Proceedings of the 13th ISTRC Symposium, 2007
pp. 755 - 762

Potential of cassava and sweetpotato leaves to contribute to the Vitamin A requirements

Mulokozi G., Mugyabuso J. and Modaha F.
Tanzania Food and Nutrition Centre, Department of Food Science and Nutrition,
P.O. Box 977, Dar es Salaam, Tanzania

Abstract. Cooked green leafy vegetables constitute a regular relish in most households of Tanzania, usually served as a side dish for the starchy based staples. Provitamin A carotenoids from these vegetables are major dietary sources of vitamin A in Tanzania. Cassava and sweetpotato are widely grown in Tanzania including the semi-arid areas due to their tolerance to harsh weather conditions. Besides caloric density provided by these tubers to the diet, their leaves also contribute to the vitamin A requirements of populations in production areas. In this study five sundried green leafy vegetables including leaves of cassava and sweetpotato were cooked without oil, with sunflower oil or with red palm oil. The total amount and *in vitro* accessibility of α-carotene and β-carotene from a portion (100g) of vegetable relish was determined. The *in vitro* method used simulated the digestion process in the gastrointestinal tract. Carotenoids released after digestion were quantified using high performance liquid chromatography. The total amount of β-carotene varied between 1211 and 3659 mg/100g among the five vegetable sources studied. From green leaves cooked without oil, 8-29% of the β-carotene content became accessible after *in vitro* digestion and 39-94% from leaves cooked with sunflower or red palm oil. Adding red palm oil instead of sunflower oil resulted in about twice as much accessible β-carotene, due to high accessibility of its β-carotene content. The red palm oil contributed also a considerable amount of α-carotene. The results showed that by eating vegetable relishes with added oil daily, it should be possible to provide the recommended intake amount of vitamin A.

Introduction

Plants and animals are two major dietary sources of vitamin A in humans. Vitamin A from animal sources is in a preformed form and therefore readily available for utilization whereas that from plant sources is in the form of provitamin A carotenoids which have to be converted to the active form of vitamin A (retinol) and its derivatives in the gut before being utilized (Booth *et al.*, 1992). This implies that, of the two major dietary sources of vitamin A, animal sources would be most preferred in terms of efficacy of vitamin A supply to humans. However, in most developing countries animal sources of vitamin A are scarce, expensive and beyond the reach of the majority due to socio-economic constraints. Thus provitamin A carotenoids from vegetables and fruits remain the major dietary source of vitamin A in these countries. In Tanzania various types of leafy vegetables including sweetpotato (*Ipomoea batatas*) and cassava *(Manihot esculenta*), are widely consumed and constitute a regular relish in many rural households.

Increased consumption of carotene rich vegetables and fruits has for a long time been recommended as the most appropriate and sustainable solution for improving vitamin A status of populations in low-income countries due to their high carotene contents. However,

it is now known that the ability of provitamin A carotenoids from leafy vegetables to improve vitamin A status is limited by their low bioavailability (de Pee *et al.,* 1995 and 1998; Castenmiller *et al.*, 1999). Bioavailability is defined as the efficiency with which provitamin A carotenoids are absorbed and converted into vitamin A in the body.Thus to maximize the benefit of provitamin A containing foods, strategies to increase the dietary intake of these foods should also include measurers to enhance carotenoid bioavailability.

This paper discusses some processing and preparation methods, which have been found to enhance the bioavailability of provitamin A carotenoids from plant sources. Results from our research study on the *in vitro* accessibility of carotenes in leafy vegetables cooked without and with sunflower or red palm oil and their estimated contribution to the vitamin A requirements support available information.

The objective of our study was to determineα- carotene and β-carotene content and the *in vitro* accessibility in five types of green leafy vegetables commonly used in Tanzania and cooked with either sunflower or red palm oil.

Materials and Methods

The study emulated the traditional method of vegetable processing commonly practiced in semi-arid areas of central Tanzania. It involves sun drying of fresh vegetables, a method commonly used to preserve vegetables for off-season consumption. The dry vegetables were cooked into a vegetable relish using a traditional recipe without and with oil (red palm oil or sunflower oil). Cooked vegetable relish samples were analyzed for the bioaccessibility (amount released from the vegetables after digestion and available for absorption in the gut) of their carotenes using an *in vitro* digestion method. The contribution of one portion of vegetable relish to the recommended daily intake (RDI) was obtained by calculation.

Five different types of freshly harvested green leafy vegetables were purchased from markets in Dar es Salaam. They included the leaves of amaranth (*Amaranthus* spp*),* cow pea (*Vigna* spp), sweetpotato (*Ipomoea batatas*), pumpkin (*Cucurbita moschata*) and cassava *(Manihot esculenta*).

The sunflower oil (Zeta) used was purchased in a supermarket and the red palm oil (Carotino, Malaysia) was taken from a large stock, which was to be used in a dietary intervention study with pregnant women in Tanzania (Lietz *et al*., 2000).

Sample preparation. After blanching in boiling water for 2-3 minutes, all the leaves were sundried in open sunlight. A portion of fresh leaves from each vegetable were blanched and then kept frozen as a reference sample. Samples were transported under cold conditions and kept in a freezer (-20 ^{o}C) before analysis. The reference sample was freeze-dried before determination of total content of α- and β- carotene.

Each type of the sundried leaves was divided into three equal portions (~10g dry weight) and cooked in the laboratory according to the traditional recipe. The leaves were soaked in tap water (1:9) for about 5 min and cooked with a small amount (~1g) of table salt for 15 min. In two of the samples sunflower oil or red palm oil was added at the end of the cooking time. The ratio of sundried vegetable leaves to oil was about 1:1, on a dry weight basis. Caution was taken to avoid excessive boiling after addition of oil. Cooked samples were kept frozen at -20 ^{0}C and later freeze-dried before analysis. For comparison of results one portion of vegetable relish was standardised to100 g, which included 10 g of sundried green leaves, water and no oil or 10 g of sunflower oil or red palm oil.

Determination of oil content in prepared vegetable relish. The oil content in the vegetables cooked with either sunflower oil or red palm oil was determined by using Soxhlet extraction (AOAC 963.15) AOAC, 1990). Extraction was performed with

petroleum ether (B.P. 40-60°C) (Merck, Darmstadt, Germany).

Determination of carotene content in vegetables. Carotenoid extractions were carried out according to a method described by Pettersson and Jonsson (1990). High Performance Liquid Chromatography (HPLC) grade solvents of methyl-t-butyl ether, methanol, acetone and petroleum ether (B.P. 40-60°C) (Merck) were used. Other chemicals used were 95% ethanol (Kemetyl AB, Stockholm, Sweden) and analytical grade sodium chloride (Merck).

The freeze-dried vegetable relish was ground in a blender for about 30 seconds. About 0.5 g of the finely ground sample (in duplicate) was accurately weighed into a flask, reconstituted with 10 ml of distilled water and allowed to stand for 5-10 min. In one of the duplicates 100 µl of an internal standard (200 µg trans-β-apo-8-carotenal/ml) (Sigma Chemicals A-9956, St Louise, MO, USA) was added. The extraction was repeatedly carried out by moderate shaking for 5 min with equal amounts (20 ml) of acetone and ethanol containing 0.1 % (w/v) butylated hydroxytoluene (BHT) (Sigma Chemicals), until the residue was colourless. The extracts were decanted into a separatory funnel containing 20 ml of petroleum ether and 20 ml of 25 % (w/v) NaCl solution. After transferring the carotenes to petroleum ether by gently swirling the separatory funnel, the lower layer was drawn off and washed repeatedly with 20 ml of petroleum ether until the extract was colourless. The pooled extracts were evaporated under nitrogen in a rotary vacuum evaporator at 35 °C. The residue was dissolved in 10 µl of mobile phase solvent and filtered through a 0.45 ìm pore size cellulose membrane filter (Sartorius Filtration AB, Sundbyberg, Sweden) before HPLC analysis.

Due to the risk of oxidation and formation of isomers during sample preparation, the extraction was performed at dimmed light and room temperature.

Determination of in vitro accessibility of carotenes. The in vitro digestion procedure developed by Hedrén *et al.* (2002), which simulates digestion in the gastrointestinal tract, was used to determine the bioaccessibility of carotenes in the vegetable relishes. Briefly, the freeze-dried vegetable relish was homogenized and reconstituted with distilled water. Digestion was carried out by acidifying the sample (pH 2), followed by treatment with porcine pepsin solution and incubation at 37°C in a shaking water bath for one hour. The pH was adjusted to 7.5 and a pancreatin and bile salt solution was added. The sample was further incubated for 30 min. The digesta was centrifuged and the aqueous fraction was extracted with petroleum ether that was evaporated to dryness. The residue was dissolved in 5 or 10 ml of mobile phase solvent and filtered through a 0.45µm pore size cellulose membrane filter before a reverse phase HPLC analysis.

HPLC determination of provitamin A carotenoids. Trans-β-apo-8-carotenal was employed as internal standard and all-trans-β-carotene (Sigma Chemicals C-9750) was used as an external standard. The concentration of ß-carotene standard working solution was confirmed using a spectrophotometer, Hitachi U-1100 (Hitachi Instruments, San Jose, USA), and using the extinction coefficient value for ß-carotene in hexane. The provitamin A carotenoids, α- and β- carotene, were determined by isocratic reversed phase HPLC using methyl-t-butyl ether: methanol: water (56:40:4) as mobile phase. The HPLC system comprised a Waters 600 dual piston solvent delivery pump (Waters, Sollentuna, Sweden) connected to a Waters 996 UV-visible photodiode array detector (Waters) and equipped with a C_{30} polymeric column (YMC Inc., Wilmington, NC, USA). The C_{30} ligands are bonded on high silica particles (5 ìm) in a stainless steel 250 mm column (4.8 mm i.d.), allowing for phase loading and polarity optimised for

separation of both polar and non-polar carotenoids and their isomers. Millenium 2010 software (Waters) was used to acquire, store and process spectra and chromatographic data. Absorption spectra of the carotenoids were recorded between 250-500 nm at the rate of 0.5 spectra per second and the resolution of 2.4 nm. The flow rate was 1 ml/min and injections were made with a 20 µl loop.

Statistical analyses. Differences in mean values of total ß-carotene content in cooked vegetable relishes were tested by analysis of variance (ANOVA). Determination of the significance of differences ($p<0.05$) among samples cooked without oil, with sunflower oil or red palm oil were obtained by Tukey´s HSD multiple rank test.

Results and Discussion

This study has shown that green leafy vegetables studied are good sources of ß-carotene with the amount in blanched leaves ranging from 399 to 664 µg/g DM (Table 1). Small proportions of α-carotene amounting to 17±5.2 and 31±2.3 µg /g DM were detected in blanched leaves of amaranth and sweetpotato respectively. Sundrying of vegetables resulted in a wide range of loss of the ß-carotene content in the different vegetables (Table 1). However, sweetpotato and cassava leaves retained higher amounts (62% and 63%) of ß-carotene than other vegetables. After sundrying no α-carotene was detected in any of the green leaves. The average amount of total ß-carotene from one portion (100g) of the vegetable relishes are shown in Table 2 and varied between 1211 and 3649 µg when cooked without oil. The oil content in the vegetable relishes cooked with either sunflower oil or red palm oil ranged from 47 to 52 %. The total amount of á- and ß-carotene in the red palm oil was 107±6.2 and 125±5.2 µg/g oil, respectively.

Of the amount of ß-carotene retained, only 8-29 % is available for absorption after cooking without adding oil as measured by an *in vitro* digestion method, which measurers the maximum amount of carotenes possible for absorption (Table 3). Adding sunflower oil in the preparation resulted in a manifold increase of the accessibility due to the reasons provided in the section on *in vitro* accessibility of carotene from cooked vegetables ahead. Red palm oil that contains both α- and ß-carotene further increased the amount of accessible carotenes to the vegetable relishes when added in the preparation. Calculations made showed that green leafy vegetables cooked without oil may only constitute from 12 to 47% of an individual's daily need of vitamin A, whereas green leaves cooked with either sunflower oil or red palm oil may provide enough daily retinol (Table 4). These findings may have

Table 1: The total amount of A- and ß-carotene in green leaves before and after sundrying, (µg/g DM)[1].

Green leaves	Blanched		Blanched and sundried [2]	Losses of ß-carotene during sundrying [2]
	ß-carotene	α- carotene	ß-carotene	(%)
Amaranth	664±7.8	17±5.2	287±18	57
Cowpea	622±14	Trace	148±1.3	76
Sweet potato	592±9.8	31±2.3	365±7.7	38
Pumpkin	590±21	Trace	121±3.9	79
Cassava	399±15	Trace	250±2.8	37

[1] Mean ± SD in duplicate samples.
[2] α- carotene was only detected in blanched green leaves.

Table 2: The total α- and ß-carotene content in each portion of vegetable relish (µg/100g)[1].

Green leaves	Without oil[2]	With sunflower oil[2]	With red palm oil	
	(ß-carotene)	(ß-carotene)	(ß-carotene)	(α- carotene)
Amaranth	2851±17[a]	2974±39[a]	4193±51[b]	1491±73
Cowpea	1475±13[a]	1418±61[a]	2836±11[b]	1196±5.8
Sweet potato	3659±106[a]	4008±90[a]	5443±296[b]	1480±76
Pumpkin	1211±39[a]	1526±36[b]	3067±17[c]	1203±16
Cassava	2233±71[a]	2728±7.0[b]	4637±47[c]	1427±8.0

[1] Mean±SD in duplicate samples. Equivalent to10 g dry weight of vegetable leaves.
[2] No α- carotene was detected in relishes cooked without oil or with sunflower oil.
[abc] Values on the same row not sharing the same superscript were significantly different ($p<0.05$).

Table 3: The in vitro accessibility of α- and ß-carotene in each portion of vegetable relish[1].

Green leaves	without oil[2]		with sunflower oil[2]		with red palm oil			
	ß-carotene		ß-carotene		α- carotene		ß-carotene	
	(µg)	(%)[3]	(µg)	(%)[3]	(µg)	(%)[3]	(µg)	(%)[3]
amaranth	512±29	18	1724±137	58	1212±72	86	2861±250	68
cowpea	426±31	29	1267±73	94	1120±33	94	2357±81	83
Sweet potato	316±37	9	1559±238	39	1343±15	90	3240±246	61
Pumpkin	125±35	10	969±146	64	1067±12	88	2468±155	81
Cassava	178±15	8	1284±29	47	793±65	56	2340±201	51

[1] Mean ± SD in two duplicate samples. Equivalent to 10 g dry weight of the vegetable leaves.
[2] No α- carotene was detected in relishes cooked without oil or with sunflower oil.
[3] Percentage in vitro accessible α- and ß-carotene, respectively.

Table 4: The percentage contribution of retinol equivalents (RE) to the recommended safe intake level[1] by one portion of vegetable relish [2].

Green leaves	Without oil *(%)*	With sunflower oil *(%)*	With red palm oil *(%)*
Amaranth	47	157	316
Cowpea	39	115	265
Sweet potato	29	143	356
Pumpkin	12	89	273
Cassava	16	116	249

[1] Recommended safe intake level of vitamin A in populations in developing countries, 550 µg RE/day (WHO/UNICEF, 1995)
[2] Assuming 100 % absorption of the in vitro accessible α- and ß-carotene and 25 and 50 % conversion to retinol in mucosa, respectively.

major implications for the design of intervention studies that use a dietary approach to combat vitamin A deficiency in developing countries.

Effect of drying on carotene contents of vegetable. Green leafy vegetables are available throughout the year for most people in Tanzania. However, during dry season in semi- arid areas people mainly rely on leaves dried in open sunlight. Exposure to light and oxygen are known to have an impact on the carotenoids through oxidation and isomerization (Thane & Reddy 1997; Mosha *et al.,* 1997). Maeda and Salunkhe (1981) obtained substantial losses of carotenes (60-94 %) when green leaves of sweetpotato, cassava and cowpea were sundried without blanching. In this study leaves meant for cooking were blanched prior to sundrying, in order to inactivate the enzyme peroxidase and thereby prevent losses of carotenes from degradation to some extent (Negi and Roy, 2000). This resulted in losses between 37 and 79 % of the ß-carotene content that is less compared with what was reported by Maeda and Salunkhe (1981). An alternative to drying in open sunlight is the solar drying technology, whereby products are protected from light and therefore retain more carotenes (Maeda and Salunkhe, 1981; Negi and Roy, 2000; Mulokozi *et al.*, 2000).

In vitro estimation of accessible carotenes from cooked vegetables. In evaluating the bioavailability of provitamin A carotenoids in plant foods two important concepts involved are their bioaccessibility and absorption. Bioaccessibility of carotenoids from food sources may be affected by factors such as food matrix, dietary lipids, particle size of the food, protein and low bile secretion. In the case of green leafy vegetables, the leafy matrix seems to be an important factor that affects the bioaccessibility and consequently the bioavailability of carotenes (van het Hof *et al.,* 1998). In green leaves, carotene molecules are organized in pigment-protein complexes located in cell chloroplasts, which have to be accessed to make the carotenes available. Cooking and reduction of particle size by grinding may reduce the matrix effects and have been reported to have the potential to increase the bioavailability of carotenoids (van het Hof *et al.,* 1998, Castenmiller *et al.,* 1999) possibly by disrupting and softening the plant cell walls and carotenoid protein complexes. However, complete disruption of the leaf matrix, for example by extensive cooking can also destroy carotenoids molecules (Rahman *et al.,* 19990).

In the present study there was a considerable range in percentage accessibility of ß-carotene (8-29 %), from different leaves cooked without oil. This might be explained by different cell matrices of the vegetable varieties. Depending on vegetable source, cooking with sunflower oil or red palm oil increased the released amount of ß-carotene by 3-20 times. It is well established that presence of dietary fat provides a hydrophobic domain within which carotenoids are solubilized and is known to stimulate bile flow, which is important for the formation of micelles from which carotenoids are absorbed into the mucosa (Parker, 1996; Furr and Clark, 1997). Enhanced bio-accessibility and absorption due to dietary fat has been shown in animal studies where consumption of cooked vegetables containing oil resulted in a significant increase in serum retinol concentrations (Jalal *et al*., 1998; Takyi *et al*., 1999; Huang *et al*., 2000). The carotenes in red palm oil seemed to be highly available for absorption, as the leaves cooked with this oil had about twice as much in vitro accessible ß-carotene as the ones cooked with sunflower oil. In the present study, depending on vegetable species, 39 to 94 % of the ß-carotene content became available for absorption after cooking with oil.

Contribution of carotenes from vegetables to vitamin A requirement. There has been an extended discussion about the impact on vitamin A status with a diet consisting of dark-green leaves. De Pee *et al.* (1995) reported that the vitamin A status was not

improved when lactating women in Indonesia received stir-fried dark-green leafy vegetables as a daily supplement for 12 weeks. In contrast, increased serum retinol concentrations were found in three studies on children after consumption of green leafy vegetables (Jayarajan *et al*., 1980; Takyi, 1999) or red sweetpotato (Jalal *et al*., 1998) prepared with cooking oil.

The conventional conversion factor adopted by FAO/WHO (1988) for converting provitamin A carotenoids to retinol assumes that 1/3 of the total carotene content is absorbed into the mucosa and that 1/2 and 1/4 of absorbed ß- and α- carotene are converted to retinol, respectively. In this study these conversion factors were used to estimate how much a portion of each vegetable relish might contribute to the recommended safe daily intake level of vitamin A, but instead of the absorption factor 1/3 the in vitro digestion data obtained in this study was used. The assumption made was that 100 % of the carotenes released after in vitro digestion is absorbed into the mucosa. The calculated amount of retinol was divided by the recommended safe daily intake level of vitamin A as adopted for planning purposes for individuals in developing countries, i.e. 550 µg RE (WHO, 1995). According to the results from present study, one portion (100 g) of green leaves cooked without oil may only constitute from 12 to 47 % of an individual's daily need of vitamin A, whereas green leaves cooked with either sunflower oil or red palm oil may provide enough daily retinol.

Conclusion

Sweetpotato leaves and cassava leaves like other dark green leafy vegetables have a potential of providing adequate vitamin A if prepared with oil. However, it is recommended that vegetables should be treated in a proper way to avoid loss of carotene content before and during cooking.

References

AOAC. 1990. Official Methods of Analysis. 15th Edn. Association of Official Analytical Chemists, Arlington, Virginia.

Booth, S.L., Johns, T. and Kuhlein, H.V. 1992. Natural food sources of vitamin A and provitamin A. Food Nutr Bulletin 14: 6-16

De Pee S, West CE, Muhilal, Karyadi, D. and Hautvast, JGAJ. 1995. Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet*:* 346:75- 81.

De Pee, S., West, C.E., Permaesih, D., Muhilal S.M. and Hautvast, JGAJ. 1998. Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and ß-carotene in schoolchildren in Indonesia. Am. J. Clin. Nutr. 68:1058-1067.

FAO/WHO 1988. Requirements of vitamin A, iron, folate, and vitamin B_{12}. Report of a joint FAO/WHO expert consultation FAO Food Nutr. Ser. no 23. Rome, Italy.

Furr, C.H. and Clark, R.M. 1997. Intestinal absorption and tissue distribution of carotenoids. Nutr. Biochem. 8:364-377.

Hedrén, E., Mulokozi, G., Svanberg, U. 2002. Estimation of carotenoid accessibility from carrots determined by an *in vitro* digestion method. Eur. J. Clin. Nutr. 56:425-430.

Huang, C.J., Tang, Y.L., Chen, C.Y., Chen, M.L., Chu, C.H. and Hseu, C-T. 2000. The bioavailability of ß-carotene in stir- or deep-fried vegetables in men determined by measuring the serum response to a single ingestion. J. Nutr. 130: 534-540.

Jalal, F., Nesheim, M.C., Agus, Z., Sanjur, D. and Habicht, J.P. 1998. Serum retinol concentrations in children are affected by food sources of ß-carotene, fat intake, and anthelmintic drug treatment. Am. J. Clin. Nutr. 68:623-629.

Jayarajan, P., Reddy, V. and Mohanram, M. 1980. Effect of dietary fat on absorption of ß-carotene from green leafy vegetables in children. Indian J. Med. Res. 71: 53-56.

Lietz, G., Henry, C.J., Mulokozi, G., Mugyabuso J., Ballart, A., Ndossi, G., Lorri, W. and Tomkins, A. 2000. Use of red palm oil for promotion of maternal vitamin A status. Food Nutr. Bulletin 21:215-218.

Maeda, E.E. and Salunkhe, D.K. 1981. Retention of ascorbic acid and total carotene in solar dried vegetables. J. Food Sci. 46:1288-1290.

Mosha, T.C.., Pace, R.D., Adeyeye, S., Laswai, H.S. and Mtebe, K. 1997. Effect of traditional processing practices on the content of total carotenoids, beta-carotene, alpha-carotene and vitamin A activity of selected Tanzanian vegetables. Plant Foods Hum. Nutr. 50: 189-201.

Mulokozi, G., Mselle, L., Mgoba, C., Mugyabuso, J.K.L. and Ndossi, G.D. 2000. Improved solar drying of vitamin A-rich foods by women groups in the Singida District of Tanzania. ICRW/OMNI Research Report series 5, International Centre for Research on Women, Washington DC.

Negi, P.S. amd Roy, S.K. 2000. Effect of blanching and drying methods on ß-carotene, ascorbic acid and chlorophyll retention of leafy vegetables. Lebensm. Wiss u-Technol. 33:295-298.

Parker, R.S . 1996. Absorption, metabolism, and transport of carotenoids. FASEB J. 10: 542-550.

Pettersson, A. and Jonsson, L. 1990. Separation of cis-trans isomers of alpha- and beta-carotene by adsorption HPLC and identification with diode array detection. J. Micronutr. Anal. 8: 23-41.

Rahman, M.M., Wahed, M.A., Ali, M. A. 1990. b-carotene loses during different methods of cooking green leafy vegetables in Bangladesh. Food Comp Anal 3:47-53.

Takyi, E.E.K. 1999. Children´s consumption of dark green, leafy vegetables with added fat enhances serum retinol. J. Nutr. 129: 1549-1554.

Van het Hof, K.H., Gärtner, C., West, C.E. and Tijburg, L.B.M. 1998. Potential of vegetable processing to increase the delivery of carotenoids to man. Int. J. Vitam. Nutr.Res. 68:366-370.

WHO/UNICEF 1995. *Global prevalence of vitamin A deficiency*. WHO Micronutrient Deficiency Information System (MDIS), Working paper no 2, WHO/NUT/95.3. Geneva: World Health Organization/ United Nation´s Children´s Fund.