PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 6 (5): 497-501, 2007
ISSN 1680-5194

Carotenoids Content of Some Locally Consumed Fruits and Yams in Cameroon

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Abstract: Vitamin A deficiency constitutes a public health problem in Cameroon. Data on provitamin A content of fruits locally consumed are scarce. To solve this nutritional problem, it is important to promote the consumption of yellow/orange fruits or green leafy vegetables which are rich sources of provitamin A. In order to achieve this, the carotenoids content of seven fruits (Mango: Mangifera indica, papaya: Carica papaya, water melon: Cucurbita moschata, pumpkin: cucurbita pepo, guava: Psidium guayava, cythere apple: Spondias cytherea and fruit of Parkia biglobosa) and two yam varieties (Dioscorea schimperiana and bulbifera) consumed in Cameroon were determined by HPLC method. Water content in the fruits varied from 56.30% (Papaya) to 93.76% (water melon), while in tubers, it varied from 62.94 (D. bulbifera) to 80.65 (D. schimperiana, red variety). Papaya solo, as well as red and yellow pumpkin contained all the five carotenoids analyzed. Quantitatively, amongst fruits, mangoes are the richest in total pro-vitamins A carotenoids with a mean content of 2854.74±429.78 µg/100 g FW, followed by the red pumpkin (1204.74±26.49). Guavas had the lowest mean values of 21.84±0.86 and 29.07±1.62 µg/100 g FW for the different harvest periods. For the fruits, ß-carotene is the highest provitamins A carotenoid. In tubers, ß-Cryptoxanthin and Zeaxanthin was mostly found. The fruit of Parkia biglobosa was very rich in α-carotene (166.34 µg/100 g FW) and was the only fruit that lacked zeaxanthin. Carotenoid content of different foods analyzed, could help improve vitamin A status among the target populations.

Key words: α-Carotene, ß-Carotene, ß-Cryptoxanthin, Zeaxanthin, lycopene, Pro-vitamin A

Introduction

Carotenoids are a group of natural pigments responsible for yellow, orange or red colour of many foods. Besides the well-known provitamin A activity of some of these pigments, they have also been associated with lowered risk of developing degenerative diseases such as cancer, cardiovascular diseases and macular degenerative. In many developing countries, green leafy vegetables and red, yellow or orange fruits constitute the main sources of carotenoids pro vitamin A. In fact, in food habits of developing countries, the vitamin A contribution from fish, meat, eggs and milk or milk products are of minor importance, because they are expensive or not accessible. Vegetables and fruits contribution to bioavailability of provitamin A are a major factor that affects the vitamin A status (Parker, 1996). At least 254 million pre-school aged children globally suffer from clinical and subclinical vitamin A deficiency (WHO, 2000). Thus, alleviation of vitamin A deficiency is a major objective particularly in poor target countries. Food based strategies are one of the best means that are used for combating VAD in developing countries (Ruel, 2001).

In Cameroon, vitamin A Deficiency constitutes a major public health problem (Gouado *et al*., 1997; 1998; 2005), a situation that affects all regions and population groups (Kollo *et al*., 2001). Solution proposed to alleviate the situation, is the distribution of Vitamin A capsules to children under five years, twice a year. Geographically, Cameroon is within the tropics where a large diversity of carotenoid rich foods are available: tropical climate favours biosynthesis of carotenoids (Nestel and Nalubola, 2003). Studies on the composition and levels of carotenoids in Cameroonian foods are not yet available. A strategy to combat and prevent this nutritional problem, would therefore require the putting in place of an effective data base comprising the different provitamin A carotenoids food source. ß-carotene, α-carotene and ß-Cryptoxanthin are the major pro-vitamin A carotenoids found in most foods. But because of its unceasing growing importance in public health, notably in the prevention of different forms of cancer like prostrate cancers (Gann and Khachik, 2003; Hardley *et al*., 2002) and problems linked to aging of the eye (Landrun and Bone, 2001), lycopene and zeaxanthin respectively are becoming of much interest nowadays. Our objective is to put in place an efficient, long term strategy based on foods to combat vitamin A deficiency in Cameroon. This study is aimed at determining the levels of major carotenoids (α-carotene, ß-carotene, ß-cryptoxanthin, zeaxanthin and lycopene) in some fruits (Guava: Psidium guayava L., Mangoes: Manguifera indica L., Cythera apple: Spondias cytherea, Water melon: Cucurbita moschata, Pumkin: Cucurbita pepo. Papaya: Carica papaya L., Néré: Parkia biglobosa) and yams (Dioscorea schimperiana and Dioscorea bulbifera) locally produced and used by the Cameroonian population.

Materials and Methods

Sampling

Fruits: Dried fruit of Parkia biglobosa (néré) was purchased in Ngaoundéré market (Adamaoua Province where we usually found out this fruit) in January 2004. The other fruits were purchased at four neighbouring villages around the volcanic region of the Littoral Province (Penja, Mbanga, Njombé and Manengolé) in February 2004 (Dry season), but Guava, mango and Solo papaya was purchased in the same locality for the second time during the rainy season in May 2004. A total of approximately 4 kg of each ripe fruit was purchased from 4 persons randomly selected. Following purchase, the fruits were transported to the laboratory where they were sorted and prepared on the same day. Thus they were washed with tap water, pealed, the seeds removed where necessary, grind and homogenized using a food blender, filled in opaque sealed plastic bags and stored at-25°C, except the néré fruit that was kept in a desiccator while awaiting analysis.

Tubers: Dioscorea schimperiana (red and yellow variety) and Dioscorea bubilfera(white variety) were purchased in January 2004 at three different places randomly selected in Dschang market (Ouest Province) and in compigna village (Littoral Province); then, carried to the laboratory for subsequent analysis.

Analysis: As soon as the sample arrived in the laboratory, the water content was determined on the same day by the method previously described by AOAC (1980).

Carotenoids content was analyzed by the method described by Taungbodhitham *et al.* (1999). Briefly, the samples were unfrozen on the day of analysis. 5 to 10 grams were introduced into a test tube, containing 3 mL of BHT 0.01% in ethanol (In the case of parkia biglobosa fruits, soaking in a small quantity of water was done to facilitate extraction) after homogenization using a vortex mixer for 1 minute, 3 mL of hexane was added to the sample and left overnight at 4°C in the dark. For carotenoids extraction, the mixture was homogenized using a vortex mixer, centrifuged at 3000 rev. min^{-1} for 5 minutes at-5°C and the hexane phase removed, using a micro pipette and introduced into a new test tube. The extraction was repeated until complete discoloration of the fruit residue. All the extract obtained in the hexane phase was pooled together and evaporated under nitrogen (to avoid any form of oxidation by air). The residue thus obtained was collected into 1 mL of acetonitrile, homogenized using a vortex mixer and soniced (Sonicator Bioblock Scientifique 88169) at 10°C for 15 to 30 minutes. 60 µL of the extract was injected into the «loop» using a micro syringe. The elution was made on the HPLC chain which consisted of: a column supelcosil LC-18 (Belfonte USA) 25 cm length and 4.6 mm diameter, particles size: 5µm, a pre column, a pomp Alltech 426 type, an integrator HP 3395, a lamp (Diode Array detector) Linear VIS 200. The following were the conditions for analyses: flow rate of the mobile phase (Acetonitrile: Dichloromethane: Methanol; 70:20: 10; v: v: v): 2 mL per minute, average pressure: 1650. Wavelength of detection: 450 nm, scale: 0.01AUFS, rise time: 0.3 second. All analysis were done under yellow light and the samples protected by aluminium foil.

Solvents used were from Carlo Erba, Italy and were of the HPLC grade. All analytical standards (α-carotene, ß-carotene, ß-cryptoxanthin, zeaxanthin and lycopene) were obtained from Sigma in Germany.

Calculation of the carotenoid levels in the fruits: For each carotenoid, a few milligrams of the standard were dissolved in 3 mL of hexane and then the dilutions of 1/10, 1/100 and 1/1000 of these solutions were made. Their respective Optical Densities (OD) were measured at 450 nm. Solution with OD between 0.1 and 0.9 were retained and their concentrations were calculated according to their coefficient of molar extinction (ε).

From the standard solution thus prepared where the concentration was determined, precise volumes of each solution of carotenoid was collected with the aim of having the final concentration of 15 pmoles in 20 µL for each carotenoid in the calibration mixture except for ß-carotene, where final concentration was 30 pmol/20 µL. Different volumes obtained were mixed together, evaporated under nitrogen and the residue collected into 500 µL of acetonitrile obtained above mentioned concentrations. Injection of the mixture on each day of analysis was necessary to standardize the equipment and adjust peaks of different carotenoids on the chromatograms obtained for the different retention times. Calculations were made taking into consideration the proportional relationship which exist between ratios of different concentrations and that of the standard and sample.

Data processing: All measurements were performed in triplicate; results are given as mean±standard error (SE).

Results

Fruits: According to Table 1, fruits were grouped into four families for a better comparison:

Table 1: Moisture content and carotenoids mean values obtained from analysis

	Mean values of carotenoids±Standard error (µg/100 g edible fresh portion)*				
Foods	Moisture content (g/100 g FM)	α-Carotene	ß-Carotene	Lycopene	β-Crypto-xanthin
A-Fruits					
1-Myrtaceae: Guava (*Psidium guayava* L)					
Group 1 (PG1)	79.87	-	14.02±1.35	87.11±3.06	7.82±0.23
Group 2 (PG2)	64.35	-	17.85±2.43	87.67±2.34	11.22±0.54
2-Cucurbitaceae					
a-Water melon (3 varieties of *Cucurbita moschata*					
Green variety	92,48	-	106.06±7.36	833.77±56.23	27.12±5.91
Spotted green variety	93,76	-	99.17±1.14	702.37±87.91	14.32±1.43
Deep green	92.72	-	629.26±93.62	1713.12±139.88	35.58±4.77
b-Pumpkin (2 varieties of *Cucurbita pepo*)					
Red variety	84.55	55.32±2.07	1133.52±39.75	7.22±0.73	15.89±4.13
Yellow variety	89.83	27.85±5.88	150.66±27.08	9.91±0.72	245.97±8.73
3-Caricacea : Papaya (2 varieties of *Carica papaya*)					
Solo: group 1	79.97	69.19±6.60	644.33±46	955.30±23.26	354.45±10.10
Solo: group 2	67.43	1.62±0.46	130.42±29.86	113.36±12.56	123.96±3.17
Americana	56.30	16.45±1.36	68.05±12.98	-	102.40±0.82
4-Anacardiaceae: Mangoes (2 varieties of *Mangifera indica L.*)					
Dame : group 1	73.64	60.13±4.61	558.79±44.23	-	1.93±0.17
Dame: group 2	68.93	44.69±2.24	2796.98±739.25	-	13.06±2.91
Books	67.20	26.80±1.81	504.39±19.85	-	8.59±0.86
5– Anarcadiaceae : Cythere apple (*Spondias cytherea*)					
Cassamango	85.98	-	6.54±1.47	-	23.61±0.085
6-Fabaceae: Fruit of *parkia biglobosa* (Nere)	6.98	166.34±2.47	11.73±0.95	2.41±0.16	1.06±0.08
B-Yams (Dioscoreacea)					
1-Dioscorea bulbifera : 2 varieties					
White variety-West (WW)	71.72	7.37±0.40	----	---	94.37±1.91
Black variety-Littoral (BL)	62.94	1.12±0.10	10.92±1.33		12.18±0.48
White variety-littoral (WL)	72.41	----	----	---	18.07±1.11
2-Dioscorea schimperiana: 2 varieties					
Yellow variety	72.86	15.64±1.60	58.93±3.38	7.12±1.08	29.05±2.95
Red variety	80.65	----	123.0±8.50	30.84±3.09	40.75±1.26

	Mean values of carotenoids±Standard error (µg $100g^{-1}$ edible fresh portion)*			
Foods	Zeaxanthin	Total provitamin A	Vitamin A contribution	Total carotenoids
A-Fruits				
1-Myrtaceae: Guava (Psidium guayava L)				
Group 1 (PG1)	3.11±0.34	21.84±0.86	1.50±0.07	112.06±1.98
Group 2 (PG2)	2.11±0.34	29.07±1.62	1.95±0.13	118.85±1.57
2-Cucurbitaceae				
a-Water melon (3 varieties of *Cucurbita moschata*				
Green variety	23.36±4.81	133.18±5.45	9.96±0.50	990.32±30.06
Spotted green variety	14.36±3.28	113.48±1.40	8.86±0.09	830.24±52.93
Deep green	0.8±0.053	664.84±55.00	54.0±4.61	2378.77±135.41
b-Pumpkin (2 varieties of *Cucurbita pepo*)				
Red variety	11.30±1.02	1204.73±26.50	97.42±2.06	1223.27±26.37
Yellow variety	302.4±6.19	424.52±23.58	23.96±1.65	736.80±25.93
3-Caricacea : Papaya (2 varieties of *Carica papaya*)				
Solo: group 1	15.63±2.32	1061.97±34.48	71.1±2.7	2038.9±44.15
Solo: group 2	19.34±0.87	256.0±17.27	16.10±1.5	388.71±24.08
Americana	4.45±0.64	186.9±6.64	10.62±0.67	191.35±7.01
4-Anacardiaceae: Mangoes (2 varieties of *Mangifera indica L.*)				
Dame : group 1	18.04±2.71	620.85±27.17	49.15±2.24	638.90±26.71
Dame: group 2	2.36±0.13	2854.73±429.8	235.48±35.7	2857.10±429.85
Books	7.57±0.79	539.78±12.10	43.51±10.12	547.36±11.69
5-Anarcadiaceae : Cythere apple (*Spondias cytherea*)				
Cassamango	5.11±0.49	30.15±0.83	1.52±0.08	35.27±0.91
6-Fabaceae: Fruit of *parkia biglobosa* (Nere)	---	179.13±3.25	7.95±0.18	181.54±3.38
B-Yams (Dioscoreacea)				
1-Dioscorea bulbifera : 2 varieties				
White variety-West (WW)	159.9±4.18	101.74±2.32	4.24±0.1	261.64±6.44
Black variety-Littoral (BL)	45.4±0.81	24.23±1.67	1.46±0.13	69.63±0.93
White variety-littoral (WL)	87.27±0.48	18.07±1.11	0.75±0.04	105.34±0.91
2-Dioscorea schimperiana: 2 varieties				
Yellow variety	4.60±0.15	103.62±3.97	6.77±0.47	115.34±2.86
Red variety	6.82±0.21	163.75±9.60	11.94±0.76	201.41±6.75

*:With the exception of the "néré" analysed in dried form, Group 1: Harvested during raining season; Group 2: harvested during dry season

(i) Myrtaceae: guava (red variety);
(ii) Anarcadiaceae: mangoes (books and dame variety) and cythere apple;
(iii) Cucurbitaceae: water melon (green, spotted green and deep green variety) and pumpkin (red and yellow variety);
(iv) Fabaceae: Parkia biglobosa fruit (Néré).

The water content varied from 56.30% (americana papaya) to 93.76% for water melon. Qualitatively, solo papaya, red and yellow pumpkin had all the five different carotenoids analysed (α-carotene, ß-carotene, ß-Cryptoxanthin, Zeaxanthin and lycopene). Mangoes were the richest in total carotenoids provitamin A with a mean of 2854.74±429.8 µg/100 g FW, followed by yellow pumpkin (1204.74±26.5). Guava had averagely the lowest level: between 21.84±0.86 and 29.07±1.62 µg/100 g FW. For all the carotenoids provitamin A, ß-carotene was the highest in the analysed fruits. For the same fruit, the levels of carotenoid varied largely and these variations were based on variety and period of harvest. The most important example was that observed for solo papaya where the α-carotene levels varied from 1.62 to 69.19 µg/100 g. Highest variation in ß-carotene was observed in the anarcadiaceaes family where cythere apple had 6.54 µg/100 g FW and the dame variety of mango 2796.98 µg/100 g FW (lot 2). Solo papaya (lot 1) was richest in ß-Cryptoxanthin (354.45 ug/100 g FW) while dame variety of mango (lot 1) had the lowest value (1.93 µg/100 g FW). Lycopene which is not a provitamin A, is found in all analysed fruits except those of the myrtaceaes family. However, the best sources are water melon where lycopene values varied from 702.37±87.91 to 1713.12±139.88 µg/100 g FW. Zeaxanthin was also found in all analysed fruits. Two extreme values were observed: 0.8 µg/100 g FW in deep green water melon and 302.4 µg/100 g FW in yellow pumpkin. In terms of the contribution of total provitamin A, lowest level was found in guava (21.84 µg/100 g MF) while dame mango had the highest level (2854.73 µg/100 g FW). For total carotenoids, cythere apple and mango had the lowest (35.27 µg/100 g FW) and highest (2857.10 µg/100 g FW) level respectively. Parkia biglobosa fruit had very low water content (6.98%) and for all samples analysed, it was the only fruit that lacks zeaxanthin, though very rich in α-carotene (166.34 µg/100 g FW).
We realized that carotenoids content of papaya, mangoes and guava from the same region, but harvested during rainy and dry seasons were different ($p<0.05$).

Tubers: Water content varied from 62.94 for D. bulbifera (D. bL) to 80.65% for the red variety of D. Schimperiana. Qualitatively, D. schimperiana (yellow variety) is the only tuber that contained all the five carotenoids analysed. D. schimperiana (red variety) is the richest in total provitamin A carotenoids (163.75 µg/100 g FW) while D. bulbifera (D. b WL). had the lowest level (18.07 µg/100 g FW). For total caroteniods, the values range in D. bulbifera from 69.63 to 261.64 µg/100 g FW for D. bulbifera (D. b BL) and D. bulbifera (D. b WW). respectively. Comparing with the fruits analysed, these tubers were having a significantly lower levels of carotenoids than the fruits.

Contribution to vitamin A: The vitamin A levels in different fruits were calculated taking into consideration that 1 µg of vitamin A is supplied by 12 µg of β-carotene or 24 µg of α-carotene or β-cryptoxanthin in a mixed diet (West *et al.*, 2002). Mangoes and pumpkin are good sources of provitamin A with 235.48±35.7 and 97.42±2.06 µg/100 g FW respectively. This is in contrast to guava, cassamango and yams with lower levels of provitamin A.

Discussion

Lycopene and zeaxanthin are the two carotenoids with no pro vitamin A activity, though they play a vital role in human health: zeaxanthin and luteine (are structural isomers) accumulates in the eye where they play a very important role in the prevention of eye aging diseases such as cataracts (Bone *et al.*, 2003). For lycopene, it serves as a very important antioxidant in the organism, that is, in the prevention of some forms of cancers and cardiovascular diseases (Agarwal and Rao, 2000). Foods like pumpkin, solo papaya and water melon, which are the best sources of these carotenoids play a double role, first as a source of provitamin A and then as an antioxidant. The best sources of carotenoids pro vitamin A in fruits are mangoes, pumpkins and water melon. Bhaskarachary *et al.* (1995) in Indonesia found average total carotenoids levels of 2210; 2760 and 50 µg/100 g FW for mangoes, papaya and guava respectively. Similar trends were also observed for ß carotene.
Tubers were comparatively poorer in carotenoids pro vitamin A than analysed fruits. Parkia biglobosa (néré) fruit was the richest in α-carotene.
Variation of different carotenoids is also found within the same fruit. The values double in some cases like β-carotene, cryptoxanthin and zeaxanthin; and between different fruits for all the carotenoids. This variation in carotenoids in different food materials is due to climate, geographic site, differences in cultivars, state of maturity, ripeness and post harvest treatment (Rodriguez-Amaya, 1993). It has been proven that the levels of carotenoids in fruits increase with the state of maturity (Mercadante and Rodriguez-Amaya, 1998). In this study, fruits used were those in the same state of maturity, ripeness and from the same region. Variations observed in different fruits are due to their differences in origin. Variations between and within the same fruit are therefore an indication of the vast diversity of sources of carotenoids caused by seasonal and regional variations. Differences observed between carotenoids content of fruits

harvested during different seasons (in the case of guava, solo papaya and mango) are probably due to climatic characteristics. In fact, several studies on others fruits (Markus *et al.*, 1999; Lima *et al.*, 2005) noticed that carotenoids content was higher in mature fruits harvested during rainy season (poor sunshine, high rainfall and low temperature) than in those harvested during the dry season. Taking into consideration the contribution of vitamin A from mango and pumpkin and with high bioavailability, these foods can therefore meet the daily requirements of vitamin A for the population concerned.

From the results of this study, we can conclude that β-carotene is the major carotenoid pigment of these fruits. Thus provitamin A value of these fruits derives principally from β-carotene content. Results will be useful for proper education of the population on cultivation and use of these fruits throughout the year. This will therefore lead to the amelioration of vitamin A status in particular and population health in general. Bioavailability and bioconversion of carotenoids provitamins A are the principal determinant of vitamin A status. However, this can be influenced by many factors. For better exploitation of these food materials, their bioavailability and bioconversion based on different forms of preparation and consumption of different carotenoids analysed are therefore required.

Acknowledgements

We thank:

- The International Foundation of Science (IFS) and Nutrition Third Word (NTW) for the grant (N°E-3584-1) offered to the first author.
- The "Agence Universitaire de la Francophonie (AUF-BAC) Bureau Afrique Centrale" for the research mission granted to the first author of this work.

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