African Journal of Plant Science Vol. 3 (11), pp. 245-253, November 2009
Available online at http://www.academicjournals.org/AJPS
ISSN 1996-0824 © 2009 Academic Journals

Full Length Research Paper

Antioxidant substances and pesticide in parts of beet organic and conventional manure

Maria Rosecler Miranda Rossetto[1], Fabio Vianello[2], Suraya Abdallah da Rocha[1]and Giuseppina Pace Pereira Lima[1]

[1]Department of Chemistry and Biochemistry, Biosciences Institute, São Paulo State University, Botucatu, São Paulo, Brazil.
[2]Department of Biological Chemistry, University of Padova, Padova, Italy.

Accepted 26 October, 2009

Organic agriculture together with sustainable management systems are growing in the world due to concerns about the environment and the health of the populace: being real and exhaustively documented the damages caused by pesticides. The aim of this work was to observe the possible differences in the concentrations of free radical scavengers and substances recently classified as functional, in different beet parts cultivated following organic and conventional procedures. Regarding the total antioxidant activity (AA%), significant differences were not observed between the two cultivation procedures, being found a high AA% in every beet part analyzed. Organic plants concentrated higher vitamin C content (6.7 - 16.6 mg/100 g) with respect to the conventional ones (4.1 - 9.1 mg/100 g); higher flavonoid content (0.5 - 5.2 mg rutin/g) and, when cooked, the pulp of organic beet maintained a higher polyamine content and higher amount of total carotenoids. No significant differences were observed for total phenolic compounds.

Key words: Vitamin C, pesticides, putrescine, spermidine, spermine, index of antioxidant activity.

INTRODUCTION

In last years, the market of organic grown products is strongly increasing, mainly in the countries presenting a higher development. The behavior of population living in such countries should be attributed to the idea that these foods can be considered free from pesticides (Roitner-Schobesberg et al., 2008). This is very close to reality and studies showed a drastic reduction of pesticides when children ingested foods of organic origin, assuring their health. According to the same authors, the presence of organochlorides, organophosphates and carbamates in foods coming from conventional manuring procedure can be the cause of a lot of diseases in the population and it was demonstrated that the chronic exposure of infants and children to pesticides resulted in serious neurological problems (Lu et al., 2006). However, organic foods are not totally free from contaminations, due to the possible microbiological contamination, in particular, if organic grown vegetables are not prepared according to the correct procedure. Therefore, good agricultural practices, as recommended by certifier organizations, can guarantee the safety of the product (Roitner-Schobesberg et al., 2008).

Some authors demonstrated that in Chinese mustard (*Brassica juncea* var. *rugosa*), lettuce (*Lactuca sativa* L.), tomato (*Lycopersicon esculentum* Mill.) and collard greens (*Brassica oleraceae* L.), the content of β-carotene and ascorbic acid was higher in comparison with that observed in conventionally grown plants (Ismail and Fun, 2003). Other works showed a higher concentration of sulphur (important for phytoalexins synthesis, as well as for glucosinolates) and a lower content of nitrates in organic foods (Siderer et al., 2005). Another general food property of great interest is the antioxidant capacity, which is peculiar of the specific vegetable, and could be used to classify foods in agreement with their functional properties. These foods are denominated as “functional”, because they act on diversified systems, such as: inhibition/activation of enzymes of antioxidant system

*Corresponding author Email: gpplima@ibb.unesp.br

(Navrot et al., 2006), eliminate reactive oxygen (ROS) and nitrogen species (RNS) (Halliwell and Gutteridge, 1999), demonstrate an anti-inflammatory action (Pietta, 2000) and act in different ways preventing the proliferation of cancerous cells (Hu et al., 2006).

Other substances, such as polyamines (PAs), especially putrescine (Put), spermidine (Spd) and spermine (Spm), which are normally found in millimolar concentrations in the nucleus, can function directly as a free radical scavenger (Ha et al., 1998), and these amines universally occurring in plant organs are involved in a wide array of processes, ranging from triggering organogenesis to protecting against stress. Comprehensive information on different aspects of polyamine roles in plant physiology is available in recent reviews (Kalac and Krausová, 2005). PAs molecules are polycationic, at physiologic pH, showing several effects on cells: they participate in the division process and cellular differentiation and they present high affinity for anionic molecules (DNA, RNA and membrane phospholipids). Furthermore, they promote the synthesis of nucleic acids and proteins; they are related to the stability of the plasmatic membrane and to different kind of cell stress, as pH, heat and osmotic shocks (Bouchereau et al., 2000). Conversely, although they do not show direct poisonous effects in foods, determinate amount of Put, Spd and Spm can potentiate the effect of tyramine, histamine and other biogenic amines. However, it seems to exist a mechanism of regulation of PAs content in healthy human cells, meanwhile public health organizations (FDA) fixed the maximum amount of these amines in the diet, mainly when they are proposed to patients suffering from neoplastic diseases (Kalac and Krausová, 2005).

The aim of the present work was to evaluate the differences in the antioxidant activity, total phenolic, flavonoid and polyamine content in peel, pulp, leaves and stalk of common beet (*Beta vulgaris esculenta* L.) cultivated using organic or conventional procedures. Furthermore, during the experimental work the effect of home-made cooking procedure and the presence of pesticides in these materials were verified.

MATERIALS AND METHODS

Materials

Beet plants (*Beta vulgaris esculenta* L.) were harvested after 70 days starting from the sowing. The vegetables constituting conventional and organic samples were obtained from the same region (São Paulo state, Brazil) (latitude 22° 53' 09'' sought, longitude 48° 26' 42'' west and altitude 804 m), however in different places. The analysis were carried out in triplicates for repetition (three for cultive). The organic ones were acquired from certified producers.

Sample preparation

Pulp, peel, leaves and stalks of organically and conventionally grown common beet were cleaned with distilled water, surface-sterilized with 1% sodium hypochlorite solution during 10 min, rinsed with water, immediately frozen in liquid nitrogen and stored at -80°C. A part of these samples was dried in a greenhouse with forced air circulation at 60°C until a constant weight was reached, according to the method suggested by the AOAC (2000) for humidity. Soon afterwards, samples were fine ground in mill type Willey (S/A-3-001-MG-Brazil) for accomplishments of dry matter analyses.

Thermal processing

About 550 g of pulp and peel from conventional and organic beet samples were subjected to cooking process in boiling water for 45 min in different containers. These samples were stored and processed in the same way as the raw ones.

Assay of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The extraction of the antioxidants was carried out in absolute ethanol, according to Brand-Williams et al. (1995). Samples were diluted to a final concentration of about 1 mg/ml. Then they were desiccated by drying in a water bath at 100°C. The dry extract was reconstituted with the same volume of absolute ethanol. Antioxidant activity was determined according to the DPPH assay in a wide range of sample concentrations (10, 50, 100, 125 and 250 µg/ml), on at least 5 repetitions. The reaction started with the addition of 1 ml of 0.3 mM DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) (Sigma-Aldrich-Brazil) in ethanol. The incubation process was carried out at room temperature for 60 min, in the dark. The negative control was prepared by substituting samples with absolute ethanol. The absorbance readings at 518 nm were used to calculate the antioxidant activity (AA), according to the following Equation:

$$AA\ (\%) = 100 - [(Abs_{sample} - Abs_{blank}) / Abs_{control}] \times 100$$

The values of substrate concentration (sample) responsible of 50% inhibition (IC_{50}: expressed in µg/ml) were calculated, according to Brand-Williams et al. (1995), by linear regression and the index of antioxidant activity (AAI were calculated according to Scherer and Godoy (2009), using DPPH at a final concentration of 118.3 µg/ml (0.3 mM).

Determination of ascorbic acid

The determination vitamin C was carried out according to the method of Terada et al. (1978), with minor modifications. Pulp, peel, leaves and stalks of conventional and organic beet (300 mg), previously triturated under liquid N_2, were homogenized in a solution of oxalic acid (0.5%, w/v) using a miniturrax (Marconi, Brazil) for 20 s. Activated charcoal (50 mg) (Sigma-Aldrish-Brazil) was added to each sample and the mixture was incubated for 150 min at 4°C. Then, the mixture was centrifuged at 12000 × g for 10 min. An aliquot of the supernatant (0.5 ml) was added to 3.5 ml of 0.5 % (w/v) oxalic acid. Ascorbic and dehydroascorbic acids were determined by addition of 150 µl of an aqueous solution containing 0.25% (w/v) 2,6-dichlorophenolindophenol (Merck-Brazil), 1 ml of solution containing 4.5 M sulphuric acid (Sigma-Aldrish-Brazil) plus 2% (w/v) 2,4-dinitrophenylhydrazine (Merck-Brazil) and 50 µl of 10% thiourea in 50 % ethanol in water. The mixture was incubated in a boiling water bath for 15 min and, after cooling, 85% H_2SO_4 (w/v) was added (5 ml) (Sigma-Aldrish-Brazil). The absorbance readings were carried out at 520 nm. Results were compared with a standard curve built, under the same conditions, with 100 µg/ml

ascorbic acid (Merck-Brazil) in 0.5% oxalic acid. Analyses were not accomplished on cooked material, since vitamin C decomposes at high temperature.

Total phenolic compounds and flavonoids

The analysis of compounds phenolic was carried out on dry matter in agreement with the spectrophotometric method of Folin-Ciocalteu (Singleton et al., 1999). Dry samples (50 mg) were resuspended in 50% aqueous acetone (v/v). After 20 min in ultrasonic bath, samples were centrifuged at 6.000 x g for 10 min and the supernatants were collected. The precipitate was re-extracted with 50% aqueous acetone (v/v) and the supernatants were pooled. The whole process was carried out at room temperature.

Measurements were carried out in triplicate. Extracts (0.1 ml) were added to the Folin-Ciocalteu reagent (0.5 ml) and followed by the addition of a 20% solution of Na_2CO_3 (2.5 ml, w/v). After 2 h, absorbance readings were acquired at 725 nm. Results were calculated on the base of a calibration curve obtained with gallic acid (100 µg/ml) (Sigma-Aldrich-Brazil) and converted to fresh weight (FW) according to humidity data. The analysis of total flavonoids was optimized according to the method described by Awad et al. (2000). Fresh sample material powdered by manual grinding in liquid nitrogen (from 100 to 150 mg), was dissolved in a solution of methanol in 10% acetic acid to(85:15, v/v) (4 ml). The extract was homogenized and subjected to ultrasonication for 30 min. The homogenates were centrifuged for 20 min at 12000 x g. A 5 % (w/v) solution of aluminum chloride in methanol (1 ml) was added to the supernatant, according to modifications of Popova et al. (2004). After 30 min, the absorbance reading was acquired at 425 nm and results were compared with a calibration curve built with rutin (100 µg/ml) (Sigma-Aldrich-Brazil). Total flavonoids were expressed as µg rutin.g^{-1} FW.

Total carotenoids

The extraction of total carotenoids was carried out on fresh material according to Sims and Gamon (2002). Fresh material (50 mg) was powdered in liquid nitrogen and suspended in a cold solution of methanol/HCl/water (90:1:1, v:v:v) (3 mL) and homogenized in mini-turrax (Marconi- Brazil) for one minute at medium speed. The extraction was carried out in an ice bath and protected from light. Soon afterwards, samples were centrifuged at 2000 x g for 5 min. Supernatants were analyzed by UV/VIS spectrophotometry (Ultrospec- 2000- Pharmacia Biotech). The absorbance readings were: 663 nm for chlorophyll a, 647 nm for chlorophyll b, 529 nm for anthocyanin and 470 nm for carotenoids and were converted in mg of total carotenoids/g fresh weight, in agreement with the formulas described by the authors: Carotenoids (µmol/mL) = {Abs_{470} - [17.1 (Cl_a + Cl_b)] - 9.479 anthocyanin} / 119.26. Chlorophyll a (µmol/mL) = 0.01373 (A_{663}) - 0.000897 (Abs_{529}) - 0.003046 (Abs_{647}). Chlorophyll b (µmol/mL) = 0.02405 (Abs_{647}) - 0.004305 (Abs_{529}) - 0.005507(Abs_{663}). Anthocyanins (µmol/mL) = Abs_{529} - 0.288(Abs_{650})

Identification of polyamines

Polyamines were determined by thin layer chromatography (TLC) according to Flores and Galston (1982) and optimized according to Lima et al. (2008). Standards were purchased from Sigma-Aldrich-Brazil.

Pesticide residue analysis

Dry vegetable samples (5 g) were subjected to an extraction process with n-hexane (20 ml). Pesticides were qualitatively identified by thin layer chromatography (20 x 20 cm plate; 0.25 µm; SiO_2 60 G- Merck-Brazil) as regards the characteristic chemical groups (carbamates, organochlorides, organophosphates and herbicides). Diazinon, Malation, Chlorpiryfos, Carbofuran and Aldica were used as standards (Moraes et al., 1991).

Statistical analyses

The experimental design constituted of 4 treatments: organic, conventional, raw and thermally processed (using three repetitions from each producer) which was entirely randomized. The extractions were analyses in triplicates. The obtained data were subjected to variance analysis (F Test) and the averages were compared by the Tukey test (*P < 0.05), by the SigmaStat 2.0 program.

RESULTS AND DISCUSSION

Free radical scavenging activity

The capacity to react with radical species determined by the DPPH method was significantly higher in conventional beet leaves if compared with organic ones (Table 1), meanwhile as regard pulp, peel and stalks, this capacity did not show significant differences among the two cultivation procedures. Furthermore, considering the antioxidant activity index (AAI), we found a significant differences in leaves of conventional carrot, but, according to classification of Sherer and Godoy (2009), both exhibited strong antioxidant activity (AAI between 1.0 to 2.0) (Table 1). However, in most of plants parts analyzed, there were no significant differences in the profile of antioxidant activity.

According to Scherer and Godoy (2009), the index of antioxidant activity (AAI) in the pulp of conventional raw beet, peel and stalks, presented a moderate value, that is, an antioxidant power, not very high. Leaves and pulp of cooked conventional beet are considered much higher (strong), by these authors. Anything significant differences were observed in pulp, peel and stalk, regarding AAI of organic plants. According to the data reported by Kahkonen et al. (1999), this antioxidant activity can be attributed to the presence of anthocyanins and proanthocyanins. Furthermore, besides prolonging the post-harvest life of vegetables and fruits, the system of organic cultivation seems to better preserve the cellular apparatus of vegetables, showing different contents of antioxidants in different parts of the plant, that is, as observed when vegetables are exposed to heat stress (Yamaguchi et al., 2001).

The data reported in Table 1 show an alteration of the antioxidant capacity in the pulp of conventional beet when subjected to cooking process. As for organic tubers, a moderate increase of AAI was observed after cooking. It seems that beet produced following organic procedures shows a more stable antioxidant defense at high temperature; however deeper studies should be accomplished. Yamaguchi et al. (2001) also reported an

Table 1. Free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl) (IC_{50} expressed as µg/ml) and antioxidant activity index [AAI = 118,26 (µg/ml) / IC_{50} (µg/ml)] in four different parts of beet (*Beta vulgaris esculenta* L.) obtained by conventional and certified organic grown culture (mean of repetitions ± standard deviation).

	Conventional				Organic			
Beet	**Mean IC_{50}**	**SD**	**Mean AAI**	**SD**	**Mean IC_{50}**	**SD**	**Mean AAI**	**SD**
Pulp	126.2[a]	10.9	0.94[a]	0.11	122.9[a]	6.4	0.96[a]	0.04
Peel	121.1[a]	15.1	0.98[a]	0.42	109.0[a]	8.2	1.09[a]	0.09
Leaves	79.3[b]	5.8	1.49[a]	0.10	99.6[a]	7.3	1.19[b]	0.09
Stalk	112.6[a]	21.5	1.05[a]	0.28	113.2[a]	4.4	1.05[a]	0.01
Cooked pulp	89.8[a]	6.8	1.31[a]	0.09	118.3[a]	27.7	1.00[a]	0.24

The letters represent significant differences (*P < 0,05) among the procedures of cultivation.

Table 2. Ascorbic acid content in different parts of beet (*Beta vulgaris esculenta* L.) obtained by conventional and certified organic grown culture (mean of repetitions ± standard deviation).

Ascorbic acid (mg/100g FW)		
Food	**Conventional**	**Organic**
Pulp of beet	9.1 ± 2.7[b]	16.6 ± 3.3[a]
Skin of beet	5.9 ± 0.7[b]	13.2 ± 2.7[a]
Leaf of beet	4.1 ± 0.6[b]	7.0 ± 1.3[a]
Stalk of beet	6.1 ± 2.0[a]	6.7 ± 1.9[a]

The letters represent significant differences (*P < 0.05) among the cultivation procedures.

increase of the antioxidant activity after cooking. Maeda et al. (1992), finding similar results, attributed the phenomenon to the increased destruction of cellular walls of vegetables, releasing active compounds and/or to the thermal reaction, which could produce different and more specific antioxidants. However, in other works by Yamaguchi et al. (2003), the decrease of the antioxidant activity in lettuce (*Lactuca sativa* L) and broccolis (*Brassica oleracea* L. var. *italica*) after cooking was also observed. In agreement with these authors, there are no apparent reasons explaining the higher antioxidant activity observed in cooked vegetables when compared to raw ones. It is assumed that these observations could be due to inactivation at high temperature of enzymes, such as polyphenol-oxidases and ascorbate oxidase.

Levels of ascorbic acid (vitamin C)

Notwithstanding, no significant differences were found in total antioxidant activity of most of beet vegetable parts (Table 1), according to the data reported in Table 2, the content of ascorbic acid was significantly higher in pulp, peel and leaves of organic beet than that observed in conventional grown plants.

Vitamin C is a potent reducing agent (E_o' = -170 mV), able to reduce most of physiologically relevant ROS/RNS (Halliwell and Gutteridge, 1999). Besides eliminating directly ROS/RNS, it regenerates α-tocoferol and, therefore, it participates to the protection mechanism against lipoperoxidation. Furthermore, besides eliminating free radicals, literature studies on cell cultures demonstrated that vitamin C can alter the expression of genes involved in the inflammatory response (Bennotti et al., 2003).

Levels of totals phenolic compounds and flavonoids

The data reported from this study showed higher concentrations of phenolic compounds in organic beet, when compared with conventional samples (Table 3). According to the data, significant differences were observed among samples and, in particular, the highest total phenolic content was observed in peel of raw organic beet, accordingly with results reported on raspberry (*Rubus idaeus* L.) and organic corn (*Zea mays* L.) by Asami et al. (2003). However, in pulp, leaves and stalks of organic vegetables, this difference was generally not significant when compared to conventional plant samples. The content of phenols was similar to the values observed in red mulberry (*Morus nigra* L.) by Anttonen and Karjalainen (2005).

Regarding the effect of cooking process on total phenol content, both pulp and peel of organic beet presented higher values than conventional samples. It should be noticed that cooking lead to a general decrease of phenol content on samples coming from the two cultivation procedures (Table 3). Accordingly, Yamaguchi et al. (2003) also observed a decrease in phenolic content in *Arctium lappa* (greater burdock) and lettuce (*Lactuca sativa* L.) after cooking in boiling water for 1 min. Meanwhile, in broccolis (*Brassica oleracea*), phenol content was constant after 15 min processing in hot water. Rocha-Guzmán et al. (2007), also observed in beans (*Phaseoulus vulgaris L.*), upon cooking, a decrease in the content of these substances. In agree-ment with Barroga et al. (1985), the effect could be explained as due to the leaching of phenolics during the cooking process in hot water, being dependent on temperature and on time of cooking.

Regarding the compositions of total phenolics, a significant

Table 3. Content of total phenolic compounds and total flavonoids in beet (*Beta vulgaris esculenta* L.) obtained by conventional and certified organic grown culture (mean of repetitions ± standard deviation).

Tissue	Total phenolic compounds (mg/g. FW)		Total flavonoids (*mg rutin/g. FW*)	
	Conventional	Organic	Conventional	ORGANIC
Pulp	12.6 ± 4.4[a]	15.6 ± 3.1[a]	1.32 ± 0.41[a]	2.73 ± 1.60[a]
Skin	24.5 ± 7.4[b]	46.0 ± 7.6[a]	1.83 ± 0.33[b]	3.00 ± 0.25[a]
Leaf	21.5 ± 6.6[a]	14.3 ± 2.4[a]	3.52 ± 0.30[b]	5.24 ± 0.24[a]
Stalk	16.7 ± 7.1[a]	19.0 ± 3.5[a]	0.82 ± 0.38[b]	1.13 ± 0.37[a]
Pulp cooking	7.4 ± 2.1[b]	11.7 ± 0.9[a]	0.98 ± 0.03[a]	0.55 ± 0.02[b]
Skin cooking	13.7 ± 0.3[b]	25.2 ± 0.3[a]	0.77 ± 0.05[b]	2.26 ± 0.03[a]

The letters represent significant differences (*P < 0.05) among the manners of cultivations. FW: fresh weight.

higher amount was observed in the peel of organic beet and an accumulation of these compounds in the corresponding organic cooked pulp. No significant differences were observed in other plants parts between the two production procedures.

Significant differences in flavonoid content were observed in most of the beet parts analyzed (Table 3). The highest concentrations were found in organic leaves, peel, pulp and stalks, respectively, accordingly with observations on tomatoes (*Lycopersicon esculentum* L. cv. Halley 3155) by Mitchel et al. (2007), reporting about 1.65 mg/g of total flavonoids in conventional fruits and 5.9 mg/g in organic ones. The values reported by these authors on organic tomatoes corroborate the hypothesis that organic vegetables accumulate high concentrations of these bioactive compounds.

Flavonoid content in beet (0.5 to 5.2 mg/g in organic and 0.7 to 3.5 mg/g in conventional) was also close to that observed in apple peel and pulp by Awad et al. (2000). The concentration of these substances in beet, reported in the present work, in apple peel, by Awad et al. (2000) and in several species of *Hibiscus*, reported by Puckhaber et al. (2002) can be attributed to plant pigmentation, as it exists a direct correlation between sample pigmentation and flavonoids concentration. However, no significant differences were observed in the pulp of samples coming from both cultivation procedures. The cooked conventional pulp showed significantly the highest flavonoid content. Conversely to what is observed regarding phenolic compounds, cooking process in boiling water significantly reduced flavonoid content in beet pulp and peel coming from both cultivation procedures, accordingly to the results reported by Ferracane et al. (2008) in artichoke (*Cynara cardunculus scolymus* L.), suggesting a loss of these compounds by solubilization or by thermal degradation, even if, each sample seems to have a certain capacity of retention of these substances (Hassimotto et al., 2007). Besides, glycosilated and/or conjugated forms of flavonoids, in general, are absorbed in small intestine (Day et al., 2003).

Table 4. Concentration of carotene in beet (*Beta vulgaris esculenta* L.) obtained by conventional and certified organic grown culture (mean of nine repetitions ± standard deviation).

Tissue	Total carotene (mg/g.FW)	
	Conventional	Organic
Pulp	4.39 ± 0.89[a]	4.38 ± 0.47[a]
Skin	6.94 ± 1.52[a]	4.99 ± 1.73[a]
Leaves	1.00 ± 0.32[a]	0.66 ± 0.29[a]
Stalk	0.36 ± 0.03[b]	0.56 ± 0.08[a]
Cooked pulp	1.72 ± 0.27[b]	2.50 ± 0.31[a]
Cooked skin	2.50 ± 0.14[a]	1.24 ± 0.35[b]

The letters represent significant differences (*P < 0.05) among the manners of cultivations. FW: fresh weight.

Total carotenoids

The highest carotenoid concentration (Table 4) was found in peel, followed by pulp, leaves and stalks and a higher carotenoid content was observed in organic stalks and cooked pulp, when compared with conventional ones, but significant difference was found just to this parts. The content of these substances in the present work was quite higher than that found in several fruits (Sant'ana et al., 2007), and similar to that found in varieties of purple and orange carrots (*Daucus carota* L.) by Alasalvar et al. (2005). Therefore, all the analyzed parts of beet seem to contain high carotenoids concentration. Interestingly, it should be noted that these substances stimulate the formation of the communicating "gap junctions" among cells, which are essential for the monitoring of biochemical functions in the organisms (El-Agamey et al., 2004).

When beet pulp and peel were subjected to the cooking process in boiling water, a decrease in carotenoid content was observed. In pulp of organic beet, carotenoid content was higher than that found in conventional beets (Table 4). The decrease of carotenoid content observed after

Table 5. Presence or absence of pesticides organo-phosphates (Ph), carbamates (C) and organo-chlorine (Cl) in conventional and organic beet (*Beta vulgaris esculenta* L.) obtained by conventional and certified organic grown culture (repetition:1, 2 and 3).

	Conventional									Organic								
	Ph			C			Cl			Ph			C			Cl		
Repetitions	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peel	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Leaf	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Stalk	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-		-	-
Cooked pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cooked peel	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Presence (+) and absence (-) of pesticides. A pesticide was considered absent if its concentration was below the detection limit of the TLC method used for its determination.

thermal treatment was also described by Bianchini et al. (1998) in yellow *Capsicum annuum*. In agreement with Sant'ana et al. (1998), the type of cooking process and the amount of water seem to influence the decomposition of these compounds in some vegetables. In *C. annuum*, Chuah et al. (2008) observed a decrease of these compounds after cooking and this attributed for the differences among studied varieties. Several authors reported in their literature that thermal treatment promotes the biodisponibilization of carotenoids in some vegetables (Sá and Rodriguez-Amaya, 2003), showing, in some cases, a more efficient extraction of these compounds at high temperature. On the other hand, other studies showed a decrease in carotenoid retention after cooking (Bianchini et al., 1998; Chuah et al., 2008). Probably, carotenoid content is differently affected by temperature, depending on the species under study.

In the case of cooked organic beet, due to the absence of pesticides (Table 5), the intake of cooking water could be a source of important chemical substances for human metabolism, as carotenoids, classified as natural antioxidant and which can collaborate, among others, against the action of certain cancers, to prevent peptic ulcer, to stimulate the immune system and also prevent cardiovascular diseases (Krinsky and Johnson, 2005).

Polyamine content

A specific pattern characterizing polyamines content among organic and conventional beet samples cannot be recognized, as shown in the data reported in Figure 1. However, it is clear that vegetables produced by organic procedure, when they are subjected to cooking, maintain a higher concentration of these substances, presenting significant differences regarding higher putrescine (Put), spermidin (Spd) and spermine (Spm) content. These data were in accord with the hypothesis that polyamines exert a protective effect against several types of stress, in particular favoring the stability of cell membranes (Bae et al., 2008).

Naturally, in the pulp, significant differences of Put and Spd were not observed; differently Spm content in the conventional grown pulp was significantly higher, suggesting a high metabolic activity in this tissue, since this polyamine is related to cellular proliferation (Kalac and Krausová, 2005). Lima et al. (2008) reported alterations in polyamine content according to the cultivation method, finding a higher content in organic vegetables.

In organic beet, a higher content of Spd was observed in the peel; meanwhile Put and Spm were found in the leaves and no significant variation of Spd and Spm in the stalks. As regards conventionally grown beet plants, a higher Put in stalks and Spd content in leaves were observed (Martínez-Romero et al., 2004), that would explain a higher stability of the product. In fact, polyamine concentration is related to youthfulness of the tissue, since in agreement with several authors, old tissues present S-adenosyl-metionine as common precursor to ethylene, the vegetable regulator related to senescence (Bouchereau et al., 1999). Accordingly, high polyamine content would indicate longer useful life of the tissue. The analysis of polyamines in foods is fundamental, since they are related with human being growth, acting possibly on cellular proliferation and differentiation (Bárdocz et al., 1993) and they are intimately linked to the growth of some tumors (Bárdocz et al., 1993; Seiler et al., 1998). In agreement with the results obtained in the present work, depending on the consumer's nutritional state, it should be suggested that the consumption of raw or cooked beet, in relation to the polyamine content, considering that some cancer therapies limit polyamine intake, or that these substances can be effective in wound healing and in promoting growth, maturation and regeneration of intestinal mucosa (Weiss et al., 2004). These results could be interesting for individuals that need a diet with a limited content of polyamines, since the organic beet contains higher amount of these substances when cooked. In spite of their beneficial effects in humans under normal physiological conditions, even considering their antioxidant actions (Bae et al., 2008), anti-cancer effects (Kalac and Krausová, 2005) the polyamines are harmful in patients affected with cancer presenting a

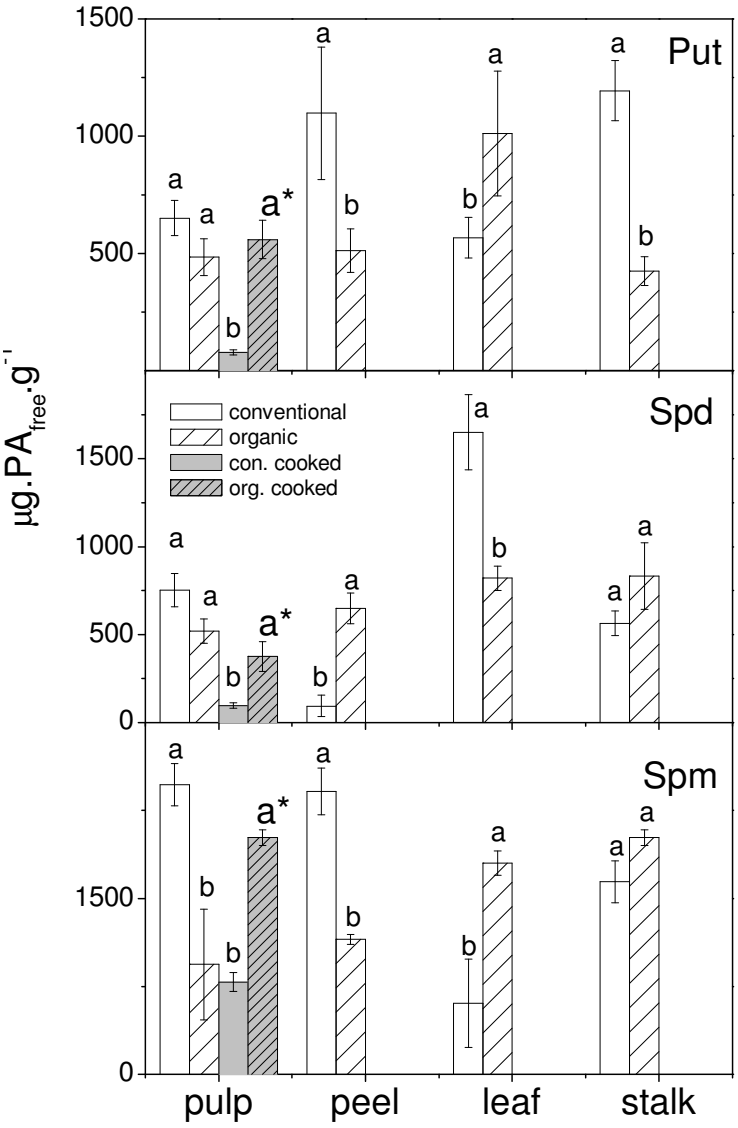

Figure 1. Content of polyamines: putrescine (PUT), spermidine (SPD) and spermine (SPM) in four differents parts of beet *(Beta vulgaris esculenta,* L.*)* obtained by conventional and certified organic grown culture (means of repetitions ± standard deviation). Different letters represent statistical differences between of organic and conventional system, and asterisk means statistical differences between cooked pulps, both *P < 0.05.

constant proliferating activity (Seiler et al., 1998).

Analysis of pesticides

The presence of pesticides in different beet parts was analyzed. The data reported in Table 5 are fundamental for the consumer preferring the intake of vegetables free from pesticides, as practiced in the organic agriculture. Of course, this preference is due to the damages produced by these substances to human health, pollution of soil, of water, decrease of the number of species of insects, among other and the lack of monitoring systems of the correct use of these products within the environment (Travisi and Nijkamp, 2008). In conventional cooked beet, the presence of pesticides was not observed, probably the thermal treatment (boiling in water) lead to the leaching of these substances. Problems could arise with the ingestion of the water used for cooking; therefore, the parts of beet such as leaf and peel are safer when cooked.

Conclusion

Beet plants, cultivated following organic procedure,

concentrated a higher content of vitamin C and flavonoids and, when subjected to cooking process, increased significantly the concentration of carotenoids and conserved constant the content of phenolic compounds and of polyamines, when compared with samples produced conventionally.

ACKNOWLEDGMENTS

The authors would like to thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for grating (06/52646-8; 08/50718-7; 07/53403-4 and 07/54368-8) and Santander.

REFERENCES

Alasalvar C, Al-Farsia M, Quanticka PC, Shahidib F, Wiktorowicz R (2005). Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. Food Chem., 89: 69-76.

Anttonen MJ, Karjalainen RO (2005). Environmental and genetic variation of phenolic compounds in red raspberry. J. Food Compost. Anal., 18: 759–769.

Asami DK, Hong YJ, Barrett DM, Mitchell AE (2003). Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry and corn grown using conventional, organic and sustainable agricultural practices. J. Agric. Food Chem., 51: 1237-1241.

Awad MA, Jager A, Van-Westing LM (2000). Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Scientia Horticulturae, 83: 249-263.

Bae H, Kim S, Kim MS, Sicher JR, Strem, MD, Natarajan SS, Bailey BA (2008). Coordinated regulation of polyamine biosynthesis in *Theobroma cacao* tissues responding to stress. Plant Physiol. Biochem., 46: 174-188.

Bardòcz S, Grant G, Brown DS, Ralph A, Pusztai A (1993). Polyamines in food – implications for growth and health. J. Nutr. Biochem., 4: 66–71.

Barroga C, Laurena AC, Mendoza EMT (1985). Polyphenols in mung bean (*Vigna radiata* (L.) Wilczek): determination and removal. J. Agric. Food Chem., 33: 1006–1009.

Bianchini R, Penteado M, Camargo V (1998). Carotenoids of yellow pepper (*Capsicum annuum* L.) characterization and verification of changes after cooking. Ciência e Tecnologia de Alimentos, 18: 283-288.

Bouchereau A, Guénot P, Larher F (2000). Review: Analysis of amines in plant material. J. Chromatogr., B. 747: 49-67.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und Technologie, 28:25–30.

Chuah AM, Lee YC, Yamaguchi T, Takamura H, Yin LJ, Matoba T (2008). Effect of cooking on the antioxidant properties of coloured peppers. Food Chem., 111: 20–28.

Crozier A, Lean MEJ, Mcdonald MS, Black C (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. J. Agric. Food Chem., 45: 590 -595.

Day AJ, Gee JM, Dupont MS, Johnson IT, Williamson G (2003). Absorption of quercitina-3-glucoside and quercitina-4-glucoside in the rat small intestine: role of lactase phlorizin hydrolase and sodium-dependent glucose tranporter. Biochem. Pharmacol., 65: 1199-206.

El-Agamey A, Lowe GM, Mcgarvey DJ, Mortensen A, Phillip DM, Truscot TTG, Young AJ (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Arch. Biochem. Biophys., 430: 37-48.

Ferracane R, Pellegrini N, Visconti A, Graziani G, Chiavaro E, Miglio C, Fogliano V (2008). Effects of different cooking methods on antioxidant profile, antioxidant capacity, and physical characteristics of artichoke. J. Agric. Food Chem., 56: 8418–8426.

Flores HE, Galston AW (1982). Analysis of polyamines in higher plant by high performance liquid chromatography. Plant Physiol., 69: 701-706.

Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero JR (1998). The natural polyamine spermine functions directly as a free radical scavenger. Proc. Natl. Acad. Sci,. 95: 11140-11145.

Halliwell B, Gutteridge JMC (1999). Free Radic. Biol. and Med., 5th ed., Claredon Press: Oxford.

Hassimotto NMA, Genovese MI, Lajolo FM (2007). Identification and characterization of anthocyanins from wild mulberry (*Morus nigra* L.) growing in Brazil. Food Sci. Technol. Int. 13: 17-25.

Hu R, Khor TO, Shen G, Jeong WS, Hebbar V, Chen C, Xu C, Reddy B, Chada K, Kong ANT (2006). Cancer chemoprevention of intestinal polyposis in ApcMin/+ mice by sulforaphane, a natural product derived from cruciferous vegetable. Carcinogenesis 27(10): 2038-2046.

Ismail A, Fun CS (2003). Determination of vitamin C, □□carotene and riboflavin contents in five green vegetables organically and conventionally grow. Malaysian J. Nutr., 9: 31-39.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47: 3954-3962.

Kalac P, Krausová P (2005). A review of dietary polyamines: formation, implications for growth and health and occurrence in foods. Food Chem., 90: 219-230.

Krinsky N, Johnson EJ (2005). Carotenoid actions and their relation to health and disease. Mol. Aspects Med., 26(6): 459-516.

Lima GPP, da Rocha SA, Takaki M, Ramos PRR, Ono EO (2008). Comparison of polyamine, phenol and flavonoid contents in plants grown under conventional and organic methods. Int. J. Food Sci. Technol., 43(10): 1838-1843.

Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R (2006). Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. Environ. Health Persp., 114(2): 260-263.

Maeda H, Katsuki T, Akaike T, Yasutake R (1992). High correlation between lipid peroxide radical and tumor-promoter effect: Suppression of tumor promotion in the Epstein-Barr virus/B-lymphocyte system and scavenging of alkyl peroxide radicals by various vegetable extracts. Japanese J. Cancer Res., 83: 923–928.

Martínez-Romero D, Serrano M, Carbonell A, Castillo S, Riquelme F, Valero D (2004). Mechanical damage during fruit post-harvest handling: technical and physiological implications, In: Dris, R., Jain, S.M. (Ed.), Production practices and quality assessment of food crops. Quality Handling and Evaluation. Kluwer Academic Publishers, Dordrecht 3, 233–252.

Mitchell AE, Yun-Jeong HI, Koh E, Barrett DM, Bryant DE, Denison RF, Kaffka S (2007). Ten-Year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. J. Agric. Food Chem. 55: 6154–6159.

Moraes ECF, Sznelwar RB, Fernicola NAGG (1991). Inseticidas organoclorados. *In:* Manual de toxicologia analítica. São Paulo: Roca, Ltda, pp. 98-99.

Navrot N, Collin V, Gualberto J, Gelhaye E, Hirasawa M, Rey P, Knaff DB, Issakidis E, Jacquot JP, Rouhier N (2006). Plant glutathione peroxidases are functional peroxiredoxins distributed in several subcellular compartments and regulated during biotic and abiotic stresses. Plant Physiol. 142: 1364-1379.

Pieta PG (2000). Flavonoids as Antioxidants. Reviews. J. Natural Prod. 63: 1035-1042.

Popova M, Bankova V, Butovska D, Petkov V, Nikolova-Damyanova B, Sabatini AG, Marcazzan GL, Bogdanov S (2004). Validated methods for the quantification of biologically active constituents of poplar-type propolis. Phytochem. Anal., 15: 235–240.

Puckhaber LS, Stipanovic RD, Bost GA (2002). Analyses for flavonoid aglycones in fresh and preserved hibiscus flowers. Trends in New Crops and New Uses, 556-563.

Rocha-Guzmán NE, González-Laredo RF, Ibarra-Pérez FJ, Nava-Berúmen CA, Gallegos-Infante JA (2007). Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars. Food Chem., 10: 31-35.

Roitner-Schobesberger B, Darnhofer I, Somsook S, Vogl CR (2008).

Consumer perceptions of organic foods in Bangkok, Thailand. Food Policy, 33: 112–121.
Sá MC, Rodriguez-Amaya DB (2003). Carotenoid composition of cooked green vegetables from restaurants. Food Chem., 83: 95-600.
Sant'ana HMP, Stringheta PC, Brandão SCC, Azevedo RMC (1998). Carotenoid retention and vitamin a value in carrot (*Daucus carota* L.) prepared by food service. Food Chem., 61: 145-151.
Seiler N, Atanossov CL, Raul F (1998). Polyamine metabolism as target fir cancer chemoprevention (review). Int. J. Oncol., 13: 993–1006.
Scherer R, Godoy HT (2009). Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chem., 112: 654–658.
Sims DA, Gamon JA (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens. Environ., 81: 337– 354.
Siderer Y, Maquet A, Anklam E (2005). Need for research to support consumer confidence in the growing organic food market. Trends Food Sci. Tecnol., 16: 332-343.
Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152-178.
Terada M, Watanabe Y, Kunitoma M, Hayashi E (1978). Differential rapid analysis of ascorbic acid and ascorbic acid 2-sulfate by dinitrophenilhydrazine method. Anal. Biochem., 84: 604-608.
Travisi CM, Nijkamp P (2008). Valuing environmental and health risk in agriculture: A choice experiment approach to pesticides in Italy. Ecol. Econ., 67: 598-607.
Yamaguchi T, Mizobuchi T, Kajikawa R, Kawashima H, Miyabe F, Terao J, Takamura H, Matoba T (2001). Radical-scavenging activity of vegetables and the effect of cooking on their activity. Food Sci. Technol. Res., 7: 250–257.
Yamaguchi T, Katsuda M, Oda Y, Terao J, Kanazawa K, Oshima S, Inakuma T, Ishiguro Y, Takamura H, Matoba T (2003). Influence of polyphenol and ascorbate oxidases during cooking process on the radical- scavenging activity of vegetables. Food Sci. Technol. Res., 9(1): 79–83.
Weiss TS, Herfarth H, Obermeier F, Ouart J, Vogl D, Schölmerich J, Jauch KW, Rogler G (2004). Intracellular polyamine levels of intestinal epithelial cells in inflammatory bowel disease. Inflamm. Bowel Dis., 10: 529–535.