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Phytochemicals Content in Italian Garlic Bulb (*Allium sativum* L.) Varieties

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Abstract

Several studies have demonstrated a wide range of therapeutic effects due to garlic high content of phytochemicals, including sulfur-containing compounds, vitamins, saponins, flavonoids and moderate levels of carotenoids. The synergistic interactions between these components seem to explain the outcomes of certain healing properties from garlic. This study evaluates the health promoting phytochemicals (ascorbic acid, flavonoids and carotenoids) content and antioxidant capacity in four “typical garlic varieties” (Rosso di Castelliri, Bianco Piacentino, Rosso di Sulmona, Rosso di Proceno), grown in different geographical areas. β-carotene content ranged from 5.68 to 7.41 μg/100g and 6.36 to 7.46 μg/100g for Viterbo and Alvito bulbs respectively. Overall, vitamin C levels were statistically higher in samples from Alvito compared with same cultivars from Viterbo; among them Rosso Sulmona and Rosso Castelliri displayed the higher content (21.59 ± 2.75 and 18.91 ± 0.34 mg/100 g respectively). FRAP values were positively correlated ($r = 0.74$ and $P = 0.03$) with vitamin C levels and highly correlated ($r = 0.86$ and $P = 0.005$) with myricetin levels. Our findings have revealed that genotype and environmental conditions (production areas and pedoclimatic factors), as well as their interaction, could influence the phytochemical composition and the antioxidant properties of Italian garlic bulb varieties.

Keywords: Italian garlic, cultivar, phytochemicals, environmental conditions

1. Introduction

Garlic has been used throughout history for both culinary and medical purposes (Hahn, 1994). Even if the garlic consumption do not affect daily food intake, raw and cooked garlic is used in many recipes related to the Mediterranean diet, as well as in many Asian, American and European dishes. Several studies (Chia-Wen Tsai et al,. 2012; Neil et al., 1996) have demonstrated a wide range of therapeutic effects due to its high content of phytochemicals, including sulfur-containing compounds, vitamins, saponins, flavonoids and moderate levels of carotenoids. The synergistic interactions between these components contribute to provide the observed health benefits from garlic as well as antibacterial, antifungal, hypolipidemic, antihypertensive, antiatherosclerotic, anticoagulant, hypoglycemic and chemopreventive (Patumraj et al., 2000; Ohaeri, 2001; Zeng et al., 2012; Rahman, 2007; Lau, 2001).

Many of these biological effects are related to the thiosulfates, volatile sulfur compounds, typical of the Allium plants, which are also responsible of their characteristic pungent aroma and taste (Arzanlou & Bohlooli, 2010). However, these compounds are unstable and give rise to transformation products. Recently the attention has been focused on other phytonutrients, representing secondary metabolites from foods that are more stable to cooking and to the storage. The main classes among these compounds including sapogenins, saponins, and flavonoids and their interaction seem to explain the outcomes of certain healing properties from garlic (Nuttakaan et al., 2006; Bozin, 2008; Queiroz et al., 2009).

Several cultivars of *Allium sativum* L. species are mostly derived from local selection and local adaptation and the bulbs are covered with a white or red tunic. Numerous factors could impact upon the composition of any plant food, including a wide range of soil textures and soil pH, fertilization, irrigation, and harvest practices different for each geographical area.

This study evaluates the health promoting phytochemicals (ascorbic acid, flavonoids and carotenoids) content and antioxidant capacity in four "typical garlic varieties" (Rosso di Castelliri, Bianco Piacentino, Rosso di Sulmona, Rosso di Proceno), grown in two different geographic locations (Viterbo and Alvito), in order to identify and valorize Italian local, traditional and certified products.

2. Material and Methods

2.1 Sample

The four typical samples named as Rosso di Castelliri, BiancoPiacentino, Rosso di Sulmona and Rosso di Proceno were grown in two different geographic areas of Lazio (Viterbo and Alvito), using the same technical/agronomic trail. A representative sample from each cultivar was homogenized in a Waring blender for 1 minute. Three replicates were prepared from each sample and each was analyzed in triplicate for the following assays polyphenols, carotenoids, vitamin C, and total antioxidant capacity as described below.

2.2 Materials

All solvent were purchased from Carlo Erba (Milan, Italy), BDH (Poole, England) and Merck (Darmstadt, Germany). 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) was from Fluka (Switzerland). Phosphate-buffered saline (PBS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and ascorbic acid were provided by Sigma–Aldrich Srl. Commercial standards were also from Sigma–Aldrich Srl (Milan, Italy). Double distilled water (Millipore, Milan, Italy) was used throughout the study.

2.3 Analytical Methods

Carotenoids were extracted using the method described by Sharpless et al. (1999) and the determination of carotenoid concentrations was carried out by high performance liquid chromatography (HPLC) techniques (Maiani, 1995). Flavonoids were extracted from garlic using the methods described by Hertog et al. (1992) and quantitative analyses was performed using an ESA 5600 eight-channel coulometric electrode array detector using 0.02 M sodium phosphate adjusted with 85% orthophosphoric acid to pH 2.8 (solvent A) and methanol (solvent B).The used linear gradient, maintaining the flow at 1 mL/min, consisted of 7%solvent B, increasing to 30% over 25 min before being held for 7 min, increasing to 40% over 5.5 min, and reaching 73% over 17.5 min before being held for 7.5 min, increasing to 95% over 4 min and reaching 100% over 4 min before being held for 4 min and returning to 7% solvent B over 5 min, where it was maintained for a further 5 min. The setting potentials were 60,120, 200, 340, 480, 620, 760 and 900 mV. Total ascorbic acid was extracted using Margolis et al (1990) method and the quantitative analyses were performed by an HPLC system equipped with a coulometric detector (ESA model 580, Chemsford, MA, USA). The setting potential was 0, 100, 200, 300 and 400 mV (v. Palladium reference electrode) and the chromatographic separation was obtained applying an isocratic elution at flow rate of 0.8 ml/min. Total Antioxidant Capacity, carotenoids, polyphenols and total vitamin C were measured as follow: total antioxidant capacity (TAC) using two different assays Ferric Reducing Antioxidant Power (FRAP) (Benzie & Strain, 1996) and Trolox Equivalent Antioxidant Capacity (TEAC) method (Pellegrini et al., 2003; Re et al., 1999).

2.4 Statistic Analysis

Results were expressed as mean ± sd and statistical data analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test (significance at $P < 0.05$). Pearson's linear correlation coefficient was used to evaluate the interactions between parameters.

3. Results and Discussion

Figure 1 shows the observed β-carotene mean levels by different cultivars and production area and no significant differences were present. β-carotene content ranged from 5.68 to 7.41 µg/100 g and 6.36 to 7.46 µg/100 g for Viterbo and Alvito bulbs respectively. Tuan et al. (2011) have reported that the leaves of garlic have the highest concentration of carotenoid compounds compared with other organs (respectively, 73.44 µg/g dry weight in the leaves respect to 2.85 µg/g dry matter in the bulbs), underlying the essential role of light in the accumulation of carotenoids.

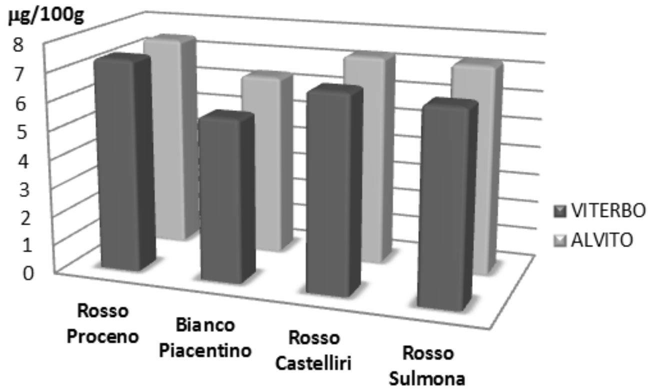

Figure1. β-carotene means values (μg/100g) by different cultivars and production area

The Figure 2 illustrates ascorbic acid content ranged from 11.01 to 21.59 mg/100 g. In the bulbs grown in Viterbo, the ascorbic acid content ranged from 11.01 ± 0.44 to 15.82 ± 1.32 mg/100 g with significantly higher levels ($P < 0.05$) in Rosso Castelliri (15.82 ± 1.82 mg/100 g) than Rosso Proceno (11.01 ± 0.44 mg/100 g) and Bianco Piacentino (12.98 ± 0.80 mg/100 g). Rosso Sulmona bulbs (15.11 ± 0.36 mg/100 g) exhibited higher vitamin C ($P < 0.05$) than Rosso Proceno ones (11.01±0.44 mg/100 g). In the bulbs grown in Alvito, the ascorbic acid content of Rosso Sulmona (21.59 ± 2.75 mg/100 g) was significantly higher (Anova: $P < 0.05$) respect to Rosso Proceno and Bianco Piacentino varieties (14.72 ± 0.36 and 14.45 ± 1.10 mg/100 g, respectively), while Rosso Castelliri (18.91 ± 0.34 mg/100 g) shows a higher ascorbic acid content than Bianco Piacentino (14.45 ± 1.10 mg/100 g). Overall, vitamin C levels were statistically higher in samples from Alvito compared with same cultivars from Viterbo, among them Rosso Sulmona and Rosso Castelliri displayed the higher content (21.59 ± 2.75 and 18.91 ± 0.34 mg/100 g respectively). Põldma et al. (2011) have reported variable amounts of ascorbic acid ranging from 5.1 mg/100 g to 11.4 mg/100 g dry weight before foliar selenium (Se) treatment of garlic in 2008 and 2009 in Estonia, while USDA database reports an average of 31 mg in 100g of raw garlic (2003 and 2004).

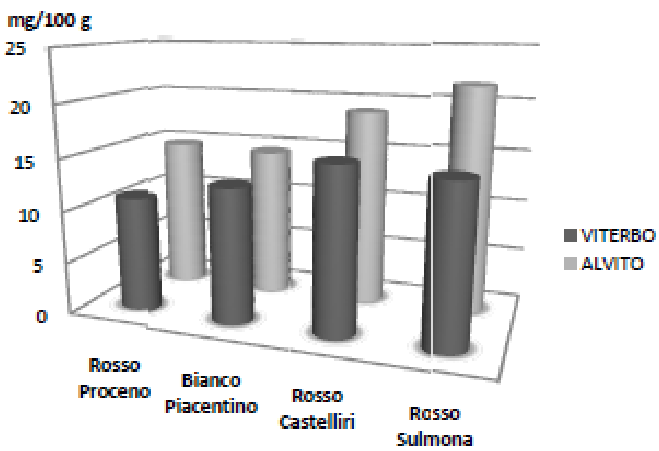

Figure 2. Vitamin C means values (mg/100g) by different cultivars and production area

The antioxidant properties of garlic have been attributed, in part, to the presence of phenolic compounds including flavonols, myricetin, apigenin, and in contrast with onions, low levels of quercetin. With regard to the quantitative analysis of flavonoids (free plus conjugated forms), Figure 3 displays the varied flavonoids content amongst different varieties and growing locations. Myricetin and apigenin were found as the most abundant flavonoid in four varieties, while a significant difference between Rosso Proceno and Bianco Piacentito was present in quercetin levels (17.69 ± 3.23 and 10.05 ± 3.77 mg/kg, respectively) in the bulbs grown in Viterbo. Quercetin content in the Alvito bulbs ranged from 10.04 ± 0.42 to 29.08 ± 0.80 mg/kg in Rosso Proceno and Rosso Castelliri, respectively.

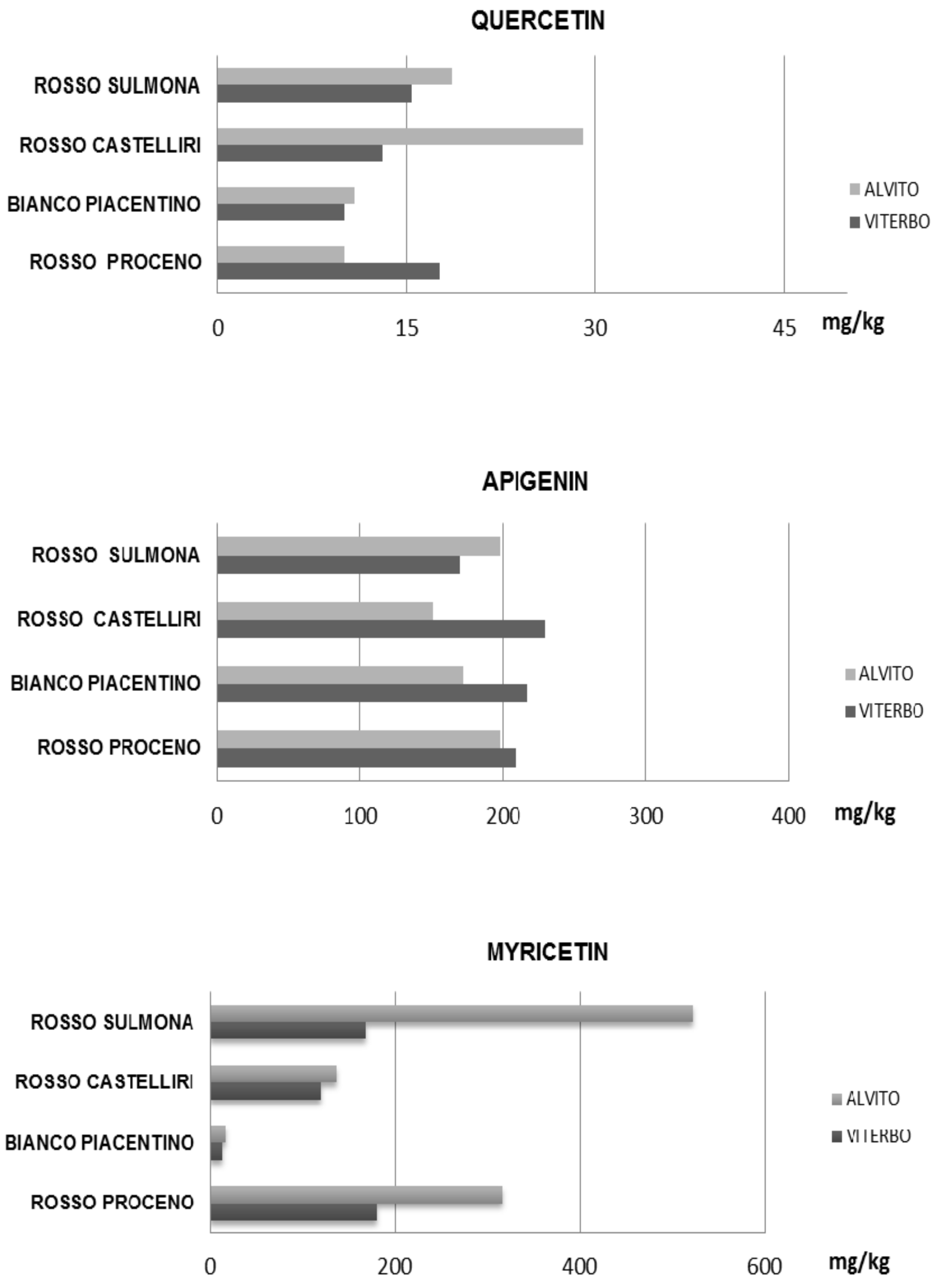

Figure 3. Flavonoids mean values (mg/kg) by different cultivars and production area

Comparing the two growing location, quercetin content was significantly higher in Alvito bulbs for Rosso Sulmona and Rosso Castelliri varieties (18.62 ± 0.36 and 29.08 ± 0.8 mg/kg, respectively). There were no significant differences in the apigenin content between cultivar and production area on average range from 150.93 ± 27.12 to 229.87 ± 38.59 mg/ kg in Rosso Castelliri respectively for Alvito and Viterbo.

The myricetin, was significantly different among cultivars by same production area, except for Rosso Proceno

(180.20 ± 16.55 mg/kg) and Rosso Sulmona (167.36 ± 26.73 mg/kg) samples grown in Viterbo. Rosso Sulmona and Rosso Proceno grown in Alvito were exhibited the highest myricetin content comparing all samples. There were no significant differences in the levels of apigenin between cultivar and production area ranging between 150.93 ± 27.12 mg/kg and 229.87 ± 38.59 mg/kg in Rosso Castelliri respectively from Alvito and Viterbo.

Koo Hui Miean and Suhaila Mohamed (2001) have reported a flavonoids content of 47.0 mg/kg, 217.0 mg/kg and 639.0 mg/kg respectively for quercetin, apigenin and myricetin.

Table 1 shows the synergistic effects of various antioxidants measured by FRAP (mmol/kg) and TEAC (mmol trolox/kg) method. Our results highlighted the highest FRAP values ($P < 0.05$) of Rosso Sulmona respect to Rosso Proceno, Rosso Castelliri and Bianco Piacentino (4.69 ± 0.65 mmol/kg *vs* 3.16 ± 0.18 mmol/kg, 2.38 ± 0.19 mmol/kg, 2.28 ± 0.33 mmol/kg) grown in Alvito, while comparing production area significant differences were recorded between Rosso Sulmona and Rosso Proceno varieties grown in Alvito respect to Viterbo ones (4.69 ± 0.65 mmol/kg *vs* 1.78 ± 0.12 mmol/kg and 3.16 ± 0.18 mmol/kg *vs* 1.70 ± 0.26 mmol/kg respectively). In addition FRAP values were positively correlated ($r = 0.74$ and $P = 0.03$) with vitamin C levels and highly correlated ($r = 0.86$ and $P = 0.005$) with myricetin levels. It could be useful to underline that the FRAP assay directly measures antioxidants with a reduction potential below the reduction potential of the Fe^{3+}/Fe^{2+} couple, but it is failed to detect other small molecular weight thiols and sulfur containing molecules of garlic.

Table 1. Total Antioxidant Capacity: FRAP (mmol/kg) and TEAC (mmol trolox/kg) values by different cultivars and production area

VARIETIES	FRAP (mmol/kg)		TEAC (mmol trolox/kg)	
	VITERBO	ALVITO	VITERBO	ALVITO
Rosso Proceno	1.70±0.26^{c}	3.16±0.18^{bd}	4.91±0.65	5.67±0.34
Bianco Piacentino	1.86±0.25	2.28±0.33^{b}	5.57±0.56	5.55±0.92
Rosso Castelliri	1.66±0.16	2.38±0.19^{b}	5.52±0.37	4.42±0.96
Rosso Sulmona	1.78±0.12^{c}	4.69±0.65^{ad}	4.82±0.30	6.27±1.80

Anova: $P < 0.05$ a *vs* b by column; c *vs* d by raw.

No difference by varieties and production area were present in TEAC (mmol trolox/kg) results. TEAC values ranged from 4.42 ± 0.96 to 5.67 ± 0.34 mmol trolox/kg in Alvito bulbs and from 4.82 ± 0.30 to 5.57 ± 0.56 mmol trolox/kg for in Viterbo ones, respectively.

4. Conclusion

In the present study we reported some phytochemicals content and total antioxidant capacity of four Italian garlic varieties grown in two different geographic locations.

Our findings have revealed that genotype and environmental conditions (production areas and pedoclimatic factors), as well as their interaction, could influence the phytochemical composition and the antioxidant properties of Italian garlic bulb varieties.

In summary, the evidence presented in this paper supports the highest content of phytochemicals in red tunic bulbs respect to white tunic ones and highlighted the site of growth of garlic as the major environmental factor in determining phytochemicals concentration and total antioxidant capacity: all parameters were highest in samples from Alvito.

Our data improve the knowledge of four Italian “typical garlic varieties” contributing to quantify and valorize the variety of antioxidant phytochemicals in garlic, which protect against disease-causing oxidative damage.

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