.

Statistical Analysis

Data were processed by analysis of variance (ANOVA), with Tukey´s HSD as a post-hoc test using Statistica v. 13.4 software (Tibco, Palo Alto, California) for volatile compounds, TPC, FRAP, DPPH and DM. Dendrograms were constructed in PAST software (Hammer et al., 2001) using the unweighted pair group method with arithmetic mean (UPGMA) algorithm and Euclidean similarity index, with bootstrapping, N = 5000. Pearson correlation coefficient was calculated for TPC, AOC and DM in Statistica v. 13.4.

Results

Bioactive Compounds Variability in Garlic Accessions on the Istrian Peninsula

Twelve garlic accessions were collected from eight different locations for the assessment of the variability of their bioactive compounds content. Mean values and standard errors for volatile compounds, TPC, AOC and DM are shown in Table 2 and Table 3. Significant difference in the content of all the analyzed compounds was found among the investigated accessions except for DADS (Table 2).

Volatile compounds from Istrian garlic ecotypes extracted by HS-SPME and identified by GC-FID and GC-MS were diallyl sulfide (DAS), methyl allyl disulfide (MADS), diallyl disulfide (DADS), methyl allyl trisulfide (MATS), and diallyl trisulfide (DATS). Cultivar 'Istarski crveni' was not significantly different from IPT 340 and IPT 342 in DAS content and from IPT 343, IPT 347 and IPT 348 in MADS content. Cultivar 'Music' had a significantly higher mean value of DATS (2.74 ± 0.08) comparing to other investigated ecotypes. The results for MATS ranged from 0.40 ± 0.08 in IPT 346 to 3.12 ± 0.10 in cv. 'Music'. DADS had the highest peak area compared to the other compounds in all the samples.

Accessions IPT 338 and IPT 340 had the highest AOC measured by the FRAP assay (94.5 ± 9.51 and 87.4 ± 3.89, respectively) compared to all the other tested accessions. The highest FRAP value of 94.5 ± 9.51 found in IPT 338 was almost three times higher than its lowest value of 32.9 ± 0.5 found in IPT 349. The accession IPT 340 was not significantly different with respect to DPPH value from IPT 338, IPT 341, IPT 343, IPT 344 and cv. 'Music'. The accessions IPT 338, IPT 340, IPT 341, IPT 343 and cv. 'Music' did not differ in the TPC content. The TPC varied ~2.5 folds among the tested ecotypes with a range from 28.6 ± 2.21 mg GAEQ/g FW in cv. 'Istarski crveni' to 70.0 ± 2.13mg GAEQ/g FW in cv. 'Music'. Mean value of DM content found in this study ranged from 33.5% in IPT 340 to 41.8% in cv. 'Music', with only the cultivar 'Music' and the accession IPT 346 having DM content above 40%.

Table 2. The content of volatiles in garlic cv. 'Istarski crveni', cv. 'Music' and accessions IPT 337 – IPT 349

Accession	DAS	MADS	DADS	MATS	DATS
			peak area x 10^4		
IPT337	8.23 ± 0.28 bc[a]	28.5 ± 0.03 bcd	893 ± 57.08	2.1 ± 0.12 abc	1.24 ± 0.03 b
IPT338	6.14 ± 1.27 c	21 ± 2.48 cd	862 ± 4.19	2.86 ± 0.17 ab	1.31 ± 0.17 b
IPT340	11.1 ± 1.58 ab	22.7 ± 3.31 cd	1495 ± 39.38	0.75 ± 0.04 c	1.60 ± 0.38 b
IPT341	8.38 ± 1.61 bc	23.3 ± 6.92 cd	1016 ± 251. 03	1.86 ± 1.01 abc	0.85 ± 0.09 b
IPT342	11.2 ± 0.49 ab	23.3 ± 0.10 cd	1045 ± 82.08	0.76 ± 0.20 c	1.30 ± 0.28 b
IPT343	8.74 ± 0.32 bc	40.8 ± 2.14 ab	1319 ± 23.62	1.71 ± 0.22 abc	1.41 ± 0.16 b
IPT344	8.18 ± 1.11 bc	17.4 ± 0.28 d	1066 ± 78.10	0.82 ± 0.12 c	1.44 ± 0.12 b
IPT345	7.47 ± 0.33 bc	22.4 ± 1.90 cd	1141 ±127.1	0.78 ± 0.02 c	1.35 ± 0.04 b
IPT346	7.56 ± 0.84 bc	15.1 ± 0.28 d	1121 ± 177.67	0.40 ± 0.08 c	1.08 ± 0.14 b
IPT347	8.03 ± 0.35 bc	40.1 ± 0.43 ab	1128 ± 114.14	1.29 ± 0.35 bc	1.09 ± 0.16 b
IPT348	7.68 ± 0.13 bc	31.9 ± 0.98 abc	1231 ± 3.15	1.02 ± 0.01 c	1.48 ± 0.09 b
IPT349	9.00 ± 0.78 bc	20.6 ± 0.07 cd	1095 ± 108.85	0.62 ± 0.06 c	1.3 ± 0.19 b
cv. 'Istarski crveni'	15.5 ± 0.25 a	44.9 ± 1.23 a	999 ± 87.40	1.29 ± 0.08 bc	0.9 ± 0.02 b
cv. 'Music'	7.2 ± 0.32 bc	23.3 ± 3.01cd	998 ± 29.32	3.12 + 0.10 a	2.74 ± 0.08 a

(Abbreviations: DAS- diallyl sulfide, MADS - methyl allyl disulfide, DADS - diallyl disulfide, MATS methyl allyl trisulfide, DATS diallyl trisulfide)

[a] Different letter within a column denotes significant difference by Tukey's HSD test at $P \leq 0.05$

Table 3. The total phenol content, antioxidant capacity and dry matter content in garlic cv. 'Istarski crveni', cv. 'Music' and accessions IPT 337 – IPT 349

Accession	TPC	FRAP	DPPH	DM
	EQ GAL (mg g^{-1} FW)	EQ FeSO4 (mmol g^{-1} FW)	EQ TX (mmol g^{-1} FW)	(%)
IPT337	39.0 ± 1.48 efg[a]	51.0 ± 1.25 bc	74.2 ± 1.81 bcde	37.8 ± 0.17 de
IPT338	69.5 ± 2.72 ab	94.5 ± 9.51 a	86.4 ± 3.64 ab	37.4 ± 0.14 def
IPT340	61.7 ± 3.63 ab	87.4 ± 3.89 a	89.8 ± 1.12 a	33.5 ± 0.16 j
IPT341	59.6 ± 1.0 abc	42.4 ± 3.54 bcd	80.8 ± 4.94 abcd	35.5 ± 0.30 i
IPT342	47.8 ± 1.90 de	49.0 ± 3.58 bcd	70.2 ± 1.93 cde	39.2 ± 0.24 bc
IPT343	63.2 ± 2.39 ab	50.2 ± 1.74 bcd	74.6 ± 4.93 abcde	35.9 ± 0.22 hi
IPT344	58.9 ± 2.16 bc	46.6 ± 2.67 bcd	77.8 ± 2.08 abcde	37.0 ± 0.03 efg
IPT345	41.7 ± 3.07 def	43.9 ± 0.43 bcd	68.6 ± 2.35 de	39.2 ± 0.21 c
IPT346	41.3 ± 3.02 def	48.1 ± 1.05 bcd	67.8 ± 1.13 def	40.3 ± 0.20 b
IPT347	39.2 ± 0.37 ef	39.1 ± 1.43 cd	70.8 ± 1.76 cde	35.4 ± 0.29 i
IPT348	49.9 ± 1.86 cd	35.4 ± 1.03 cd	65.6 ± 0.85 ef	38.2 ± 0.11 cd
IPT349	35.4 ± 1.32 fg	32.9 ± 0.50 d	66.0 ± 1.40 def	36.6 ± 0.32 fgh
cv. 'Istarski crveni'	28.6 ± 2.21 g	45.4 ± 3.63 bcd	52.6 ± 4.79 f	36.1 ± 0.20 ghi
cv. 'Music'	70.0 ± 2.13 a	59.0 ± 3.05 b	84.8 ± 3.07 abc	41.8 ± 0.23 a

(Abbreviations: TPC- total phenol content, FRAP - Ferric Reducing Antioxidant Power, DPPH –radical scavenging, DM - dry matter)

[a]Different letter within a column denotes significant difference by Tukey's HSD test at $p \leq 0.05$

Cluster Analysis

Cluster analysis was carried out based on UPGMA and two cluster tree diagrams were plotted. The first was based on the content of garlic volatiles, while the second was based on the values of TPC, FRAP, DPPH and DM (Fig. 1 and Fig. 2).

According to the cluster analysis based on volatile compounds, twelve accessions and cultivars were clustered into two main groups (Fig. 1). The first group included only the commercial cultivar 'Music' and the second group included cv. 'Istarski crveni' and all the other investigated accessions.

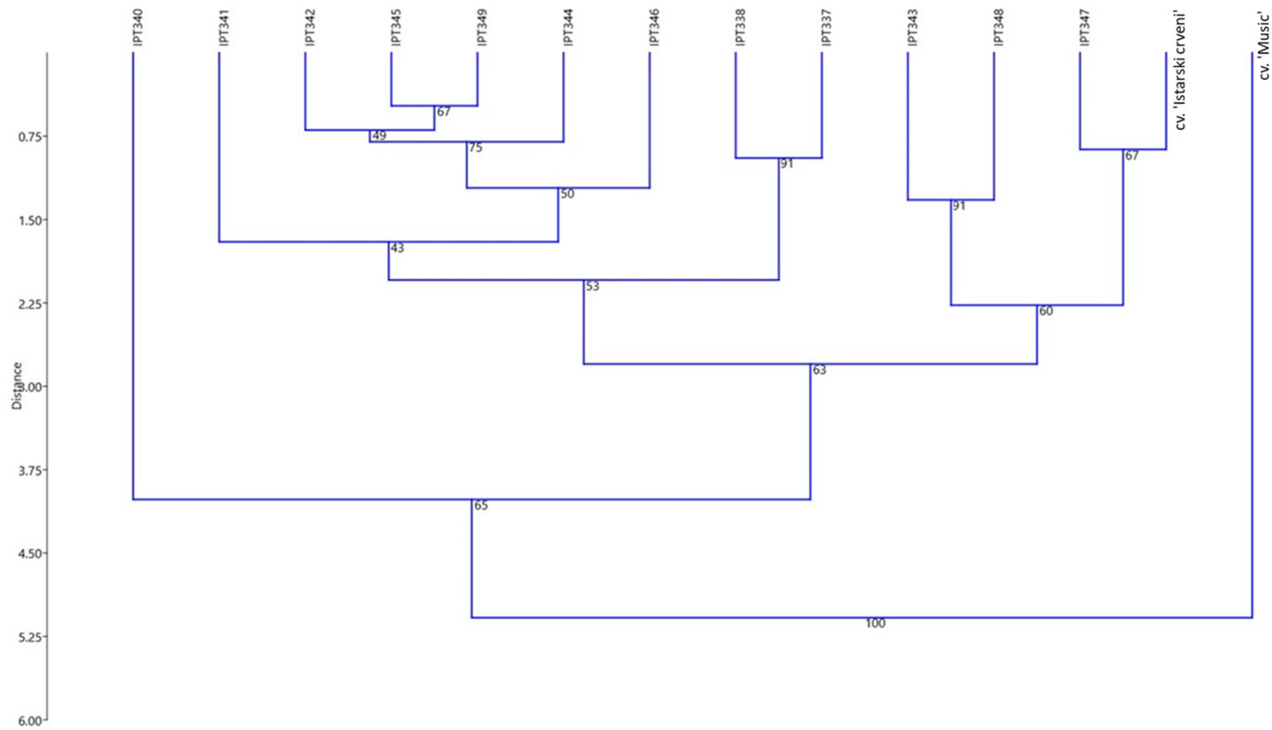

Figure 1. Cluster analysis of garlic cultivars and accessions based on the content of DAS, MADS, DADS, MATS and DATS. Unweighted pair group method with arithmetic mean (UPGMA) algorythm with Euclidean similarity index, with bootstrapping, N = 5000 (abbreviations: DAS – diallyl sulfide, MADS – methyl allyl disulfide, DADS – diallyl disulfide, MATS – methyl allyl trisulfide, DATS – diallyl trisulfide)

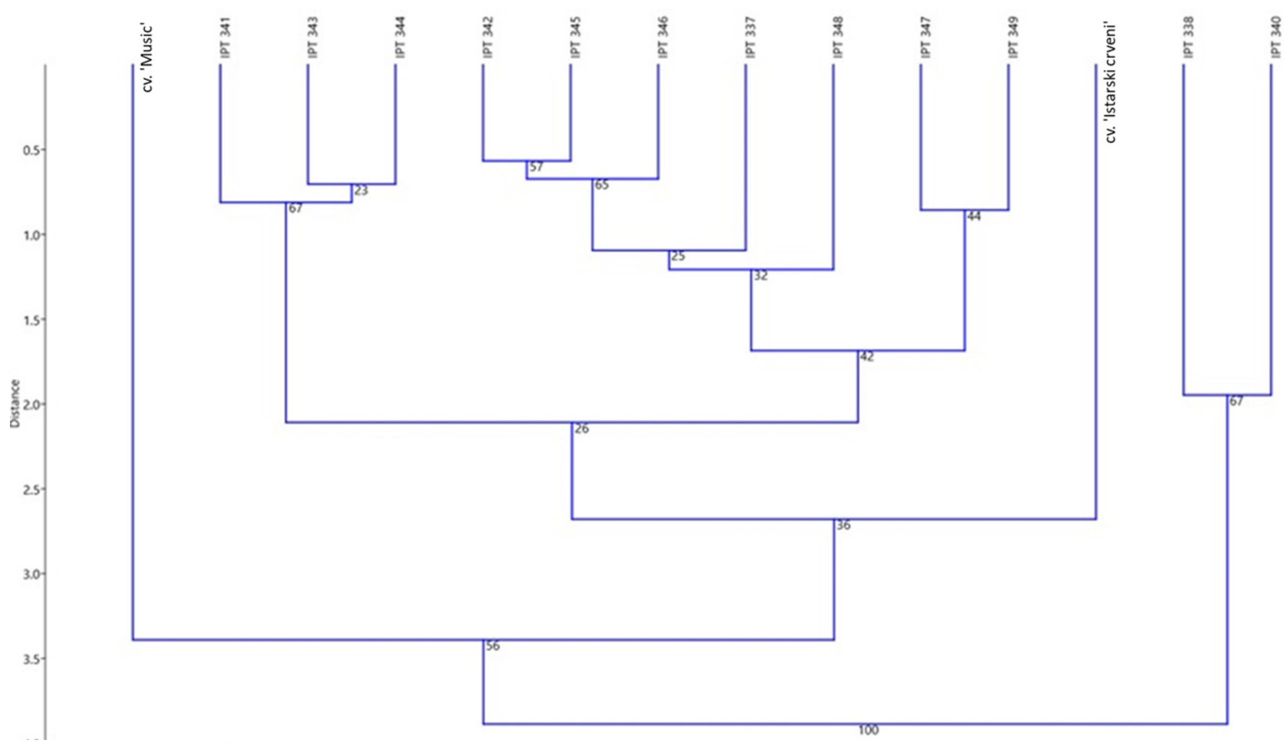

Figure 2. Cluster analysis of garlic cultivars and accessions based on TPC, FRAP, DPPH and DM. Unweighted pair group method with arithmetic mean (UPGMA) algorythm with Euclidean similarity index, with bootstrapping, N = 5000 (abbreviations: TPC – total phenol content, FRAP – Ferric Reducing Antioxidant Power, DPPH – radical scavenging, DM – dry matter)

The second group was divided into two subgroups (Fig. 1). The first subgroup contained cv. 'Istarski crveni' and eleven accessions, IPT 347, IPT 348, IPT 343, IPT 337, IPT 338, IPT 346, IPT 344, IPT 349, IPT 345, IPT 342 and IPT 341, while the second subgroup consisted only of IPT 340. The volatile profile of cv. 'Music' was different from all the other accessions, whereas accession IPT 340 was the most distinguished among ecotypes from Istria with respect to the volatiles content. 'Music' had high values of MATS and DATS and lower values of DAS and DADS, contrary to IPT 340, which had low values of MADS and DATS and higher values of DAS and DADS. In the first subgroup, IPT 345 and IPT 349 were the most similar accessions based on the volatile content. High similarity with respect to the volatiles content was found between cv. 'Istarski crveni' and IPT 347, IPT 348 and IPT 343, and between IPT 337 and IPT 338. Cultivar 'Istarski crveni' and IPT 337, which were considered the most similar, were not grouped close by cluster analysis.

Cluster analysis based on the content of phenolics, AOC and DM clustered accessions into two main groups (Fig. 2). The first group included the accessions IPT 338 and IPT 340, while the second group contained cv. 'Istarski crveni', cv. 'Music' and all the other accessions. The ecotypes from the first group stood out with higher AOC and TPC than those from the second group. Accessions from the second group were divided into two subgroups. The first subgroup contained cv. 'Istarski crveni' and ten accessions: IPT 349, IPT 347, IPT 348, IPT 337, IPT 346, IPT 345, IPT 342, IPT 344, IPT 343 and IPT 341. Cultivar 'Istarski crveni' and IPT 337, grown at different locations, were clustered into the first subgroup. Among the accessions from the first subgroup, IPT 342 and IPT 345 were the most similar, while notable similarities were observed between IPT 343 and IPT 344, as well as between IPT 347 and IPT 349. The second subgroup included only the cultivar 'Music' which was different from all the other accessions, probably due to high TPC and DM content found.

Discussion

The twelve garlic accessions grown on the Istrian peninsula were evaluated for the content of volatiles, TPC, AOC and DM to determine the extent of bioactive compounds variation.

Five volatile compounds were detected using HS-SPME in combination with GC-FID and GC-MS, namely DAS, MADS, DADS, MATS and DATS, among which DADS had the highest peak area. Based on the review from Abe et al. (2020), volatile compounds detected in our study may be classified as monosulfides, such as DAS, disulfides, such as MADS and DADS, and polysulfides, such as MATS and DATS. The volatile DADS had the highest peak area also in the study of Galoburda et al. (2013), where garlic cultivars originating from Latvia and France were studied. DADS was also the major compound in *Allium roseum* L. where its amount, along with that of MADS, was influenced by sulfur fertilization (Imen et al., 2013). The volatiles DAS, MADS, DADS, MATS and DATS were also detected in accessions originating from Greece (Avgeri et al., 2020). In our study, the concentration of TPC ranged from 28.6 ± 2.21 to 70.0 ± 2.13 mg GAEQ/g FW, which was a larger variation than that found by Chen et al. (2013), who investigated 43 garlic cultivars and found concentrations varying from 17.16 to 42.53 mg GAE/g FW. Our results are also in agreement with those of Beato et al. (2011) who found a notable variation in TPC among the investigated garlic cultivars collected from different parts of Spain. FRAP and DPPH values measured in this study were higher than those found by Major et al. (2018) for local shallot accessions.

Garlic secondary metabolites, sulfur volatiles and phenolics, along with AOC and DM, were used for the assessment of the variability in garlic phytochemical composition previously (Beato et al., 2011; Chen et al., 2013; Galoburda et al., 2013, Barboza et al., 2020). Factors such as genotype, cultivation practices and environmental conditions could influence the diversity of constituents of garlic plants grown at different locations, particularly organosulfur content (Kamenetsky et al., 2005), phenolic composition (Tomás-Barberán and Espin, 2001) and morphological parameters (Polyzos et al., 2019). It was found previously that cultivars may differ regarding their sulfur uptake and its metabolism (Randle and Lancester, 2002), and that environmental factors can influence the quality of garlic flavor due to their impact on its secondary metabolism during plant growth (Kamenetsky et al., 2005). Different expression of phenolics in plants is probably due to variable activity of the phenylalanine ammonia lyase (PAL) enzyme, responsible for phenolic biosynthesis (Beato et al., 2011; Tomás-Barberán and Espin, 2001). It depends on the expression of PAL gene which could be induced by different environmental stress conditions (Beato et al., 2011; Tomás-Barberán and Espin, 2001). The variability found in biochemical profiles among the accessions analyzed in this study could be probably ascribed to those factors. Two accessions that were supposed to be the same variety grown under different locations, cv. 'Istarski crveni' and IPT 337, were similar in morphological traits according to the results from Prekalj et al. (2019), which, however, was not in line with our results for their biochemical content (Fig. 1 and Fig. 2). Accession IPT 347 was the most similar to cv. 'Istarski crveni', IPT 348 to IPT 343 and IPT 345 was the most similar to IPT 349 (Fig. 1), belonging to a group which was given a local name *istarski crveni* or red – type garlic. Another two accessions very similar with respect to their volatiles content, IPT 337 and IPT 338, locally named *istarski crveni* and *istarski bijeli* (white-type garlic), were collected from the same growing location. Among the investigated Istrian accessions, IPT 340 was the most distinguished and had low values of MADS and DATS, and higher values of DAS and DADS compared to other accessions.

The grouping based on TPC, AOC and DM was different from that based on the content of volatile compounds. Accessions IPT 343 and IPT 344, which were grown at the same location but were named differently as *istarski crveni* and *istarski bijeli*, showed similarity in the content of TPC, AOC and DM, and were clustered together by cluster analysis. Accessions IPT 347 and IPT 349, both named *istarski crveni* by local farmers, which were geographically very close, were also very similar with respect to morphological parameters (Prekalj et al., 2019). The only two accessions clustered in the first group, IPT 338 and IPT 340, with different local names *istarski bijeli* and *istarski crveni*, were not geographically close and were not highly similar, probably due to differences in DM content.

All the accessions including cultivar 'Istarski crveni' had different volatile profile, TPC, AOC and DM with respect to the commercial variety 'Music'.

Garlic is rich in phenolics which strongly contribute to its antioxidant capacity regardless of the particular phenolic profile (Beretta et al., 2017). Antioxidative capacity, often related to the phenolic content, is commonly evaluated by at least two methods to reflect garlic antioxidant system more accurately (Prior et al., 2005; Chen et al., 2013). A significant positive correlation was found between TPC and DPPH (r=0.734, p<0.001) and TPC and FRAP (r=0.569, p<0.05), respectively. Such significant positive correlation between TPC and DPPH or FRAP was also found by Chen et al. (2013) and Avgeri et al. (2020).

The content of DM in garlic is important for processing, especially for dehydration, since it enables longer shelf-life and it is usually associated with flavor intensity (Sance et al., 2008, Barboza et al., 2020). Rosen and Tong (2001) found that scape removal had significant impact on DM content, which was higher when the scape was not removed. Based on the results from Nabi et al. (2013) different storage conditions and curing methods significantly influenced the DM content and showed that cold storage and drying with leaves gave higher percentage of DM in onion. Dry matter content of bulbs decreased with increasing level of irrigation in the research from Biswas et al. (2010). It can be noted that 'Music' and IPT 346 had DM content higher than 40% and were considered more suitable for dehydration and longer shelf life compared to the other accessions. Different cultivation practices were probably applied on different locations, which could have contributed to the observed difference in total DM between the accessions and cultivars analyzed in this study.

There is a high interest for preservation and evaluation of local ecotypes due to their adaptation to different agroclimatic conditions. Several authors have reported about the diversity of important traits of locally grown plant material as a large potential for breeding purposes (Baghalian et al., 2006; Shaaf et al., 2014; Wang et al., 2014). It was found that some local genotypes could have superior traits compared to imported cultivars (Avgeri et al., 2020). Major et al. (2018) found local shallot accessions with higher AOC and diverse phenolic profile compared to particular commercial cultivars. The research carried out by Fernandes et al. (2020) showed that local Spanish onions had different quantitative and qualitative composition of volatiles. The Istrian garlic ecotypes in our study have shown high variability in the content of volatiles, TPC, AOC and DM, and were different from the commercial cultivar 'Music'.

Conclusion

Garlic accessions collected from Istria have shown variability in the content of volatiles, TPC, AOC and DM. The lack of conventional breeding methods renders the improvement of garlic economically important traits difficult. Studied accessions can be used further in breeding programs for selection of cultivars with higher content of secondary metabolites. This is the first study on the characterization of Istrian garlic ecotypes based on phytochemical content. Further investigation is warranted to evaluate the collected accessions under the same agro-climatic conditions.

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References

Abe K., Hori Y., Myoda T. (2020). Volatile Compounds of Fresh and Processed Garlic (Review). Exp Ther Med 19: 1585-1593. doi: 10.3892/etm.2019.8394

Avgeri I., Zeliou K., Petropoulos S. A., Bebeli P. J., Papasotiropoulos V., Lamari F. N. (2020). Variability in Bulb Organosulfur Compounds, Sugars, Phenolics, and Pyruvate among Greek Garlic Genotypes: Association with Antioxidant Properties. Antioxidants 9: 967. doi: 10.3390/antiox9100967

Baghalian K., Naghavi M. R., Ziai S. A., Badi H. N. (2006). Post-Planting Evaluation of Morphological Characters and Allicin Content in Iranian Garlic (*Allium sativum* L.) Ecotypes. Sci Hortic 107 (4): 405-410. doi: 10.1016/j.scienta.2005.11.008

Barboza K., Salinas M. C., Acuña C. V., Bannoud F., Beretta V., García-Lampasona S., Burba J. L., Galmarini C. R., Cavagnaro P. F. (2020). Assessment of Genetic Diversity and Šopulation Structure in a Garlic (*Allium sativum* L.) Germplasm Collection Varying in Bulb Content of Pyruvate, Phenolics and Solids. Sci Hortic 261:108900. doi: 10.1016/j.scienta.2019.108900

Bažon I., Lukić I., Ban D., Horvat I., Prekalj B., Goreta Ban S. (2020). Volatile Compounds of Garlic cv. 'Istarski crveni' at Different Harvesting Dates. J Cent Eur Agric 21 (2): 333-337. doi: 10.5513/JCEA01/21.2.2728

Beato V. M., Orgaz F., Mansilla F., Montaño A. (2011). Changes in Phenolic Compounds in Garlic (*Allium sativum* L.) Owing to the Cultivar and Location of Growth. Plant Foods Hum Nutr 66 (3): 218-223. doi: 10.1007/s11130-011-0236-2

Benzie I. F. F., Strain J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of 'Antioxidant Power': the FRAP Assay. Anal Biochem 239: 70-76. doi: 10.1006/abio.1996.0292

Beretta H. V., Bannoud F., Insani M., Berli F., Hirschegger P., Galmarini C. R., Cavagnaro P. F. (2017). Relationships between Bioactive Compound Content and the Antiplatelet and Antioxidant Activities of Six *Allium* Vegetable Species. Food Technol Biotechnol 55 (2): 266-275. doi: 10.17113/ftb.55.02.17.4722

Biswas S., Khair A., Sarker P., Alom M. (2010). Yield and Storability of Onion (*Allium cepa* L.) as Affected by Varying Levels of Irrigation. Bangladesh J Agric Res: 35(2)

Block E. (1985). The Chemistry of Garlic and Onions. Sci Am 252(3): 114-118. doi: 10.1038/scientificamerican0385-114

Block E. (2005). Biological Activity of *Allium* Compounds: Recent Results. Acta Hortic 688: 41-58. doi: 10.17660/actahortic.2005.688.4

Brand-Williams W., Cuvelier M. E., Berset C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT- Food Sci Technol 28: 25-30. doi: 10.1016/S0023-6438(95)80008-5

Bravo L. (2009). Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. Nutr Rev 56(11): 317-333. doi: 10.1111/j.1753-4887.1998.tb01670.x

Chen S., Shen X., Cheng S., Li P., Du J., Chang Y., Meng H. (2013). Evaluation of Garlic Cultivars for Polyphenolic Content and Antioxidant Properties. PLoS One 8(11): e79730. doi: 10.1371/journal.pone.0079730

Croatian Bureau of Statistics. (2021) Zagreb, Croatia. Available at: https://www.dzs.hr/ [Accessed 01 03. 2021]

Fernandes S., Gois A., Mendes F., Perestrelo R., Medina S., Câmara J. S. (2020). Typicality Assessment of Onions (*Allium cepa*) from Different Geographical Regions Based on the Volatile Signature and Chemometric Tools. Foods 9(3): 375. doi:10.3390/foods9030375

Fritsch R. M., Friesen N. (2002). Evolution, Domestication and Taxonomy. In: *Allium* Crop Science: Recent Advances (Rabinowitch H.D., Currah

L., eds.), CABI Publishing, Wallingford, United Kingdom, pp. 5-30. doi: 10.1079/9780851995106.0005

Galoburda R., Bodniece K., Talou T. (2013) *Allium sativum* Flavour Compounds as an Indicator for Garlic Identity and Quality Determination. J Food Eng 3: 226-234

Hammer Ø., Harper D. A. T., Ryan P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol Electron 4(1)4: 9

Imen A., Najjaa H., Neffati M. (2013). Influence of Sulfur Fertilization on S-Containing, Phenolic, and Carbohydrate Metabolites in Rosy Garlic (*Allium roseum* L.): A Wild Edible Species in North Africa. Eur Food Res Technol 237(4): 521–527. doi: 10.1007/s00217-013-2025-7

Ipek M., Sahin N., Ipek A., Cansev A., Simon P. W. (2015). Development and Validation of New SSR Markers from Expressed Regions in the Garlic Genome. Sci Agric 72(1): 41-46. doi: 10.1590/0103-9016-2014-0138

Isah T. (2019). Stress and Defense Responses in Plant Secondary Metabolites Production. Biol Res 52(1). doi: 10.1186/s40659-019-0246-3

Kamenetsky R., London Shafir I., Khassanov F., Kik C., Van Heusden A. W., Vrielink-Van Ginkel M., Burger-Meijer K., Auger J., Arnault I., Rabinowitch H.D. (2005). Diversity in Fertility Potential and Organo-Sulphur Compounds among Garlics from Central Asia. Biodivers Conserv 14(2): 281-295. doi: 10.1007/s10531-004-5050-9

Lachowicz S., Kolniak-Ostek J., Oszmiański J., Wiśniewski R. (2016). Comparison of Phenolic Content and Antioxidant Capacity of Bear Garlic (*Allium ursinum* L.) in Different Maturity Stages. J Food Process Preserv 41(1): e12921. doi: 10.1111/jfpp.12921

Lanzotti V. (2006). The Analysis of Onion and Garlic. J Chromatogr A 1112(1-2): 3-22. doi: 10.1016/j.chroma.2005.12.016

Major N., Goreta Ban S., Urlić B., Ban D., Dumičić G., Perković J. (2018). Morphological and Biochemical Diversity of Shallot Landraces Preserved along the Croatian Coast. Front Plant Sci 9: 1749. doi:10.3389/fpls.2018.01749

Martins N., Petropoulos S., Ferreira I. C. F. R. (2016). Chemical Composition and Bioactive Compounds of Garlic (*Allium sativum* L.) as Affected by Pre- and Post-Harvest Conditions: A Review. Food Chem 211: 41-50. doi: 10.1016/j.foodchem.2016.05.029

Nabi G., Rab A., Sajid M., Farhatullah, Abbas S. J., Ali I. (2013). Influence of Curing Methods and Storage Conditions on the Post-Harvest Quality of Onion Bulbs. Pak J Bot 45(2): 455-460

Polyzos N., Papasotiropoulos V., Lamari F. N., Petropoulos S. A., Bebeli P. J. (2019). Phenotypic Characterization and Quality Traits of Greek Garlic (*Allium sativum* L.) Germplasm Cultivated at Two Different Locations. Genet Resour Crop Evol 66 (2):1671–1689. doi: 10.1007/s10722-019-00831-4

Pooler M. R., Simon P. W. (1993). Characterization and Classification of Isozyme and Morphological Variation in a Diverse Collection of Garlic Clones. Euphytica 68: 121-130. doi: 10.1007/BF00024161

Prekalj B., Franić M., Ban D., Bažon I., Cvitan D., Goreta Ban S. (2019). Bulb Morphological Characteristics of Garlic Ecotypes from Istria. In: Proceedings from 54th Croatian & 14th International Symposium on Agriculture (Mioč B., Širić I., eds), University of Zagreb, Faculty of Agriculture, pp. 291-295

Prior R. L., Wu X., Schaich K. (2005). Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. J Agric Food Chem 53(10): 4290-4302. doi: 10.1021/jf0502698

Randle W. M., Lancaster J. E. (2002). Sulphur Compounds in Alliums in Relation to Flavour Quality. In: *Allium* Crop Science: Recent Advances (Rabinowitch H.D., Currah L., eds.), CABI Publishing, Wallingford, United Kingdom, pp. 5-30. doi: 10.1079/9780851995106.0329

Rosen C. J., Tong. C. B. S. (2001). Yield, Dry Matter Partitioning and Storage Quality of Hardneck Garlic as Affected by Soil Amendments and Scape Removal. Hort Science 36(7): 1235–1239. doi: 10.21273/HORTSCI.36.7.1235

Sance M. M., González R. E., Soto V. C., Galmarini C. R. (2008). Relationships between Antiplatelet Activity, Dry Matter Content and Flavor in Onion Cultivars. J Food Agric Environ 6: 41-46

Shaaf S., Sharma R., Kilian B., Walther A., Özkan H., Karami E., Mohammadi B. (2014). Genetic Structure and Eco-Geographical Adaptation of Garlic Landraces (*Allium sativum* L.) in Iran. Genet Resour Crop Evol 61(8): 1565-1580. doi: 10.1007/s10722-014-0131-4

Singleton V. L., Rossi J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic 16: 144-158

TIBCO Software Inc. (2018). TIBCO Statistica, Version 13.4.0. Palo Alto, California, USA

Tomás-Barberán F. A., Espín J. C. (2001). Phenolic Compounds and Related Enzymes as Determinants of Quality in Fruits and Vegetables. J Sci Food Agric 81(9): 853-876. doi: 10.1002/jsfa.885

Volk G. M., Henk A. D., Richards C. M. (2004). Genetic Diversity among U.S. Garlic Clones as Detected Using AFLP Methods. J. Am Soc Hortic Sci 129(4):559-569. doi: 10.21273/JASHS.129.4.0559

Wang H., Li X., Shen D., Oiu Y., Song J. (2014). Diversity Evaluation of Morphological Traits and Allicin Content in Garlic (*Allium sativum* L.) from China. Euphytica 198(2): 243-254. doi: 10.1007/s10681-014-1097-1

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