

Full Length Research paper

Evaluating Total Carotenoid and β -Carotene Degradation in Varieties of Bitter Yellow Cassava (*Manihot esculenta* Crantz)

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The assessment of the variability of total carotenoid, β -carotene, all-*E*, and 13 and 9-*Z*- β -carotene isomers in twelve bitter yellow cassava was carried out, as well its degradation in five varieties after flour processing and during storage. HPLC and UV/ visible spectrophotometry were used in sample analyses. Varieties of bitter yellow cassava presented variation in the total carotenoid contents from 1.97 - 16.33 $\mu\text{g}/\text{bg}$ and the β -carotene contents varied from 1.37 - 7.66 $\mu\text{g}/\text{g}$. The 13, 9-*Z* and all-*E*- β -carotene isomers were found in all varieties, being all -*E*- β -carotene the predominant one. The mean degradation among the roots after processing was of 50%. Total carotenoid complete degradation was observed with less than 30 storage days. It was also observed that the total carotenoid contents in yellow bitter cassava roots varied, probably due to the characteristics of the used varieties. Heat during processing, light and oxygen might have been the agents that most contributed to flour total carotenoids degradation.

Key words: Bitter yellow cassava, β -carotene, carotenoid, flour.

INTRODUCTION

Cassava plant (*Manihot esculenta* Crantz) belongs to the *Euphorbiaceae* family, being originated from South America where it was cultivated by the Indians who were responsible for its dissemination almost all over America. Portuguese spread it to other continents, especially Africa and Asia. Nowadays, in the African, Latin American and Asian continents it is still one of the main caloric food to nearly 500 million people, mainly in under developed countries (Maduagwu et al., 2002; FAOSTAT, 2008).

In Brazil it is estimated that 80% of the total cassava root production is designated for cassava flour, an average of 23 million tonnes. The lack of official statistics and the existence of small and informal producers cause

a bias in the estimate of total production. The variability of the different types of cassava flour in Brazil is extremely large, making its commercialization very difficult, as there is yellow cassava flour in the North Region and white cassava flour in the rest of the country. The consumption of fresh cassava traditionally occurs throughout the country. Nowadays cassava is frozen, fried or boiled (Silva Souza and Faria, 2005; FAO, 2008).

The products used mainly for human consumption and derived from yellow bitter cassava in the world are traditional flour (peeled roots are grated, pressed and the residue dried in special device at 160°C for 50 min.), gari flour (similarly processed as the traditional flour where unpeeled roots are fermented instead of peeled root), cassava dried starch (peeling, washing, grating, extraction of starch, drying, milling) for bread and snacks industries, cassava chips (slices of 0.1 cm of peeled root were fried in oil at 180°C) and the tapioca obtained from cassava starch and used in the preparation of puddings and infant

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feed (Oliveira et al., 2008; Silva et al., 2008; Gameiro, 2002).

In the last ten years, expressive efforts have been made aiming the identification of new varieties with high provitamin A (β -carotene) carotenoid contents which might contribute to the improvement of the nutritional status of populations located in the tropics particularly in the Brazilian Northeast, with severe malnutrition issues, where bitter cassava constitutes one of the main cultivations, being almost their only nutrient source. Thus, besides carbohydrates, yellow bitter cassava may be an excellent source of provitamin A carotenoid (Iglesias et al., 1997; Bedoya, 1999; Chávez et al., 1999). Studies on carotenoids in yellow cassava roots revealed β -carotene contents above 90%, becoming an important finding as it can be transformed by human organism into vitamin A (Devlin, 1997). β -carotene can also be found in bitter cassava leaves (Ortega-Flores et al., 2003). Besides the high carotenoid levels, bitter cassava has demonstrated to have promising quantities of proteins and minerals (Bedoya, 1999; Chávez et al., 1999; Devlin, 1997; Pereira et al., 2005; Ceballos et al., 2006; Oliveira et al., 2008a and b). In order to minimize malnutrition, HarvestPlus, an international program that involves a global alliance of research institutions and implementing agencies in developed and in developing countries and seeks to reduce micronutrient malnutrition by harnessing the power of plant breeding to develop staple food crops rich in micronutrients, supports many researches in order to enhance the carotenoid content of yellow bitter cassava roots varieties in Brazil as well as other potential sources of micronutrients (maize, rice, orange flash sweet potato, common beans and wheat).

Studies regarding the assessment of total carotenoid and β -carotene before and after the processing and home cooking methods of sweet and bitter yellow cassava roots are being carried out (Oliveira et al., 2008b; Nascimento et al., 2008; Kimura et al., 2008; Rangel et al., 2008). On the other hand, many other studies were carried out in order to use the biofortified yellow cassava flour to produce non expanded pellets (Gameiro, 2002), bread (sandwich loaves) and English cakes with wheat flour mixed with cassava flour to improve the nutritional value of processed food (Rangel et al., 2008; Clydesdale et al., 1970).

The objectives of the present work were to assess the variability of total carotenoid and β -carotene contents in 12 varieties of raw yellow bitter cassava roots, the all-*E*- β -carotene and 13 and 9-*Z*- β -carotene isomers, to determine the total carotenoid degradation in five varieties after the toasted flour process and the heat treatment effect on degradation.

MATERIALS AND METHODS

Materials

Seventeen bitter yellow cassava roots obtained by conventional

breeding, cultivated at Embrapa cassava and tropical fruits, located at Cruz das Almas, State of Bahia, Brazil were used. Harvest was done after 12 months of planting. Roots were washed to remove dirt excess, being dried and immersed in liquid paraffin at 50 - 60°C for 45 s. The procedure was repeated three times to form a thick paraffin layer.

Experiments were carried out at Embrapa Food Technology, located at Rio de Janeiro, Rio de Janeiro State, Brazil where the roots were received and maintained under refrigeration at 4°C in order to guarantee their preservation.

Twelve varieties of yellow bitter cassava: 61 (Crueira), 878 (Cachimbo I), 893 (Imari III), 949 (Pretinha II), 991 - IM 222 (Juba), 1138 - IM 147, 1140 - IM 217, 1700 (Varejão), 1704 - IM 936 (Canço), 1705 (Juriti), 1711 (Arari), and 1785 (Flor do Brasil) were assessed regarding total carotenoid, β -carotene and their all(*E*), 13 and 9-*Z*- β -carotene isomers contents.

The following five varieties were used for toasted flour processing: 1783, 1757 (Anajazinha), 1752 (Flor do Brasil1), 1740 (Najacaiá), and 1751 (Bonita), which were assessed with regard to the total carotenoid contents in raw roots, as well their degradation in storage times: Day 0 (processing day), 5th, 12th, 19th, and 26th days, respectively.

Isolation of β -carotene standard for HPLC quantification

β -carotene standard was isolated from carrot (*Daucus carota* L.), recognizably one of the best α and β -carotene sources by open column chromatography (Ihl et al., 1998; Dutta et al., 2005; Rodriguez-Amaya and Kimura, 2004).

Carrot was peeled, sliced with an inox knife and ground in a vertical mixer (Black and Decker, Kmvsb40t model, São Paulo, Brazil). About 50 grams of homogeneous sample were weighed in a mortar and 15 grams of celite 454 (Hyflosupercel, Tedia, Ohio, USA) were added.

Carotenoid extraction was performed with the addition 25 ml of cold acetone (Tedia, HPLC grade, Ohio, USA), being grounded until a homogenized paste was formed. Then, this extract was filtered through a sintered glass funnel coupled to a 250 ml Büchner flask and filtered under vacuum. This procedure was repeated three times.

The extract was transferred to a 500 ml separatory funnel with a Teflon stop-cock, containing 40 ml petroleum ether (Tedia, HPLC grade, Ohio, USA) and ultra-pure water (MilliQ[®] Millipore, Massachusetts, USA) was added (about 200 ml). This procedure was repeated four times.

Extract was transferred to a 250 ml volumetric flask with the help of a funnel containing about 15 g of anhydrous sodium sulphate to remove residual water and evaporated until 3 ml in a rotary evaporator (Jank KI, Tkunkel, Germany) coupled to a water-bath under a temperature less than 36°C.

For carotenoid separation, a glass column for open chromatography was prepared by mixing equal parts (1:1) of magnesium oxide (Across Organics, Geel, Belgium) and celite 454. The resulting mixture was put for four hours in an oven at 110°C for its activation and further glass column packaging (25 x 300 mm) containing glass wool. The resulting mixture was let to cool and transferred to the column until it completed $\frac{2}{3}$ of the height. After the mixture was compacted in the column, a Buchner flask was adapted under vacuum for one hour. At the end of packaging, anhydrous sodium sulphate was added to complete 1 cm of height.

The concentrated carotenoids extract was quantitatively transferred to the open column top and petroleum ether was slowly added under vacuum until α - carotene (orange) was separated from β - carotene (orangish yellow).

After the initial fraction (head) and end (tail) withdrawal, β -carotene fraction was filtered through a 5 μ m sintered glass funnel coupled to a Büchner flask under vacuum for separation of

celite/magnesium oxide and β -carotene, extracted with acetone until it was colorless. β -carotene fraction was transferred from Büchner flask to a separatory funnel containing petroleum ether (40 ml) and washed three times with water. The extract was transferred to a 50 ml volumetric flask in a funnel containing 15 g of anhydrous sodium sulphate. In order to avoid β -carotene extract oxidation, 0.05 g of butylated hydroxytoluene (BHT) (0.1%) was added. Absorbance was measured at 450 nm and the concentration was calculated according to the formula below:

$$C (\mu\text{g/g}) = \frac{A \times 10^4}{A_{1\%}^{1\text{cm}}}$$

where: A = absorbance, $A_{1\%}^{1\text{cm}} = 2592$ (Absorption coefficient of β -carotene in petroleum ether).

A 2 ml aliquot from β -carotene extract was taken for verification of its purity, being dried under N_2 , dissolved in 1 ml of acetone, and injected in the chromatograph (HPLC).

Purity determination of β -carotene extract was performed according to the formula below:

$$\% \text{ Purity} = \frac{\text{Standard peak area} \times 100}{\text{Total area}}$$

β -carotene extract concentration was corrected according to the formula below:

$$C (\mu\text{g/g}) = \frac{C (\mu\text{g/g}) \times \% \text{ Purity}}{100}$$

A standard calibration curve was constructed, in triplicate, with β -carotene extract aliquots of 1, 2, 3, 4 and 5 ml. The aliquots were dried under N_2 , and 1 ml of acetone was added for the injection of 10 μl in triplicate.

Sample preparation

The bitter yellow cassava samples were placed at room temperature for approximately 10 min; the layer of paraffin was removed and peeled.

Then, the samples were divided in four parts through two longitudinal cuts, from one extremity to its opposite, and four sections were obtained. Among these, two opposite sections were discarded and the remaining were used for analysis, and homogenized in a vertical mixer (Black and Decker, model Kmvsb04t, São Paulo, Brazil) for obtainment of a homogenous mass (Davies, 1976).

Total carotenoid and β -carotene analyses

Total carotenoid contents analysis was carried out by UV-visible spectrophotometry (Specord 210, Analytikjena model Torre Boldone BG, Italy) and the absorbance was taken at 450 nm.

β -carotene content analyses of the standard and samples, as well as their all-*E* and *Z* isomers, were conducted by high performance liquid chromatography (HPLC) in a Waters 2695 Chromatograph - Alliance Model, Milford, USA, and a model 2966 Waters UV/Visible photodiode array detector controlled by a Empower[®] software. The absorbance was taken from 350 - 600 nm. The reversed-phase column used for the analyses was the C₃₀ YCM[®] Carotenoid S-3 (4.6 x 250 mm; particle size, 5 μm) from

water. The mobile phase was composed of 80% of methanol (Tedia, HPLC grade, Ohio, USA) and 20% of methyl tert-butyl ether (MTBE) (Tedia, HPLC grade, Ohio, USA). Analysis conditions were: 0.8 ml/min flux, 25 μl automatic injection of the sample extract, temperature of 30°C and total analysis time of 60 min. The same procedure described for total carotenoids and β -carotene isomers extraction was employed for the samples.

The extract was transferred to a 500 ml separatory funnel with a Teflon stop-cock, containing about 40 ml of petroleum ether. Acetone removal from the extract was performed with ultra pure water, slowly added to avoid emulsion formation. The aqueous phase was discarded and this procedure was repeated four times until no acetone residue was left.

The petroleum ether phase was transferred to a 50 ml volumetric flask in a funnel containing 15 g of anhydrous sodium sulphate to remove the residual water. The carotenoid extract was made up to volume with petroleum ether and the absorbance was taken at 450 nm. The total carotenoid content was calculated according to Davies, 1976. The formula to determine the total carotenoid content is shown below:

$$\text{Content of carotenoids } (\mu\text{g/g}) = \frac{A \times V (\text{mL}) \times 10^4}{A_{1\%}^{1\text{cm}} \times W (G)}$$

where: A= absorbance, V= total extract volume, W = sample weight, 2592 (β -carotene absorption coefficient in petroleum ether).

β -carotene isomers quantification

For β -carotene and its 9 and 13-*Z* and all-*E* isomers quantification, a 2 ml extract aliquot was withdrawn and dried in an amber vessel under nitrogen flux. The sample (dried extract) was diluted in 100 μl of acetone under stirring in vortex (Genie 2 - Scientific Industries, Bohemia, NY, USA), and transferred to a 2 ml amber vessel for HPLC analyses.

Yellow bitter cassava flour processing

Cassava flour was processed in pilot scale, with capacity for 5 kg of roots by batch. The processing unit was equipped with a rotary grinder, a manual press, a gas oven (propane/butane), a rotary wooden palette and a copper bowl. Oven temperature varied from 120 - 150°C during 30 min until the obtainment of toasted flour.

Flour processing had the following steps: (a) Washing of the roots for withdrawal of the field derived dirt, (b) Manual peeling with inox knives, (c) Washing followed by immersion of the roots in water until processing, in order to avoid enzymatic oxidation (darkening of the roots) after peeling, (d) Roots were grated and a humid mass was obtained and then transferred to polyethylene bags, which allowed water flowing, (e) Manual press for water withdrawal, (f) the resulting pie was crumbled with the help of a grater, (g) Drying and toasting steps.

Degradation kinetics assessment

In order to observe the total carotenoids degradation kinetics in the flour, the following formula was applied: $dC = -K \cdot C \cdot dt$. Integrating the above formula: $\text{Log} (C_0/C) = K / 2.303 \cdot t$.

Statistical analysis

All analyses were carried out in triplicate. The results are given as

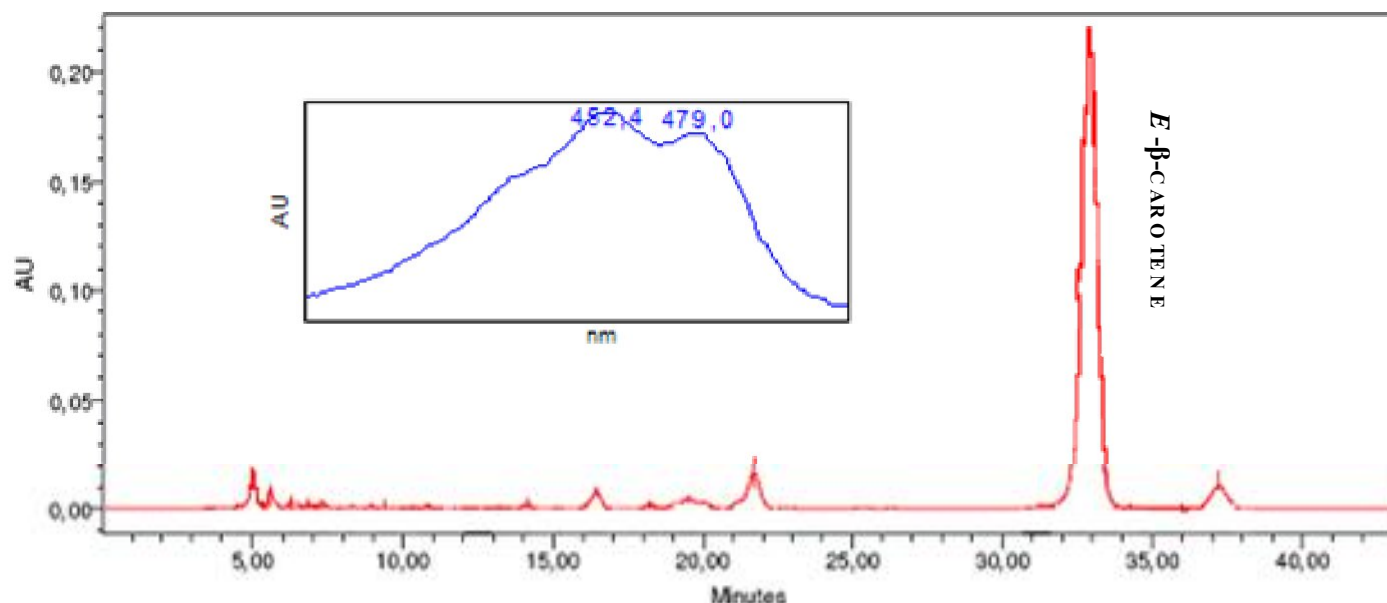


Figure 1. Chromatogram of the β -carotene standard extracted from *Daucus carota* L. determined by HPLC and all-*E*- β -carotene UV/visible spectrum.

means \pm standard deviation (SD). Tukey test was used for comparison between two means and a one-way analysis of variance (ANOVA) was used for comparison of more than two means. A difference was considered statistically significant when $p \leq 0.05$. Pearson correlation at $p < 0.01$ was used for concentration reduction along the storage time by ANOVA in flour cassava samples.

RESULTS AND DISCUSSION

β - carotene standard

The β -carotene standard from carrot (*D. carota* L) presented 93.99% chromatographic purity verified by HPLC (Figure 1). According to Rodriguez-Amaya and Kimura (2004), β -carotene standards must contain a 90% purity level, thus the result of the present work was compatible and satisfactory.

The obtained equation from a tendency line with linear regression generated a calibration curve equal to $Y = 4.05 \cdot 10^{-5} \cdot X + 5.52 \cdot 10^{-4}$ with 0.9826 (R^2). This correlation is in accordance to Rodriguez-Amaya and Kimura (2004) for carrot.

Raw yellow bitter cassava total carotenoid and β -carotene isomers

In Table 1, total carotenoid, total β -carotene, 13 and 9-*Z* and all-*E*- β -carotene isomers contents of the twelve varieties of raw yellow bitter cassava roots may be observed as well as in Figure 2, the chromatogram of the 1138 - IM 147 sample.

The raw yellow bitter cassava varieties presented total carotenoid contents ranging from 1.97 (1785 - Flor do Brasil) to 16.33 $\mu\text{g/g}$ (878 - Cachimbo I), respectively. The 1785 - Flor do Brasil variety presented a much lower total carotenoid content when compared to the other varieties. Excluding the 1785 - Flor do Brasil variety, the range of total carotenoid would be comprised in a smaller interval, thus varying from 8.13 (1705 - Juriti) to 16.33 $\mu\text{g/g}$ (878 - Cachimbo I). The total carotenoid content of the 949 - Pretinha II raw roots (13.36 $\mu\text{g/g} \pm 0.55$) was similar to ones found by Oliveira et al. (2008) for the same variety (12 $\mu\text{g/g}$).

It can be observed that total β -carotene content (sum of the contents of the all-*E*, 13 and 9-*Z*- β -carotene) varied from 1.37 (1785 - Flor do Brasil) to 7.66 $\mu\text{g/g}$ (991 - IM 222 - Juba), respectively. Similar results were found by Thakkar et al. (2007) in ten different genotypes of yellow fleshed cassava.

Despite the fact that the highest total carotenoid content was found in the 878 (Cachimbo I), this variety was not the one which presented the highest total β -carotene content.

Although a larger amount of samples was used in this study, the variation ranges of total carotenoid (1.97 - 16.33 $\mu\text{g/g}$) and β -carotene (3.11 - 7.66) were lower than those reported by Chávez et al. (2007) who, while assessing three clones of yellow bitter cassava roots (CM2772-3, MBRA1324 and MCOL2401), found total carotenoid variation from 10 - 22 $\mu\text{g/g}$ and β - carotene from 7 - 13 $\mu\text{g/g}$, respectively with indexes from 60 - 70% of β - carotene in relation to total carotenoid. These differences may be due to the fact that clones with an

Table 1. Total carotenoids and total β -carotene, all-*E*, 13 and 9-*Z*- β -carotene isomers in contents¹ ($\mu\text{g/g} \pm \text{SD}$) in raw bitter yellow cassava roots.

Samples	Total carotenoids ($\mu\text{g/g}$) \pm SD	Total β -carotene ($\mu\text{g/g}$) \pm SD	All- <i>E</i> - β -carotene ($\mu\text{g/g}$) \pm SD	13- <i>Z</i> - β -carotene ($\mu\text{g/g}$) \pm SD	9- <i>Z</i> - β -carotene ($\mu\text{g/g}$) \pm SD
878 - Cachimbo I	16.33 \pm 0.87 (a)	4.13 \pm 0.04 (b)	3.04 \pm 0.03 (c)	0.81 \pm 0.10 (d)	0.28 \pm 0.04 (e)
991 - IM 222 - Juba	15.15 \pm 0.01 (a)	7.66 \pm 0.45 (b)	5.11 \pm 0.34 (c)	1.24 \pm 0.06 (d)	1.32 \pm 0.05 (e)
1140 - IM 217	14.85 \pm 0.21 (a)	5.84 \pm 0.83 (b)	3.91 \pm 0.52 (c)	0.93 \pm 0.23 (d)	1.00 \pm 0.08 (d)
949 - Pretinha II	13.36 \pm 0.55 (a)	4.44 \pm 0.14 (b)	2.75 \pm 0.01 (c)	0.93 \pm 0.15 (d)	0.76 \pm 0.02 (e)
61 - Crueira	12.62 \pm 0.03 (a)	4.66 \pm 0.37 (b)	3.07 \pm 0.04 (c)	0.69 \pm 0.17 (d)	0.90 \pm 0.16 (e)
1711 - Arari	12.16 \pm 0.39 (a)	3.25 \pm 0.03 (b)	2.40 \pm 0.04 (c)	0.80 \pm 0.01 (d)	0.05 \pm 0.01 (e)
1700 - Varejão	11.40 \pm 0.93 (a)	4.16 \pm 0.23 (b)	2.69 \pm 0.08 (c)	0.70 \pm 0.27 (d)	0.78 \pm 0.03 (d)
893 - Imari III	11.29 \pm 0.03 (a)	6.05 \pm 0.14 (b)	3.49 \pm 0.08 (c)	0.94 \pm 0.01 (d)	1.61 \pm 0.05 (e)
1138 - IM 147	10.17 \pm 0.65 (a)	4.34 \pm 0.55 (b)	2.68 \pm 0.35 (c)	0.93 \pm 0.10 (d)	0.73 \pm 0.10 (e)
1704 - IM 936 - Caniço	8.95 \pm 0.29 (a)	3.11 \pm 0.03 (b)	2.10 \pm 0.01 (c)	0.50 \pm 0.03 (d)	0.51 \pm 0.01 (d)
1705 - Juriti	8.13 \pm 0.23 (a)	3.17 \pm 0.53 (b)	2.26 \pm 0.39 (c)	0.49 \pm 0.08 (d)	0.42 \pm 0.06 (d)
1785 - Flor do Brasil	1.97 \pm 0.20 (a)	1.37 \pm 0.01 (b)	0.76 \pm 0.02 (c)	0.22 \pm 0.01 (d)	0.39 \pm 0.01 (e)

¹ β -Carotene and isomers analyzed by HPLC and total carotenoid by UV/ vis. spectrophotometry. The results are the mean of duplicate.

HPLC condition : C₃₀ YCM® Carotenoid S-3 column, mobile phase methanol: methyl tert-butyl ether (MTBE) (80:20), 0.8 ml/min flux, 25 μL and 30°C temperature.

SD = standard deviation.

Same letters in the same line, indicate no significant difference at 0.05 level (Tukey test).

The statistical analysis were performed by OriginPro 8 software, One-Way ANOVA, Tukey Test - significance level (0.05).

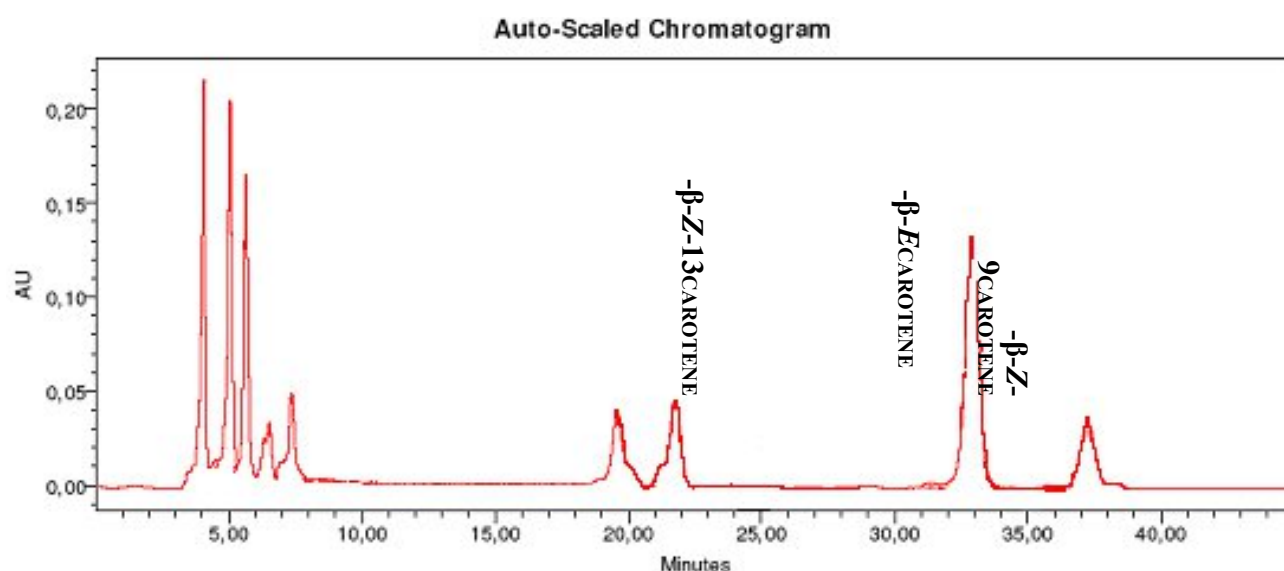


Figure 2. Chromatogram of the yellow bitter cassava 1138 - IM 147 with the 13-*Z*, all-*E* and, 9-*Z*- β -carotene isomers.

intenser color were selected for assessment, whereas in the present work there was the preoccupation of assessing a higher amount of varieties without a previous coloration selection according to Chávez et al. (1999). They also observed that the yellow intensity of the roots has a correlation with the total carotenoid content.

It can be considered that other factors, besides the root color, may quantitative and qualitatively affect the total carotenoid content in the same varieties such as the maturation stage, weather conditions, type of cultivation

soil, and planting conditions (Davies, 1976). On the other hand, the β -carotene percentages were lower than those in yellow bitter cassava roots (83%), cv. BRA 005771 (Chávez et al., 1999).

The all-*E*- β -carotene contents varied from 0.76 (1785 - Flor do Brasil) to 5.11 $\mu\text{g/g}$ (991 - IM 222 - Juba), respectively. All-*E*- β -carotene was the most predominant isomer in all the varieties assessed.

The 13-*Z*- β -carotene contents varied from 0.22 (1785 - Flor do Brasil) to 1.24 $\mu\text{g/g}$ (991 - IM 222 - Juba),

Table 2. Total carotenoids content¹ (µg/g ± SD) in raw bitter yellow cassava and flour.

Storage time	1757	1783	1752	1740	1751
	Anajazinha		Flor do Brasil I	Najacaiá	Bonita
Raw roots	3.65 ± 0.32	9.61 ± 0.06	11.63 ± 0.06	16.32 ± 0.01	18.92 ± 0.01
0 day - process	1.05 ± 0.29	6.43 ± 0.01	6.07 ± 0.01	8.23 ± 0.01	9.07 ± 0.01
5 days - storage	0.42 ± 0.18	1.63 ± 0.21	5.02 ± 0.01	2.20 ± 0.02	2.17 ± 0.01
12 days - storage	0.25 ± 0.06	nd	1.80 ± 0.21	0.88 ± 0.01	1.20 ± 0.03
19 days - storage	nd	nd	0.16 ± 0.09	nd	nd
26 days - storage	nd	nd	nd	nd	nd

nd: not detectable.

¹Total carotenoid contents were analyzed by spectrophotometry. The results are the average duplicate.

Table 3. Total carotenoids flour degradation (%) after process and during storage.

Storage time	0 day - Flour process	5 days	12 days	19 days	26 days
1757 Anajazinha	71 ^(a)	60 ^(a)	76 ^(a)	99 ^(a)	-
1783	33 ^(b)	75 ^(b)	89 ^(b)	-	-
1752 - Flor do Brasil I	48 ^(c)	17 ^(c)	70 ^(c)	86 ^(c)	-
1740 - Najacaiá	50 ^(d)	74 ^(d)	89 ^(d)	98 ^(d)	-
1751 - Bonita	52 ^(e)	76 ^(e)	87 ^(e)	97 ^(e)	-

a, c, d and e : samples have no Pearson correlation at $p < 0.01$ were found despite the occurrence of concentration reduction along the storage time by ANOVA statistical analysis.

b: samples were a Pearson correlation of $R^2 = 0.91$ with $p < 0.01$ were found due to the occurrence of the total carotenoid concentration reduction along the time by ANOVA statistical analysis.

respectively, and those of 9(Z) from 0.05 (1711 - Arari) to 1.61 µg/g (893 - IMARI III), respectively.

Variety 991 - IM 222 - Juba was the one which presented the highest β-carotene isomers contents among all varieties. Similarly, Kimura et al. (2007) reported the presence of the same isomers in the yellow bitter cassava BRA 005771 variety, despite not having quantified them, being the all-*E*-β-carotene isomer prevalence also observed in relation to the 13 and 9-Z isomers.

Besides the natural occurrence of these isomers in the studied varieties, isomerizations may occur during the analyses, which would justify the content of isomers found in the 1785 (Flor do Brasil) variety. This occurrence was suggested by Schieber (2005), Giuliano (2002) and Aman et al. (2005).

Total carotenoid degradation kinetics in yellow bitter cassava flour

The total carotenoid contents varied from 3.65 (1757 - Anajazinha) to 18.92 µg/g (1751 - Bonita), respectively. It was observed that despite the fact that the 1751 - Bonita variety had presented a higher total carotenoid content, its percentage of degradation was 87% on the 12th storage day. The results of the assessment of total

carotenoid degradation on a dry weight basis, may be observed in Table 2.

Mean total carotenoid degradation in all the roots was 50% after processing. Exposure to heat during flour processing was the main agent of total carotenoid degradation (oxidation) since the temperature was in the 120 -150°C range for about 30 min. It is well knowing that carotenoid degradation occurs in temperatures close or superior to 40°C (Hiane et al., 2003; Thakkar et al., 2009).

Degradation of total carotenoid was continuous even during storage in the absence of light, being extinguished on the 12th day in the flour of the 1783 variety, on the 19th day in the 1757 (Anajazinha), 1740 (Najacaiá), and 1751 (Bonita) varieties, and on the 26th day in the 1752 - Flor do Brasil variety, showing about 90% of degradation on the 19th day (Table 3).

Oliveira et al. (2008) observed total carotenoid degradation on traditional flours from Gema de Ovo, Dourada, 1709, and 949 varieties after the 15th storage day.

Other factor which may have influenced the carotenoid degradation, could be the stage of grinding/grating which exposes their cellular content to the environment (oxygen) in the passage through the grater, thus facilitating the oxidative process.

In a general way, the scarcity of literature regarding the

total carotenoid and β -carotene degradation in yellow bitter cassava made it difficult to compare the results in the present work. Nonetheless some authors reported deleterious effects of the oxygen on these micronutrients in other raw materials.

Hiane et al. (2003) observed that after the Bacuri fruit (*Scheelea phalerata*, Mart.) flour processing by drying the pulp with ventilation, and temperature of 60°C for two days, a lower β -carotene (38%) percentage loss was observed as regards the fruit in *natura*. Since the temperature was lower, the carotenoid degradation in the present work occurred due to the temperature of 150°C used in the bitter cassava flour process.

Total carotenoid degradation in the samples of yellow bitter cassava flour was continuous along the time. This behaviour was also observed in other matrixes following a first order model (Tsimidon and Biliadesis, 1993 - in saffron; Moreno-Alvarez et al., 2000 - in papaya; Gabas et al., 2003 - in plums).

Degradation of total carotenoid in relation to the storage time by the ANOVA statistical analysis revealed that in four varieties: 1740 (Najacaiá), 1751 (Bonita), 1752 (Flor do Brasil), and 1783, a correlation at $p < 0.01$ was not found despite the occurrence of concentration reduction along the storage time.

In the 1757 (Anajazinha) variety a Pearson correlation of $R^2 = 0.91$ with $p < 0.01$ was found, due to the occurrence of the total carotenoid concentration reduction along the time.

Conclusion

On account of the results we may presume that other factors influenced the total carotenoid degradation such as package permeability to oxygen since the samples had not been wrapped up under vacuum, maintenance of the samples under refrigeration, and temperature of the storage room. On the other hand, these factors were not taken into account as the proposition of the study was based on the commercial conditions of storage of this type of product. The total carotenoid contents of raw yellow bitter cassava roots varied due to the characteristics of the used varieties. The all-*E*, 13 and 9-*Z*- β -carotene isomers were found in all raw yellow bitter cassava roots and the all-*E*- β -carotene isomer was predominant with respect to the others, but the contents of the 13 and 9-*Z*- β -carotene isomers were expressive in relation to total carotenoid of some varieties. The total carotenoid degradation in yellow bitter cassava flour was completed between the 12th and 19th days of storage in four of the five analyzed varieties. Heat, light and oxygen may have been the agents which contributed to degradation being the oxidation process not interrupted during storage. Hence, there is the necessity to optimize the drying process which will minimize the total carotenoid loss taking into account that this is a largely consumed staple food by the low income Brazilian

population, that not only presents deficiency of these nutrients but also has less access to other sources of Vitamin A carotenoid precursors.

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