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Analytical Methods

Garlic (*Allium sativum* L.) and ready-to-eat garlic products: *In vitro* antioxidant activity

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ABSTRACT

Garlic contains polyphenol and sulphur compounds, which are responsible for its antioxidant activity (AA). This study aimed at evaluating the AA of fresh garlic and its commercialised products and their shelf life. Fresh garlic (FG) and its products, i.e. chopped with salt (CGS), chopped without salt (CG), fried (FRG) and mixed garlic (FG with dehydrated garlic; MG) antioxidant activity was evaluated by three different methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, β-carotene/linoleic acid assay and Rancimat® method. Amongst all the analysed products, fried garlic presented the highest antioxidant activity. The free radical-scavenging activity decreased during the shelf life of all analysed products that correlated with the decrease in the total polyphenol content. Our findings suggest that some compounds other than phenol may have contributed towards this outcome.

1. Introduction

Habitual consumption of fruits and vegetables provides significant benefits for human health (Lampe, 1999). Amongst them, garlic (*Allium sativum* L.) is of particular interest due to its prophylactic and therapeutic actions (Bhagyalakshmi, Thimmaraju, Venkatachalam, Murthy, & Sreedhar, 2005; Kabasakal et al., 2005; Lawson & Gardner, 2005).

In Brazil, the consumption of processed garlic products (e.g. chopped and fried) has considerably increased over the last few years, probably due to its easy of use, when comparing to fresh garlic (Oliveira, Souza, & Yuri, 2004).

Sulphur and polyphenols present in garlic respond to the antibacterial, antifungal and antioxidant activity was carefully studied in a previous reports (Benkeblia, 2004, 2005; Bozin, Mimica-Dukic, Samojlik, Goran, & Igic, 2008; Chung, 2006; Gorinstein, Leontowicz, Drzewiecki, et al., 2006a; Gorinstein, Leontowicz, Leontowicz, et al., 2006b; Nuutila, Puupponen-Pimia, Aarni, & Oksman-Caldentey, 2003; Pedraza-Chaverrí, Medina-Campos, & Segoviano-Murillo, 2007; Sener et al., 2005; Tsai, Tsai, & Ho, 2005). However, antioxidant activity of fruits and vegetables is affected by several factors as processing, the presence of additives, nutrients interactions, amongst others (Cardelle-Cobas, Moreno, Corzo, Olano, & Villamiel, 2005; Nicoli, Anese, & Parpinel, 1999; Pedraza-Chaverrí et al., 2007). Heat process, for example, may increase or decrease garlic antioxidant activity, depending if polyphenolic antioxidant compounds are degraded or if Maillard reaction antioxidant products are generated during the thermal processing (Yilmaz & Toledo, 2005).

The antioxidant activity of fresh garlic was already documented, but there is no data concerning the antioxidant activity of different commercialised garlic products and the possible changes in antioxidant activity during the shelf life. This study reports the polyphenol contents and antioxidant activity of garlic and its read-to-eat products and the possible changes due to aim of this work was to evaluate the antioxidant activity of fresh garlic and ready-to-eat garlic products during the shelf life.

2. Materials and methods

2.1. Reagents and solvents

Methanol and sodium carbonate (Na_2CO_3) were purchased from CAAL (São Paulo, Brazil). Folin–Ciocalteu and Tween 40 from Merck (Darmstadt, Germany), Gallic acid, β-carotene, Linoleic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma–Aldrich (Steinheim, Germany). Chloroform from Synth (Diadema, Brazil).

2.2. Plant material

Fresh garlic (*A. sativum* L.) was purchased in a City-hall food market of São Paulo, January 2005 and processed at Fresh Garlic Co., (São Paulo, Brazil) in order to obtain five garlic products: (1) fresh (raw) garlic (FG): bulbs were manually peeled and chopped

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in a processor (Waring Inc., United States); (2) fried garlic (FRG): fresh garlic was fried at 180 °C for 2 min, using a 1:1 mixture of soybean oil and hydrogenated vegetable fat; (3) mixed garlic (MG): a mixture of fresh garlic (20%) with dehydrated garlic (80%); (4) chopped garlic with salt (CGS): fresh chopped garlic with 2% (w/w) salt; and (5) fresh chopped garlic with no salt (CG). MG, CGS and CG were supplemented with food additives (citric acid 8.0%; sodium metabisulfite 0.15%; and sodium benzoate 0.06%) and the others products were stored without additives.

Garlic products were stored in plastic packages (200 g) at room temperature.

2.3. Experiment design

The products were processed and stored at room temperature for 60, 90 or 180 days, due to the differences in the shelf life of each product type. Analysis were performed at the first day of storage (T1), at the halfway from the expiration date (T2) to a day before the deadline (T3), as depicted in Table 1.

2.4. Extraction of the polyphenol fraction

About 1.5 g of each garlic product was extracted with methanol (10 mL) under magnetic stirring for 1 h. After 20 min under ultrasonic vibration. The extract was centrifuged at 3000 rpm for 20 min, and the supernatant was filtered. The extraction procedure was repeated once and supernatants were pooled together. In order to standardise the concentrations at 1 mg/mL, the weight of the dry matter of the extracts was determined gravimetrically.

2.5. Determination of total phenol content

The amount of total phenolics was determined using Folin–Ciocalteu reagent (Singleton & Rossi, 1965). Four hundred microlitres of extract was diluted with the same amount of methanol. Four hundred microlitres Folin–Ciocalteu reagent and 2 mL 20% Na_2CO_3 were added. The tubes were mixed and 800 μL 20% Na_2CO_3 were added. After they were centrifuged for 3 min at 14,000 rpm and allowed to stand for 20 min in the dark at room temperature. The absorbance was measured at 735 nm. The standard curve was plotted using gallic acid. The amount of total phenolics was calculated as gallic acid equivalents (GAE) in micrograms per milligram of extract (dry matter).

2.6. Determination of the antioxidant activity

2.6.1. DPPH assay

The free radical-scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) according to Yamaguchi, Takamura, Matoba, & Terao, 1998. In brief, 1 mL of each extract containing 1.0 mg of soluble solids/mL was added to 1.5 mL of 20 mg/mL methanol solution of DPPH. After 20 min incubation period at room temperature, the absorbance was read at 517 nm. The DPPH scavenging activity was expressed as the inhibition of free radical DPPH in percent (*I*%).

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. Nine replicates tests were carried out for each extract.

2.6.2. β-Carotene/linoleic acid assay

The inhibition of linoleic acid peroxidation (volatile organic compounds and conjugated diene hydroperoxides) was determined according to Miller (1971).

A stock solution of β-carotene/linoleic acid mixture was prepared as follows: 20 mg of β-carotene was dissolved in 1 mL of chloroform. About 28 μL of 20 mg/mL β-carotene methanolic solution was mixed with 28 μL of linoleic acid and 200 mg of Tween40. The chloroform present was completely evaporated and 140 mL of distilled water saturated with oxygen (30 min, 100 mL/min) was added. After vigorous shaking, 5 mL of the reaction mixture were dispensed to test tubes and 1 mL portions of the diluted extracts (1 mg/mL) were added. After shaking the mixtures, the absorbance was measured at 470 nm. The tubes were placed in a water bath at 50 °C and next measurements were made every 15 min for 2 h.

2.6.3. Preservation against induced oxidative rancidity (Rancimat® method)

The test was carried out using partially hydrogenated vegetable fat as the matrix supplemented with the extracts as described by Antolovich, Prenzler, Patsalides, McDonald, and Robards (2002). The exact volume of each extract necessary to achieve a final concentration of 0.03% (w/w) of soluble solids: partially hydrogenated vegetable fat oil were dispensed into the Rancimat® reaction vessels and dried under nitrogen. Then, 3.0 g of vegetable fat oil were introduced into the vessels. Distilled water was added and the measuring cells were connected in the equipment ensuring freedom of air bubbles. Vegetable fat oil alone was added to a reaction vessel as a control. The apparatus used was a Rancimat Metrohm® (Herisau, Switzerland) operating at 110 °C and airflow rate of 20 L/h. Six replicate tests were carried out for each extract. The induction period (IP) (h) was recorded automatically. The protection factor (PF) was calculated according to the following formula: ($PF = IP_{extract}/IP_{control}$).

The protection factor may be used as a criterion for the effectiveness of antioxidants.

2.7. Data analysis

Data were expressed as mean ± standard deviation. ANOVA ($P < 0.05$) and Tukey's posteriori test were employed to verify if

Table 1
Experimental design.

Garlic products	Shelf life (months)	Time of assays (days)[a]		
		T1	T2	T3
Fresh garlic (FG)	2	1	30	60
Chopped garlic without salt (CG)	3	1	45	90
Chopped garlic with salt (CGS)	3	1	45	90
Mixed garlic (MG)	3	1	45	90
Fried garlic (FRG)	6	1	90	180

[a] T1, 1st day of storage; T2, halfway from the expiration date; T3, on a day before the deadline date.

Table 2
Total polyphenol content in fresh garlic and its processed products[A,B].

Garlic products[C]	Polyphenol content (μg/mg)		
	Time 1	Time 2	Time 3
FG	$6.99^{b,x} \pm 0.39$	$7.23^{c,x} \pm 0.33$	$8.70^{c,y} \pm 1.83$
FRG	$8.32^{c,y} \pm 1.32$	$7.07^{c,x,y} \pm 0.64$	$6.45^{b,x} \pm 2.15$
CG	$6.36^{b,z} \pm 0.54$	$2.95^{b,y} \pm 0.38$	$2.55^{a,x} \pm 0.35$
CGS	$4.78^{a,z} \pm 0.37$	$2.44^{a,y} \pm 0.17$	$2.10^{a,x} \pm 0.19$
MG	$6.21^{b,z} \pm 1.10$	$3.15^{b,y} \pm 0.65$	$2.74^{a,x} \pm 0.47$

[A] Values are means ± standard deviation ($n = 9$).
[B] Different letters in the same column (a–d) are significantly different ($P < 0.05$). Different letters in the same row (x–z) are significantly different ($P < 0.05$).
[C] FG, fresh garlic; FRG, fried garlic; CG, chopped garlic without salt; CGS, chopped garlic with salt; MG, mixed garlic.

there were significant differences ($P < 0.05$) amongst the products. SPSS software version 10.0 was used.

3. Results and discussion

The total polyphenol content at T1 varied from 4.78 (chopped garlic with salt) to 8.32 μg/mg (fried garlic) in the analysed products (Table 2). Interestingly, fried garlic presented higher content of polyphenols than fresh garlic (from 6.99 to 8.32 μg/mg), unlike the behaviour observed by Lanzotti (2006) for quercetin.

There was a significant decrease in the total phenolic content due to the storage at the halfway to the expiration date (T2) for the chopped garlic (with and without salt) and the mixed garlic (49%, 54% and 49%, respectively), as already described (Gorinstein, Leontowicz, Drzewiecki, et al., 2006). Polyphenol content decreased from T2 to the expiration date for all the garlic products extracts. The decreasing of polyphenol during the storage can be explained by the possible oxidation of polyphenols to quinones, considering their action as antioxidants (Gorinstein, Leontowicz, Drzewiecki, et al., 2006; Jastrzebski et al., 2007).

The free radical-scavenging capacity was significantly different between the fresh garlic and the ready-to-eat garlic products extracts (Table 3). Frying increased the free radical-scavenging activity, what might be due to: (1) the highest total phenol content amongst all the analysed samples, indicating a positive correlation between the total phenol content and the DPPH assay, as already observed by other authors Shahidi and Wanasundara (1992) and Schlesier, Harwat, Böhm, and Bitsch (2002). (2) Garlic was fried in soybean oil, which contains natural and added antioxidants, some of which could have been incorporated to garlic during the frying process. (3) The exposure to high temperature might have contributed to the generation of Maillard reaction products that possess antioxidant activity (Nicoli et al., 1999; Peralta et al., 2008).

Fried garlic extract also protected the linoleic acid from peroxidation (%LAP) more efficient than the other garlic ready-to-eat products (Table 3). However, fresh garlic (FG) extract was the least efficient in inhibiting linoleic acid peroxidation (39.84% for T2 and 44.97% for T3). In the study of Kaur and Kapoor (2002) the fresh garlic inhibited lipid oxidation by 62.1% (ethanol extract) and 61.8% (aqueous extract). The differences between our data and the findings of Kaur and Kapoor (2002) can be explained by factors such as differences in experimental parameters and the natural qualitative and quantitative variability in the raw material.

The capacity of inhibiting linoleic acid peroxidation increased during the course of storage for all the garlic products, in opposition to the behaviour observed for the free radical-scavenging activity DPPH assay at Table 3, suggesting that some compounds other than phenols, such as sulphur compounds or melanoidins might had been generated produced during the storage period.

The significant increases in the levels of β-carotene/linoleic acid in chopped garlic (CG and CGS) and mixed garlic (MG) extracts could be explained by additions of sodium metabisulfite and citric acid to garlic ready-to-eat products might contribute to the antioxidant activity since these substances can quelate metal ions, acting synergistically with other antioxidant compounds in foods. Similar finds are reported by Fagundes and Ayub (2005) studying the storage of kaki (*Diospyros kaki*, L.).

In the Rancimat® method, garlic and ready-to-eat garlic products presented antioxidant activity, expressed as protection factors (PF) as shown in Fig. 1.

Amongst the products studied, the fried garlic (FRG) presented a better performance in protecting the vegetable fat against oxidation, what might be related to its total polyphenol content.

During shelf life, there was a slight increase in the protection factor (Fig. 2) for all garlic products, as observed in the β-carotene/linoleic acid assay.

In summary, our results show that fresh garlic and ready-to-eat garlic products present antioxidant properties that are affected by

Table 3
Antioxidant activity of garlic products[A], determined by the DPPH assay[B] and by the inhibition of linoleic acid peroxidation (% LAP)[C].

Garlic products	DPPH scavenging activity (I%)[D]			(%) LAP		
	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
FG	$21.69^{a,u} \pm 3.22$	$19.78^{b,u} \pm 6.95$	$21.68^{b,u} \pm 1.76$	$35.74^{d,e,x} \pm 3.65$	$39.84^{d,y} \pm 2.12$	$44.97^{d,z} \pm 2.06$
FRG	$60.85^{c,u} \pm 6.03$	$46.04^{c,v} \pm 3.60$	$49.83^{c,v} \pm 1.23$	$79.09^{f,x} \pm 2.97$	$85.49^{g,y} \pm 0.87$	$78.03^{f,x} \pm 3.20$
CG	$28.80^{b,a,u} \pm 4.11$	$5.63^{a,v} \pm 0.45$	$7.30^{a,v} \pm 0.98$	$34.42^{d,x} \pm 4.47$	$65.14^{e,y} \pm 3.13$	$68.16^{e,y} \pm 3.62$
CGS	$23.11^{a,u} \pm 1.78$	$6.87^{a,v} \pm 0.54$	$7.76^{a,v} \pm 0.23$	$38.11^{d,e,x} \pm 2.56$	$69.85^{f,y} \pm 3.08$	$78.82^{f,z} \pm 7.07$
MG	$24.92^{a,b,u} \pm 4.76$	$9.04^{a,v} \pm 3.42$	$9.57^{a,v} \pm 0.86$	$40.94^{e,x} \pm 4.94$	$72.03^{f,y} \pm 4.26$	$82.25^{f,z} \pm 5.18$

[A] Values are means ± standard deviation ($n = 9$). FG, fresh garlic; FRG, fried garlic; CG, chopped garlic without salt; CGS, chopped garlic with salt; MG, mixed garlic.
[B] Different letters within a column (a–c) are significantly different ($P < 0.05$). Different letters within a row (u–v) are significantly different ($P < 0.05$).
[C] Different letters within a column (d–g) are significantly different ($P < 0.05$). Different letters within a row (x–z) are significantly different ($P < 0.05$).
[D] DPPH scavenging activity of a 1 mg/mL methanolic extract.

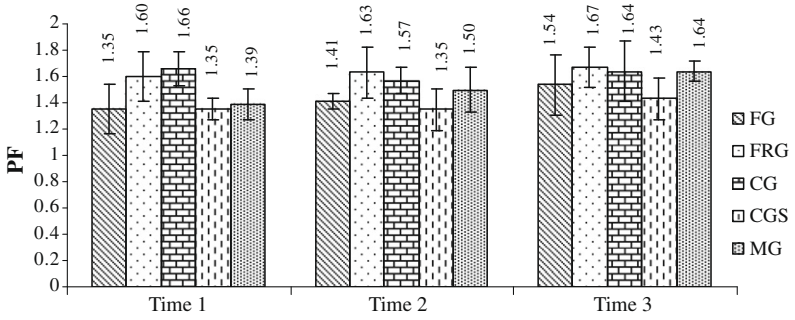

Fig. 1. Protection factor (PF) in fresh garlic and ready-to-eat garlic products, as measured by the Rancimat® method. FG, fresh garlic; FRG, fried garlic; CG, chopped garlic without salt; CGS, chopped garlic with salt; MG, garlic mixed. Values are means ± standard deviation ($n = 6$).

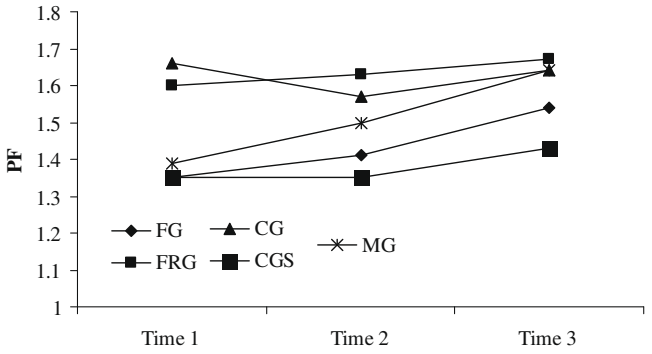

Fig. 2. Protection factor (PF) in fresh garlic and ready-to-eat garlic products during shelf life, as measured by the Rancimat® method. FG, fresh garlic; FRG, fried garlic; CG, chopped garlic without salt; CGS, chopped garlic with salt; MG, mixed garlic. Values are means ± standard deviation (n = 6).

the process and to the storage time. Fried garlic extract exhibited the best performance in all tests. Nevertheless, the results presented here should not be automatically applied in human. *In vivo* studies are needed for better comprehension of the potential benefits and risks of the bioactive compounds in garlic, in relation to human health.

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