

Volatile aroma and bioactive compounds diversity in wild populations of rosemary (*Salvia rosmarinus* Schleid.) from Italy cultivated under homogeneous environmental conditions

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Abstract

Current and potential uses of Rosemary (*Salvia rosmarinus* Schleid.) in the food, cosmetic and pharmaceutical industry are based on the sensory properties and biological activities related to Volatile Organic Compounds (VOCs) and non-volatile bioactive compounds, such as phenolic diterpenes and acids. Fifty-six rosemary plants from the CREA germplasm collection, representing 14 wild populations (4 plants for each population) collected in different geographical areas across Italy, were characterised for VOCs and phenolic diterpenes and acids. Three chemotypes were identified and associated to different geographical areas, with marked differences in the levels of key odorants, such as 1,8-cineole, verbenone and camphor, and key antioxidant compounds, such as carnosic acid. Genotypes characterised by potentially better flavour profile seemed also to be associated with enhanced level of bioactive compounds and related functional properties. This diversity can be exploited to design specific products for the food and flavouring industry, with improved flavour quality and functional properties.

Keywords: *Rosmarinus officinalis*, aroma compounds, essential oil, antioxidants, biodiversity

Introduction

Rosemary (*Salvia rosmarinus* Schleid.) is an aromatic plant native of the Mediterranean basin which is traditionally used for culinary and medicinal purposes, but also increasingly used nowadays in the food and cosmetic industry. Key chemical constituents for such uses are volatile organic compounds (VOCs), which form the essential oil, and non-volatile bioactive compounds, such as phenolic diterpenes and acids, which attract increasing interest related to their potential health-protecting properties and application as food additive [1]. While VOCs play a main role in determining rosemary flavour, also phenolic diterpenes and acids may contribute to it, by imparting a mild bitter taste and by preventing the formation of off-flavours due to their strong antioxidant activity [2].

A germplasm collection of rosemary has been established at the CREA-Research Centre for Vegetable and Ornamental Crops (Sanremo, Italy), where spontaneous plants collected across the Mediterranean basin are maintained ex situ and are available to investigate genetic and phenotypic biodiversity within the species [3, 4]. The purpose of the collection is to preserve and study the biodiversity within the species, also in view of promoting its conservation through use. The aim of the present study was to characterise the chemical composition of rosemary plants from 14 wild populations collected in Italy and conserved in such germplasm collection, by analysing both VOCs, by GC-MS, and phenolic diterpenes and acids, by HPLC-DAD.

Experimental

Plant material

Rosemary plants belonging to wild populations collected in 14 natural areas distributed across Italy (Figure 1) and conserved ex situ at the CREA-Research Centre for Vegetable and Ornamental Crops (Sanremo, Italy) were tested in the present study. All plants were cultivated for four years at the CREA-Research Centre under similar environmental conditions, climate and substrate, in order to minimise environmental influences and highlight genetic related differences in chemical composition. For each population 4 individual plants, for a total of 56 plants, were characterised for VOCs and phenolic diterpenes and acids content in the dried leaves.

Chemical analyses

For VOCs determination a 0.5-g sample of powdered dried rosemary leaves was extracted by 5 mL of tert-butyl methyl ether solution containing 0.25 µL/mL butyl benzene as internal standard, for 24 h at ambient temperature, and then filtered through anhydrous Na₂SO₄ and silica gel 60. Quantification of VOCs was carried out by a GC-

MS analytical system (6890-5973N Agilent Technologies, Palo Alto, CA, USA) according to the following conditions: analytical column, Rxi-5Sil MS (30m, 0.25 mm, 0.25 μ m); temperature ramp: 50 (1 min), 5°C/min to 250°C, then 20°C/min to 315°C (15 min); He, carrier gas at 43 cm/s; 1 μ L of extract injection; split injection, with 5:1 split ratio, at 250°C; transfer line at 325°C; MS spectrometer operating by the EI mode at 70 eV with a scan range of 30-250 amu. The identification of VOCs was based on comparison of mass spectra with NIST/Wiley libraries, and retention indices. Quantification data were calculated as percentage of total VOCs content.



Figure 1: map of Italy indicating the collection sites of the 14 wild populations of rosemary plants. Red points: North Western Italy; green points: Sardinia; blue points Central and Southern Italy.

For determination of phenolic diterpenes and acids, 200 mg of powdered rosemary dried leaves were dissolved in a 70% ethanol extracting solution, stirred and then sonicated for 30 minutes. After centrifugation and filtration, 10 μ L were injected into an Agilent 1100 HPLC system, the column used being a Zorbax XDB C18 (25cm x 4.6 mm x 5 μ m) thermostated at 30 °C. HPLC conditions: gradient elution with soln. A (H₂O acidified with 1% v/v of acetic acid) and soln. B (methanol) as mobile phases; readings at 280 and 330 nm. Quantification was carried out by using calibration curves obtained by standard solutions of pure compounds; results were expressed as mg/g of dry matter.

Statistical analyses

Principal Components Analysis (PCA) and Agglomerative hierarchical clustering (AHC) analyses were performed by the XLStat software package (2020.4.1 version). AHC analysis was performed on average data (n=4) obtained on each population.

Results and discussion

As regards VOCs, 32 individual compounds were quantified, belonging to the following groups: 11 monoterpene hydrocarbons, 7 oxygenated monoterpenes, 6 sesquiterpenes, 2 oxygenated sesquiterpenes and 6 from other minor groups. Carnosic acid and carnosol were determined for phenolic diterpenes and rosmarinic acid for phenolic acids. AHC analysis grouped all populations into three clusters representing different chemotypes, as shown by the dendrogram reported in Figure 2: populations from Sardinia (green) with high verbenone levels, from Central and Southern Italy (blue) with high 1,8-cineole levels, and from North Western Italy (red) with relatively high camphor levels. Populations assigned to the three clusters seemed to belong to the three main chemotypes previously described in the literature, the verbenoniferum, the cineoloferum and the camphoriferum [3]. The profile plot also suggested that the verbenone level is particularly low in the cluster from Central and Southern Italy and in that one from North Western Italy. Average level of verbenone in populations from Sardinia amounted to 30.5%, whereas in the cluster from North Western Italy it reached 5.5% and in the third cluster its content was very low (<0.1%). This point could be of particular interest, because high levels of verbenone have been reported to be associated with better flavour properties [2]. Moreover, the cluster from Sardinia was also characterised by the highest content of carnosic acid, with an average content of 55 mg/g d.m., followed by the North Western Italy cluster with an average level of 41 mg/g d.m. One particular population, SPIE, showed an average carnosic acid level of 77 mg/g d.m., higher than in accessions previously selected for high level in carnosic acid [5]. As a whole, classification of the examined populations according to VOCs and bioactive compounds reflected results from a previous genetic characterisation of the same populations [4].

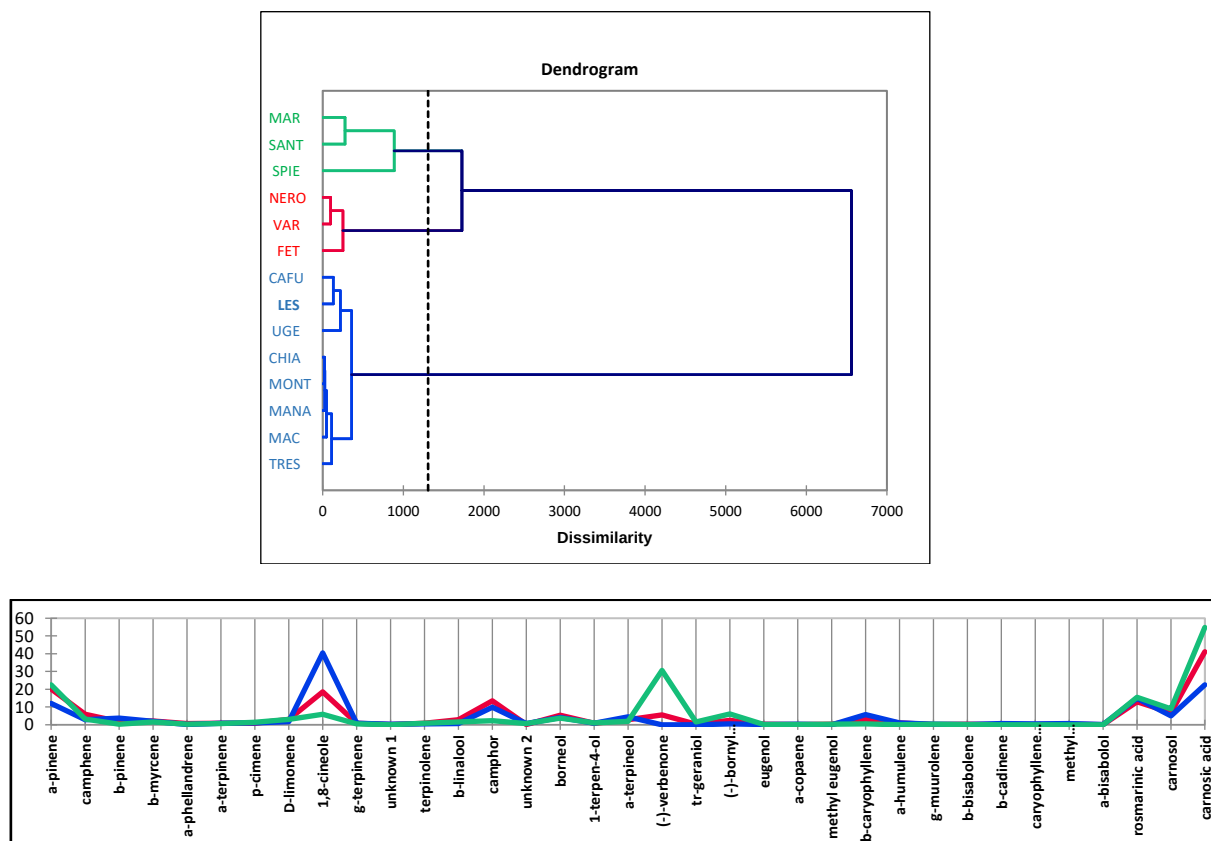


Figure 2: Dendrogram and profile comparing the means of the different classes that have been created, as obtained by Agglomerative Hierarchical Clustering (AHC) analysis.

PCA analysis further supported the chemotype classification highlighted by the AHC analysis (Figure 3). All plants from the Central and Southern Italy cluster (blue labels), were associated with positive values of the first PC, whereas at negative values of the first PC, plants from the two other clusters (North Western Italy in red and Sardinia in green) tended to group apart from each other, with minor exceptions (Figure 3: Observations). Interestingly, the plot of variables also showed that levels of the bioactive phenolic diterpenes carnosic acid and carnosol are high in genotypes characterised by high verbenone and a-pinene content as main VOCs (Figure 3: Variables), suggesting that the accumulation of both group of compounds seemed to be controlled in a similar way by genetic factors. Pearson's correlation coefficients highlighted significant (at $p < 0.05$) linear correlation between verbenone and carnosic acid content ($R = 0.768$) and inverse correlation between 1,8-cineole and carnosic acid content ($R = -0.778$).

Conclusion

The highlighted chemical diversity can be usefully exploited to improve the flavour of rosemary based products and to design specific products for the food industry, such as essential oils for flavourings, characterised by geographically distinctive aroma notes, thus providing flavour diversity. Interestingly, genotypes characterised by potentially better flavour profile seemed also to be associated to enhanced level of bioactive compounds and related functional properties.

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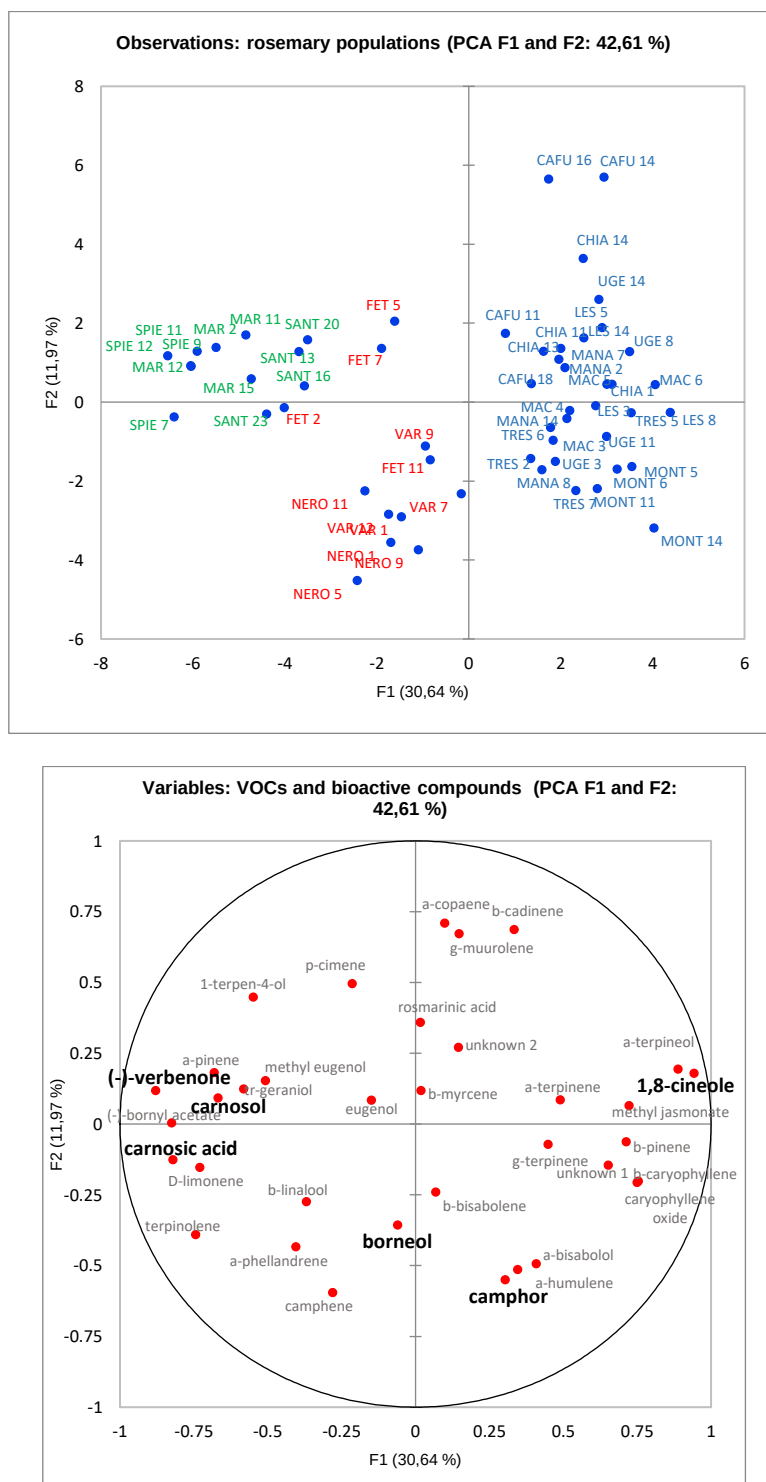


Figure 3: Score plot (Observations: rosemary populations) and loadings plot (Variables: VOCs and bioactive compounds) of the PCA analysis: PCs 1 and 2. PCA was carried out on the dataset formed by experimental data of 32 VOCs and 3 bioactive compounds determined on 56 individual rosemary plants. In the score plot the label denotes a single plant (acronym: population; number: individual plant within the population).