



## Carotenoids in the pulp and peel of bananas from 15 cultivars in two ripening stages<sup>1</sup>

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### ABSTRACT

The current study aims to evaluate the occurrence and concentration of carotenoids in the pulp and peel of 14 banana and one plantain cultivars in two ripening stages. We analyzed the occurrence and content of lutein,  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and lycopene – by high performance liquid chromatography with UV-visible detection, the content of total carotenoids by spectrophotometry. In the unripe pulp, the ‘Ouro’ cultivar stood out with lutein concentration 13 times higher than that of the ‘Marmelo’. As for  $\alpha$ -carotene and  $\beta$ -carotene, ‘Terrinha’ plantain stood out with mean concentration of 1195.30 and 1126.11  $\mu\text{g}$  (100 g MF<sup>-1</sup>), respectively. Total carotenoids ranged from 159.66 to 2553.51  $\mu\text{g}$  (100 g MF<sup>-1</sup>) in ‘Caipira’ and ‘Terrinha’, respectively. In the ripe pulp there was 36% increase in the lutein content in comparison to the unripe pulp and there was 7.3 and 8.5% reduction in  $\alpha$ -carotene and  $\beta$ -carotene levels, respectively. The total carotenoid concentration in the ripe pulp was 17% higher than that found in the unripe pulp. The unripe peel showed lutein predominance, although ‘Terrinha’ stood out with higher  $\alpha$ -carotene and  $\mu$ -carotene concentrations than those found in the other cultivars. Lutein concentration hardly changed due to fruit ripening; however, there was slight reduction in  $\alpha$ -carotene and  $\beta$ -carotene concentrations.

**Keywords:** *Musa* spp.; provitamin A; lutein;  $\alpha$ -carotene;  $\beta$ -carotene.

### RESUMO

#### Carotenoides em polpas e cascas de bananas de 15 cultivares em dois estágios de maturação

Este estudo objetivou caracterizar a dinâmica, além das mudanças estruturais e florísticas em uma comunidade arbAvaliou-se a ocorrência e a concentração de carotenoides na polpa e na casca dos frutos de 14 cultivares de bananeira e um de plátano em dois estágios de maturação. Luteína,  $\alpha$ -caroteno,  $\beta$ -caroteno,  $\beta$ -criptoxantina e licopeno foram quantificados por cromatografia líquida de alta eficiência (CLAE), carotenoides totais por espectrofotometria. Na polpa verde, a banana ‘Ouro’ destacou-se com concentração de luteína 13 vezes maior que a da ‘Marmelo’. Para  $\alpha$ -caroteno e  $\beta$ -caroteno, o plátano ‘Terrinha’ se destacou com concentração média de 1.195,30 e 1.126,11  $\mu\text{g}$  (100 g MF<sup>-1</sup>), respectivamente. Os carotenoides totais oscilaram de 159,66 a 2.553,51  $\mu\text{g}$  (100 g MF<sup>-1</sup>), para ‘Caipira’ e ‘Terrinha’, respectivamente. Na polpa madura, houve acréscimo de 36% no teor médio de luteína em relação à polpa verde e redução de 7,3 e 8,5% nos teores médios de  $\alpha$ -caroteno e de  $\beta$ -caroteno, respectivamente. A concentração média de carotenoides totais na polpa madura foi 17% superior à da polpa verde. Na casca verde houve predominância da luteína, embora a ‘Terrinha’ tenha se destacado com concentrações de  $\alpha$ -caroteno e  $\beta$ -caroteno superior às dos demais cultivares. A concentração de luteína praticamente não se alterou com o amadurecimento dos frutos, no entanto houve discreta redução nas concentrações de  $\alpha$ -caroteno e  $\beta$ -caroteno.

**Palavras-chave:** *Musa* spp.; provitamina A; luteína;  $\alpha$ -caroteno;  $\beta$ -caroteno.

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## INTRODUCTION

Carotenoids are known for their wide distribution, structural diversity and multiple functions. Animals are unable to biosynthesize carotenoids. Thus, they depend on dietary provitamin A carotenoids, which can be absorbed and converted into vitamin A after ingestion (Rodríguez-Amaya & Kimura, 2004). Besides being vitamin A precursors, some carotenoids present a number of health benefits such as the prevention of certain cancer types, the ability to prevent photosensitization in certain skin diseases, the increased immune response to certain infection types as well as anti-aging properties (Kurahashi *et al.*, 2009).

The main carotenoids found in food are  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin, lutein, lycopene and zeaxanthin (Rodríguez-Amaya & Kimura, 2004). Among them, only  $\beta$ -carotene,  $\alpha$ -carotene and  $\beta$ -cryptoxanthin may convert into vitamin A in the body (Davey *et al.*, 2009; Newilah *et al.*, 2009). The presence of lutein,  $\alpha$ -carotene and  $\beta$ -carotene has been reported in bananas (Wall, 2006; Davey *et al.*, 2009; Newilah *et al.*, 2009; Ekese *et al.*, 2012; Lokesh *et al.*, 2014). Thus, two provitamin A carotenoids may be found in bananas, in addition to lutein, which is known as macular carotenoid (Davey *et al.*, 2009).

Vitamin A deficiency is a major public health issue worldwide. It is more pronounced in developing countries and it mainly affects children, women in reproductive age and pregnant women (Davey *et al.*, 2009; Ekese *et al.*, 2012). Thus, yellow and red fruits with high carotenoid concentration play a key role in preventing this deficiency, especially in regions where there is little food diversification or in those lacking provitamin-carotenoids-rich foods. One of the sustainable ways to mitigate the vitamin A deficiency issue is by encouraging the consumption of natural carotenoids-rich foods such as fruits and dark-green-leaf vegetables (Ekese *et al.*, 2012).

Banana production in Brazil is mainly concentrated in approximately six cultivars: 'Prata-Anã', 'Nanica', 'Nanicão', 'Pacovan' and to a lesser extent, 'Maçã' and 'Ouro'. However, there is huge diversity of cultivars known by the population, which are no longer cultivated, or which are cultivated in subsistence scale due to unfavorable agronomic features. Most of these cultivars, especially in Brazil, were not evaluated for the chemical composition of the fruits. Thus, it is imperative to evaluate the occurrence and to quantify the carotenoids concentrations found in a reasonable number of cultivars. In addition, studies evaluating the composition of banana cultivars in Brazil quantify total carotenoids alone (Amorim *et al.*, 2009; 2011). Thus, it is not possible to express the vitamin A value.

Most studies evaluate just the ripe pulp and they do not provide information about the concentrations of different carotenoids in the unripe pulp, and in the unripe and ripe peels. It demonstrates the need for studies with bigger number of cultivars in different ripening stages, since concentrations may range depending on the cultivar, on the management conditions, on the cultivation regions and on the fruit ripening stage.

Thus, the current study aims to evaluate the occurrence and concentration of carotenoids in the pulp and peel of 14 banana and one plantain cultivars in two ripening stages.

## MATERIAL AND METHODS

Bunches of Ouro (AA), Nanica (AAA), Nanicão (AAA), Caru-Verde (AAA), Caru-Roxa (AAA), Caipira (AAA), Prata (AAB), Prata-Anã (AAB), Maçã (AAB), Mysore (AAB), Pacovan (AAB), Marmelo (ABB), Prata-Graúda (AAAB) and Caju (non-defined genomic group) banana and of Terrinha (AAB) plantain cultivars were harvested from 3.5m x 2.5 m spaced plants in a six-year-old experimental orchard at Federal University of Viçosa (FUV), Viçosa, Minas Gerais, Brazil, located at 648 m altitude.

The bunches were harvested when the first signs of yellow peel were found in the fruits from each cultivar. The second, third and fourth hands were removed from each bunch and they were immediately transported to the Fruit Analysis Laboratory at the FUV campus. The fingers were cut off the hands and the damaged, sick and malformed fingers were discarded. Subsequently, they were washed with running water and left to rest on paper towels for a few minutes to coagulate the latex. Next, 12 fruits in color stage 1 (dark-green peel color) were randomly selected and six of them were immediately processed. The remaining six fruits were immersed in ethephon solution ( $1.2 \text{ g L}^{-1}$ ) for 8 min to achieve uniform ripening. After they were air dried for 15 min, they were dipped in Prochloraz fungicide solution ( $0.49 \text{ g L}^{-1}$ ) for 5 minutes. After this time, the six fruits were placed in plastic boxes and kept at room temperature until they reached color stage 6 (fruits with completely yellow peel) which occurred within approximately 4 days.

The unripe and ripe pulp and peel samples were placed in aluminum wrap, weighted in *semi-analytical balance*, identified, subjected to freezing in liquid nitrogen, and placed in *ultra-low temperature freezer* at  $-80 \text{ }^{\circ}\text{C}$  until the time of the analysis, which occurred within approximately 180 days.

The  $\alpha$ -carotene,  $\beta$ -carotene, lutein,  $\beta$ -cryptoxanthin and lycopene occurrence and concentration in the unripe and ripe pulp and peel of 15 cultivars were evaluated.

Carotenoids were extracted according to the method suggested by Rodriguez-Amaya (2001) with modifications. Five grams of plant material were weighed in appropriate extraction tubes, protected from direct light with aluminum foil, added with 60 mL cooled PA acetone (divided in three 20 mL volumes) and processed in Ultra-Turrax homogenizer (T18 basic model) for 6 min. Then, the extract was vacuum filtered on Buchner funnel using filter paper. After filtration, the three extract fractions were transferred to separatory funnel containing 20 mL cold PA petroleum ether. Each fraction was washed with distilled water to completely remove acetone PA, anhydrous sodium sulfate was added to the extract in petroleum ether in order to remove any residual water from the extract. Subsequently, the extract in petroleum ether was transferred to a 25.0 mL volumetric flask, and the volume was completed with cold petroleum ether. The samples were not subjected to the saponification process.

Regarding the carotenoid analysis, 5.0 mL aliquots of the extract from each fruit part and ripening stage were evaporated under nitrogen gas flow, and the dry residue was recovered using 2.0 mL HPLC grade acetone. Then, the extracts were filtered in filtering units with 0.45  $\mu$ m (Millipore) porosity, and 100  $\mu$ L of them were injected into the chromatographic system to be analyzed.

Carotenoid analyses were performed by high-performance liquid chromatography with diode array detection (HPLC-DAD), according to the chromatographic conditions developed by Pinheiro-Sant'Ana *et al.* (1998). The equipment used in the analysis consisted of a Shimadzu gas chromatograph equipped with high-pressure pump (LC-10ATVP model), automatic injector (SIL-10AF model) and diode array detector (SPD-M10A model), and it was controlled by the Multi System software, Class VP 6.12. The separation was performed on an RP-18 chromatographic column (Phenomenex Gemini, 250 x 4.6 mm, with 5  $\mu$ m internal particle) provided with guard column (Phenomenex ODS (C18), 4 mm x 3 mm), and the detection was done at 450 nm. The current study used mobile phase composed of methanol: ethyl acetate: acetonitrile (80:10:10, v/v/v), HPLC grade (Tedia, Brazil), with 2.0 mL min<sup>-1</sup> flow and run time of 13 minutes.

The peaks of interest were identified by comparing the retention times of both the standard and the samples and, mainly, by comparing the absorption spectrum of authentic samples and standards analyzed under the same conditions. The  $\alpha$ -carotene was identified by means of the absorption spectrum. Quantification was performed by external standardization, by means of analytical curves, using regression equations obtained through the concentration correlation *versus* the standards' peak areas. The results were expressed in  $\mu$ g (100 g)<sup>-1</sup> of each plant material, in the wet basis.

The vitamin A value was calculated according to the recommendations from the Institute of Medicine (2001), wherein 1 Retinol Activity Equivalent (RAE) corresponds to 1  $\mu$ g retinol, and it was calculated as follows:  $\mu$ g of  $\beta$ -carotene/12 +  $\mu$ g of  $\alpha$ -carotene/24, with data expressed in  $\mu$ g (100 g)<sup>-1</sup> of each plant part and ripening stage, based on the wet matter.

Total carotenoids were estimated according to the methodology suggested by Higby (1962), and the results were expressed in  $\mu$ g (100 g<sup>-1</sup>) of pulp and peel.

The current study adopted a completely randomized design in a 15 x 2 factorial scheme, being 15 cultivars and two maturing stages (unripe and ripe), with four replications (bunches), and six fruits per sampling unit. Pulp and peel was analyzed as an individual test. Comparisons among fruit parts were made through descriptive statistics.

Data from the variables related to the comparison among cultivars were subjected to variance analysis and the means were grouped according to the Scott-Knott criterion ( $p < 0.01$ ), using the System for Statistical and Genetic Analyses – SAEG (Sistema para Análises Estatísticas e Genéticas) 9.1.

## RESULTS AND DISCUSSION

The  $\beta$ -cryptoxanthin and lycopene carotenoids were not identified in any fruit part or ripening stage. Lutein,  $\alpha$ -carotene and  $\beta$ -carotene were identified and quantified, fact that was also observed in bananas by other authors (Wall, 2006; Davey *et al.*, 2009; Newilah *et al.*, 2009; Ekesa *et al.*, 2012; Lokesh *et al.*, 2014) (Table 1). The cultivar and the fruit parts, as well as the ripening stage, influenced the evaluated carotenoid concentrations. Figure 1 shows the chromatographic typical profile of the carotenoids found in the samples (Terrinha plantain).

Carotenoid concentrations in the unripe pulp varied greatly among cultivars. By analyzing just the overall mean, it was possible to see that there was slight  $\beta$ -carotene predominance in comparison to the other studied carotenoids, agreeing with Lokesh *et al.* (2014) and Englberger *et al.* (2010). As for lutein, five mean groups were formed. 'Ouro' cultivar belonged to the group showing the highest mean, followed by the group 'Nanica', 'Caipira', 'Mysore' and 'Marmelo', which showed lutein concentration around the half of the presented by 'Ouro'. In addition, lutein concentration exceeded that of  $\alpha$ -carotene and  $\beta$ -carotene in Ouro, Caipira, Prata, Maçã, Pacovan, Prara Graúda and Caju cultivars. Newilah *et al.* (2009) also observed this trend when they evaluated 19 banana cultivars and plantains in Cameroon. As for  $\alpha$ -carotene and  $\beta$ -carotene, there was variation among cultivars. Eight cultivars showed  $\alpha$ -carotene concentration

higher than that of  $\beta$ -carotene, in the unripe pulp (Table 1).

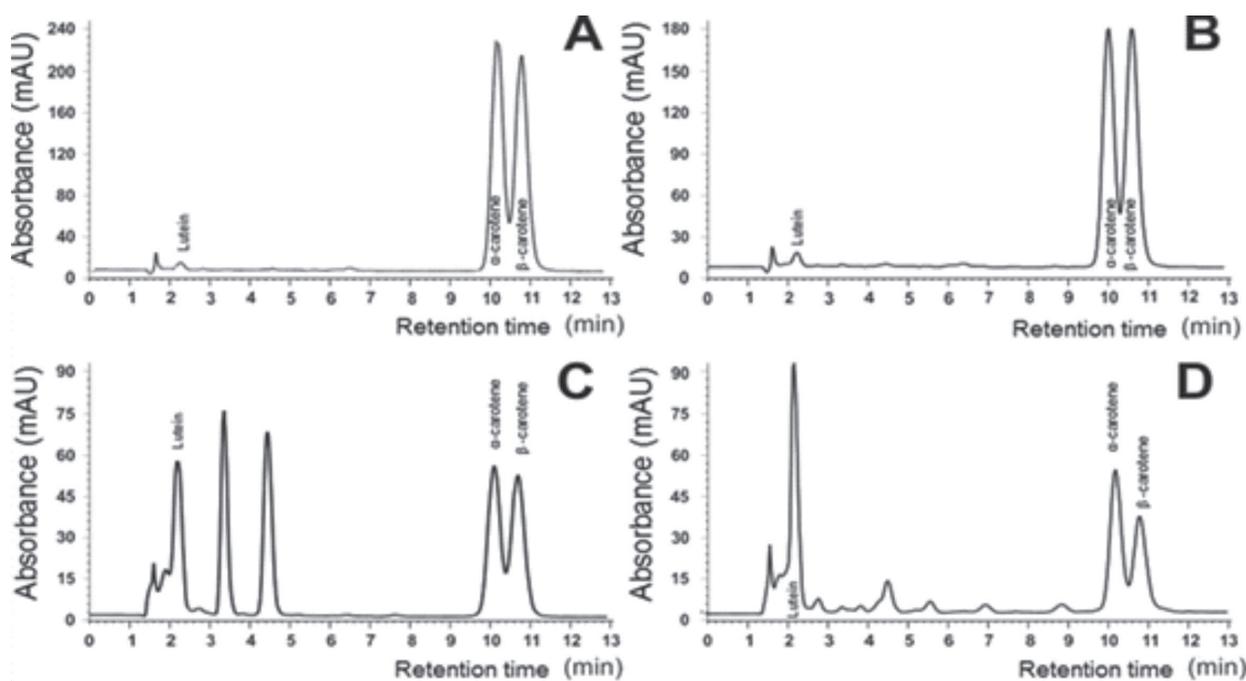
There was 36% increase in lutein concentration in the ripe pulp (mean of all cultivars) in comparison to the unripe pulp. There was increased lutein concentration in the pulp of all tested cultivars due to ripening (from stage 1 to 6), except for 'Ouro', 'Caru-Roxa' and 'Caju' (Table 1). Similarly, Newilah *et al.* (2009) observed significant lutein concentration increase in 16 out of 19 banana cultivars evaluated from stage 1 to 7. The lutein content in Nanicão Caru-Roxa, Caipira, Maçã and Caju cultivars did not differ with the ripening of fruits. In addition, the ripe pulp of all cultivars, except for 'Caru-Roxa', 'Caru-Verde' 'Terrinha' and 'Caju', showed higher lutein concentration than that of the other evaluated carotenoids (Table 1). It corroborates the study done by Wall (2006), Newilah *et al.* (2009) and Lokesh *et al.* (2014) who also found higher lutein concentrations in comparison to the other carotenoids found in bananas. However, Davey *et al.* (2009) and Ekesa *et al.* (2012) found lower lutein concentration than that of the other carotenoids in ripe banana pulp.

Lutein is a yellow carotenoid and it does not have provitamin A activity (Davey *et al.*, 2009). However, it plays important roles in the human body. Known as macular carotenoid, lutein - along with zeaxanthin - accounts for the yellowish color in the macula, which is a high visual acuity region responsible for the clear view of images (Yeum *et al.*, 1995). Lutein and zeaxanthin are the only carotenoids found in the eye in much higher amount than

in any other human tissue (Yeum *et al.*, 1995). In addition, lutein is linked to other benefits, such as decreased risk of developing age-related macular degeneration (AMD), and it also shows beneficial effects on the protection against atherosclerosis, cataract, cancer and other diseases (Koh *et al.*, 2004). Furthermore, lutein presents antioxidant activity, and it protects cells from oxidative damages.

For the  $\alpha$ -carotene, there was no difference in the content with the ripening of the pulp for any cultivar evaluated, except for 'Terrinha', where the unripe pulp content was higher (Tabela 1). 'Terrinha' stood out with significant  $\alpha$ -carotene and  $\beta$ -carotene concentrations in comparison to the other cultivars in both ripening stages. In addition, it is worth emphasizing that the  $\alpha$ -carotene and  $\beta$ -carotene concentrations in 'Terrinha' unripe pulp were, respectively, approximately 4 and 3 times higher than those found in 'Caru-Roxa' and 'Caru-Verde'. It indicates 'Terrinha' cultivar great potential as source of these pigments. 'Caipira' showed the lowest  $\alpha$ -carotene and  $\beta$ -carotene concentrations in both ripening stages although it had been grouped together with other cultivars. Newilah *et al.* (2009) also found in 'Caipira' pulp in Cameroon very low concentrations of  $\alpha$ -carotene (62 and 106  $\mu\text{g } 100 \text{ g}^{-1}$ , unripe and ripe pulp, respectively) and  $\beta$ -carotene (13 61 and 100  $\mu\text{g } \text{g}^{-1}$ , unripe and ripe pulp, respectively), compared with 18 other cultivars.

Similarly to what was seen in the unripe pulp, 'Terrinha' showed wide  $\alpha$ -carotene predominance in the ripe pulp than the other cultivars, and its concentration was approximately 144 times higher than that found in 'Marme-



**Figure 1:** Chromatographic profiles of the lutein,  $\alpha$ -carotene and  $\beta$ -carotene carotenoids extracted from 'Terrinha' plantain. (A) unripe pulp (B) ripe pulp (C) unripe peel and (D) ripe peel. The chromatographic conditions are described in Materials and Methods.

**Table 1:** Mean carotenoid concentrations and retinol activity equivalents (RAE) based on fresh matter and respective coefficients of variation (CV %) of unripe and ripe fruit pulp from 15 banana and plantain cultivars grown in Viçosa County, Minas Gerais State

Cultivars	Lutein		$\alpha$ -carotene		$\beta$ -carotene		Total carotenoids		RAE (in 100 g)	
	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
	(µg 100 g <sup>-1</sup> )									
Ouro	442.97aA	303.15aB	137.60cA	162.39cA	142.03cB	209.00cA	809.47bA	819.34dA	17.56cB	24.18cA
Nanica	86.63eB	151.74cA	104.24cA	134.22dA	58.35dA	103.10eA	384.62cA	501.47eA	9.20dA	14.18dA
Nanicão	122.35dA	142.04cA	130.61cA	106.45dA	66.37dA	63.97fA	447.21cA	476.74eA	10.97dA	9.76eA
Caru-Verde	132.05dB	219.28bA	255.25bA	281.79bA	290.79bA	307.18bA	895.10bB	1.075.53cA	34.86bA	37.34bA
Caru-Roxa	237.86bA	229.30bA	286.26bA	252.19bA	362.92bA	323.55bA	978.80bB	1.296.58bA	42.17bA	37.47bA
Caipira	65.71eA	101.26cA	6.95dA	13.89gA	3.61dA	15.29fA	159.66dA	183.88fA	0.59dA	1.85fA
Prata	132.16dA	133.42cA	103.78cA	57.66eA	116.68cA	61.36fB	462.50cA	398.41eA	14.04cA	7.51eB
Prata-Anã	104.34dB	238.97bA	124.59cA	75.99eA	149.41cA	65.93fB	493.71cA	563.40eA	17.64cA	8.66eB
Maçã	164.31cA	205.41bA	47.63dA	8.34gA	39.16dA	17.26fA	279.66dB	496.18eA	5.24dA	1.78fA
Mysore	70.38eB	184.05cA	94.89cA	117.32dA	174.73cA	152.14dA	407.09cB	604.92eA	18.51cA	17.56dA
Pacovan	137.86dB	220.79bA	104.70cA	73.21eA	99.32cA	51.65fA	437.05cA	563.29eA	12.64cA	7.35eA
Terrinha	99.31dB	192.49bA	1.195.30aA	1.073.03aB	1.126.11aA	1.051.84aB	2.553.51aA	2.583.23aA	143.64aA	132.36aB
Marmelo	33.33eB	111.28cA	7.28dA	7.41gA	91.60cA	26.76fB	167.20dA	279.79fA	7.93dA	2.53fA
Prata-Graúda	112.13dB	280.51aA	51.05dA	53.04fA	42.07dA	44.76fA	289.62dB	488.96eA	5.63dA	5.94eA
Caju	175.91cA	169.16cA	89.97cA	121.82dA	71.42dA	98.97eA	443.93cA	429.98eA	9.70dA	13.32dA
Mean	141.15	192.19	182.67	169.24	188.97	172.85	613.94	717.44	23.35	20.61
CV (%)	19.73	16.57	26.83	13.76	24.73	16.20	16.56	12.16	24.27	13.26

Means followed by the same uppercase within a column and lowercase within a line, for each variable, belong to the same group, by the Scott-Knott criterion at 5% probability.

lo', 128 times higher than that found in 'Maçã', and 77 times higher than that found in 'Caipira' (Table 1). In addition, 'Nanica', 'Nanicão', 'Prata-Anã', 'Pacovan', 'Terrinha', 'Prata-Graúda' and 'Caju' showed slightly higher  $\alpha$ -carotene concentration than that of  $\beta$ -carotene. As for the  $\beta$ -carotene, six mean groups were formed and 'Terrinha' stood out in comparison to the other cultivars once more. In addition, the concentrations of eight cultivars were grouped with the lowest means. Oliveira *et al.* (2011) found  $\alpha$ -carotene concentrations of 366.3; 548.6; and 1557.1  $\mu\text{g}$  (100g MF)<sup>-1</sup> in other fruits, such as 'Paluma' guava, 'Formosa' papaya and 'Tommy Atkins' mango, respectively. They are carotenoid sources for human diet and demonstrate the potential of cultivars such as 'Caru-Roxa' and 'Caru-Verde' - with similar concentrations - and 'Terrinha' - with higher concentration than that of guava and papaya.

As for the ripe pulp, there was 7.3 and 8.5% reduction in the mean  $\alpha$ -carotene and  $\beta$ -carotene concentrations, respectively, due to fruit ripening. There was no difference in the  $\beta$ -carotene content between ripening stages for most cultivars, except for 'Prata', 'Prata-Anã', 'Terrinha' and 'Marmelo', with higher levels of unripe pulp. For the 'Ouro', the content of the unripe pulp was lower, differing from the content of the ripe pulp (Table 1). The decreased concentrations of these carotenoids during ripening may due to their degradation or to the synthesis of other carotenoids such as lutein (Newilah *et al.*, 2009). On the other hand, Mata *et al.* (2011) found that ripening led to increased  $\beta$ -carotene content in 'Ubá' mango; whose content was approximately 3.4 times lower in the unripe fruit.

Similarly to what was seen in the unripe pulp, eight ripe pulp of the analyzed cultivars showed higher mean  $\beta$ -carotene concentrations than those of  $\alpha$ -carotene (Table 1), which was also observed by Englberger *et al.* (2003; 2010), Ekesa *et al.* (2012) and Lokesh *et al.* (2014) (cultivars not evaluated in this current study). However, Wall (2006) and Newilah *et al.* (2009) found higher  $\alpha$ -carotene concentrations than those of  $\beta$ -carotene (cultivars not evaluated in this study). This fact shows the importance of assessing carotenoids occurrence and of quantifying their concentrations in order to provide more accurate nutritional information about each cultivar as well as to encourage the consumption of cultivars with higher nutritional potential.

As for the total carotenoids found in the unripe pulp, four mean groups were formed. The concentrations ranged from 159.66 to 2553.51  $\mu\text{g}$  (100 g FM<sup>-1</sup>) in 'Caipira' and in 'Terrinha', respectively (Table 1). The scientific literature shows few studies focused on evaluating the different carotenoids in unripe banana pulp.

The mean total carotenoid concentration in the ripe pulp was 17% higher than that found in the unripe pulp, and the

concentrations ranged from 183.88 to 2583.23  $\mu\text{g}$  (100 g FM<sup>-1</sup>). There was no difference between ripening stages, except for the cultivars Caru-Roxa, Caru-Verde, Maçã, Mysore and Prata-Graúda, with the highest levels observed in the ripe pulp. (Table 1). 'Terrinha' also showed high carotenoid concentration, which was 14 times higher than that found in 'Caipira', and nine times higher than the concentration found in 'Marmelo', which resulted from high  $\alpha$ -carotene and  $\beta$ -carotene concentrations. Amorim *et al.* (2011) evaluated 62 banana accessions in Brazil - including improved and wild diploids, triploids and tetraploid hybrids - and found total carotenoid concentration ranging from 140 (AAA genotypes) to 1924  $\mu\text{g}$  (100 g FM<sup>-1</sup>) (AAB genotypes). The lowest concentration was found in 'Caipira'.

The current study found that the total carotenoid concentration in banana ripe pulp varied in a wide range according to the cultivar, as it was also observed by Amorim *et al.* (2009) who evaluated 42 banana accessions in Brazil and found total carotenoid concentrations ranging from 106 ('Nanica') to 1924  $\mu\text{g}$  (100 g FM<sup>-1</sup>) ('Saney') in white - and orange-pulp bananas, respectively. On the other hand, Englberger *et al.* (2010) conducted a study in Micronesia (Oceania) and found concentrations ranging from 130 to 9400  $\mu\text{g}$  (100 g FM<sup>-1</sup>). Lokesh *et al.* (2014) conducted a study in India and found total carotenoids concentrations in bananas ranging from 60 to 2100  $\mu\text{g}$  (100 g FM<sup>-1</sup>).

The current study found that orange pulp cultivars showed the highest carotenoid concentration both in unripe and in ripe fruits in comparison to white or cream pulp cultivars, similarly to what was verified by other authors (Amorim *et al.*, 2009; Davey *et al.*, 2009; Fungo & Pillay, 2011) in bananas.

The RAE in the unripe and ripe pulp followed the  $\alpha$ -carotene and  $\beta$ -carotene concentration trend, and 'Terrinha' stood out in comparison to the other evaluated cultivars (Table 1). The RAEs were close to those reported by Newilah *et al.* (2009) in unripe pulp. According to the authors their data ranged from 16.02 to 147.99  $\mu\text{mg}$  (100 g FM<sup>-1</sup>) in the 'Grande Naine' cultivar and in the 'Mbouroukou' plantain, respectively.

There was a difference between ripening stages only for the cultivars Ouro, Prata, Prata-Anã and Terrinha, similar to that observed in  $\beta$ -carotene. The mean vitamin A value found in the ripe pulp - expressed in RAE - was 12% lower than that found in the unripe pulp (Table 1) due to the reduced  $\alpha$ -carotene and  $\beta$ -carotene concentrations and to the change in the relationship between the two carotenoids, which have different weights in the conversion into vitamin A. 'Terrinha' showed high vitamin A value in the ripe pulp, but the concentration was 17% lower than that found in the unripe pulp. The vitamin A value found in most cultivars in the current study is close

to that reported by Englberger *et al.* (2010) who found values ranging from 8 to 435  $\mu\text{g}$  (100 g FM<sup>-1</sup>). However, Newilah *et al.* (2009) found values ranging from 9.74 to 78.63  $\mu\text{g}$  (100 g FM<sup>-1</sup>) and Lokesh *et al.* (2014) found values ranging from 4 to 114.6  $\mu\text{g}$  (100 g FM<sup>-1</sup>), therefore, lower than that found in 'Terrinha' in the current study.

Although it is not an eating habit, consuming 100 g unripe pulp of the 'Terrinha' cultivar analyzed in the current study may provide 20.5% and 16.0% Dietary Reference Intake (DRI) of vitamin A to adult men and women, respectively. The same amount of ripe pulp from the same cultivar may provide 18.9% and 14.7% RDI of vitamin A to men and women, respectively, based on the RDI of 700 and 900  $\mu\text{g day}^{-1}$  for men and women (19 to 50 years old), respectively (Institute of Medicine, 2001). (Table 1).

Great lutein prevalence was found in comparison to that of the other carotenoids in the unripe and ripe peel. Lutein concentration hardly changed in the ripe peel, only in 'Ouro', 'Terrinha' and 'Caju' did the lutein content differ between maturation stages. 'Ouro' stood out with the highest lutein concentration in the unripe peel, whereas 'Maçã' and 'Pacovan' showed lower concentrations than the other cultivars (Table 2). Delgado-Pelayo *et al.* (2014) also observed this trend when they evaluated apple peels. According to Pogson *et al.* (1998), lutein is the most abundant carotenoid found in the photosynthetic tissues of plants. It represents up to 50% carotenoid concentration in the leaves, and its synthesis is evolutionarily conserved in both terrestrial plants and in green algae. Matos *et al.* (2009) reported that lutein was the main carotenoid found in coffee leaves, with concentration 16% higher in leaves subjected to more radiation. These results corroborate those found by Matsubara *et al.* (2011) who reported that lutein plays an important role in the photoprotection of avocado leaves and that it works in heat dissipation, although its mechanisms are still unknown.

In the peel there was slight reduction in  $\alpha$ -carotene and  $\beta$ -carotene concentrations due to the fruit ripening. This reduction may be due to the synthesis of other unidentified and non-quantified carotenoids in the current study, as it can be seen in the unripe peel chromatograms (C) in comparison to those of the ripe peel (D) (Figure 1). The synthesis of other carotenoids due to fruit ripening influenced the total carotenoids concentration, which showed discreet increase from stage 1 to 6 (Table 2). The content of  $\alpha$ -carotene varied considerably between cultivars and ripening stages, and in the cultivars Nanicão, Caru-Verde, Caru-roxa, Caipira, Maçã, Prata-Graúda and Caju, there was a significant reduction in the content, differing from the unripe peel. In addition, similarly to what was found in the pulp, the  $\beta$ -carotene concentration was slightly higher than that of  $\alpha$ -carotene, except for 'Ouro', 'Nanicão', 'Terrinha' and 'Caju' cultivars. The  $\beta$ -carotene

concentration was higher than that of  $\alpha$ -carotene in all genotypes, except for 'Nanicão', 'Maçã' and 'Terrinha' (Table 2).

'Terrinha' stood out with higher  $\alpha$ -carotene and  $\beta$ -carotene concentrations than those found in other cultivars in the peel. In addition, the  $\alpha$ -carotene concentration in the unripe peel was higher than that found in the unripe pulp, except for 'Caru-Roxa', 'Caru-Verde', 'Prata', 'Prata-Anã', 'Pacovan' and 'Terrinha'. The  $\beta$ -carotene concentration in the peel was higher than that found in the pulp, except for 'Caru-Roxa', 'Caru-Verde' and 'Terrinha' (Table 2). 'Terrinha' and 'Ouro' stood out with high  $\beta$ -carotene concentrations, whereas 'Caipira', 'Prata', 'Prata-Anã', 'Maçã', 'Pacovan', 'Marmelo' and 'Prata-Graúda' cultivars showed the lowest  $\alpha$ -carotene and  $\beta$ -carotene concentrations (Table 2).

As for the total carotenoids in the unripe peel, five groups were formed. 'Ouro' cultivar belonged to the group that showed the highest mean concentrations, with concentrations 3.19 and 3.88 times higher than that found in the unripe and in the ripe pulp of the same cultivar, respectively. The lowest concentrations were found in the peel of 'Marmelo', 'Pacovan' cultivars (Table 2). In relation to the ripening stage, there was a low variation between the contents, with the exception of the cultivars Ouro, Terrinha and Caju, where the contents of the ripe peel were higher and 'Caru-Verde' and 'Caru-Roxa' with higher contents in the unripe peel (Table 2).

The total carotenoid concentration in the ripe peel was higher than that found in the other fruit parts and ripening stages, except for unripe and ripe pulp for 'Terrinha', which showed higher concentration than that found in the ripe peel. It demonstrates this cultivar potential as carotenoids provider. There was 7.2% increase in the ripe peel in comparison to the unripe peel, and 'Ouro' cultivar stood out (Table 2). Ajila *et al.* (2007) conducted a study in India and found total carotenoid concentration 4 to 8 times greater in mango ripe peel than that found in the unripe peel.

The mean vitamin A value in the unripe peel was similar to that found in the unripe pulp. Four groups were formed, and 'Terrinha' stood out again with the highest value in comparison to the other cultivars. However, it is noteworthy that eight of the cultivars were grouped with the lowest means due to the low provitamin A carotenoids concentration presented by these cultivars. Vitamin A value was higher in the unripe peel than in the unripe pulp, except for 'Caru-Roxa', 'Caru-Verde', 'Prata', 'Prata-Anã', 'Pacovan' and 'Terrinha'. For the ripening stage, there were higher contents of unripe peel, with the exception of 'Ouro'. Behavior similar to that observed in the contents of  $\beta$ -carotene in the peel and ripening stages, because of this carotenoid present larger weight in the conversion in vitamin A.

**Table 2:** Mean carotenoid concentrations, retinol activity equivalents (RAE) based on fresh matter and the respective coefficients of variation (CV %) of unripe and ripe peel from 15 banana and plantain cultivars grown in Viçosa County, Minas Gerais State

Cultivars	Lutein		$\alpha$ -carotene		$\beta$ -carotene		Total carotenoids		RAE (in 100 g)	
	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
	----- (µg 100 g <sup>-1</sup> ) -----									
Ouro	2310.31aB	2587.93aA	222.62cA	248.77bA	209.73cB	461.98aA	2566.86aB	3182.13aA	26.75cB	48.86aA
Nanica	1832.04bA	1682.72bA	178.34cA	178.12cA	185.89cA	190.61dA	2007.93cA	2012.60dA	22.92dA	23.30cA
Nanicão	1561.22cA	1579.17bA	201.30cA	161.66cB	195.79cA	159.78dA	1778.91dA	1897.14eA	24.70cA	20.05cB
Caru-Verde	1806.19bA	1587.27bA	183.01cA	106.33dB	206.78cA	201.35dA	2052.37cA	1796.38eB	24.85cA	21.21cA
Caru-Roxa	1780.92bA	1612.19bA	216.64cA	135.64dB	283.68bA	273.55cA	2328.36bA	1996.32dB	32.66bA	28.45bA
Caipira	1802.93bA	1579.95bA	113.37dA	51.34eB	146.56dA	96.81eB	1802.69dA	1785.88eA	16.93dA	10.20dB
Prata	1801.94bA	1653.52bA	82.16eA	57.27eA	118.81dA	93.05eA	1802.44dA	1723.19eA	13.32dA	10.14dA
Prata-Anã	1293.88cA	1248.62cA	80.21eA	56.80eA	163.93cA	88.76eB	1355.64eA	1438.12fA	17.00dA	9.76dB
Maçã	1282.40dA	1317.00cA	89.35eA	54.34eB	118.14dA	52.22eB	1338.07eA	1453.70fA	13.57dA	6.62dB
Mysore	1593.81cA	1555.30bA	138.81dA	107.86dA	244.69bA	212.65dA	1740.63dA	1898.04eA	26.17cA	22.22cA
Pacovan	1195.89dA	1004.57cA	72.98eA	57.31eA	117.71dA	72.12eB	1106.52fA	1216.26fA	12.85dA	8.40dA
Terrinha	1121.18cB	1431.92bA	389.95aA	398.62aA	360.48aA	395.10aA	1664.55dB	2247.69cA	46.28aA	49.53aA
Marmelo	994.88cA	995.10cA	62.88eA	49.50eA	185.14cA	96.70eB	1099.68fA	1260.88fA	18.04dA	10.12dB
Prata-Graúda	1273.13cA	1400.89bA	87.27eA	40.56eB	170.25cA	89.86eB	1365.22eA	1434.67fA	17.82dA	9.17dB
Caju	1948.47bB	2529.79aA	266.70bA	154.48cB	246.05bA	268.95cA	2264.94bB	2813.35bA	31.61bA	28.84bA
Mean	1573.27	1584.39	156.17	124.57	196.90	183.56	1751.65	1877.09	22.92	20.45
CV (%)	11.65	12.33	16.19	15.36	17.59	14.04	8.42	7.19	16.48	12.03

Means followed by the same uppercase within a column and lowercase within a line, for each variable, belong to the same group, by the Scott-Knott criterion at 5% probability.

The vitamin A value in the ripe peel was similar to that found in the ripe pulp, with 11% decrease in comparison to the unripe peel. 'Ouro' and 'Terrinha' stood out with the highest values, and 'Caipira', 'Prata', 'Prata-Anã', 'Maçã', 'Pacovan', 'Marmelo' and 'Prata-Graúda' cultivars showed the lowest values, since they presented low  $\alpha$ -carotene and  $\beta$ -carotene concentrations (Table 2).

The results found in the current study indicated the potential use of certain banana cultivars in promoting health due to the presence of important carotenoid pigments. Some cultivars such as 'Caru-Roxa', 'Caru-Verde' and 'Ouro' and the 'Terrinha' plantain must be highlighted due to their high carotenoids concentration. In addition, the consumption of fruits from these cultivars should be encouraged. Furthermore, they can be used in breeding programs in order to obtain biofortified cultivars and to help increasing carotenoid intake by people living in regions at risk of vitamin deficiency.

It is worth developing recipes using banana unripe pulp and peel in order to take advantage of the nutritional potential of those parts, which are not usually eaten by the population. However, it is necessary to conduct further studies in order to investigate the anti-nutritional factors and the methods to inactivate them or to reduce them in these fruit parts.

## CONCLUSION

Lutein,  $\alpha$ -carotene and  $\beta$ -carotene are the carotenoids present in the pulp and peel unripe and ripe.

There is an increase in lutein content and reduction in the content of  $\alpha$ -carotene and  $\beta$ -carotene with ripening of the pulp.

In the peel, the lutein content hardly change due to fruit maturation, however, there was a reduction in the content of  $\alpha$ -carotene and  $\beta$ -carotene.

The mean total concentration of carotenoids increases in the pulp and peel due to fruit ripening.

It is worthwhile to increase or implement the cultivation of the cultivars Terrinha, Caru-Roxa, Caru-Verde and Ouro in regions deficient in vitamin A, due to the higher value of vitamin A.

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