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A N I N T E R N A T I O N A L J O U R N A L

Free-Radical Scavenging Capacity and Antioxidant Properties of Some Selected Onions (*Allium cepa* L.) and Garlic (*Allium sativum* L.) Extracts

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ABSTRACT

The radical scavenging activity (RAS), chain-breaking activity, H_2O_2-scavenging, reducing capacity and total phenolics of four types of onions (Green onion, Yellow, Red and Purple) and garlic were investigated. Total phenolics varied from 30 mg (green onion) to 49 mg.100 g^{-1} fresh weight (garlic). Garlic extract showed the highest RAS, while green onion showed the lowest one. The chain-breaking activity of green onion extract was higher (0.48) than garlic extract (0.029). Chain-breaking activity of yellow, red and purple onion extracts was 0.19, 0.048 and 0.032 respectively. However, heating treatment (90 ºC, 3h) caused an increase in this activity. Low ability of green onion extract to scavenge hydrogen peroxide was noted (35%), whereas high ability was noted in other onion and garlic extracts and ranged from 60 to 90%. The lowest reducing capacity was noted in green onion extract (18%), whereas the highest in garlic extract (196%). Statistically, high significant correlations were observed between total phenolics content and reducing power, scavenging of hydrogen peroxide and chain-breaking activity of extracts.

Key words: Radical scavenging activity, chain-breaking activity, H_2O_2-scavenging, reducing capacity, *Allium cepa*, *Allium sativum*

INTRODUCTION

Onion and garlic were one of the first cultivated crops due to their long storage and portability. They could be dried and preserved for several months. At the present time, the *Allium* family has over 500 members, each differing in taste, form and color, but close in biochemical, phytochemical and neutraceutical content. *Alliums* were revered to possess anti-bacterial and anti-fungal activities, and contain the powerful antioxidants, sulfur and other numerous phenolic compounds which arouse great interests. Oxidation of unsaturated fatty acids leads to the formation of compounds that are undesired from the point of view of both taste and toxicity (Frankel, 1984). Free radicals are among the main products of lipid oxidation and have been implicated in playing a role in over 100 diseases including cancer, atherosclerosis and arthritis (Thomas, 1995).

During the last 20 years, *Allium* spices were among the most studied vegetables and aroused great interest for food industries. These interests arose from the encouraging results of the antioxidant capacity of some of their compounds, which have been to be comparable to and sometimes higher than that of synthetic antioxidants used in food industry particularly

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BHA (butylated hydroxyanisole,) and BHT (butylated hydroxytoluene) (Barlow, 1990). However, both consumer preference and toxicological investigations diverted the interest in the research and use of natural plant antioxidants used long time before under raw forms. Antioxidants are thought to reduce the risk of these diseases by lowering the concentration of free radicals. At first, attention was paid to the natural compound e.g. vitamin C, E and carotenoids, and during the recent years the powerful antioxidant capacity of phenolics aroused more interests (Shahidi et al., 1992). Although onion and garlic have been used for centuries in herbal and traditional medicine, it is only the last 20 years that some of the health claims have been tested rigorously for legitimate scientific merit (Lawson, 1998). Despite the large data available on the antioxidant properties of the *Allium* plant extracts particularly oil extracts, further investigations are needed to determine the antioxidant properties of different cultivars of extracts of the numerous *Allium* species and cultivars cultivated throughout the world.

The purpose of this study was to investigate the radical scavenging activity, chain-breaking activity, the scavenging of H_2O_2 and the reducing capacity of four types of onions and garlic extracts.

MATERIALS AND METHODS

Materials

Four type of onions (*Allium cepa*) – green onion (Grn) (var. Premier), yellow (Ylw) (var. Jaune d'Espagne), red (Rd) (var. Rouge Amposta) and purple (Prl) (var. Rouge), and garlic (*Allium sativum* L. var. Cristo) were selected for this investigation. Onion and garlic samples (freshly harvested) were sorted for uniformity and absence of defects and stored at 4 ºC prior analyses.

The folin-ciocalteu reagent (purity: 99.8%), chlorogenic acid (95%), quercetin (99%), rutin (99%), ascorbic acid (99%) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) (92%) were purchased from Sigma Chemical Co. (St Louis, MO, USA), and all other chemicals were purchased from Merck (Darmstadt, Germany) and are of high-grade purity (> 98%).

Extraction

Samples (50 g of tissues) were homogenized in 100 mL of methanol using a Waring blender at high speed for 1 min at 4 °C. The extract was stirred 10 min at 4 °C and filtered through four layer of cheesecloth and the residue was re-extracted under the same condition with 100 mL of methanol. The combined filtrate was concentrated under vacuum at 65 ºC to dryness and the dry residue was dissolved in 10 mL of methanol. These methanolic extracts were used for the determination of total phenolics, radical scavenging activity, chain-breaking activity, H_2O_2-scavenging and reducing capacity.

Total phenolics determination

Total phenolics of extracts were quantified colorimetrically using Folin-Ciocalteu reagent and chlorogenic acid as standard (Horwitz, 1984). Five milliliters of Folin-Ciocalteu (diluted ten fold in distilled water), 2 mL of sodium bicarbonate (200 $g.L^{-1}$) and 2 mL of distilled water were added to 1 mL of extract. After 15 min incubation at room temperature, the absorbance was read at 730 nm using an UVmini-1240 recording spectrophotometer (Shimadzu, Kyoto, Japan). Results are expressed in chlorogenic acid equivalents (mg CAE. 100 g^{-1} fresh weight).

Radical Scavenging Activity (RAS)

The radical scavenging activity was examined by the reduction of DPPH in methanol. To methanolic solution (2 mL) of DPPH (4 10^{-4} $M.L^{-1}$) was added 200 µL of extract and the mixture wax vortexed. The decrease in absorption at 515 nm was measured in 1-cm quartz cell during 300 min using an UVmini-1240 recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH was estimated from the following equation:

$$\%inhibition = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

Chain-breaking activity assessment

The chain-breaking activity was based on the methodology of Brand-Williams et al. (1995) and assessed as described by Manzocco et al. (1998). In order do determine this activity after severe conditions, extracts were heated at 90 ºC for three hours. Then, a volume of 3 mL of 6.1×10^{-5} M DPPH in methanol was added to 10 µL of extracts.

After 60 min incubation at room temperature, absorbance was read at 515 nm using an UVmini-1240 recording spectrophotometer (Shimadzu, Kyoto, Japan). The chain-breaking activity was expressed by the reaction rate *k* and calculated by the following equation:

$$\frac{1}{A^3} - \frac{1}{A_0^3} = -3kt$$

where A_0 is initial optical density, A is optical density at increasing time, t. The reaction rate was expressed as $k.mL^{-1}$ ($-OD^{-3}.min^{-1}.mL^{-1}$), assuming that all extracts possess antioxidant properties.

Scavenging of hydrogen peroxide (Svg)

The ability of extracts to scavenge hydrogen peroxide (H_2O_2) was assessed by the method of Ruch et al. (1989). Hydrogen peroxide solution (2 $mM.L^{-1}$) was prepared in phosphate-buffered saline (PBS, pH 7.4). Hydrogen peroxide (H_2O_2) concentration was determined spectrophotometrically from absorption at 230 nm with the molar absorptivity of 81 $(M.L^{-1})^{-1}.cm^{-1}$. One milliliter of extract was added to H_2O_2 solution (0.6 mL) and absorbance of the hydrogen peroxide at 230 nm was read after 10 min against a blank solution containing extract (1 mL) in PBS without H_2O_2. The scavenging of hydrogen peroxide was determined as follow:

$$\%Svg = \left[\frac{A_m}{A_b}\right] \times 100$$

A_m = Absorbance of reaction mixture
A_b = Absorbance of blank mixture (extract in PBS without H_2O_2)

Quercetin, rutin and ascorbic acid solutions (200 $mg.L^{-1}$ in methanol) were used as comparative standard molecules.

Reducing capacity assessment

The reducing capacity (*RP*) of the extracts was assessed as described by Oyaizu (1986). Two milliliters of extracts were added to potassium ferricyanide (2.5 mL, 10 $g.L^{-1}$) and the mixture incubated at 50 ºC for 20 min. Trichloroacetic acid (2.5 mL, 100 $g.L^{-1}$) was added to the mixture, which was then centrifuged at 650 × *g* for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 1 $g.L^{-1}$). The absorbance was read at 700 nm. Higher absorbance indicated greater reducing capacity which is calculated as follow:

$$RP = \left[\frac{A_m}{A_b} - 1\right] \times 100$$

A_m = absorbance of reaction mixture
A_b = absorbance of blank mixture (distilled water instead extract)

Statistical analysis

All determinations were conducted in triplicate, and experiment was run in duplicate. Data (n = 6) were treated by analysis of variance (ANOVA) and computed using *Statistica* 5.0 software (StatSoft, Maisons-Alfort, France). Differences among means were determined by the least significant difference (*LSD*) test with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

Total phenolics

Total phenolics content of extracts varied from 30 to 40 mg. The lowest content was found in green onion (30 $mg100\ g^{-1}$ fresh weight) while highest content in garlic (49 $mg.100\ g^{-1}$ fresh weight). Contents in other *Allium* were 34.7, 44 and 47.3 $mg.100\ g^{-1}$ fresh weight in yellow, red and purple onion bulbs, respectively (Fig. 1). Phenolics content in onions or *Allium* plants varies considerably particularly with cultivar (Bajaj et al., 1980). Total phenolics of red onion var. Rouge Amposta were reported by Benkeblia (2000) from 18 to 20 $mg.100\ g^{-1}$ fresh weight. Nuutila et al. (2003) reported that the amount of total phenolics varied widely in the *Allium* extracts and ranged from 845 to 2075 $mg.kg^{-1}$ and from 75 to 115 $mg.kg^{-1}$ of lyophilized tissues of different onions and garlics, respectively.

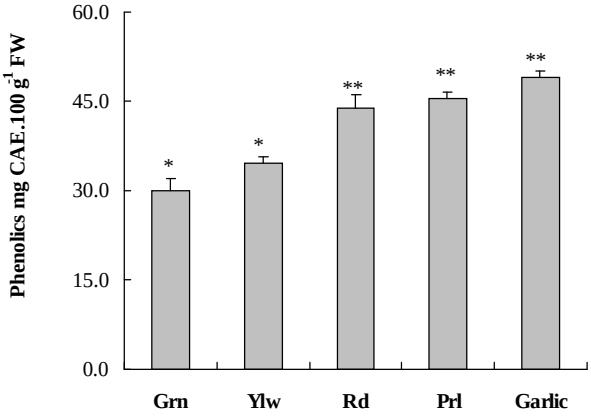

Figure 1 - Total phenolics content of onions and garlic extracts. Means followe'd by different symbol are significantly different at $P < 0.05$.

Radical scavenging activities (RAS) of the extracts are shown in Fig. 2. Among the extracts tested, garlic extract reacted faster than other extracts and was the most effective DPPH radical scavenger, followed by purple, red and yellow onion extracts, while green onion extract showed the lowest RAS. Comparatively, rutin showed close DPPH radical scavenging activity to garlic extract, while quercetin showed the highest scavenging activity.

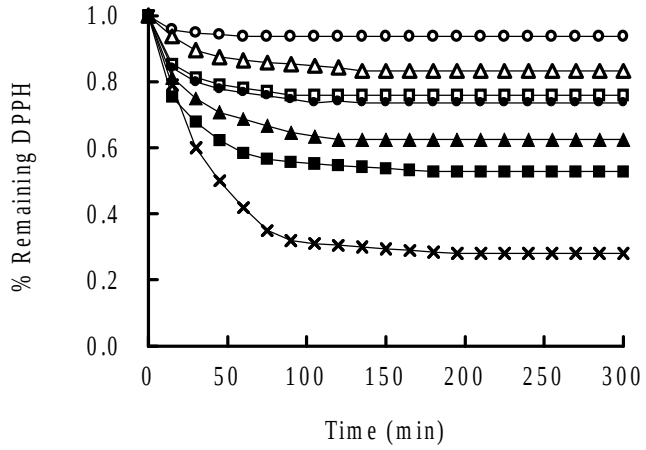

Figure 2 - Radical scavenging activity on DPPH of *Allium* extracts (○ green onion, △ yellow onion, □ red onion, ● purple onion, ▲ garlic, ■ rutin, x quercetin) (errors bars were not shown to aid clarity in the figure)

The high antioxidant activity of *Alliums* and especially high RAS of garlic were reported by numerous investigators (Velioglu et al., 1998; Yin and Cheng, 1998; Miller et al., 2000). However, RAS activity depended on both phenolics and sufur compounds of *Alliums*. On the other hand, Nuutila et al. (2003) reported that the lowest antioxidant activity was detected in garlic.

Table 1 shows the chain-breaking activity of the extracts. It was interesting to note that heating caused an increase in chain-breaking activity which was very high in case of ascorbic acid. Comparatively with *Allium* extracts, initial chain-breaking of ascorbic acid was from 12 to 208 fold higher. After three hours at 90 ºC, *k* value was from 15 to 298 fold higher. These results indicated

that the decrease in this activity was associated with a corresponding increase in reducing capacity and scavenging of H_2O_2. The gain of these activities was also associated to the increase in total phenolics content of the extracts. It is known that heating induces non-enzymatic browning, and promotes polymerization of phenolic compounds to form brown-colored macromolecules.

Chain-breaking of *Allium* extracts was not subject of large investigation. However, Manzocco et al. (1998) reported similar results on tea extracts. These authors reported also that thermal treatment enhanced chain-breaking activity and decreased oxygen uptake of the extracts. On the other hand, the presence (as additive) of six *Allium* members in food significantly delayed lipid oxidation. However, this antioxidant activity was lost progressively with high temperatures (65 and 100 ºC) (Yin and Cheng, 1998).

Ability of the investigated *Allium* extracts to scavenge hydrogen peroxide is shown in Fig. 3.

Table 1 - Chain-breaking activity of *Allium* extracts. Means within same column followed by different letters are significantly different at $P < 0.05$.

	Chain-breaking activity ($-O.D.^{-3}. min^{-1}.mL^{-1}$)	
	Crude extract	**Heated extract (90 ºC – 3h)**
Grn	0.48 ± 0.05^{a}	$1.2\ 0.\pm 13^{a}$
Ylw	0.19 ± 0.02^{b}	0.59 ± 0.11^{b}
Rd	0.048 ± 0.07^{c}	$0.14\ \ 0.07^{c}$
Prl	0.032 ± 0.006^{c}	0.071 ± 0.011^{d}
Garlic	0.029 ± 0.004^{cd}	0.063 ± 0.009^{d}
AA	6.01 ± 0.96^{e}	18.53 ± 1.63^{e}

Grn= Green, Ylw= Yellow, Rd= Red, Prl= Purple, AA= Ascorbic Acid

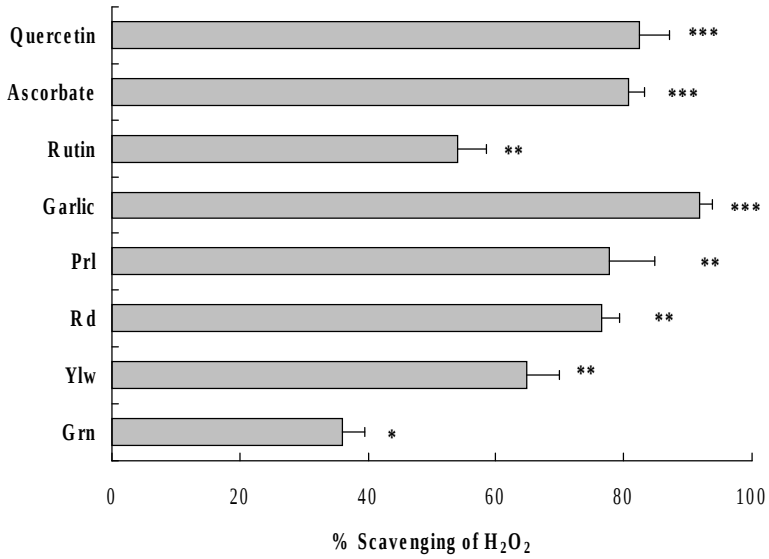

Figure 3 - Scavenging of hydrogen peroxide of onions and garlic extracts. Means followed by different symbol are significantly different at $P < 0.05$

Green onion showed low scavenging activity (35.9%), whereas other extracts and garlic showed high ability with 64.8, 76, 77 and 91% for yellow, red, purple and garlic, respectively. Scavenging activity of standard molecules showed that rutin possesses low activity (54%), when ascorbic acid and quercetin possess high activity with 80.73 and 82.35%, respectively.

The H_2O_2-Scavenging activity ratio of the extracts to the standard molecules showed that garlic

extract possessed the highest relative ratio among the other extracts as shown in Table 2. The scavenging of hydrogen peroxide of *Allium* extracts is not well documented. Nevertheless, Duh et al. (1999) reported similar results for *Chrysanthemum morifolium* with high relationship between phenolics content and scavenging activity of the water extracts. This ability to scavenge hydrogen peroxide could be an efficient assessment method to evaluate antioxidant property of water extracts, and could be compared to antioxidant activity (AOX) of extracts despite the difference between the two methodologies.

As illustrated in Fig. 4, reducing capacity of green onion extract was the lowest (18%), followed by yellow and red onion extracts with 24 and 58%, respectively. Purple onion and garlic showed the highest reducing capacity with 107 and 196%, respectively. The relationship between reducing power and total phenolics content was highly significant with a determination coefficient (r^2) of 0.93.

Reducing capacity of *Allium* extracts was not investigated, however, high antioxidant activity of red onion scales (Velioglu et al., 1998) and garlic (Yin and Cheng, 1998) was reported.

Non-enzymatic antioxidant activity of garlic extract was also reported by Yin et al. (2002). However, according to some authors (Amagase et al., 2001; Imai et al., 1994), this antioxidant activity (AOX) was more attributed to the organosulfur compounds.

Table 2 - Scavenging of H_2O_2 ratio (scavenging of extract/scavenging of standard. Means within same column followed by different letters are significantly different at $P < 0.05$.

	Sg_e/Sg_Q	Sc_e/Sg_R	Sg_e/Sg_{AA}
Grn	1.44 ± 0.27^{ac}	0.96 ± 0.10^{a}	0.94 ± 0.05^{a}
Ylw	1.2 ± 0.13^{a}	0.8 ± 0.13^{b}	0.76 ± 0.03^{b}
Rd	0.67 ± 0.11^{b}	0.44 ± 0.09^{c}	0.44 ± 0.11^{c}
Prl	1.42 ± 0.36^{ac}	0.95 ± 0.08^{a}	0.93 ± 0.21^{a}
Garlic	1.7 ± 0.24^{c}	1.14 ± 0.21^{a}	1.11 ± 0.14^{a}

Sg subscripts indicate: e: exctract, Q: Quercetin, R: Rutin and AA: Ascorbic Acid

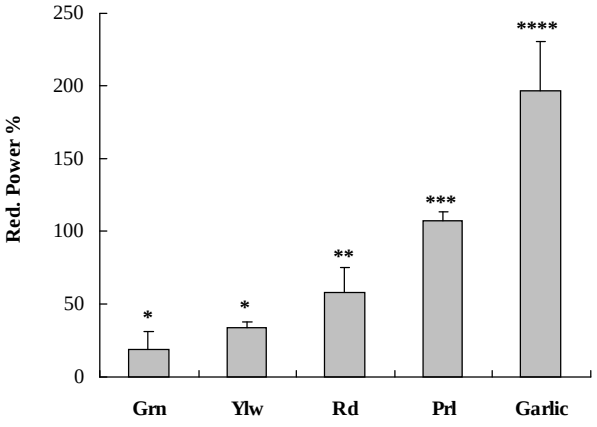

Figure 4 - Reducing capacity of onions and garlic extracts. Means followed by different symbol are significantly different at $P < 0.05$.

Statistical analysis also showed that radical scavenging activity, reducing capacity, scavenging of hydrogen peroxide and chain-breaking activity (initial and after heating) were highly correlated with total phenolics content of extracts, and coefficients of determination (r^2) ranged from 0.90 and 0.95.

CONCLUSION

Finally, it could be concluded that studied *Allium* extracts possessed variable but interesting antioxidant properties. These properties were significantly correlated to total phenolics content which were high in red, purple onions and garlic. However beside phenolic compounds, sulfur compounds could be involved in the assessment of the antioxidant properties. Heat treatment reduced the antioxidant activity of the extracts; however, heating should be carefully considered when *Allium* plants are used in food preparation or cooking for antioxidant protection

From the general point of view, the activity of these plants must be tested individually in different food systems and breakdown products under different conditions must be investigated. It could also be necessary that full structural identification of the active components of antioxidant compounds of plant foods is, therefore, required and their toxicological properties be investigated.

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