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ORIGINAL PAPER

Biochemical, antioxidant properties and antimicrobial activity of different onion varieties in the Mediterranean area

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Abstract
Onion (*Allium cepa* L.) is a very important vegetable crop cultivated worldwide. The bulb is a major source of phytochemicals useful for human health and rich in sulphur compounds responsible for their typical odour and flavour. In this study, we investigated the volatile compounds and biochemical properties besides the antimicrobial activity of onion *Montoro* and *Alife* varieties, and of two ecotypes of *Vatolla* variety (spinning top and tapered shape), cropped in the southern Mediterranean area (Italy). For all investigated onions, the organosulphur compounds, mainly di- and trisulfides, are the most abundant compounds. *Alife* variety showed higher polyphenols amount (8.2 GAE mg/g dw) with respect to the lowest one (3.9 GAE mg/dw g) in spinning top *Vatolla*, as well as an higher antioxidant activity (42.37 µmol TE/g dw) about two-fold higher than those detected in the other varieties. All the onions showed low pungency level, confirming their popular classification as a sweet onion. The total content of soluble sugars ranged from 461 to 624 mg/g dw; malic acid was the major organic acid in *Alife* and *Montoro* varieties instead for both *Vatolla* ecotypes citric acid was the most abundant ones. The biochemical characterization highlighted the three onion varieties as a good source of bioactive compounds. The antimicrobial activity of the onion extracts pointed out an effective action against three Gram-positive species (*B. cereus, L. innocua, S. aureus*) and P. *aeruginosa*; consequently, they could represent a new source of natural antimicrobial agents.

Keywords Onion · Sulphur compounds · Antioxidant activity · Polyphenols · Antimicrobial activity

Introduction

Onion (*Allium cepa* L.) is one of the most ancient and cultivated vegetable used worldwide mainly for its ability to enhance the flavour of a great variety of dishes.

The flavour of onion arises after the breakage of the tissues caused by cutting, mastication, and cooking. In particular, when onion tissues are cut or disrupted, the enzymatic hydrolysis of the *S*-alk(en)yl-l-cysteine sulfoxides (ACSOs) by alliinase or S-alk(en)yl cysteine sulfoxides lyase starts producing sulfenic acids, ammonia and pyruvic acid [1].

The major flavour compounds are generated by spontaneous reactions of the sulfenic acids that immediately produce thiosulfinates by a condensation reaction. Thiosulfinates are very unstable compounds and give rise to further rearrangements leading to a wide variety of derived Sulphur compounds including thiosulfonates, mono-, di-, and trisulfides as well as specific compounds such as the lachrymatory or tear factor, thiopropanal S-oxide [2].

Thiosulfinate compounds extracted from onions have been shown to have interesting antibacterial and antioxidant properties from both the technological point of view and the human health. Crude onion extract is effective in vitro against many bacteria species including *Bacillus subtilis, Salmonella*, and *E.coli* [3, 4]. The main compounds responsible for the antibacterial activities are mainly due to thiosulfinate compounds. The antioxidant activities in *Allium cepa* tissues extract have been observed the particular interest because of the relationship between oxidative stress and pathologies such as atherosclerosis, cancer, and aging, in which free radicals and reactive oxygen species are implicated [5]. Also, in this case, the thiosulfinates or related organosulphur components are primarily responsible for

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the observed antioxidant effects [6, 7]. In addition, phenolic compounds already present in the onion bulb have antioxidant properties. Two flavonoid subgroups are found in onion: anthocyanins impart a red/purple colour to some varieties, whereas flavanols such as quercetin and its derivatives are responsible for the yellow and brown skins of many other varieties [8].

Another parameter related to the number of volatile S-compounds enzymatically produced from ACSOs is the pungency [9]. The chemistry of the S-volatiles is complicated and unstable therefore, the pyruvic acid produced enzymatically in onion juice, which is stable and easy to measure, is highly recommended and used as an indicator of pungency [10].

Consumers trend is geared towards varieties of sweet and slightly pungent onions but with high nutritional and health properties. Food vegetable market is constantly in search of novel onion varieties and local ones possess quality parameters which are able to satisfy the consumer's demand. Different studies [11, 12] have been pointed out that the variability in the contents of phytochemicals and health-promoting compounds, among the cultivars and the seasonal variation within the cultivar are considered.

The aim of this work is to study and investigate the main chemical and biological properties of onion varieties cropped in the southern Mediterranean area (Italy) which may represent a valid and potential alternative to the common onions varieties from those already available in the market.

Materials and methods

Materials

Cultivated bulbs of onion, *Allium cepa* L. of the three varieties were provided from local factories of Campania, South part of Italy. *Montoro* onion (MO) also named "*Cipolla ramata*" (coppery onion) for the colour of the external skin of the bulb, while the internal part is white with longitudinal purple shades. *Alife* onion variety (AO) is slightly flattened in shape and an intense copper-coloured skin. *Vatolla* onion exists in two ecotypes: spinning-top (STVO) and tapered shape (TSVO), the colour is straw/pink, whilst the bulb is whitish with pink shades. The onion varieties were shown in Fig. 1.

Extraction of volatile compounds and GC-MS analysis

One hundred grams of the edible part of onion sample were homogenized in an Ultra-Turrax blender (T25, IKA Werke, Staufen, Germany) at room temperature. The homogenized was transferred into a flask with 50 mL of distilled water and 1 µL of 2-octanol, as an internal standard, sonicated in cold conditions at 50 Hz for 30 min. In the flask containing the homogenized onion, fresh dichloromethane was added three times. The organic phases were collected, dehydrated with Na_2SO_4 anhydrous, filtered with filter paper and the solvent was removed under reduced pressure.

The identification and determination of volatile compounds were performed by GC–MS (Trace MS plus, Thermo Finnigan, USA) equipped with a capillary column (Supelcowax 10; 60 m, 0.25 mm, 0.25 µm, Supelco, USA). Chromatographic separation and the identification of volatile compounds were carried out according to Liguori et al. [13].

Extracts preparation and determination of antioxidant activity

Onions extracts were carried out according to Sharma et al. [12]. Antioxidant activity was determined by measuring the free radical scavenging activity (FRSA) of the onion extract in 1,1-diphenyl-2-picrylhydrazyl (DPPH) methanol solution. The absorbance was recorded at 517 nm to determine the remaining DPPH.

The efficient concentration (EC_{50}) is a parameter widely used to measure the antioxidant activity. It represents the amount of antioxidant necessary to decrease the initial DPPH concentration of 50%. EC_{50} was expressed in terms of the concentration of sample extract used for the test (mg/mL), from the lower level of EC_{50}, to the higher level of antioxidant activity.

Antioxidant activity was also expressed as Trolox equivalent, according to the literature [14, 15]. Values were determined by a calibration curve of Trolox standard solutions (10–600 µM), performed following the same procedure as the extracted sample. Results were expressed as µmole Trolox equivalent per gram of dry weight of onion (µmoL TE/dw g).

Quali-quantitative profile of polyphenols

Total phenols were estimated by Folin–Ciocalteu colourimetric assay [16, 17] and results were expressed as mg gallic acid equivalent (GAE)/g dw. The absorbance of solutions was read at 765 nm using a UV–Vis spectrophotometer (Lambda Bio 40; PerkinElmer, Waltham, MA, USA), after 2 h of incubation in the dark at room temperature.

The identification of phenolic compounds in onion extracts was carried out by HPLC, using an Agilent 1100 chromatograph (Agilent, Santa Clara, USA). Chromatographic separation was performed on an RP-Amide column (5 µm × 150 mm × 4.6 mm) (Phenomenex, Torrance, USA). The operating conditions were the same as described previously [18].

Fig. 1 Representative pictures of **a** MO: Montoro Onion; **b** AO: Alife Onion; **c** STVO: spinning top Vatolla Onion; **d** TSVO: tapered shape Vatolla Onion

Pungency analysis

The pungency of onions was determined through an enzymatically process (alliinase) produced pyruvate (EPY) by colourimetric analysis according to Schwimmer and Weston [10] with slight modifications.

The determination of the total and basal concentration of pyruvate alliinase produced which was then measured by using PerkinElmer Lambda 25 UV–Vis spectrometer at 420 nm. Enzymatically (alliinase) produced pyruvate (EPY) in each sample was calculated from the difference of total and basal concentration of pyruvate. Standards were prepared with sodium pyruvate solution, ranging from 20 to 100 µM.

Sugars and organic acids analysis

Sugars were determined by HPLC (Hewlett Packard, mod. 79852, USA) equipped with 4.6 × 250 mm (60°A, 4 µm) carbohydrate cartridge column (Waters, USA) and a refractive index detector (Hewlett Packard, mod. 100, USA). The quality-quantitative sugar profile was performed basing on the procedures described by Liguori et al. [18].

For the analysis of organic acids, 1 g of fresh onion was added to distilled water up to 10 mL and homogenized in an UltraTurrax blender (T25, IKA Werke, Staufen, Germany) for 2 min. The samples were centrifuged at 4000 rpm for 10 min and filtered with 0.45 µm syringe cellulose filter (Millipore, USA) before ion exchange chromatography analysis. The apparatus (Dionex Corp., USA) was equipped with an ED 500 electrochemical detector, Ionpac AS11 column (250 × 4 mm), and Ionpac AS11 Guard (50 × 4 mm). The operating conditions were set according to the literature [19]. A calibration curve of organic acid standards was obtained and used for quantitative analysis.

Antimicrobial activity

The antibacterial activity of the onion extracts was assayed by the inhibition halo test on agar platesagainst *Bacillus cereus* (DSM 4313 and DSM 4384), *Staphylococcus aureus* (DSM 25923), *Listeria innocua* (DSM 20649), *Escherichia coli* (DSM 8579) and *Pseudomonas aeruginosa* (DSM 50071), all provided by Leibniz-Institut DSMZ-(Braunschweig, Germany). Each strain was incubated at 37 °C for 18 h in Luria Bertani broth (Sigma–Aldrich, Italy). Sterile paper filter disks (⌀ = 5 mm), previously impregnated with samples (ranging from 2 to 8 µg/disk) and dried at room temperature for 60 min, were individually placed on the inoculated plates containing microbial suspensions at 1×10^8 cfu/mL. Plates were then incubated at 37 °C for 24–48 h, depending on the strain. The activity of compounds was evaluated by the diameter (mm) of the inhibition zones around the disks. Sterile DMSO was used as negative control. Tetracycline (7 µg/disk) was the reference sample.

Minimum inhibitory concentration

The resazurin microtitre-plate assay [20] modified following Caputo et al. [21] was used to evaluate the minimal inhibitory concentration (MIC) of the four extracts. Briefly, different volumes of the test material were pipetted in a multiwell with different volumes of Muller-Hinton broth (Sigma Aldrich, Italy). Two-fold serial dilutions were performed, such that each well contained 50 µL of the test material in serially descending concentrations. 30 µL of 3.3 × strength isosensitised broth (Sigma Aldrich) and 5 µL of resazurin indicator solution was added in each well, to reach a final volume/well of 240 µL. Finally, 10 µL of bacterial suspension was added to each well to achieve a concentration of approx. 5×10^5 cfu/mL. Ciprofloxacin (3 µM in DMSO) and DMSO were used as positive and negative controls, respectively. Plates were covered with cling film and incubated at 37 °C for 24 h. The lowest concentration at which a colour change occurred indicated the MIC value.

Statistical analysis

All the analysis were performed in triplicates. Results were reported as means of standard deviation and data were subjected to analysis of variance (ANOVA). The least significant differences were obtained using an LSD test ($p \leq 0.05$). Principal component analysis (PCA) was applied to describe the relationship between the volatile compounds. Statistical analysis of data was performed using the SPSS software package, Version 20.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Volatile compounds profile

A total of 39 volatile compounds (Table 1) were identified in onion samples belonging to the following chemical classes: sulphur-containing compounds (S-compounds), aldehydes and alcohols.

There were no significant differences among MO and the other two *Vatolla*'s ecotypes (STVO, TSVO), which showed a concentration of total volatiles about 1350 mg/kg dw, while lower amount ($p \leq 0.05$) was found in AO onions.

The total volatiles found in all samples were considered in accordance with the report of other authors [18, 22] who investigated the volatile profile of fresh onions. For all the investigated samples and, according to the literature [1, 8, 23], S-compounds have been the main and the most abundant volatiles of onion volatile profile accounting for about 85% of the total identified compounds. Among the detected S-compounds di- and trisulfides accounted for 55% in MO, 60% in TSVO, 47 and 50%in STVO and AO respectively. The S-volatile profile detected in the samples was in agreement with Lanzotti [24] and Liguori et al. [18], who found that di-and trisulfides were the main compounds in the sulphur volatile fraction of the onion extract.

Other compounds, aldehydes and alcohols were found in *Alife, Montoro* and *Vatolla* onions in a smaller amount ranging between 7.5–10.6% and 1.9–4.5% of the total volatile compounds, respectively. For the aldehydes class, five compounds were detected in the volatile pattern of onion varieties except for furfuraldehyde which was not detected in AO sample.

Table 1 Volatile compounds of onion varieties (MO: Montoro Onion; AO: Alife Onion; STVO: spinning top Vatolla Onion; TSVO: tapered shape Vatolla Onion)

PCA Code	Ritention time (min)	Volatile compound (mg/kg dw)	MO	AO	STVO	TSVO
Sulphur compounds						
A1	8.3	1-Propanethiol	63.47 ± 1.84^{d}	10.96 ± 0.82^{a}	40.88 ± 0.60^{c}	23.28 ± 0.68^{b}
A2	19.1	Diallyl disulphide	8.31 ± 0.36^{a}	47.96 ± 3.76^{c}	15.20 ± 0.96^{b}	19.76 ± 1.15^{b}
A3	19.4	2 Methyl-1-propanethiol	69.78 ± 11.30^{a}	95.92 ± 8.50^{ab}	122.35 ± 16.36^{b}	188.79 ± 30.56^{c}
A4	20.2	2,4-Dimethylthiophene	74.79 ± 10.36^{b}	44.90 ± 0.33^{a}	115.80 ± 9.74^{c}	68.95 ± 10.13^{b}
A5	20.6	Trans propenyl methyl disulphide	0.00 ± 0.00^{a}	6.11 ± 0.36^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
A6	23.4	Isopropyl methyl disulphide	10.47 ± 0.92^{b}	8.48 ± 0.94^{ab}	32.12 ± 1.40^{c}	7.44 ± 0.65^{a}
A7	24.6	Dipropyl disulphide	39.59 ± 1.73^{b}	66.16 ± 0.68^{c}	30.11 ± 0.88^{a}	92.33 ± 5.37^{d}
A8	25.0	Dimethyl-trisulphide	0.00 ± 0.00^{a}	4.52 ± 0.20^{b}	0.00 ± 0.00^{a}	55.27 ± 4.03^{c}
A9	26.7	*Trans*-propenyl propyl disulphide	95.06 ± 5.53^{b}	59.32 ± 4.94^{a}	80.41 ± 8.34^{b}	119.74 ± 9.30^{c}
A10	27.3	*Cis*-propenyl propyl disulphide	15.91 ± 1.57^{c}	5.59 ± 0.49^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
A11	28.6	Methyl-2-propenyl disulphide	2.82 ± 0.00^{b}	0.00 ± 0.00^{a}	2.91 ± 0.00^{c}	4.08 ± 0.00^{d}
A12	29.4	Isopentyl pentyl sulphide	24.72 ± 0.36^{d}	8.37 ± 0.46^{b}	21.51 ± 0.31^{c}	0.00 ± 0.00^{a}
A13	29.8	2-Methyl-2-hexanethiol	4.94 ± 1.48^{b}	11.53 ± 2.10^{c}	14.06 ± 1.46^{d}	0.00 ± 0.00^{a}
A14	30.0	Methyl propyl trisulphide	136.02 ± 3.80^{d}	38.39 ± 0.78^{a}	62.10 ± 1.40^{b}	103.20 ± 0.16^{c}
A15	30.7	Dithio (1-propenyl) propionate	19.80 ± 1.15^{d}	8.89 ± 0.66^{a}	16.68 ± 0.73^{b}	17.78 ± 0.26^{c}
A16	30.9	Dithioallylpropionate	8.08 ± 0.80^{c}	0.59 ± 0.07^{a}	9.54 ± 0.28^{d}	6.28 ± 0.27^{b}
A17	32.3	Methyl allyl sulphide	1.54 ± 0.07^{a}	13.53 ± 1.41^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
A18	36.6	1,2,4-Trithiolane	113.47 ± 7.91^{b}	62.40 ± 5.42^{a}	121.15 ± 4.57^{b}	106.63 ± 7.77^{b}
A19	37.6	2-Ethylidene-(1–3) dithiane	12.63 ± 0.92^{b}	8.52 ± 0.94^{a}	38.85 ± 1.52^{c}	15.33 ± 1.12^{b}
A20	37.7	*Cis* propenylpropyl trisulphide	43.91 ± 1.91^{d}	4.96 ± 0.55^{a}	39.44 ± 1.72^{c}	23.39 ± 1.71^{b}
A21	38.3	*Trans* propenyl propyl trisulphide	$108.21 \pm 7.89^{b,c}$	85.17 ± 5.35^{a}	$93.98 \pm 4.14^{a,b}$	118.99 ± 8.67^{c}
A22	39.2	2-Methyl-2-hexanethiol	16.31 ± 1.19^{a}	0.00 ± 0.00^{a}	97.54 ± 14.53^{b}	0.00 ± 0.00^{a}
A23	39.6	*Trans*-2-ethyl-3-methylthiophane	119.75 ± 26.22^{b}	98.54 ± 9.86^{b}	36.03 ± 5.37^{a}	17.82 ± 7.77^{a}
A24	42.6	2 methylthiociclohexa(c)thiophene	18.61 ± 1.36^{c}	4.65 ± 0.34^{a}	12.66 ± 1.89^{b}	4.09 ± 0.78^{a}
A25	42.8	Diallyl trisulphide	9.07 ± 0.53^{b}	4.29 ± 0.37^{a}	5.72 ± 0.85^{a}	5.14 ± 0.99^{a}
A26	44.6	3,4-Dimethyl-2,5-dihydrothiophene 1,1-dioxide	161.61 ± 31.01^{b}	58.28 ± 6.26^{a}	124.36 ± 18.52^{b}	51.84 ± 9.95^{a}
A27	45.4	*Cis*-2-ethyl-3-methylthiophane	17.70 ± 3.40^{b}	8.14 ± 0.65^{a}	15.14 ± 2.25^{b}	15.53 ± 2.98^{b}
A28	59.6	Propylene sulphide	36.21 ± 6.95^{b}	14.18 ± 1.29^{a}	23.55 ± 3.51^{a}	24.04 ± 4.61^{a}
		Total of sulfur compounds	1127.24 ± 235.33^{b}	813.19 ± 57.51^{a}	1168.09 ± 149.60^{b}	1160.38 ± 108.91^{b}
Aldehydes						
A29	7.2	Propionaldehyde	65.44 ± 0.95^{d}	43.23 ± 0.58^{b}	38.33 ± 0.28^{a}	47.81 ± 0.69^{c}
A30	16.9	2-Methyl-2-pentenal	40.40 ± 3.54^{c}	39.66 ± 1.84^{c}	31.60 ± 0.46^{b}	23.44 ± 0.34^{c}
A31	28.5	2-Methylpentanal	17.98 ± 0.78^{c}	7.13 ± 0.49^{a}	24.33 ± 2.52^{d}	12.98 ± 1.91^{b}
A32	28.7	2-Isononenal	16.72 ± 2.46^{b}	2.09 ± 0.10^{a}	25.89 ± 0.75^{c}	17.87 ± 0.26^{a}
A33	43.6	Furfuraldehyde	1.35 ± 0.00^{c}	0.00 ± 0.00^{a}	0.87 ± 0.00^{b}	1.18 ± 0.00^{d}
		Total of aldehydes	140.54 ± 7.73^{d}	92.10 ± 3.01^{a}	120.15 ± 4.02^{c}	102.10 ± 3.20^{b}
Higher alcohols						
A34	22.4	2-Hexanol	0.00 ± 0.00^{a}	5.81 ± 0.33^{b}	13.79 ± 0.61^{c}	18.58 ± 2.45^{d}
A35	24.0	3-Pentanol	8.68 ± 1.28^{b}	2.27 ± 0.08^{a}	26.77 ± 0.39^{c}	23.24 ± 3.41^{c}
A36	47.7	Furfuryl alcohol	30.83 ± 5.92^{b}	10.51 ± 1.11^{a}	16.51 ± 2.46^{a}	19.97 ± 3.83^{a}
		Total of higher alcohols	39.51 ± 7.19^{b}	18.60 ± 1.52^{a}	57.06 ± 3.46^{c}	61.79 ± 9.70^{d}
Other compounds						
A37	7.9	Z-3,4-dimethyl-2-pentene	0.00 ± 0.00^{a}	11.12 ± 4.73^{b}	35.23 ± 1.54^{d}	22.63 ± 1.62^{c}
A38	50.0	Nonanoic acid	3.64 ± 0.70^{a}	13.63 ± 1.46^{b}	12.62 ± 1.88^{b}	21.72 ± 4.17^{c}
A39	52.1	2-Hexyl-5-methyl(2H)-furan-3-one	11.85 ± 2.27^{b}	15.25 ± 1.66^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
		Total of volatile compounds	1322.79 ± 253.22^{b}	963.90 ± 69.88^{a}	1393.15 ± 156.48^{b}	1368.63 ± 127.60^{b}

Different superscript letters (a, b, c, d) in the same row mean significant differences ($p \leq 0.05$)

Aldehyde contents were different ($p \leq 0.05$) not only for the three onion varieties investigated but also between the ecotypes of *Vatolla* onion. Accordingly, to different authors [23, 25], *propionaldehyde* and *2-methyl-2-pentenal*were the most abundant aldehydes in the onion samples. These two compounds are both breakdown products from the sequential transformation from 1-propenylsulfenic acid to thiopropanal-S-oxide (the lachrymatory factor) [22]. As regards alcohol compounds both *Vatolla* ecotypes showed highest amounts, referred to the sum of the three alcohol detected, compared to MO and AO samples. The volatile compounds variability in onion samples were analysed by PCA approach (Fig. 2). Two principal components are necessary to explain the total variability of the characteristics analysis, using the cross-validation technique. The eigenvalues of the covariance matrix showed that the 71.03% of the total variability in volatiles response was explained by the first two principal components (41.02 and 30.01%, respectively).

The variables were distinctly oriented towards four PCA quadrants as shown in Fig. 2. The most compounds were positively correlated with PC1 and PC2.

The onion samples were grouped differently in 2D-PCA plot. Only MO was plotted in the PCs positive. In particular, MO and *Vatolla* ecotypes showed a separation by PC2; AO was separated from the other onion varieties by PC1 and showed high correlation with *trans propenyl methyl disulfide, 2-Methyl-2-hexanethiol* and *Methyl allyl sulfide*. MO onions showed higher correlation with the most of the volatile S-compounds detected than other varieties.

The data obtained by PCA confirmed as already reported in literature [5] that is the volatile fraction of onion governed by genetic factors within the onion. Besides genetic differentiation, the growing environment plays an important role in onion flavour. In fact, Lancaster et al. [26] indicated that the same cultivar, grown in different locations, had different flavours. Furthermore, environmental factors such as sulphur fertility, nitrogen concentration, irrigation, and temperature affect flavour intensity and environmental conditions under which the onions grow [27].

Biochemical properties of onion varieties

The total phenols, quantified in the onion samples, were reported in Table 2. Significant differences ($p < 0.05$) were found among the varieties with a minimum of 3.9 mg GAE/g extract in STVO to a maximum of 8.2 mg GAE/g extract in AO. The results were consistent with a previous study on white onion Italian landraces [18] and with Lisanti et al. [11], who found 6.53 and 4.71 mg GAE / g dw in two copper Italian onion varieties.

The phenolic profile (Table 3) of the three onion varieties showed 7 phenolic acids and 4 flavonoids, i.e. kaempferol, catechin and quercitin in glycoside and aglycone form. The sum of the phenolic acids was comparable for all onion samples, which showed gallic acid in the highest amounts except for TSVO, which presented ferulic acid as the most abundant phenolic acid. Similar phenolic acids profile was reported by Gorinstein et al. [28] in white and red onions, who detected the total amount of two magnitude orders lower than that herein reported. On the contrary, Prakash et al. [29] investigated four varieties of *Allium cepa* with different colour and the amount of ferulic and gallic acids were consistent with these results.

Quercetin, as quercetin aglycone conjugated to sugar moieties such as rutinose, was found the most abundant flavonoid in the analyzed onion samples [30]. Quercetin has

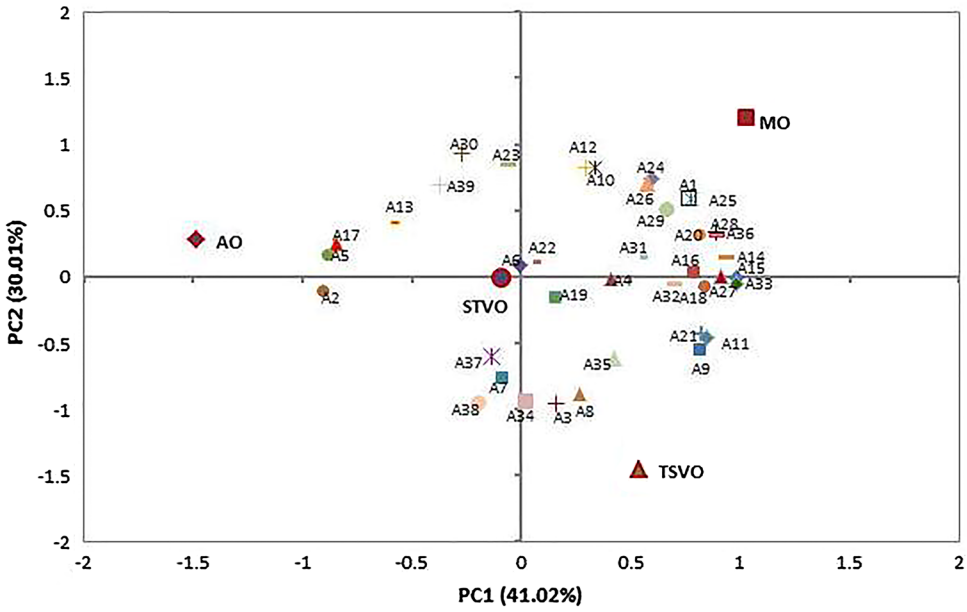

Fig. 2 PCA plot of volatile compounds of onion varieties (MO: Montoro Onion; AO: Alife Onion; STVO: spinning top Vatolla Onion; TSVO: tapered shape Vatolla Onion)

Table 2 Nutritional properties of onion varieties (MO: Montoro Onion; AO: Alife Onion; STVO: spinning top Vatolla Onion; TSVO: tapered shape Vatolla Onion)

Parameters	MO	AO	STVO	TSVO
Polyphenols (GAE mg /g dw)	6 ± 0.01^{b}	8.2 ± 0.06^{d}	3.9 ± 0.06^{a}	7.6 ± 0.07^{c}
Fructose (mg/g dw)	250.84 ± 1.25^{b}	240.67 ± 12.00^{b}	240.99 ± 1.03^{b}	179.29 ± 8.53^{a}
Glucose (mg/g dw)	297.83 ± 1.88^{b}	301.07 ± 39.20^{b}	232.89 ± 8.00^{a}	209.84 ± 6.07^{a}
Sucrose (mg/g dw)	75.82 ± 0.10^{ab}	64.14 ± 10.43^{a}	88.17 ± 10.13^{b}	72.41 ± 6.24^{ab}
Ratio (F + G)/S	7.24	8.45	5.37	5.37
Pungency (EPY µmol/ g fw)	1.33 ± 0.04^{b}	1.00 ± 0.05^{b}	0.65 ± 0.23^{a}	1.14 ± 0.22^{b}
Antioxidant activity – EC_{50} (mg/mL)	159.7 ± 6.3^{b}	121.2 ± 12.2^{a}	145.3 ± 2.2^{b}	150.0 ± 4.8^{b}
Antioxidant activity (µmol TE / g dw)	23.53 ± 6.3^{a}	42.37 ± 4.1^{b}	23.46 ± 2.6^{a}	29.48 ± 3.2^{a}

Different superscript letters (a, b, c, d) in the same row mean significant differences ($p \leq 0.05$)

Table 3 Polyphenols profile of onion varieties (MO: Montoro Onion; AO: Alife Onion; STVO: spinning top Vatolla Onion; TSVO: tapered shape Vatolla Onion)

Polyphenols (mg/100 g dw)	MO	AO	STVO	TSVO
Gallic acid	74.95 ± 1.51^{d}	72.28 ± 1.40^{c}	61.92 ± 0.44^{b}	41.55 ± 1.33^{a}
Vanillic acid	13.03 ± 0.91^{a}	18.95 ± 0.43^{b}	20.97 ± 1.15^{c}	26.91 ± 1.28^{d}
p-coumaric acid	17.30 ± 0.93^{a}	33.00 ± 0.11^{c}	28.22 ± 1.37^{b}	18.20 ± 0.80^{a}
Cinnamic acid	7.04 ± 0.27^{a}	8.82 ± 0.23^{a}	8.39 ± 1.82^{a}	12.30 ± 1.40^{b}
Chlorogenic acid	17.95 ± 0.27^{c}	8.75 ± 0.62^{b}	3.74 ± 0.76^{a}	3.92 ± 0.46^{a}
Caffeic acid	10.87 ± 1.50^{c}	9.24 ± 1.37^{c}	4.00 ± 0.27^{a}	5.37 ± 0.50^{b}
Ferulic acid	32.00 ± 0.24^{b}	23.00 ± 1.55^{a}	47.27 ± 2.98^{c}	71.06 ± 1.27^{d}
Quercetin	43.84 ± 0.64^{c}	47.84 ± 1.20^{d}	34.23 ± 0.79^{b}	26.66 ± 0.47^{a}
Total phenolic acids	173.14 ± 5.63^{a}	174.04 ± 5.71^{a}	174.51 ± 8.79^{a}	179.31 ± 7.04^{a}
Kaempferol	3.15 ± 0.12^{a}	3.09 ± 0.40^{a}	3.28 ± 0.11^{a}	2.84 ± 0.13^{a}
Catechin	7.45 ± 0.45^{a}	10.26 ± 0.24^{b}	7.13 ± 0.29^{a}	4.71 ± 0.51^{a}
Rutin- quercetin-3-O-rutinoside	73.21 ± 0.35^{d}	41.06 ± 1.80^{a}	64.92 ± 0.22^{c}	43.27 ± 0.25^{b}
Total quercetin	117.05 ± 0.99^{d}	88.90 ± 3.00^{b}	99.15 ± 1.01^{c}	69.93 ± 0.72^{a}

Different superscript letters (a, b, c, d) in the same row mean significant differences ($p \leq 0.05$)

been reported to exhibit antioxidative, anti-carcinogenic, anti-inflammatory, anti-microbial, anti-viral, antiaging, and vasodilating effects [31]. Moreover, the bioavailability of quercetin is higher for the glycoside form, compared to quercetin aglycone [32].

The sum of quercetin-3-O-rutinoside (rutin) and free quercetin varies from a maximum of 117.05 mg/100 g dw for MO to a minimum of 69.93 mg/100 g dw in TSVO. Similar results were found by different authors who detected total quercetin in white and red onions [28] and in six Tenerife (Spain) traditional onion cultivars [33]. Higher amount, about 286 mg/100 g dw was found by Tedesco et al. [34], who detected five quercetin glucosides and quercetin free in *Montoro* onion.

Furthermore, the glycoside form was detected in higher amount than the free form in all onion samples, according to previous authors [33, 35]; who investigated the flavonols profiles in different onion varieties. Kaempferol amount was significantly different ($p < 0.05$) in the investigated onion varieties ranging from 1.89 to 3.20 mg/100 g dw [28, 33, 34].

The onion varieties were also studied for their antioxidant activity expressed as EC_{50} and Trolox equivalent (TE). EC_{50} and TE were inversely correlated. The EC_{50} in AO extracts was found higher than the other varieties with an antioxidant activity, about two-fold higher than those detected in the other samples such as MO, STVO, and TSVO (Table 3). These data were consistent with previous results by Lisanti et al. [11], who used the same method and units. Their results highlighted the antioxidant activity of three Italian onion cultivars, ranging from 15.8 to 22.90 µmol TE/g dw.

Commonly, a classification of onion varieties is based on its levels of pungency: sweet (0–3 µmoL pyruvic acid/g fw), medium pungency (3–7 µmoL pyruvic acid/g fw) and high pungency (above 7 µmoL pyruvic acid/g fw) [36]. All the onion samples showed a distinctly low pungency level, confirming their popular classification as a sweet onion with a delicate taste. In order to define the sweetness of onions, the sugars profile was also investigated. Fructose, glucose, and sucrose were detected in the onion varieties (Table 2), with fructose and glucose higher ($p \leq 0.05$) than sucrose in all samples. The total content of soluble sugars changed from

624 to 461 mg/dw g, according to the ranges reported in the literature [18, 37]. Significant differences ($p \leq 0.05$) in total soluble sugars were found among the investigated varieties: MO and AO, which showed the highest content. Between the two ecotypes of *Vatolla* variety, the TSVO sample showed the lowest sugar content. The difference found in the quantitative profile of the onion varieties influences the sweet sensation perceived by the consumers. Crowther et al. [38] reported that the pungency values lower than 4 µmoL/g fw could allow appreciating sweetness differences during taste.

Onions are also a good source of organic acids [39] and in this study, five organic acids were identified and quantified (Table 2). The results were in accordance with those found in Recs cv [37], in four onion Greek cvs [40] and, in six Spanish cvs [39]. Malic acid was the major organic acid in all onion varieties except for both *Vatolla* ecotypes, where citric acid was the most abundant organic acid. Significant differences ($p \leq 0.05$) were found among all varieties for organic acids amount, probably due to a different activation level of multiple metabolic pathways, among which the Cycle of Krebs is the main one.

Regarding ascorbic acid, it contributes to the nutritional value of the onion with respect to the other acids; the highest amount was found in MO followed by AO, TSVO and STVO onions. In addition, ascorbic acid concentration ranged from 17.09 to 37.40 mg/100 g fw, according to different *Bianca di Pompei* ecotypes (14.80 to 21.65 mg/100 g fw) [18], but higher than those reported in other onion cultivars (range 1.2–6 mg/100 g fw) [38].

Antimicrobial activity

The data on antimicrobial activity were shown in Table 4. Polyphenol extracts of the onion varieties exhibited different antimicrobial activity against all the strains used as testers. A good activity of the ethanolic extracts against *B. cereus, S. aureus, L. innocua* and *P. aeruginosa*, and the other microbial strains used in the experiments was found. This could be due to flavonoids which, as indicated by Hendrichet al. [41], are capable to interacts with lipid bilayers as well as biological membranes of some bacteria, affecting their physiology and metabolism, until their death. In this study, the four onion extracts were effective against the three Gram-positive species used (exhibiting the following global efficacy scale: *Bacillus cereus* > *Listeria innocua* > *Staphylococcus aureus*), as can be also evident by the MIC data (Table 5). Also, they were effective against the Gram-negative *Pseudomonas aeruginosa* but ineffective against the other Gram-negative *Escherichia coli*, against whom generally the ethyl acetate or chloroform extracts show more efficacy [42]. The obtained results were also consistent with those reported by Dziri et al. [43], which did not detect antimicrobial activity by a North African variety of *Alliaceae* against *E.coli*. As previously reported for other vegetal extracts [44], the inhibitory

Table 4 Antimicrobial activity of the polyphenol extracts of onion varieties

	B. cereus 4313			*B. cereus* 4384			*P. aeruginosa*		
	2 µg	4 µg	8 µg	2 µg	4 µg	8 µg	2 µg	4 µg	8 µg
MO	8.66 ± 0.57^{cF}	9.66 ± 0.57^{aF}	11.33 ± 1.14^{aG}	9.66 ± 0.57^{bF}	11 ± 1^{aG}	11.33 ± 1.14^{aH}	4.66 ± 0.57^{aF}	10 ± 0^{aG}	12 ± 0^{aH}
AO	8.33 ± 0.57^{cF}	10.33 ± 0.57^{aF}	13 ± 1.73^{abG}	5.66 ± 0.57^{aF}	11.33 ± 0.57^{aG}	12.33 ± 0.57^{aG}	6.33 ± 0.57^{bF}	12 ± 1^{bG}	15.66 ± 0.57^{bH}
STVO	4.66 ± 0.57^{aF}	10 ± 0^{aG}	10.66 ± 0.57^{aG}	10 ± 0^{bF}	13.66 ± 0.57^{bG}	14.33 ± 0.57^{bG}	9 ± 1^{cF}	14.66 ± 0.57^{cG}	16.33 ± 0.57^{bH}
TSVO	6.66 ± 0.57^{bF}	10.66 ± 0.57^{aG}	14.33 ± 0.57^{bH}	5.33 ± 0.57^{aF}	10.66 ± 1.15^{aG}	14.33 ± 0.57^{bH}	10.33 ± 0.57^{dF}	16 ± 0^{dG}	16.66 ± 0.57^{bG}
DMSO	0 ± 0			0 ± 0			0 ± 0		
Tetracycline (7 mg/disk)	10.33 ± 0.57			9 ± 0.57			10.33 ± 0.57		

	E. coli			*S. aureus*		
	2 µg	4 µg	8 µg	2 µg	4 µg	8 µg
MO	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	4.33 ± 0.57^{bG}	4.66 ± 0.57^{bG}
AO	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	4.33 ± 0.57^{bG}	5 ± 0bH
STVO	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	4.33 ± 0.57^{bF}	4.66 ± 0.57^{bF}	6.33 ± 0.57^{cG}
TSVO	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}	0 ± 0^{aF}
DMSO	0 ± 0			0 ± 0		
Tetracycline (7 mg/disk)	12.67 ± 0.57			11.33 ± 0.57		

Data are expressed in mm. Results are shown as the mean ± SD (n = 3). Bc 4313: *Bacillus cereus* 4313; Bc 4384: *Bacillus cereus* DSM 4384; Psa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Sa: *Staphyloccus aureus*; Li: *Listeria innocua*. Tetracycline (7 µg/disk) and DMSO were used as positive and negative controls, respectively

Different superscript lowercase letters (a, b, c, d) in the same column mean significant differences ($p \leq 0.05$); different superscript capital letters (F, G, H) in the same row mean significant differences ($p \leq 0.05$)

Table 5 Minimal Inhibitory Concentration (MIC, µg/well) of the polyphenol extracts of onion varieties

MIC	*B. cereus* 4313 (µg)	*B. cereus* 4384 (µg)	*P. aeruginosa* (µg)	*E. coli* (µg)	*S. aureus* (µg)	*L. innocua* (µg)
MO	> 0.8	> 0.8	> 1	> 10	> 2	> 2
AO	> 0.8	> 1	> 1	> 10	> 2	> 5
STVO	> 1	> 0.4	> 0.8	> 10	> 1	> 1
TSVO	> 1	> 1	> 0.8	> 10	> 10	> 0.8

effect could be strain-specific: the two strains of *B. cereus* showed a different resistance/sensitivity, mainly when they were treated with the extract of STVO. In fact, the strain *B. cereus* DSM 4384 resulted in more sensitive respect to *B. cereus* 4313 and, already using 2 µg of extracts, the halos of inhibition showed a different diameter (4.66 mm and 10 mm, respectively). This was also confirmed by the MIC assay (Table 5): in fact, the minimal inhibitory concentration of such extract was > 1 µm for *B. cereus* 4313 and just > 0.4 µm for *B. cereus* 4384. The extracts, particularly STVO extract, showed effectiveness against the methicillin-resistant strain of *S. aureus*, with MIC doses not superior to 3.2 µg (with the exception of TSVO extract). This could open new perspectives in the use of onion extracts as natural antimicrobial agents, due to the ever-increasing urgency to overcome the greater resistance exhibited by *S. aureus*, in respect to conventional antimicrobial drugs. In fact, staphylococcal intoxication can be responsible for almost 300 food borne outbreaks/year, basically due to the presence of the heat-stable enterotoxins [45, 46]. Thus, onion extracts could represent natural antimicrobial agents suitable to increase the food safety and improving the shelf life of perishable foods and thus satisfy the increasing demand by consumers to eat food without synthetic additives [47].

Conclusions

The present study reports that the chemical and biochemical parameters affecting the quality of three onion varieties. The volatile compounds were mainly dominated by sulphur compounds with quantitative differences among the varieties, likely due to genetic and growing environmental factors. The biochemical characterization regarding the polyphenols profile and antioxidant activity also highlighted the three onion varieties as a good source of bioactive compounds. Moreover, the antimicrobial activity of the onion extracts has pointed out an effective action against mainly Gram-positive bacteria used in the experiments and consequently could represent a new source of natural antimicrobial agents. Finally, the low pungency level, highlighted in all three varieties, coupled with a good presence of sugars, makes these onions a potential alternative for the consumers who prefer a more delicate taste.

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