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Cooked garlic and antioxidant activity: Correlation with organosulfur compound composition

D.A. Locatelli [a,b], M.A. Nazareno [c], C.M. Fusari [a,b], A.B. Camargo [a,b,d,*]

[a] Instituto de Biología Agrícola de Mendoza (IBAM) – CONICET, Mendoza, Argentina
[b] Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Alte. Brown 500, 5505, Chacras de Coria, Mendoza, Argentina
[c] Laboratorio de Antioxidantes y Procesos Oxidativos (CIDSE-CONICET-UNSE), Santiago del Estero, Argentina
[d] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina

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ABSTRACT

The antioxidant properties and the main beneficial organosulphur compounds of home-cooked garlic samples were studied in order to establish relationships between them. Antioxidant activity was tested by free radical scavenging against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\cdot}$) and 2,2′-azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS$^{+\cdot}$), Fe(III) reducing ability (FRAP) and linoleic acid co-oxidation initiated by soybean lipoxygenase in a micelle system. DPPH, ABTS and FRAP assays showed the highest activity for raw garlic samples, while β-carotene bleaching assay yielded the highest activity for stir-fried garlic. Pure organosulphur compounds tested by DPPH, FRAP and β-carotene bleaching assays showed that allicin had an antiradical action mechanism, as well as iron reducing capacity; while antioxidant activity was the main mechanism for ajoenes and 2-VD. To our knowledge, this study is the first demonstration that home-cooked garlic retains its antioxidant activity, and, at the same time, elucidates the mechanisms involved in this activity.

1. Introduction

This work is a direct continuation of a previous publication and, naturally, both studies are interlaced at some points (Locatelli, Altamirano, González, & Camargo, 2015). The main objective of the present study was to investigate if home-cooking processes affect the antioxidant properties of garlic. In this context, our previous publication demonstrated that the bioactive organosulfur compounds of garlic suffer transformations, volatilizations, leaching, etc., according to pre-cooking and cooking treatments. However, and surprisingly, organosulphur compounds (OSCs) of proven beneficial biological importance were detected in all cooked garlic samples. From this fact, it was possible to conclude that cooked preparations have potential beneficial effects. Building on that previous research, the present work was focused on finding evidence of antioxidant activity (AOA) in cooked garlic samples and the mechanisms involved for each OSC.

Garlic (*Allium sativum* L.) has been used in food as an ingredient and for seasoning, as well as for medicinal purposes, since ancient times. Several studies have shown that garlic has numerous biological properties (Fujisawa, Suma, Origuchi, Seki, & Ariga, 2008; Iciek, Kwiecien, & Wloder, 2009; Lee, Lee, Kim, Rhee, & Pyo, 2015; Santhosha, Jamuna, & Prabhavathi, 2013) thanks to the presence of phytochemicals, which make garlic a healthy food. These compounds include OSCs that are synthesized using sulfate absorbed by the roots as a source of sulfur. Since the first OSC is formed in the plant, until the garlic bulb is ready for consumption, OSCs suffer biological transformations resulting in different products or chemical compounds (Block, 2010). The first group of OSCs generated when the garlic tissue is broken is the thiosulfinates, with allicin being the most abundant in fresh garlic. These first compounds are responsible for the characteristic pungency of garlic. Allicin is an unstable and highly reactive molecule that transforms into a series of OSCs. The most stable group of compounds is the polysulfides, which are the last set of transformations. Among the OSCs found, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoenes and vinyldithiins (2-VD) have been mentioned as having important biological activities. These compounds can occur during the cooking or distillation of garlic, or may also be present in aged garlic extracts (Kamel & Saleh, 2000; Kim, Wu, Kobayashi, Kubota, & Okumura, 1995; Weinberg, Manier, Richardson, & Haibach, 1993; Yu, Lin, & Ho, 1994; Yu, Wu, & Ho, 1993), each treatment resulting in a different OSCs profile (Locatelli et al., 2015). Sensory as well as biological or

* Corresponding author at: Instituto de Biología Agrícola de Mendoza (IBAM) – CONICET, Mendoza, Argentina.
E-mail address: acamargo@fca.uncu.edu.ar (A.B. Camargo).

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functional characteristics are determined by the OSCs profile in each garlic preparation (Amagase, Petesch, Matsuura, Kasuga, & Itakura, 2001; Fujisawa et al., 2008; Liang et al., 2015).

Since antioxidant activity is one of the properties attributed to these compounds, AOA evaluations are significant for both human health and food technology. In the first case, antioxidants in garlic are studied for their potential to help protect the human body against oxidative damage mediated by reactive oxygen species (ROS). In the food industry, these compounds and their antioxidant activity are studied because garlic can be used as an additive for controlling rancidity development, delaying the formation of toxic oxidation products, maintaining nutritional quality, and extending the shelf-life of products (Shahidi & Ambigaipalan, 2015).

Most studies on this subject have been limited to raw, boiled and microwave-treated extracts of whole garlic cloves, and raw garlic heated extract (Pedraza-Chaverri, Arriaga-Noblecía, & Medina-Campos, 2007). The OSCs studied were: allicin, alliin, S-Allyl Cysteine, DAS, DADS, DATS (Argüello-García, Medina-Campos, Perez-Hernandez, Pedraza-Chaverri, & Ortega-Pierres, 2010; Wang & Huang, 2015). The scavenging mechanisms were measured against hydroxyl and superoxide radicals (Chung, 2006; Pedraza-Chaverri, Medina-Campos, Avila-Lombardo, Zuñiga-Bustos, & Orozco-Ibarra, 2006) and hypochlorous acid (Argüello-García et al., 2010; Wang & Huang, 2015), showing different patterns of antioxidant activities. After analyzing these previous reports, it can be noted that they reached limited conclusions. The garlic samples consisted of whole cloves instead of chopped garlic (which is how this vegetable is usually treated before being cooked and consumed). During heating, the OSCs transform into other OSCs of important biological activity, and these were not determined (such as ajoenes and vinyldithiins). In addition, these studies measured the antioxidant activity by only three mechanisms.

These considerations motivated carrying out the present study, in order to obtain a more comprehensive approach. The aims of this work were to assess the antioxidant power of garlic after home-cooking treatments, and to evaluate the role of pure OSCs and their contribution to diverse antioxidant mechanisms.

This paper shows the results for antioxidant activity evaluated by five mechanisms in home-cooked garlic samples and pure OSCs of biological importance. From the new correlations with valuable results obtained, it is possible to elucidate changes in antioxidant activity.

To our knowledge, this is the first demonstration that home-cooked garlic retains its antioxidant activity and, at the same time, the study sheds some light on the mechanisms involved in this activity.

2. Materials and methods

2.1. Plant materials

Red garlic clone "Rubí" from the germplasm collection of Instituto Nacional de Tecnología Agropecuaria (INTA) was used in all experiments. Garlic was grown at INTA's experimental field located in La Consulta, Mendoza, Argentina (33° 44′ S, 69° 07′ W), during 2013. Bulbs were harvested when the leaves had senesced and afterwards they were fully cured. During postharvest, the bulbs were stored at ambient conditions in sheds for 2 months.

2.2. Sample preparation

As mentioned in the introduction, the present work is the continuation of a previous study (Locatelli et al., 2015). That paper showed the results of the OSCs profiles determined for each combination of pre-treatment (tissue disruption grade) and treatment (cooking processes). The conclusion reached was that chopped garlic evidenced the highest quali-quantitative levels of OSCs. For that reason, chopped garlic was selected as pre-treatment prior to cooking for the current work. Table 1 details the data corresponding to the OSCs profiles of the samples used in the present study.

In brief, four kilograms of cloves from several bulbs were pooled and peeled. Sub-samples of 150 g were prepared with the combination of pre-cooking/cooking treatments mentioned in Table 1. The pre-cooking treatment involved a disruption of tissues: chopped garlic (using a garlic press). These samples were kept standing for 15 min in order to promote allicin formation prior to the cooking treatments.

All samples were cooked in a gas-stove (Longvie-2600) until the end point for each preparation. Chopped garlic samples were cooked by simmering (HS), rolling boil (RB) and stir-fry (SF) methods, while raw garlic (R) was taken as the control. The end point for each treatment was considered when samples reached the colour and texture of home-cooked products. An informal testing panel, which included three trained panelists, determined such a point.

All garlic samples were frozen in liquid nitrogen, and freeze dried at −58 °C for 72 h under vacuum (FreeZone 2.5, Freeze Dry Systems, LabConco, Missouri, USA). The resulting lyophilized material was ground using a pestle and mortar and then stored at −80 °C.

2.3. Standards and reagents

DAS (97%) and DADS (80%) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). Chromatography grade acetonitrile (ACN), methanol (MeOH), acetone, hexane, dichloromethane (DCM), 2-propanol and chloroform were purchased from Merck (Kenilworth, NJ, USA). Ultrapure water (18 MΩcm) was obtained from a Milli-Q water purification system (Millipore, Molsheim, France). Allicin was synthesized by oxidation of diallyl disulfide (DADS) with hydrogen peroxide, following a previously reported paper by the group (González, Camargo, & Burba, 2007). To obtain *E-Z* Ajoene isomers, synthesized allicin was heated while being stirred in an acetone-water mixture (40:60 v/v) (Block, Ahmad, Catalfamo, Jain, & Apitz-Castro, 1986; Locatelli, Altamirano, Luco, Norlin, & Camargo, 2014; Soto, Camargo, González, & Galmarini,

Table 1
OSCs levels in chopped garlic (ChG) subjected to different cooking treatments: simmering (HS), rolling boil (RB), stir-frying (SF) and raw garlic (R).

Chopped garlic samples	OSCs levels [μg g^{-1}][1]					
	Allicin	E-Z ajoene	2-VD	DAS	DADS	DATS
R	6771.03 ± 93.9^{c}	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]
HS	274.30 ± 5.9^{a}	41.60 ± 3.3^{a}	31.20 ± 2.6^{a}	9.20 ± 3.4^{a}	4.80 ± 0.3^{a}	222.70 ± 8.2^{a}
RB	1574.60 ± 23.9^{b}	142.80 ± 7.3^{a}	166.70 ± 39.9^{a}	nd[2]	8.90 ± 1.1^{a}	415.20 ± 22.9^{a}
SF	nd[2]	644.20 ± 4.1^{a}	2964.50 ± 60.0^{a}	517.30 ± 88.5^{b}	92.70 ± 2.7^{b}	1324.30 ± 45.6^{b}

Values followed by the same letter within each column were not significantly different according to the Tukey's test ($p < 0.05$).
Cooking treatments: R, raw; HS, simmering (boil water with a minimum heat input); RB, rolling boil (boil water with a high heat input); SF, stir-frying.
[1] Results expressed as mean ± SD (n = 5); 95% confidence interval; μg g^{-1} DW.
[2] Not detectable.

2007). 2-VD compounds were synthesized by heating allicin in an acetone-methanol mixture (60:40 v/v) following the procedure described by Iberl, Winkler, and Knobloch (1990), with slight modifications in temperature (84 °C) and time (3.5 h). Linoleic acid (99% v/v), potassium persulfate (99% m/v), trichloroacetic acid (99% m/v), Tween® 20 (97% v/v) and 2,2′-azino-bis-(3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). Gallic acid (98% m/v), soybean lipoxidase (LOX) type 1-S (46,000 units/mg solid), trans-β-carotene (95%), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) (98%) and iron(III) chloride (99% m/v) were purchased from Sigma Aldrich (Buenos Aires, Argentina). Potassium phosphate buffer (98% m/v), Folin-Ciocalteu's phenol reagent, sodium borate anhydrous, sodium carbonate p.a. and ferrous sulfate were acquired from Biopack (Buenos Aires, Argentina).

2.4. Extraction of OSCs

One gram of lyophilized garlic powder was reconstituted with 30 ml of distilled water. Then, it was extracted with 50 ml of dichloromethane. The extraction was repeated two more times. The organic phases of each extraction were combined and concentrated almost to dryness by means of a rotary evaporator (DecalabFbr®, Buenos Aires, Argentina). The remaining extract (ca. 500 μl) was reconstituted in acetonitrile to reach a final volume of 2 ml. Then, it was filtered and analyzed by HPLC.

2.5. Antioxidant activity evaluation

2.5.1. Total phenolic content (TPC)

TPC was measured by Folin-Ciocalteu assay (Ismail, Marjan, & Foong, 2004) with slight modifications. In brief, 100 μl of extract were transferred into a test tube, and 0.65 ml of Folin-Ciocalteu reagent and 3.15 ml of 20% (w/v) sodium carbonate were added and mixed gently. The mixture was allowed to stand at 50 °C for 10 min. UV–vis absorption measurements were carried out at 725 nm using a UNICAM UV2 spectrophotometer (UNICAM, Cambridge, United Kingdom). The standard calibration curve (0.0002–0.0061 mg/ml) was plotted using gallic acid in methanol. The TPC was expressed as gallic acid equivalents (mg GAE/g^{-1} dry weight).

2.5.2. DPPH· scavenging assay

Free radical scavenging activity of garlic samples was measured by the DPPH· bleaching method (Brand-William, Cuvelier, & Berset, 1995). An aliquot of the extract was added to 3 ml of DPPH· methanolic solution in a cuvette and spectrophotometrically monitored at 515 nm (initial absorbance at 515 nm ca. 1.00). The decrease in absorbance was determined by monitoring the absorbance changes in cycles of 30 s during 10 min. Antiradical activity (ARA) was calculated according to Burda and Oleszek (2001) as shown in Eq. (1), where A_{SS} is the absorbance of the solution at the steady state and A_0 is the absorbance of the DPPH· solution before the antioxidant addition. A_{SS} was estimated by mathematical fitting of kinetic curves performed with Origin Pro 8.0 software.

$$ARA\% = \left(\frac{1 - A_{ss}}{A_0}\right) \times 100 \quad (1)$$

ARA was also expressed as gallic acid equivalents (mg GAE/g^{-1} dry weight). All determinations were performed in triplicate for each extract.

2.5.3. $ABTS^{+\cdot}$ scavenging assay

ABTS was dissolved in distilled water to yield a 7 mM solution. The radical solution was prepared by incubating the ABTS solution with a 2.45 mM potassium persulfate solution for 16 h in the dark at room temperature, and this was subsequently diluted with water to a final absorbance of 1.00 ± 0.01 AU at 734 nm. For ARA determinations, an aliquot of garlic extract was added to 3 ml of the $ABTS^{+\cdot}$ solution in a cuvette. The decrease in absorbance was monitored in cycles of 30 s for 10 min by spectrophotometry. All determinations were performed in triplicate for each extract. The percentage inhibition of $ABTS^{+\cdot}$ by the samples was calculated according to Eq. (1). Additionally, the gallic acid equivalent was calculated (mg GAE/g^{-1} dry weight).

2.5.4. Ferric reducing capacity assay (FRAP)

The ability to reduce ferric ions was measured following the procedure described in Marazza, Nazareno, de Giori, and Garro (2012). An aliquot of 1 ml of sample was mixed with 1 ml of phosphate buffer (0.2 M) pH 6.6 and 1 ml of potassium ferricyanide (1% w/v). The mixture was incubated at 50 °C for 20 min. Finally, 1 ml of trichloroacetic acid was added. The mixture was centrifuged at 15,900*g* for 10 min at 4 °C. The supernatant (1.5 ml) was mixed with 0.3 ml ferric chloride (1% w/v), and 1.5 ml of deionized water was added. After 10 min, the absorbance at 700 nm was measured. The ferric cation reducing power was expressed in Trolox® equivalent antioxidant capacity (TEAC) in mM TEAC/10 mg dry weight. The percentage of ferric reduction was calculated according to Eq. (2), where C_0 is the concentration of $FeSO_4$ (μM) with absorbance equal to 1.00 and C_s is the equivalent concentration of $FeSO_4$ (μM) found in each plant extract.

$$Reducing\,capacity\ (\%) = 100 - \left(\frac{C_0 - C_s}{C_0} \times 100\right) \quad (2)$$

2.5.5. β-carotene bleaching assay

The antioxidant activity (AOA) of the extracts and fractions was determined by the enzymatically induced β-carotene bleaching method, according to Chaillou and Nazareno (2006). An aliquot of 500 μl of a saturated stock solution of β-carotene in chloroform was mixed with 500 μl of Tween® 20. The mixture was evaporated using a nitrogen stream for 15 min to remove the chloroform. The final solution was obtained by adding 0.01 M borate buffer (pH 9) up to an absorbance value of 1.3 ± 0.01 AU at 460 nm. Linoleic acid was prepared by mixing 50 μl of this compound with 200 μl of Tween® 20 and diluted with 0.01 M borate buffer (pH 9). Lipoxygenase (LOX) solution was obtained by dissolving 10 mg of the enzyme in 0.01 M borate buffer (pH 9) up to a 10 ml solution. Spectrometric measurements were carried out in a glass cuvette at 460 nm. For the control assay, a volume of 2 ml of diluted β-carotene solution was placed with 300 μl of linoleic acid solution, and then mixed with 300 μL of 0.01 M borate buffer (pH 9). Finally, 400 μl of LOX solution were added to initiate the reaction. Determinations of AOA in garlic extracts were performed for every sample. All assays were carried out in triplicate at room temperature (25 ± 1 °C). AOA was calculated as suggested by Burda and Oleszek (2001), as the percentage inhibition of β-carotene bleaching of the samples compared to that of the control as described below in Eq. (3). A_s^0 and A_c^0 are the absorbance values measured at the initial incubation time for the samples and control, respectively. Parameters A_s^∞ and A_c^∞ are the absorbance values at the steady state measured for the samples and control, respectively, which were estimated by mathematical fitting of kinetic curves performed with Origin Pro 8.0 software.

$$AOA\% = 100 \times \left(1 - \frac{A_s^0 - A_s^\infty}{A_c^0 - A_c^\infty}\right) \quad (3)$$

Table 2
Total phenolic compounds, antiradical activities, antioxidant activity and iron reducing ability of different garlic samples.

Chopped garlic samples	TPC	DPPH		ABTS		FRAP		β-carotene bleaching assay
	mg GAE/100 g DW	%ARA/100 mg DW	mg GAE/100 g DW	%ARA/100 mg DW	mg GAE/100 g DW	%FRAP/100 mg DW	[mM] TEAC/ 10 mg DW	%AOA/100 mg DW
R	11.21 ± 2.65^{d}	19.10 ± 1.3^{c}	0.58 ± 0.04^{c}	17.60 ± 0.7^{c}	0.11 ± 0.01^{c}	708.00 ± 11^{c}	164.80 ± 23.2^{c}	24.90 ± 1.5^{a}
HS	1.69 ± 0.25^{a}	4.44 ± 0.3^{a}	0.09 ± 0.01^{a}	14.10 ± 0.4^{b}	0.03 ± 0.01^{a}	57.90 ± 0.4^{a}	13.60 ± 0.1^{a}	107.70 ± 3.5^{c}
RB	4.02 ± 0.89^{c}	6.66 ± 0.8^{b}	0.16 ± 0.03^{b}	13.60 ± 0.8^{b}	0.02 ± 0.02^{a}	52.00 ± 0.1^{a}	12.30 ± 0.1^{a}	71.40 ± 2.3^{b}
SF	2.43 ± 0.5^{b}	4.89 ± 0.4^{a}	0.05 ± 0.01^{a}	9.30 ± 0.5^{a}	0.07 ± 0.01^{b}	107.60 ± 5.3^{b}	24.50 ± 1.2^{b}	123.20 ± 2.7^{d}

Results expressed as mean ± SD (n = 5); 95% confidence interval; mg g^{-1} DW.
Values followed by the same letter within each column were not significantly different according to the Tukey's test ($p < 0.05$).
Cooking treatments: R, raw; HS, simmering (boil water with a minimum heat input); RB, rolling boil (boil water with a high heat input); SF, stir-frying.

Table 3
Pearson correlation coefficients among antioxidant activity, antiradical activities, total phenolic compounds and OSCs levels from garlic samples.

Correlation	R (p-value <0.05)
TPC × ARA (DPPH)	0.92
TPC × ARA (ABTS)	0.81
TPC × AOA	−0.84
TPC × ARA (FRAP)	0.86
Allicin × ARA (DPPH)	0.99
Allicin × ARA (ABTS)	0.76
Allicin × AOA	−0.92
Allicin × ARA (FRAP)	0.95
Allicin × TPC	0.95
Ajoene × AOA	0.65
2-VD × AOA	0.62
DAS × AOA	0.60
DADS × AOA	0.64
DATS × AOA	0.71

2.6. Statistical analysis

All data were expressed as mean ± standard deviation (SD). The data were analyzed by ANOVA using INFOSTAT software. The mean of each treatment group was compared by Tukey's test. P-values <0.05 were considered to be significant.

3. Results and discussion

3.1. Determination of antiradical activity (ARA) and antioxidant activity (AOA) in cooked garlic samples

Table 2 shows the percentage of antiradical activity (ARA%) measured by the scavenging of DPPH· and $ABTS^{+\cdot}$ radicals, the percentage of antioxidant activity (AOA%), and the percentage of Fe^{3+} reduction capacity for the different extracts from the garlic samples (R, HS, RB and SF) and control. The analysis of variance evidenced that HS, RB and SF samples presented the lowest ARA %, while the highest was found for the R extract, measured both by DPPH· and $ABTS^{+\cdot}$ radical sequestration (19.1 ± 1.3 and 17.6 ± 0.7%, respectively). These results are consistent with those expressed by Rabinkov et al. (1998), who describe the effectiveness of allicin to scavenge free radicals. It should be noted that allicin was the dominant OSC in R garlic samples (Table 1) in Locatelli et al. (2015). Similarly, concerning the percentage of Fe^{3+} reducing capacity, R extracts had the highest value (708 ± 110.3%), followed by SF (with 107.7 ± 3.5%), while HS and RB presented the lowest values (57.9 ± 0.4 and 52 ± 0.1%, respectively).

Finally, SF samples exhibited a good AOA% (123.2 ± 2.7%), possibly due to the high levels of ajoene and 2-VD. These results agree with those obtained when the AOA% was individually tested in pure compounds (Table 4), where it can be observed that 2-VD followed by ajoene were the analytes with the highest AOA. Furthermore, the lowest AOA% values were for R extracts (24.9 ± 1.5%), which corresponded to samples where neither ajoene nor 2-VD could be detected, also in agreement with the results for AOA% individually tested in pure OSCs.

Table 3 includes analysis of results showing that there are significant correlations between the values of the antioxidant activities analyzed and contents of certain OSCs in garlic samples. Almost all the correlations were positive, indicating a direct relationship between compounds and activities.

The phenolic compounds present in garlic samples show a strong and positive correlation with the free radical scavenging activity (DPPH· and $ABTS^{+\cdot}$) as well as with the iron reducing ability. In contrast to this finding, AOA was inversely and strongly related to the phenolic compound contents. Similarly, allicin

Table 5
Organosulphur compound composition for synthetic solution of stir-fried garlic.

Samples	Allicin %	Ajoene %	2-VD %	DAS %	DADS %	DATS %
SF	0.00	11.51	54.23	7.82	1.72	24.71
SS	0.00	13.48	60.65	2.13	3.18	20.57

SF: stir-fried chopped garlic.
SS: synthetic solution of stir-fried chopped garlic.

Table 4
Total phenolic compounds, antiradical activities, antioxidant activity and iron reducing ability of different individual OSCs.

OSCs	TPC	DPPH		FRAP		β-carotene bleaching assay
	mg GAE/100 g DW	%ARA/10 mg DW	mg GAE/100 g DW	%FRAP/10 mg DW	[mM] TEAC/10 mg DW	%AOA/10 mg DW
Allicin	52.84 ± 1.51^{a}	13.93 ± 0.14^{c}	40.10 ± 0.09^{c}	123.92 ± 3.95^{a}	28.86 ± 0.61^{a}	3.99 ± 0.09^{a}
Ajoene	4394.50 ± 97.0^{b}	8.18 ± 0.91^{b}	24.32 ± 2.76^{b}	4471.82 ± 137^{b}	1024.99 ± 25.1^{b}	445.10 ± 5.93^{b}
2-VD	2234.86 ± 40.3^{c}	13.50 ± 1.08 ^{c}	39.17 ± 3.15^{c}	8207.46 ± 101^{c}	1985.51 ± 183 ^{c}	1472.57 ± 67.1^{c}
DAS	7.30 ± 0.02^{a}	0.85 ± 0.02^{a}	0.13 ± 0.003^{a}	20.12 ± 4.46^{a}	4.97 ± 0.59^{a}	1.61 ± 0.00^{a}
DADS	19.46 ± 0.18^{a}	0.63 ± 0.07^{a}	0.08 ± 0.01^{a}	10.90 ± 5.43^{a}	2.49 ± 1.24^{a}	1.61 ± 0.00^{a}
DATS	13.80 ± 0.61^{a}	1.63 ± 0.02^{a}	0.22 ± 0.003^{a}	19.87 ± 1.17^{a}	4.53 ± 0.26^{a}	0.09 ± 0.01^{a}

Results expressed as mean ± SD (n = 5); 95% confidence interval; mg g^{-1} DW.
Values followed by the same letter within each column were not significantly different according to the Tukey's test ($p < 0.05$).

Table 6
Comparison between AOA and ARA measurements in SF and SS samples.

Samples	TPC	DPPH		FRAP		β-carotene bleaching assay
	mg GAE/100 g DW	%ARA/10 mg DW	mg GAE/100 g DW	%FRAP/10 mg DW	[mM] TEAC/10 mg DW	%AOA/10 mg DW
SS	9.37 ± 0.30[a]	0.60 ± 0.04[a]	0.14 ± 0.01[a]	12.86 ± 1.80[a]	29.50 ± 4.50[a]	8.85 ± 1.30[a]
SF	2.43 ± 0.50[b]	0.49 ± 0.04[b]	0.05 ± 0.01[b]	10.76 ± 0.50[a]	24.50 ± 1.20[a]	12.32 ± 2.30[a]

Results expressed as mean ± SD (n = 5); 95% confidence interval; mg g^{-1} DW.
Values followed by the same letter within each column were not significantly different according to the Tukey's test ($p < 0.05$).
SF: stir-fried chopped garlic.
SS: synthetic solution of stir-fried chopped garlic.

presented a strong but inverse correlation with AOA. This inverse relationship would indicate that the higher content of allicin did not exert protection, but actually promoted oxidation. This finding agrees with Block (2010), who found that sulfur compounds not only behave as antioxidants, but they can also act as pro-oxidants, depending on specific conditions. The pro-oxidant compounds are chemicals that induce oxidative stress, either by creating ROS or inhibiting the antioxidant system. Also, they can have redox potentials: they are oxidized by ROS and, to regenerate them, the phenolic compounds (which are more potent antioxidants) are consumed; thus, antioxidants reduce the antioxidant share of the total extract (antagonistic effect in a mixture) (González & Nazareno, 2011).

Furthermore, the allicin levels present in raw garlic samples showed strong relationships with ARA, iron reducing ability, and TPC; this may occur because allicin also has the ability to reduce the Folin-Ciocalteu reagent, which constitutes an interference of this analytical method. This reaction was tested for the pure compound and showed positive results (which are detailed in Section 3.2 of this paper). This leads to the conclusion that, when the TPC has high values in raw garlic, it can be attributed not only to the presence of phenolic compounds, but also to the capacity of allicin to react with the Folin-Ciocalteu reagent. This fact is consistent with results obtained in Section 3.2, which includes the tests of pure compounds (Table 4), and where the TPC yielded an overestimated value in raw samples.

In addition, the levels of ajoene, 2-VD, DAS, DADS and DATS of the different garlic extracts used in this study showed that the OSCs present in this vegetable have a moderate positive relationship with the AOA as measured by the protection against substrate oxidation.

3.2. Determination of antiradical (ARA) and antioxidant (AOA) activities of the main OSCs present in garlic

The TPC, ARA, AOA and iron reducing ability for different pure OSCs are shown in Table 4. In general, the polysulfides DAS, DADS and DATS were the OSCs with the lowest levels of AOA in all trials. This can be explained because these polysulfides are unable to form sulfenic acid (Block, 2010). Besides, these results contradict those expressed by Higuchi, Tateshita, and Nishimura (2003), who show that DATS has AOA inhibiting the formation of lipid hydroperoxide (LOOH) in low density lipoproteins in humans. This activity may be due to the non-bonding electrons being associated with the double bonds in sulfur, which can improve the antioxidant activity. Furthermore, the antioxidant activity depends on the number of sulfur atoms in the molecules because trisulfides generally exhibit a higher activity than disulfides (Higuchi et al., 2003).

This finding was unexpected and indicated that all pure standards were able to reduce the Folin-Ciocalteu reagent, giving positive values for TPC. From this, it is possible to state that the sulfur compounds present in garlic can reduce this reagent and, therefore, generate a response that overestimates the total content of phenolic compounds in garlic samples.

On the other hand, it can be observed that ajoenes and vinyldithiins have the highest values in most of the determinations. This result may be explained by the fact that the double bonds associated with the non-bonding electrons in sulfur may enhance AOA. Besides, 2-VD has a conjugate pair of non-bonding electrons in sulfur in a ring double bond system, which appears to play an important role in improving AOA (Higuchi et al., 2003).

Finally, to find out if OSCs are the main bioactive compounds responsible for the AOA evidenced in garlic samples, a synthetic solution of stir-fried garlic (SS) was prepared and assayed. Table 5 shows the percentage composition of such SS. Table 6 includes the results of the Student's *t*-test ($p < 0.05$) performed for mean differences, in order to compare the values of the activities obtained for each sample. The analysis of results shows that the activities measured by the ferric reducing ability and the β-carotene bleaching test revealed no significant differences between the sample and the synthetic solution (SS). Moreover, the TPC and the activity values measured by DPPH$^{\cdot}$ scavenging assay were significantly different from $p < 0.05$. In summary, considering that no significant differences were found by FRAP and *β*-carotene bleaching assay, and although fried garlic samples have in their composition other bioactive compounds (for instance, phenolic compounds), their contribution is apparently not significant for AOA. Lastly, in fried garlic samples, antioxidant activities are related to the content of sulfur compounds.

4. Conclusions

Cooked garlic samples proved to have certain antioxidant capacity against all methods tested. The mechanism of action which characterizes the antioxidant activity of raw garlic samples was scavenging of free radicals (DPPH$^{\cdot}$ and ABTS$^{+\cdot}$) in addition to iron ion reduction by hydrogen atom or electron transfer. For stir-fried samples, the mechanism of action was found to be a mainly inhibitory activity of the pro-oxidant enzyme, as well as the ability to break radical chain propagation reactions.

In the case of individual OSCs, it can be concluded that DAS, DADS and DATS showed a lower antioxidant activity, while allicin, ajoenes and 2-VD showed antioxidant capacity for all protocols tested. Allicin presented a good antiradical action mechanism, which was demonstrated by its ability to scavenge DPPH$^{\cdot}$ and ABTS^{+} free radicals, as well as an iron reducing capacity. In the case of ajoene and 2-VD, the main mechanism present was antioxidant activity, evidenced by the *β*-carotene protection against bleaching as well as a good ability to reduce iron.

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