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Stereochemical isomers composition and vitamin A value of Brazilian palm oils

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RESUMEN. Composición de isómeros estereoquímicos y de vitamina A en aceite de palma de Brasil. Se analizaron los carotenoides en aceites crudos y refinados de palma brasileña. El beta-caroteno fue la fracción principal detectada en todas las muestras, variando de 142,60 µg/g a 314,25 µg/g en aceites crudos de palma, de 132,55 µg/g a 612,16 µg/g en frutos de palma brasileña y de 139,4 a 168,90 µg/g en aceites refinados de palma. Los niveles de alfa-caroteno fueron de 44,67 µg/g a 125,32 µg/g en frutos de palma brasileña y de 52,22 µg/g a 63,91 µg/g en aceites refinados de palma. El beta-caroteno todo trans fue el isómero más importante: también se encontraron 9-cis y 13-cis alfa y beta-caroteno. El contenido de vitamina A tuvo valores de 2065 a 10255 Equivalentes de Retinol por 100 g de muestra.
Palabras Claves: Aceite de palma, estereoisomeros, alfa-caroteno, beta-caroteno.

SUMMARY. Carotenoids pigments in crude and refined oils from Brazilian palm were analyzed. Beta-carotene was the principal fraction detected in all samples, varying from 142.6 µg/g to 314.25 µg/g in crude palm oils; from 132.55 µg/g to 612.16 µg/g in Brazilian palm kernels; and from 139.4 to 168.90 µg/g in refined palm oils. Alfa-carotene levels were 44.67 µg/g and 125.32 µg/g in the crude palm oil; 85.99 µg/g to 240.99 in palm kernel and 52.22 to 63.91 µg/g in refined palm oils. All-trans beta-carotene was the most important isomer; 9-cis and 13-cis alfa and beta-carotenes were also found. Vitamin A values were 2,065 and 10,255 RE in 100 g sample.

INTRODUCTION

Carotenoids are widely distributed in nature. They are founded in photosynthetic and non photosynthetic tisues, roots, seeds, fruits and bacteries. The orange-yellow colour of some birds, lobsters and carps are due to carotenoids (1,2). As in most developing countries, vitamin A deficiency has been considered a serious nutritional problem in Brazil, especially in Northeast region (3,4,5), were mega doses of vitamin A are administrated sistematically to children to minimize this problem (6,7,8,9).

Palm oil is obtained from the mesocarp of oil palm trees (*Elaeis guineensis*), and it is known in Brazil as «óleo ou azeite de dendê». It's orange-red colour, is due to a large amount of carotenoids. In Brazil three varietiees of palm oil trees are cultivated: dura, tenera and psifera. Among them Tenera is the most important commercial variety (10,11,12,13).

Determination of the real provitamin A content available in foods, requires separation of different pigments and identification of each fraction with provitamin A activity. Data on the provitamin A content of some Brazilian leafy vegetables, roots and fruits were reported by many workers (14,15,16,17,18).

Very little data are available on the beta and alfa carotene content of palm oils. Generally data on carotenoids are expressed as total carotenoids and most of them are not complete, they do not refer stereochemical studies. The majority of data available on palm oils, are based on determination of total beta carotene wave lenght, thus overestimation of the contribution of some inactive carotenoids (19,11,20,21,22,13).

In palm oil beta carotene is the most important pigment with provitamin A activity amounting between 60 and 80 % of total carotenoids. Large amounts of alfa carotene are also found. Fractions like gama and zeta carotenoids, criptoxanthyn, lycopene and a large amount of xantophylls are also reported (19,23,20,22,13).

The objective of this study was to determine the composition of alfa and beta carotene and their stereochemical isomers and accurate vitamin A values of differents palm oils.

Identification of the carotenoids: To identify the carotenoids parameters as Uv/vis absorption spectra, position of the fraction in the column. TLC values, iodine catalyzed cis-trans isomerization and identification of the epoxide group were made. TLC plates of silica gel, were developed with 3 % of methanol in benzene. The possible occurence of epoxide groups was tested by exposition of the chromatograms to HCl gas for a few seconds. Iodine catalized cis-trans isomerization was undertaken by dissolving cristals of iodine in petroleum ether and adding 3 drops of this solution to the pigment dissolved in petroleum ether and recording the absorption spectrum after 3 minutes.

Quantitative determination: The pigment of each concentration was calculated using its absorption coefficient and the maximum absorbance (24).

Vitamin A calculation: Vitamin A values, were calculated in Retinol Equivalents % considering biopotency of each isomers (5).

Experimental: All solvents were of analytical grade and purchased from Merck, except aceton redestilled in laboratory. The adsorbents MgO and $Ca(OH)_2$ were purchased from Riedel and Malinkrodt, respectively. Hyflo-supercel was obtained from Carlo Erba.

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UV/Visivel absorption spectra was recorded with spectrophotometer Beckman Model DU70, between wavelengths 320 to 510 nm. The MgO and $(OH)_2$ columns, were dry packed using glass columns diameter depending on carotenoids concentration of the samples.

Samples description: Crude palm oils (four samples) were obtained from different, regions of Brazil, while refined palm oils (two samples), were obtained on the market in São Paulo, Brazil. Besides these, three samples of Brazilian Palm fruits (*Elaeis guineensis, Elaeis guineensis* X *Elaeis oleifera* and *Elaeis melanococa*), were collected in the Experimental Station of EMBRAPA (Empresa Brasileira de Pesquisa Agropecuaria) in Manaus-Amazon. These fruits were whashed and blanched in hot water (75 °C for 45 minutes), and the oil was extracted from fruits by treating with hot water (95 °C) for one hour. The oil suspended in the supernatant was collected and the aqueous solution removed by centrifugation at 3000 rpm in a heated centrifuge (45 °C).

Spectra of carotenoids: The separation of carotenoids was done according to the method of Rodríguez et al 925), modified for oily fruits (28,23,20,22). Each oil was placed in a warm water bath (ca 45 °C) until become a homogeneous liquid. A 1,0 g aliquot was drawn and mixed with 200 ml of 50 % KOH. The saponification reaction was allowed to proceed for 12 hours in the dark under nitrogen, before stirring in a magnetic stirrer for 2 hours. The saponification mixture was extracted with successively five times with 30 ml portions of petroleum ether until the organic layer became colourless. The pooled pigment was washed several times with destiled water dried over anhydrous sodium sulfate. The pigment extract was filtred with common paper and saponified for 2 hours. The pigment extract was evaporated to a volume of 5 ml at 35-40 °C and stored at 15 °C, under nitrogen atmosphere until analysis.

Preparative chromatography was accomplished in a MgO: Hyflosupercel (1:1 and 1:2), and developed with acetone in petroleum ether (1-10 %). Alfa and beta-carotene isomers, were separated on $Ca(OH)_2$ columns, using pure petroleum ether. All stages of analysis were carried out with protection of the pigment against direct sun light using an aluminium foil.

RESULTS AND DISCUSSION

In crude, Brazilian palm fruits and refined palm oils, alfa and beta carotene as vitamin A precursors were identified. Three beta and alfa-carotene isomers were also identified. Three isomers from alfa and one beta-carotene were no unidentified. The main UV/Vis absorption peaks are shown in Tables 1 and 2.

TABLE 1
Characteristics of alfa and beta-carotene of Brazilian palm oils

Fraction 1	Alfa-Carotene		
Wave lenght in petroleum ether (nm)	(418-420)	439-441	469-475
Rf in TLC silica gel		0,99	
Fraction 2	Beta-Carotene		
Wave lenght in petroleum ether (nm)	(423-426)	443-46	470-473
Rf in TLC silica gel		0,99	

( ) means inflection in place of the peak

TABLE 2
Characteristics of alfa and beta-carotene isomers

Isomers	Identification	Absorption in petroleum ether (nm)	Iodine Reaction
Alfa-Carotene			
1.1	13-cis-alfa carotene	466-440-417	Cis+
1.2	Alfa- carotene t- trans	472-443-420	Trans+
1.3	9-cis-alfa carotene	465-438-418	Cis+
1.4	Isomer 1	463-435-413	Cis+
1.5	Isomer 2	464-437-414	Cis+
Beta-Carotene			
2.1	13-cis- beta- carotene	466-443 (412)	Cis+
2.2	Beta-carotene t-trans	475-447 (423)	Trans+
2.3	9-cis-beta-carotene	466-441 (420)	Cis+
2.4	Unidentified isomer	470-445 —	Cis+

( ) means inflection in place of the peak

Identification of cis-isomers were carried out comparing absorption spectra before and after iodine catalysed isomerization. Trans isomers showed a decrease in absorbance maxima of about 2 nm while, cis form showed an increase or no change (26,27). There weren't presence of substitutes in carotene, confirmed by TLC on silica gel plates.

Tables 3,4,5,6,7 and 8, show the quantitative composition of alfa and beta-carotene and their stereochemical isomers. Beta carotene was the principal (132,55 to 612,16 µg/g), followed by alfa-carotene 44,67 µg/g to 240,99 µg/g. In crude palm oil, ratio between alfa and beta-carotene was 1/2,5 and 1/3,94; oils from Brazilian palm fruits showed ratio variyng from 1,47 to 1/1,54 and in refined oils this ratio was 1/2,26. Higher amounts of alfa and beta-carotene were determined in caiaué oil (Brazilian palm fruit 7) (240,99 to 612,16 µg/g), respectively.

TABLE 3
Alfa-carotene isomers content[2] of Brazilian crude palm oils (µg/g)

Isomer Crude Oil	13 cis	Alfa-carotene t-trans	9-cis	Unidentified isomer	Total
Oil 1	10.24	37.97*	*6.66	1.79*	56.60
DP	0.89	2.41	1.12	0.50	4.06
Oil 2	15.67	89.18	16.44	4.03	125.32
DP	3.18	6.33	2.31	0.50	5.54
Oil 3	5.78	36.20	1.56	-	44.67
DP	1.28	3.40	0.41		3.46
Oil 4	9.82	43.46	3.38	-	56.66
DP	2.60	2.43	1.42	-	5.16

1 DP standard deviation $p<0,05$
- undetected
2 Average represents means of four determinations

TABLE 4
Beta-carotene isomers content[2] of Brazilian crude palm oils (μg/g)

Isomer Crude Oil	13 cis	ß-carotene t-trans	9-cis	Unidentified isomer	Total
Oil 1	37.48*	70.66*	27.66*	6.55*	142.60*
DP	3.52	2.32	2.51	1.42	6.90
Oil 2	69.90	168.42	61.97	13.85	314.15
DP	4.26	6.00	3.89	1.31	4.90
Oil 3	35.79	82.42	30.52	4.12	152.82
DP	3.92	2.90	5.54	1.91	11.53
Oil 4	39.98	138.26	39.27	5.86	223.36
DP	4.00	15.89	3.80	1.60	13.38

1 DP standard deviation p<0,05
- undetected
2 Average represents means of four determinations

TABLE 5
Alfa-carotene isomers content[2] of Brazilian palm fruits (μg/g)

Isomer Crude Oil	13 cis	α-carotene t-trans	9-cis	Isomer 1	Isomer 2	Total
Oil 5	—	77.95*	8.07	2.87*	3.15	88.99*
DP[1]		11.62	4.42	1.40	0.66	12.73
Oil 6	9.66	59.15	12.98	3.54	—	85.82
DP	1.11	11.54	4.49	1.63		15.17
Oil 7	11.04	193.77	30.64	11.52	—	240.99
DP	1.27	22.46	3.22	2.35	—	24.19

P<0,005
1 DP standard deviation
- undetected
2 Average represents means of four determinations

TABLE 6
Beta-carotene isomers content[2] of Brazilian crude palm fruits (μg/g)

Isomer Crude Oil	13 cis	ß-carotene t-trans	9-cis	Unidentified isomer	Total
Oil 5	20.48*	92.00	20.08	—	132.55*
DP	3.38	23.61	8.58		28.35
Oil 6	27.24	140.38	45.71	14.97*	224.21
DP	3.42	17.68	16.83	0.98	28.20
Oil 8	41.05	409.46	137.58	28.89	612.16
DP	3.66	38.09	40.40	6.44	65.42

P<0,05
1 DP standard deviation
- undetected
2 Average represents means of four determinations

TABLE 7
Alfa-carotene isomers content[2] of Brazilian refined palm oils (μg/g)

Oil Isomer	13 cis	α-carotene t-trans	9-cis	Isomer 1 and 2	Total
Oil 8	12.45*	46.29	5.08	—	63.91
DP	1.82	2.94	2.72		3.23
Oil 9	3.38	43.05	6.55	4,24	57.22
DP	0.78	8.05	0.81	1,07	7.70

P<0,05
1 DP standard deviation
- undetected
2 Average represents means of four determinations

TABLE 8
Beta-carotene isomers content[2] of Brazilian refined palm oils (μg/g)

Isomer Refined Oil	13 cis	ß-carotene t-trans	9-cis	Unidentified isomer	Total
Oil 8	34.20*	102.34	27.34*	4.92*	168.80
DP	1.88	5.72	2.50	0.79	5.41
Oil 9	23.59	106.09	8.22	2.04	139.94
DP	2.35	2.39	1.48	0.88	6.72

P<0,05
1 DP standard deviation
- undetected
2 Average represents means of four determinations

In spite of the fact that the fractions 1,4 and 1,5 from alfa 2,4 from beta-carotene (Table 2) were unidentified were included in the quantification of vitamin A because its cis-structure. There was pronounced statistically diference among all samples studied (p<0,005). In relation to stereochemical isomers, the results are showed in Table 3 to 8. Trans-beta-carotene was the main active form detected in all samples reaching up to 75,87% of the total beta-carotene. Alfa-trans carotene amounted 86,67 % of total alfa-carotene.

Vitamin A values (Table 9), were calculated considering isomer studied (8,9) and the result varied between 2,065 to 10,225 RE % and 20,650 UI to 102,250 UI, confirming palm oil as an excellent source of vitamin A (14,15,1,23,7,12,2).

TABLE 9
Retinol Equivalent (RE/100 g) and International Units (IU/100 g) of Brazilian crude palm oils

Palm Oil	Vitamin A IU	Value* ER
Crude 1	20,650	2,065
Crude 2	27,380	2,738
Crude 3	42,380	4,238
Crude 4	33,470	3,347
Brazilian Palm fruit 5	24,290	2,493
Brazilian Palm fruit 6	36,060	3,606
Brazilian Palm fruit 7	102,250	10,225
Refined 8	26,330	2,633
Refined 9	24,030	2,403

* Quantification of Vitamin A value included alfa, beta carotene and its stereochemical isomers

CONCLUSIONS

- Alfa and beta-carotene were the main vitamin A precursor found in palm oils studied.
- Alfa and beta-carotene were isomeric mixtures, and trans form was predominant.
- 13-cis and 9-cis alfa and beta-carotene were isolated.
- Pronunced quantitation diferences were seen among stereochemical isomers in all oils studied.
- Palm oils are very rich sources of carotenes with high provitamin A values, mainly *caicaué oil* (Brazilian palm fruit 7).

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