Extracts of red peppers: antioxidant activity and sensory evaluation

Neuza Jorge, Carolina Médici Veronezi and Danusa Cassiano Pereira
Department of Food Engineering and Technology, São Paulo State University – UNESP, São José do Rio Preto, Brazil

Received 19 August 2015
Revised 13 October 2015
7 November 2015
30 November 2015
Accepted 3 December 2015

Abstract

Purpose – This study aims to deal with the evaluation of the antioxidant capacity of lyophilized hydroalcoholic extracts of red peppers in *natura*. Furthermore, preference was evaluated for the taste and color of soybean oil added red pepper extracts.

Design/methodology/approach – The antioxidant capacity was determined by four methods. The content of phenolic compounds, carotenoids and ascorbic acid in the extracts was determined by chromatographic, spectrophotometric and titration methods, respectively.

Findings – The results showed that the highest antioxidant capacity was found in Malagueta pepper extract through reducing power (FRAP) method. In this same extract, high amount of phenolic compounds was found. However, the extracts of Bode and Dedo-de-moça peppers had higher amounts of carotenoids and ascorbic acid, respectively. Sensorially, the oil added extracts were preferred.

Practical implications – Red peppers are very popular and consumed worldwide, besides being constituted of important phytochemicals. Results showed high antioxidant activity in the extracts of peppers, and high content of phenolic compounds, carotenoids and ascorbic acid mainly in chili. This study highlights the importance of the extracts of red peppers, genus *Capsicum*, as a source of antioxidants, in addition to vegetable oils.

Originality/value – It is important to check the acceptance of the application of extract in vegetable oil, so it can be marketed as a natural antioxidant. This study provides valuable information about the antioxidant capacity of extracts of red peppers and its acceptance.

Keywords Antioxidant, Ascorbic acid, Phenolic compounds, Carotenoids, Sensory

Paper type Research paper

Introduction

Degenerative diseases and, consequently, the aging of living tissue are related to oxidation of compounds present in the human body. These compounds, called free radicals, are formed in excess, especially when the body is subjected to intense stress (Melo *et al.*, 2011). Thus, the use of substances with antioxidant capacity has been increasing, as they are of great importance in the prevention of diseases associated with increased oxidative stress (Shi and Niki, 2001).

Antioxidants can be synthetic or naturally present in food. Synthetic antioxidants are relatively inexpensive, colorless, tasteless and odorless, and mostly used by

Nutrition & Food Science
Vol. 46 No. 2, 2016
pp. 228-236

0034-6659
DOI 10.1108/NFS-08-2015-0094

The National Council of Scientific and Technological Development – CNPq for sponsoring the research and its productivity.

industries (Castelo-Branco and Torres, 2011). However, they have problems of solubility and some of them contribute to off-flavor development and are highly toxic (Pokorný, 2007). Therefore, their use is restricted in many countries. Among the natural antioxidants, the carotenoids, ascorbic acid and phenolic compounds stand out (Pokorný, 2007).

Antioxidants may have synergistic effect and act by transferring a hydrogen atom and/or an electron. Thus, there are several different methods for the evaluation of the effectiveness of antioxidants to protect the food from oxidation (Tsao and Deng, 2004).

There is evidence that vegetable consumption is associated with the decreased risk of cancer, heart disease and degenerative diseases associated with aging because of the presence of phytochemicals with antioxidant properties (Charles, 2013). Among the vegetables, there are the red peppers which originate in plants of the genus *Capsicum* and are quite popular in the world. There are 31 species, 4 of which are classified as domesticated: *C. annuum* L. (pepper, sweet pepper), *C. chinense* Jaqc., *C. frutescens* L. (chili) and *C. baccatum* L. are largely produced and consumed in Brazil (Lannes *et al.*, 2007).

These peppers are often consumed *in natura*, although they are also sold in the form of paprika paste, dehydrated and in ornamental cans. They are rich sources of phenolic compounds, carotenoids, ascorbic acid and Vitamin A, although the levels of these compounds vary according to the genotype and degree of ripeness of the peppers (Davis *et al.*, 2007).

It is necessary to conduct studies to assess the composition of pepper extracts and their acceptance when used in vegetable oils, as it is an alternative to reduce the use of synthetic antioxidants. The objective of this study was to analyze lyophilized hydroalcoholic extracts of ripe fruits of red peppers of the genus *Capsicum* and evaluate the preference for flavor and color of soybean oil added Malagueta pepper extract in different concentrations because of the fact that this pepper presents high content of phenolic compounds and antioxidant activity.

Materials and methods

Extract of peppers

Ripe fruits of Malagueta (*C. frutescens*), Cumari (*C. baccatum* var. *praetermissum*), Bode (*C. chinense*) and Dedo-de-moça (*C. baccatum* var. *pendulum*) peppers were bought locally in the city of São José do Rio Preto-SP, while Cumari was provided by the company Fogo Mineiro (Carmo do Rio Claro-MG). Each fruit (5 kg) was bought in March 2012. The fruits that were whole and fully ripened were selected, washed in running water and dried at room temperature.

The hydroalcoholic extracts were prepared according to the methodology of Costa *et al.* (2010). The fruits (20 g) were maintained under vigorous agitation with a hydroalcoholic solution (200 ml) at room temperature, for 30 min; they were centrifuged at 3,000 rpm for 10 min. The supernatant was filtered and the solvent used was removed under reduced pressure at 45°C. The extracts obtained were stored at −32°C and inertized for 50-56 h. Then, they were lyophilized (Liotop, Model L101, Brazil) for 26 h and stored at −18°C until analysis.

Vegetable oil

Refined soybean oil was used without the addition of synthetic antioxidants (tert-butyl hydroquinone – TBHQ and citric acid), ceded by Cargill Agricola S/A, Mairinque-SP, Brazil.

Antioxidant activities

Determination by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The free radical-scavenging activity of hydroalcoholic extracts was determined according to Brand-Williams *et al.* (1995) and modified according to Mensor *et al.* (2001). Briefly, the sample stock solutions (4000 μg/mL) were diluted with ethanol to final concentrations of 50, 100, 200 and 400 μg/mL. Each of the sample solutions (1.5 mL) at different concentrations was mixed with 1.0 mL of $DPPH^{\cdot}$ solution (40 μg/mL). After 30 min, the absorbance was measured at 517 nm using a spectrophotometer (Shimadzu, model UV–VIS mini 1240, Japan). For obtaining the control value, a hydroalcoholic solution 96°Gay-Lussac was used in the place of the extract.

β-carotene/linoleic acid system. The β-carotene/linoleic acid system was determined by the method described by Marco (1968). An emulsion of β-carotene/linoleic acid (1 mL solution of β-carotene, 25 μL of linoleic acid, 200 μL of Tween 40 and 50 mL of distilled water) was prepared. The emulsion (5 mL) was added to 1 mg of lyophilized hydroalcoholic extract of four varieties of peppers. These were heated to 50°C for 15 min and cooled for 30 min. After this, the absorbance was measured using a spectrophotometer (Shimadzu model UV–VIS mini 1240, Japan) at a wavelength of 470 nm in 15-min intervals to complete two h. A blank was made with only 5 mL of the solution of β-carotene/linoleic acid emulsion. The activity was determined as a percentage of antioxidant activity.

Determination of reducing power (FRAP). The reducing power of the extracts was determined according to the method by Szydłowska-Czerniak *et al.* (2008). First, 90 μL of the sample was transferred into test tubes, and to this was added 270 μL of distilled water and 2.7 mL of FRAP reagent (25 mL of 0.3 M acetate buffer, pH 3.6; 2.5 mL of a 10 mM ferric-tripyridyl triazine solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3.H_2O$). This mixture was kept in bain-marie for 30 min at 37°C and the absorbance was measured at $\lambda = 595$ nm using a spectrophotometer (Shimadzu, model UV–VIS mini 1240, Japan). The results were expressed as μM Trolox/mg.

2,2-azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) assay. The $ABTS^{\bullet+}$ radical-scavenging activity was determined according to Re *et al.* (1999). The $ABTS^{\bullet+}$ radical (10 mL solution of 7 mM $ABTS^{\bullet+}$ and 176 μL 140 mM solution of potassium persulfate) was kept in the dark for 16 h. This mixture was diluted with ethyl alcohol to obtain absorbance from 0.70 ± 0.05 nm to 734 nm. A 30 μL aliquot of each sample was mixed with 3 mL of $ABTS^{\bullet+}$ and maintained in the dark for 6 min. The absorbance was measured using a spectrophotometer (Shimadzu, model UV–VIS mini 1240, Japan) at 734 nm. A standard Trolox solution (0.025 g of Trolox in ethyl alcohol) was used. The antioxidant activity was expressed in μM Trolox/100 g.

Determination of total phenolic compounds

The identification of phenolic compounds was performed using high-performance liquid chromatography, according to the method described by Kim *et al.* (2006). A liquid chromatograph (Shimadzu Prominence model, Japan) and 5C18 (250 × 4.6 mm, 5 mM

particles) column was used. The mobile phase was composed of 2 per cent acetic acid in water (v/v) and methanol. The flow was 1.2 mL/min and the injection volume was 20 μL. The quantification of each isomer was performed by external standardization based on the peak areas, using standards salicylic acids, epicatechin and quercetin, detected at 280 nm, and of p-coumaric acids at 320 nm, all with purity above 99 per cent. The phenolic compounds were expressed in mg/kg.

Determination of total carotenoids

To obtain the total carotenoid content, the method of Rodriguez-Amaya (1999) was used. An ethanolic solution with the concentration of 1,000 μg/mL of pepper extract was prepared and the absorbance was read using a spectrophotometer (Shimadzu, model UV–VIS mini 1240, Japan) at 450 nm. Quantification of carotenoids was calculated with absorptivity value of 2,620 in ethanol, expressed in micrograms of β-carotene per gram of extract (μg of β-carotene/g).

Determination of ascorbic acid

Ascorbic acid was measured by titration according to standard AOAC (2005) and modified by Benassi and Antunes (1988). A solution with the concentration of 2000 μg/mL of pepper extract was prepared with 2 per cent of oxalic acid and transferred into 10 mL Erlenmeyer flask for titration with 0.01 per cent solution of 2,6-dichlorophenolindophenol sodium (DCFI). The content of this compound was calculated as milligrams of ascorbic acid per 100 g of extract (mg/100 g). The volume of DCFI pattern was obtained by titrating 10 mL of ascorbic acid standard solution.

Sensory analysis

For sensory analysis, soybean oil with and without extracts of Malagueta pepper (*Capsicum*) was used, according to formulation of oils: control (soybean oil); $extract_{100}$ (soybean oil + 100 mg/kg pepper extract) and $extract_{200}$ (soybean oil + 200 mg/kg pepper extract).

Malagueta pepper extract was chosen as a natural antioxidant to be added in soybean oil submitted for sensory analysis because of the fact that this pepper presents a high content of phenolic compounds and antioxidant activity, besides being well appreciated in Brazil. Concentrations of 100 and 200 mg/kg were used because these are the standard quantities of synthetic antioxidants permitted in Brazil (Agência Nacional de Vigilância Sanitária, 2005).In the preference test for flavor, three samples oils were offered simultaneously in orange plastic cups, two of which were added pepper extracts. Tasters were offered toast along with the samples, which were placed beside the toast. Tasters should try them and rank them in the order of preference.

For the preference test for color, the three samples (two with pepper extract) were examined simultaneously in Petri dishes, and volunteers were asked to rate them according to their preference.

In the preference tests for color and flavor, 110 volunteer tasters who did not have any food restrictions regarding the foods offered were employed. Among the tasters, 47 (42.7 per cent) were female and 63 (57.3 per cent) were male, aged between 14 and 56. The project was approved by the Research Ethics Committee of the Universidade Estadual Paulista (opinion 187,476). For data analysis, the values 1, 2 and 3 were assigned, and the sum of these values was calculated for each sample of all tasters. The Friedman test (ASTM, 1968) and table of

sorting test by Newell and Mac Farlane (1987), which presents the critical differences between the total of the sums of ordination, were applied.

Statistical analysis. The experiment was conducted in a completely randomized design. The results of analytical determinations in triplicate were subjected to analysis of variance and differences between means were tested at 5 per cent probability by Tukey test via STAT program, version 2.0. In the sensory analysis tests, delineation in complete and balanced blocks was used.

Results and discussions

Antioxidant activity

The results for antioxidant activities of different pepper extracts are shown in Table I. Malagueta extract showed higher antioxidant capacity in DPPH$^{\bullet}$, 59.3 per cent, and, consequently, lower in EC_{50} (313.5 μg/mL). Oliveira (2011) found higher antioxidant capacity for ethanol extract of Malagueta compared to aqueous extract of pepper. Costa *et al.* (2010) found less effective concentration for hydroalcoholic extracts of Malagueta pepper (EC_{50} = 180.0 μg/mL).

It was observed that in the β-carotene/linoleic acid system, the extract of Cumari pepper had higher percentage of antioxidant activity (Table II). The presence of antioxidants in the system protects the linoleic acid, prolonging the period of formation of free radicals (Huang and Wang, 2004).

In the FRAP method, Malagueta pepper extract (143.1 μM Trolox/mg) showed highest value, followed by Cumari, Bode and Dedo-de-moça extracts. However, in the ABTS$^{\bullet+}$ method, it was observed that the level of antioxidant activity in Dedo-de-moça

	Malagueta	Cumari	Bode	Dedo-de-moça
DPPH$^{\bullet}$ (%)	59.3 ± 0.3a	53.7 ± 0.3b	26.3 ± 0.2d	45.4 ± 0.5c
EC_{50} (μg/mL)	313.5 ± 1.5d	352.1 ± 4.9c	782.9 ± 17.9a	438.9 ± 6.6b
β-carotene/Linoleic acid (%)	23.5 ± 8.3bc	41.6 ± 7.4a	9.5 ± 4.3c	33.9 ± 4.0ab
FRAP (μM Trolox/mg)	143.1 ± 2.6a	136.2 ± 3.0b	74.5 ± 1.4c	48.9 ± 1.6d
ABTS$^{\bullet+}$ (μM Trolox/100 g)	50.0 ± 0.2d	69.9 ± 0.3c	81.3 ± 0.1b	93.4 ± 0.2a

Note: Means ± standard deviations of triplicate determinations followed by the same letters in the lines do not differ by Tukey test ($p > 0.05$).

Table I. Antioxidant activities in methods DPPH$^{\bullet}$, EC_{50}, sistem β-carotene/linoleic acid, FRAP and ABTS$^{\bullet+}$

Determinations	Malagueta	Cumari	Bode	Dedo-de-moça
Phenolic compounds (mg/kg)	32.5	23.2	27.7	5.0
Epicatechin	14.2 ± 0.7a	16.1 ± 0.7a	tr	5.0 ± 1.4b
p-coumaric acid	6.7 ± 0.2a	tr	tr	tr
Salicylic acid	6.0 ± 0.5b	7.1 ± 0.0b	27.7 ± 1.1a	tr
Quercetin	5.6 ± 0.3a	nd	tr	nd
Total carotenoids (μg/g)	1,749.4 ± 1.1c	1,975.0 ± 2.2b	2,140.3 ± 5.9a	1,632.3 ± 0.1d
Ascorbic acid (mg/100 g)	248.9 ± 1.8c	249.4 ± 2.0c	296.6 ± 2.0b	450.9 ± 2.0a

Notes: Means ± standard deviations of triplicate determinations followed by the same letters in the lines do not differ by Tukey test ($p > 0.05$); tr = less than 5 mg/kg; nd = not detected

Table II. Phenolic compounds, total carotenoids and ascorbic acid of pepper extracts

pepper extract (93.4 μM Trolox/mg) was higher than in the others. Determination of the antioxidant capacity of oils may depend on the reaction mechanisms and on the generation of oxidant free radical that were used in that measurement (Castelo-Branco and Torres, 2011). Deng *et al.* (2013) studied the antioxidant capacity of 56 plants by $ABTS^{\bullet+}$ method and found 1,030-1,130 μM Trolox/100 g for the pepper extracts of *Capsicum frutescens*.

Phenolic compounds, total carotenoids and ascorbic acid

The results of the levels of phenolics, carotenoids and ascorbic acid are shown in Table II. The presence of four different phenolic compounds was observed.

These phenolic compounds are present in most of the common oils and hence they were used as standards. The Malagueta pepper extract has higher amount (32.5 mg/kg) and variety of phenolic compounds. In extracts of Malagueta and Cumari peppers, the value of epicatechin was higher with 14.2 and 16.1 mg/kg, respectively. Epicatechin concentration of Dedo-de-moça pepper extract was low compared to others, and the extract of Bode pepper presented only salicylic acid (27.7 mg/kg). Zhuang *et al.* (2012) found content of 69.0 mg/kg of salicylic acid in *Capsicum frutescens* extract, which is higher than those found in the present study.

All extracts of peppers showed high amounts of carotenoids, Bode pepper extract showed higher value with 2,140.3 μg of β-carotene/g. The variation in the amount of carotenoids present in the extracts may be because of several factors, such as variety, degree of ripeness, climate, soil type, growing conditions and harvest, geographical area of production, processing and storage (Shils *et al.*, 2003). Other researchers, analyzing five cultivars of *Capsicum annuum* in Turkey, found high levels of carotenoids (2,310-2,390 mg/kg) in two cultivars (Topuz and Ozdemir, 2007). Blanco-Ríos *et al.* (2013) studied the red and orange peppers and concluded these peppers contain the highest levels of total carotenoids.

Regarding the content of ascorbic acid, it was observed in the present study that Dedo-de-moça pepper extract showed higher value with 450.9 mg/100 g. In the extracts of Malagueta (248.9 mg/100 g) and Cumari (249.4 mg/100 g) peppers, the lowest values were found. Topuz and Ozdemir (2007) obtained 15.2 mg of ascorbic acid/100 g for the peppers of variety Amazon F1. Nazzaro *et al.* (2009) studied two varieties of sweet pepper of *Capsicum annuum* L. species and detected 7.9 mg of ascorbic acid/100 g.

Correlation

Correlation analysis was performed between the antioxidant activities and contents of total phenolic compounds, total carotenoids and ascorbic acid, as shown in Table III.

Table III. Correlation coefficients between antioxidant activity and total phenolic, carotenoids total and ascorbic acid

Antioxidant activity	Phenolic compounds	Total carotenoids	Ascorbic acid
$DPPH^{\bullet}$	0.67	−0.60	−0.27
β-carotene/Linoleic acid	0.29	−0.61	−0.88*
FRAP	0.99*	−0.01	0.82*
$ABTS^{\bullet+}$	−0.89*	−0.47	0.13

Note: *Significance ($p < 0.05$)

It is observed that there was a significantly positive correlation between FRAP with phenolic compounds (0.99) and ascorbic acid (0.82), indicating that the contents of these compounds may contribute greatly to the antioxidant activity by FRAP mechanism. Similar results were found in the study of Deng *et al.* (2013), as FRAP presented a correlation of 0.94 with phenolic compounds. Higher values of negative correlation were found for $ABTS^{\bullet+}$ and phenolic compounds (−0.89), besides β-carotene/linoleic acid and ascorbic acid (−0.88).

Sensory analysis

In the flavor test, the sum 178 was obtained for the control, 250 for $xtract_{100}$ and 232 for $extract_{200}$. According to the table of sorting test by Newell and Mac Farlane (1987), for 110 tasters, it was observed that there was significant difference ($p < 0.05$) between the control and the both concentration of extracts. The extracts were preferred and compared to the control, although there was no significant difference between the $extract_{100}$ and $extract_{200}$.

In the color test, the sum 146 was obtained for the control, 255 for $extract_{100}$ and 259 for $extract_{200}$. According to the table of sorting test by Newell and Mac Farlane (1987), for 110 tasters, a significant difference ($p < 0.05$) was also found between the control and the extracts at concentrations of 100 and 200 mg/kg, in which the extracts were preferred over the control, but there was no significant difference between the extracts. Many tasters reported in the analysis forms that the color intensity is directly related to the concentration of pepper extract in oil and, consequently, a spicy trace.

Ravelli (2011) evaluated sensory characteristics such as color, aroma and flavor of soybean oil added TBHQ and extracts of rosemary, oregano, thyme and sage, with concentration of 100 mg/kg of phenolic compounds, and concluded that there was significant preference by consumers regarding the characteristics of color, aroma and flavor for soybean oil added hydroalcoholic rosemary extract at a concentration of 100 mg/kg. In this study, it was observed that, after the addition of TBHQ and hydroalcoholic rosemary extract, the oil remained clear and transparent. Furthermore, it was apparent that sage and thyme oil extracts interfered by increasing the green color intensity, and the oil added oregano extract became more opaque. In the present study, the orange tint of the oil added extract pleased tasters. It was observed that not only the spicy flavor is expected from oil added pepper extract but also the characteristic color of the pepper.

Regarding the consumption attitude scale of samples, it was observed that 6.2 and 5 per cent of tasters would only consume the control samples and the $extracts_{100}$ and $_{200}$, respectively, "if forced". Thus, it can be observed that there was significant preference for soybean oil added Malagueta pepper extract because of the sensory characteristics of flavor and color compared to the control. Between the extracts, there was no significant difference in preference for the concentrations of 100 and 200 mg/kg.

Conclusions

Malagueta pepper extract showed higher antioxidant activity by $DPPH^{\bullet}$ and FRAP methods and considerable content of phenolic compounds when compared to other extracts and thus was chosen as natural antioxidant to be added in soybean oil submitted to sensory analysis.

Although the soybean oil added pepper extract presented a mild spicy flavor, by means of the sensory analysis, it is possible to conclude that the samples of extracts

added to soybean oil were more pleasant for consumers, for flavor and color, when compared to control. Finally, the peppers are good sources of phenolic compounds, carotenoids and ascorbic acid. Also, they have high antioxidant activity and are acceptable when added to soybean oil.

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Corresponding author
Neuza Jorge can be contacted at: njorge@ibilce.unesp.br

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