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Antioxidants and chlorophyll in cassava leaves at three plant ages

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The aim of this study was to quantify antioxidant substances and chlorophyll content, as well as to measure the antioxidant activity in cassava leaf flour (CLF) of different cultivars at several plant ages, in order to lead to a higher utilization of these leaves, and consequently to an enhancement of this agricultural by-product. The contents of antioxidant substances (vitamin C, polyphenols and β-carotene) were regarded as high and increased as the plants matured. The chlorophyll content decreased with plant maturity and presented a negative correlation with antioxidant substances, which indicates that the highest antioxidant levels are found when the plant presents low chlorophyll levels. CLF showed a high antioxidant activity when the lipid oxidation inhibition method (β-carotene/linoleic acid) was used, and moderate when the free radical capture method (ABTS) was used. The main contribution to the CLF antioxidant activity seems to be provided by vitamin C, which presented the best correlation with the ABTS test. Out of the ages studied, that of 14 months presented the highest antioxidant levels; Mocotó and Pão da China cultivars stood out the most.

Key words: Cassava leaf flour, antioxidant activity, antioxidant substances.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial, bushy plant, of the Euphorbiaceae family. Originating on the American continent, probably in Central Brazil, it is cultivated all over the world, mainly in poor areas, where its cultivation constitutes one of the main agricultural activities, having a high social importance as a main carbohydrate source for more than 500 million people, essentially in developing countries (Brazilian Company of Agricultural Research-EMBRAPA, 2010). In recent years, the aerial part of the plant, which had been treated as an agricultural by-product, but that nutritionally, presents great potential for human and animal consumption, has been gaining prominence. Those leaves are rich in proteins and vitamins A and C (Corrêa et al., 2004; Wobeto et al., 2006), and minerals, especially Mg, Fe, Zn and Mn (Wobeto et al., 2006), obtained at a low cost, when compared to conventional leafy vegetables. Furthermore, their use can provide an extra income to various producers that live on the cassava culture.

In the search for natural substances that can bring benefits, mainly to health, aggregating value to this agricultural by-product can contribute to a greater use of the cassava leaves in feeding and in many other applications.

Experimental and epidemiological studies have been demonstrating that people who consume foods rich in

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antioxidants could have a reduced risk of many diseases, such as cancer, cardiovascular diseases, chronic diseases and aging, among others.

Antioxidants are substances that combat free radicals, which are extremely reactive species that cause oxidation of various biomolecules present in the organism. Moreover, besides the problems found with the synthetic antioxidants used in food conservation and their high production costs, it has also been demonstrated, by toxicological studies, that they can provoke undesirable effects in human and animal organism. Therefore, the search for natural substances that is efficient antioxidant sources, and less expensive than synthetic antioxidants, has been the objective of various studies (Gan et al., 2010; Surveswaran et al., 2007; Wojdylo et al., 2007).

In plant leaves, chlorophyll, the photosynthetic pigment, is directly related to their nutritional state. The amount of that pigment has been used as an evaluation index of the nutritional state for several types of cultures (Argenta et al., 2001). Variables, such chlorophyll and antioxidant levels, can be directly correlated, demanding studies to confirm that correlation and, after being confirmed, can serve as a parameter for obtaining antioxidant substances in cassava leaves.

Therefore, this work was conducted with the objective of quantifying antioxidant substances, chlorophyll and to measure the antioxidant activity in cassava leaf flour of different cultivars, at several plant ages.

MATERIALS AND METHODS

Samples

Mature leaves of four cassava cultivars, 'Mocotó ', 'Ufla ', 'Pão da China ' and 'Ouro do Vale', obtained from the Agriculture Department of Federal University of Lavras, were picked in the morning, from plants at three different ages (TPA), in December (10 months), February (12 months) and April (14 months), in three repetitions. Intact leaves, free from diseases and pests, were selected, washed in running water and distilled water and, soon afterwards, dried in a forced-air oven, for 48 h, at temperatures ranging from 30 to 35°C. After drying, they were ground (without petioles) in a Willy type mill and the cassava leaf flours (CLF) were stored in hermetically sealed flasks under refrigeration, until the analyses were performed.

Analyses

Determination of antioxidant substances

Vitamin C: The extraction of vitamin C for chromatographic analyses was conducted in oxalic acid, according to Strohecker and Henning (1967). A Shimadzu LC 200A liquid chromatography with UV-VIS detector, wavelength detection at 254 nm; quaternary pump, degasser, and automatic injection was used. A C_{18} Nucleosil (250 × 4.6 mm × 5 µm) column and a C_{18} (15 × 3.2 mm × 7.5 µm) pre-column were used. As movable phase a pH 6.7 buffer was used, containing sodium acetate 0.04 mol L^{-1}, EDTA 0.05 mol L^{-1}, tributyl ammonium phosphate isocratic mode, flow rate 0.6 ml min^{-1}, 15 min running time for each sample.

Phenolic compounds: The extraction of phenolic compounds was carried out with 1 g of sample in 50 ml of 50% methanol, under reflux for three consecutive times, at 80°C, and the extracts were collected, evaporated up to 25 ml and submitted to phenolic compound measurement, using Folin-Denis reagent, and tannic acid as a standard (Association of Official Analytical Chemists - AOAC, 2011).

β-carotene: The extraction of β-carotene was carried out with 0.5 g of sample in a 40 ml extraction solution of isopropyl alcohol:hexane 3:1. The content was transferred to a 125 ml separation funnel wrapped in aluminum, where the volume was completed with distilled water. It was left at rest for 30 min, followed by washing of the material and discard of the aqueous phase. This operation was repeated three more times. The content was filtered with cotton sprayed with anhydrous sodium sulphate 99% to a 25 ml volumetric flask wrapped with aluminum, where 5 ml of acetone were added and the volume completed with hexane. Then, absorbance readings were taken in a spectrophotometer at four wavelengths (453, 505, 645 and 663 nm) (Nagata and Yamashita, 1992) and the results expressed in mg 100 g^{-1}, calculated by the formula:

$$\beta\text{-carotene (mg 100 g}^{-1}) = 0.216\ A_{663} - 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453}.$$

Antioxidant activity

Extract preparation: For the obtention of the extracts, the CLFs were maintained under maceration using 50% ethanol, 1:40 (w/v), for 30 min and soon afterwards centrifuged at 2,500 g, for 15 min. The supernatant was collected and the precipitate was again submitted to the previously described extraction process, substituting 50% ethanol for 70% acetone; the supernatants were collected and then subjected to the detection of antioxidant activity by the methods described subsequently.

ABTS method: The methodology used was developed by Rufino et al. (2007). Four different dilutions were made from the obtained extracts for the assays and subsequent construction of analytical curves. Six point analytical curves were made with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (100 to 2,000 µmol L^{-1}) and with ascorbic acid (10 to 200 mg mL^{-1}), in addition to tests for comparison with the patterns BHT (butylhydroxytoluene–synthetic antioxidant) and with rutin and quercetin, that are flavonoids with proven antioxidant activity; these standards were prepared at a concentration of 200 mg L^{-1}.

β-carotene/linoleic acid method: The methodology used was developed by Rufino et al. (2006). The test was applied to the extracts, at a concentration of 10,000 mg L^{-1}.

For the preparation of the β-carotene/linoleic acid solution system, 50 µl of β-carotene diluted in chloroform (20 g L^{-1}) were used, to which 40 µl of linoleic acid were added, as well as 530 µl of tween 20 (emulsifier) and, for solubilization, 1 ml of chloroform. In a flask covered with aluminum for protection against light, chloroform was evaporated in a rotary-evaporator and 100 ml of oxygen saturated water (distilled water treated with oxygen for 30 min) were added, and the combination was agitated until the solution system presented a yellow-orange coloration. In test tubes, 2.5 ml of that solution system were added to 0.2 ml of each sample dilution used for the test. Control tubes were made containing 2.5 ml of the solution system with 0.2 ml of 2,6-di-tert-butyl-p-cresol (BHT), quercetin and rutin, all at the concentration of 200 mg L^{-1}. In laboratory tests, it was found that the concentration of 200 mg L^{-1} BHT is the one that provides the greatest protection for the system, when compared to others; therefore, its use is suggested. After

Table 1. Antioxidant levels in cassava leaf flour of four cultivars, at three plant ages.

Antioxidant substances	Cultivar	10 months	12 months	14 months
Vitamin C (mg 100 g^{-1})	Mocotó	281.53±14.84^{aB}	294.77±5.47^{aB}	521.19±31.60^{bA}
	Ufla	149.29±3.84^{dC}	249.68±1.32^{cB}	474.05±30.07^{cA}
	Pão da China	244.74±8.58^{bC}	276.97±11.50^{bB}	568.64±7.71^{aA}
	Ouro do Vale	171.44±2.58^{cC}	310.88±26.16^{aB}	463.69±2.40^{cA}
Polyphenols (mg g^{-1})	Mocotó	29.48±0.56^{aC}	52.12±1.13^{bB}	55.06±0.30^{aA}
	Ufla	29.14±0.19^{aC}	54.53±1.58^{aA}	50.14±0.66^{bB}
	Pão da China	29.18±0.30^{aB}	55.57±1.54^{aA}	56.21±1.39^{aA}
	Ouro do Vale	16.46±0.16^{bC}	31.73±0.86^{cB}	36.26±0.67^{cA}
β-carotene (mg 100g^{-1})	Mocotó	50.20±1.60^{bB}	70.31±0.24^{aA}	65.92±2.08^{aA}
	Ufla	53.08±3.21^{bB}	70.07±3.59^{aA}	65.60±1.39^{aA}
	Pão da China	65.90±0.83^{aB}	72.72±1.09^{aA}	61.63±1.38^{aB}
	Ouro do Vale	51.82±4.59^{bB}	68.79±4.74^{aA}	51.44±6.67^{bB}

Data are the mean of three replicates ± standard deviation. Lowercase letters in columns compare among cultivars and uppercase on the lines compare among ages. Same letters do not differ among themselves by the Scott-Knott test at 5% probability.

homogenization, their readings were taken in a spectrophotometer at 470 nm, using water to calibrate the spectrophotometer; this was considered to be the reading at time zero (initial). The tubes were placed in a water bath, at 40°C and readings were taken after 2 h.

Chlorophyll determination

The chlorophyll determination was conducted with Minolta SPAD-502 equipment, directly on the leaf still attached to the plant, moments before the collection, according to the methodology described by Argenta et al. (2001). Each SPAD reading is equivalent to the result obtained by the measurement performed on six leaves, with five measurements taken on each leaf, which were performed at points situated in half to two thirds of the sampled leaf length. The results were expressed in SPAD units, which are equal to the average of thirty readings.

Statistical analysis

The experiment was conducted in a completely randomized design, in a 4 × 3 factorial outline, with four cassava cultivars and three plant ages, and three repetitions. The statistical analysis was conducted using R (version 2.15.2) statistical software (R Core Team, 2012). Averages were compared by the Scott-Knott test, at 5% probability. Pearson correlation analysis was conducted among the antioxidant substances, antioxidant tests and chlorophyll, using the Statistical Analysis System program (1999).

RESULTS AND DISCUSSION

Antioxidant substances

Table 1 shows vitamin C, polyphenol and β-carotene levels of CLF in the four cultivars, at TPA. It was observed that vitamin C levels increased with plant maturity, for all cultivars. Among the cultivars, 'Pão da China' (14 months) presented the highest vitamin C level. Carvalho et al. (1989) found an increase in vitamin C levels for cassava leaves dried at 45°C, up to 14 months of age. It was also certified by Wobeto et al. (2006) who, analyzing five cassava cultivars, in TIP, observed an increase in vitamin C with plant maturity. These results are consistent with the observations made in the present study.

Higher vitamin C levels were found in this study, when compared to Wobeto et al. (2006) who, analyzing cultivars Mocotó and Ouro do Vale at 12 months of age, found levels of 55.72 and 64.12 mg 100 g^{-1} of dry matter (DM) for Mocotó and Ouro do Vale, respectively. In the present study, it was found, at the same age, levels of 294.77 and 310.88 mg 100 g^{-1} DM for Mocotó and Ouro do Vale, respectively. These differences may have occurred as a result of the analysis method used, which, in this study, was the chromatographic method, while Wobeto et al. (2006) used the colorimetric method, besides other factors, such as manuring, climatic conditions, among others.

When compared with other unconventional foods, CLF at 14 months presented average levels of vitamin C superior to those found in 100 g, of carrot leaves (203.70 mg) (Pereira et al., 2003). However, in relation to the fruits, those levels are also superior to those found in 100 g of fresh matter (FM) for orange (66 mg) and papaya (149 mg) (Hernández et al, 2006). Nevertheless, they are inferior to those of fruits considered rich in vitamin C, such as acerola, that contains 1,500 mg in 100 g^{-1} FM. With the discovery of the antioxidant action of this vitamin, ingestion of substances with high vitamin C content has been recommended. As such, those plants are shown as good vitamin C sources with potential for use as antioxidants.

It was possible to verify that, in general, polyphenol levels increased with plant maturity. Cultivars Pão da China and Mocotó, at 14 months of age, did not differ significantly amongst themselves and they presented the highest polyphenol levels. Ouro do Vale, at the three analyzed ages, was that which presented significantly lower polyphenol levels.

Wobeto et al. (2007), studying CLF from five cultivars, in TIP, also observed an increase in polyphenol levels with plant maturity. In that study, the authors also observed higher polyphenol levels for cultivar Ouro do Vale (61.49 mg 100 g^{-1} DM) and lower for Mocotó (44.13 mg 100 g^{-1} DM) at 12 months of age, when compared to those recorded in this study at the same age, for Ouro do Vale (31.73 mg 100 g^{-1} DM) and Mocotó (52.12 mg 100 g^{-1} DM). These different results are probably due to factors related to manuring, climatic conditions, among others.

Polyphenol levels found in this study, independent of age and cultivar, are higher than those found in mg 100 g^{-1} FM for some vegetables, such as broccoli (0.68), onion (1.13) and cabbage (0.67) and for some fruits, such as pineapple (0.85), banana (2.16), orange (1.14), papaya (0.15) and mango (1.10) according to Faller and Fialho (2009). These are within the range related by Asolini et al. (2006) who, analyzing phenolic compounds in plants used as tea, found levels between 15 (mate) to 56 (lemongrass) mg g^{-1} DM.

Phenolic compounds act as antioxidants, due to their redox properties that allow them to act as reducing agents, hydrogen donors and metal chelators. Besides their role as antioxidants, these compounds present a wide spectrum of medicinal properties, such as antiallergic, anti-inflammatory, anti-bacterial and anti-thrombotic, plus they present cardioprotective and vasodilator effects (Balasundram et al., 2006), showing a broad field of application for the phenolics in these plants.

It can be noticed that there is an increase in β-carotene levels until the age of 12 months and that those levels did not differ significantly from those at 14 months, except for Ouro do Vale and Pão da China cultivars, that presented a reduction in β-carotene levels between 12 and 14 months of age.

Wobeto et al. (2006), analyzing five cassava cultivars, in TIP, also found, at 12 months of age, higher β-carotene levels. However, these authors observed a decline in these levels with plant maturity.

Lower β-carotene levels were found in this study, when compared to Wobeto et al. (2006) who, analyzing Mocotó and Ouro do Vale cultivars at 12 months of age, found levels of 126.57 and 124.24 mg 100 g^{-1} DM for Mocotó and Ouro do Vale, respectively. In the present study, we found, at the same age, levels of 70.31 and 68.79 mg 100 g^{-1} DM for Mocotó and Ouro do Vale, respectively. Corrêa (2004), analyzing different forms of cassava leaf drying (sun-dried, shade-dried and oven-dried at 30°C and 40°C), observed that these forms of drying cause significant differences in β-carotene levels for CLF from Baiana cultivar. The highest levels are found for the oven-drying process at 30°C (84.83 mg 100 g^{-1} DM) and the lowest ones for the shade-dried leaves (64.88 mg 100 g^{-1} DM). Consequently, the different levels of this substance can be probably attributed to plant age, cultivars and also to the forms of drying.

The average β-carotene levels in the CLF studied, independent of cultivar and plant age, ranged from 50.20 to 72.72 mg 100 g^{-1} DM and, when compared to those found in 100 g DM of other green leaves, unconventionally used, are comparable to those of sweet potato (40 to 120 mg) (Almazan and Begun, 1996), superior to those of carrot (8.70 mg) (Pereira et al., 2003) and lower than those of peanut leaves (100 to 140 mg) (Almazan and Begun, 1996). However, in relation to the levels in 100 g FM of green vegetables, these are higher than those of lettuce (1.37 mg), watercress (5.26 mg), green onion (2.31 mg) and parsley (3.82 mg) (Campos et al., 2003).

The consumption of natural antioxidants, such as vitamin C, polyphenols and β-carotene present in most plants, has been associated to a lower incidence of diseases caused by free radicals. Consequently, the levels of these substances found in these CLFs can contribute to their antioxidant capacity.

Antioxidant activity

The CLF antioxidant activity (AA) determined by the ABTS method for four cultivars, at TPA, is shown in Table 2.

It was possible to observe that AA by the ABTS method increased with plant maturity for all cultivars, in equivalent of trolox, as well as ascorbic acid, and it can be explained by an increase in vitamin C and polyphenol levels with cultivar maturity, which is substances that have a strong action in capturing ABTS. The highest AA was observed at 14 months for Pão da China cultivar, in equivalent of trolox (680.62 µmol L^{-1} g^{-1}), as well as ascorbic acid (102.42 mg g^{-1}), probably due to higher levels of vitamin C and phenolic compounds in their leaves at this age. However, Ouro do Vale presented the lowest AA, in equivalent of trolox, as well as ascorbic acid, at TPA, which can be explained by lower levels of antioxidants found in the flour of that cultivar.

It is observed that the CLF antioxidant potential by the ABTS method, when compared to BHT and rutin standards, in equivalent of trolox, as well as ascorbic acid, is considered of moderate potential, because at 14 months it reached on average, almost 50% of the potential of those standards, except for Ouro do Vale cultivar. In relation to quercetin, cultivar potential at any age was very inferior to the antioxidant potential of this standard.

The good antioxidant potential shown by CLF is

Table 2. Antioxidant activity of cassava leaf flour of four cultivars, by the ABTS method, at three plant ages.

Cultivar	μmol trolox L^{-1} g^{-1}			mg of vitamin C g^{-1}		
	10 months	12 months	14 months	10 months	12 months	14 months
Mocotó	238.62±1.05^{bC}	320.25±1.02^{cB}	636.12±1.41^{cA}	38.54±0.04^{bC}	49.11±0.10^{cB}	100.83±0.25^{cA}
Ufla	359.85±0.86^{aC}	444.65±0.59^{aB}	658.71±1.84^{bA}	50.11±0.12^{aC}	52.07±0.20^{aB}	101.20±0.03^{bA}
Pão da china	240.25±0.68^{bC}	338.15±0.67^{bB}	680.62±0.30^{aA}	38.79±0.03^{bC}	50.45±0.11^{bB}	102.42±0.58^{aA}
Ouro do Vale	155.90±2.39^{cC}	266.83±0.64^{dB}	327.27±0.08^{dA}	24.51±0.09^{cC}	44.60±0.13^{dB}	50.85±0.18^{dA}
BHT		1,418.81±97.98			259,55±3.27	
Quercetin		7,491.59±119.04			942,96±1.89	
Rutin		1,055.50±13.39			161,29±0.30	

Data are the mean of three replicates ± standard deviation. Lowercase letters in columns compare among cultivars and uppercase on the lines compare among ages. Same letters do not differ among themselves by the Scott-Knott test at 5% probability.

Table 3. Antioxidant activity of the cassava leaf flour of four cultivars, at three plant ages, in % inhibition by the β-carotene/linoleic acid method.

Cultivar	% inhibition*		
	10 months	12 months	14 months
Mocotó	93.04±3.14^{aA}	94.05±0.20^{aA}	89.81±0.19^{aB}
Ufla	85.17±0.96^{bA}	86.47±0.33^{bA}	84.61±0.66^{bA}
Pão da china	81.47±1.27^{cB}	85.51±0.63^{bA}	79.79±1.28^{cB}
Ouro do Vale	82.79±0.35^{cB}	85.69±0.80^{bA}	78.87±2.25^{cC}
BHT (200 mg L^{-1})	93.60±0.56		
Quercetin (200 mg L^{-1})	76.20±1.15		
Rutin (200 mg L^{-1})	19.21±0.88		

Data are the mean of three replicates ± standard deviation. Lowercase letters in columns compare among cultivars and uppercase on the lines compare among ages. Same letters do not differ among themselves by the Scott-Knott test at 5% probability. *The test was applied to the extracts at a concentration of 10,000 mg L^{-1}.

evidenced when compared to other studies, in which, independently of the extract, surpassed that AA found by Kuskoski et al. (2005), in frozen fruit pulps, in μmol trolox L^{-1} g^{-1} FM: acerola (66.5), mango (11.8), grape (8.5), guava (7.2) and passion fruit (1.02); that of two grape peels in DM: 'Isabel' (89.22) and 'Niágara' (157.31) according to Soares et al. (2008) and that of wine agroindustrial residues: (98.9), according to Cataneo et al. (2008). It also surpassed that detected by Bouayed et al. (2007) in several parts of medicinal plants, in mg g^{-1} vitamin C: 2.8 (*Alcea kurdica* flowers), 7.36 (*Valerian officinalis* root), 15.4 (*Stachys lavandulifolium* flowers), 19.2 (*Lavandula officinalis* leaves) and 19.3 (*Melissa officinalis* leaves), and the ones detected by Wojdylo et al. (2007) who, in 32 Polish herbs, verified potentials between 0.0045 (*Archangelica officinalis*) and 3.46 (*Syzygium aromaticum*) μmol trolox g^{-1}.

The lipidic oxidation inhibition results for CLF from four cultivars, by the β-carotene/linoleic acid method, at TPA after 2 h of reaction, are shown in Table 3. Plant maturity had little influence on the CLF AA, by the β-carotene/linoleic acid method. The greatest inhibition potential of lipid oxidation occurred at 12 months of age for all cultivars, which can be explained by higher levels of β-carotene (nonpolar antioxidant) in CLF at this age, since the β-carotene/linoleic acid method shows a better response to antioxidants with apolar character. Among them, 'Mocotó ' was that which presented the highest oxidation inhibition potential, at the TPA.

The inhibition potential of lipid oxidation presented by CLF was considered high, because, compared to the BHT standard, it was verified that Mocotó cultivar, at TPA, showed AA similar to that standard. However, other cultivars presented lower AA in relation to BHT. In relation to rutin and quercetin standards, the cultivars, at TPA, presented higher AA than those standards. Many authors relate antioxidant potential above 70% as optimum for lipid oxidation inhibition. All cultivars, at TPA, reached AA above 70% and, as such, present high lipid oxidation inhibition potential.

Melo et al. (2006) analyzing lipid oxidation inhibition potential of some plants, found an inhibition potential of 70% for broccoli in relation to BHT, but in other plants the potential was below 60%, emphasizing the high antioxidant

Table 4. Average levels of chlorophyll (SPAD units) for cassava leaf flour of four cultivars, at three plant ages.

Cultivar	Months		
	10	12	14
Mocotó	565.60±3.05^{dA}	479.80±9.07^{dB}	429.60±8.68^{dC}
Ufla	719.80±7.63^{bA}	631.20±5.72^{bC}	677.00±11.51^{bB}
Pão da China	589.40±10.43^{cA}	491.60±1.52^{cB}	468.40±13.67^{cC}
Ouro do Vale	771.00±5.20^{aA}	743.20±4.38^{aC}	758.40±0.63^{aB}

Data are the mean of three replicates ± standard deviation. Lowercase letters in columns compare among cultivars and uppercase on the lines compare among ages. Same letters do not differ among themselves by the Scott-Knott test at 5% probability.

Table 5. Correlation among antioxidant substances with antioxidant activity and chlorophyll in cassava leaf flour at three plant ages.

Antioxidant substances	Vitamin C	Polyphenols	β-carotene
ABTS (trolox)	0.78*	0.75*	0.28^{ns}
ABTS (vit C)	0.84*	0.70*	0.25^{ns}
β-carotene/linoleic acid	-0.20^{ns}	0.18^{ns}	0.20^{ns}
Chlorophyll	-0.40*	-0.71*	-0.45*

*Significant at 5% probability; ns = not significant.

potential of CLF in relation to other plants.

Chlorophyll

The SPAD-502 equipment has been investigated as an instrument for fast diagnosis of the nutritional state of various cultures, adding advantages such as simplicity of use, besides enabling a non-destructive evaluation of the foliar tissue substituting, with good precision, the traditional chlorophyll level determination in plants (Argenta et al., 2001).

Chlorophyll reading results for CLF at TPA, using the SPAD-502 portable meter, are in Table 4. It was verified that the highest chlorophyll levels were found at 10 months of age and that there was a tendency of reduction of those levels with plant maturity, Ouro do Vale, at TPA, was the one which presented the highest chlorophyll levels. Mocotó cultivar, at TPA, was that which showed significantly lower chlorophyll levels.

Correlation among antioxidant substances with AA and chlorophyll

Results of the correlations among antioxidant substances (Table 1) with AA (Tables 2 and 3) and with chlorophyll (Table 4) in CLF, at TPA, are shown in Table 5. The ABTS antioxidant tests (equivalent to trolox and vitamin C) presented a high positive correlation with vitamin C and with polyphenols, but there was no correlation with β-carotene. For the β-carotene/linoleic acid test, there was no correlation with antioxidant substances. The antioxidant substances presented a negative correlation with chlorophyll, that is, when antioxidant levels increased, chlorophyll contents decreased in the plant. This is easily explained for β-carotene, because, with the increase in maturity, chlorophyll begins to disappear and carotenoids protrude.

Results of the correlations indicate that vitamin C and polyphenols are the antioxidant substances that most contributed to the increase in AA for CLF; vitamin C stood out, therefore it presented the highest correlation coefficients, 0.78 and 0.84, for equivalent of trolox and vitamin C, respectively. β-carotene levels did not contribute to the CLF AA increase. The negative correlation among the antioxidant substances levels and chlorophyll indicate that antioxidant substances are found in a higher amount when cassava leaves present low chlorophyll levels.

Cataneo et al. (2008) and Soares et al. (2008) also observed a positive correlation between total phenols and AA by the ABTS test, equivalent to trolox, in studies performed with wine agroindustrial residues and grape skins of two varieties, respectively, indicating that polyphenols are substances with a high antioxidant potential and are one of the main antioxidants present in medicinal plants and food.

Kubola and Siriamornpun (2008) and Melo et al. (2008) found no correlation between the β-carotene/linoleic acid test and polyphenol contents in fruits. Duarte-Almeida et al. (2006) found that acerola, rich in vitamin C, showed a low AA by the the β-carotene/linoleic acid test, while the DPPH test (method of scavenging free radicals, which has the same principle of the ABTS test), showed a high AA, which demonstrates that vitamin C little contributes to AA in the β-carotene/linoleic acid test, confirming the results of the present study, in which a negative correlation between antioxidants (polyphenols and vitamin C) was also observed, as the β-carotene/linoleic acid test, which can be explained by the fact that the β-carotene/linoleic acid test shows a better response to nonpolar antioxidants, thus polar antioxidants (vitamin C and polyphenols), and has little contribution in the inhibition of lipid oxidation.

Conclusions

CLFs are rich in antioxidant substances and show high antioxidant capacity in the protection of lipid peroxidation; however, they present moderate capacity in the capture of free radicals. Cassava leaves at 14 months of age present the highest antioxidant levels, and Mocotó and Pão da China cultivars stood out. The highest antioxidant levels (Vitamin C, polyphenols and β-carotene) are found when the cassava leaf presents low chlorophyll levels.

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