American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 4 (4): 170-176, 2009
ISSN 1557-4571

Steamed-Dried Squashes (*Cucurbita* sp.) Can Contribute to Alleviate Vitamin A Deficiency

[1]A. Demasse Mawamba, [1]I. Gouado, [1]M. Leng,
[2]I. Somé Touridomon and [3]F. Tchounanguepo Mbiapo
[1]Laboratory of Nutrition and Public Health, Department of Biochemistry,
Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon
[2]Health Sciences, Unit, Laboratory of Analytical Chemistry, University of Ouagadougou,
P.O. Box 03, BP 7021, Ouaga 03, Burkina, Faso
[3]Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon

Abstract: To promote the consumption of squashes flesh, the vitamin A potential of steamed-dried squashes from Cameroon was evaluated in determining the beta-carotene content through HPLC method in raw, steamed and steamed-dried peeled flesh of three squashes species: *Cucurbita moshata* cv. Dickinson, *Cucurbita maxima* cv. Hungarian Blue and *Cucurbita pepo* cv. Sacred Indian Rattle. The vitamin C and total lipids contents were also titrated with 2, 6 dichlorophenol indophenol dye and extracted with hexane in a soxhlet apparatus for 6 h, respectively. The moisture content was estimated by drying in an oven at 105°C until constant weight. The beta-carotene contents of dried steamed squashes were 2834.75±11.22; 3043.91±1.65 and 5917.83±720.49 µg/100 g serving of *C. pepo*, *C. moshata* and *C. maxima*, respectively. The vitamin C contents ranged from 5.70±0.32 µg/100 g serving (*C. moshata*) to 11.81±0.19 µg/100 g serving (*C. maxima*). Total lipids ranged from 6.22±0.00 g/100 g serving (*C. pepo*) to 7.09±0.11 g/100 g serving (*C. moshata*) and the water remaining ranged from 6.39±1.18 g/100 g serving (*C. maxima*) to 8.19±0.70 serving (*C. pepo*). Drying of steamed squashes seemed to result in a significant concentration of beta-carotene content (71 and 89 times higher than those of steamed squashes). The same effect was observed for the vitamin C content (about 1.7 times) and the total lipid content (6 to 12 times). These results suggest that as a ready to eat product, steamed-dried squashes could contribute to fight against vitamin A deficiency if they are well conserved.

Key words: Squashes flesh, steamed-dried, beta-carotene, vitamin C, total lipids

INTRODUCTION

Vitamin A Deficiency (VAD) is the most important preventable cause of morbidity, mortality and childhood blindness. West (2002) indicated that close to 127 million of preschool age children and more than 7.2 millions women were vitamin A deficient. About 250 000 to 500 000 children of preschool age become blind each year (Humphrey *et al*., 1996). The increase risk of diseases like measles and malaria is also attributed to this deficiency.

VAD is widespread in low income countries (Hussey and Klein, 1990). In Cameroon, earlier research showed that VAD is a public health problem (Gouado *et al*., 1998, 2005). Until now, the main approach for the prevention and control of the deficiency is the periodic supplementation of vitamin A capsules to infant less than five years twice a year. It is known that the cause of VAD is the insufficient intake of foods rich in vitamin A or provitamin A carotenoïds to meet the requirements for growth, infections, pregnancy and lactation. The periodic supplementation of vitamin A capsules is therefore a short term solution which need to be completed by food based approaches.

Corresponding Author: Gouado Innocent, Laboratory of Nutrition and Public Health, Department of Biochemistry, Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon

The addition of food based approaches to supplementation for controlling VAD is important because the consumption of vitamin A rich foods brings in the organism other nutrients of interest (like proteins, zinc) necessary for the better use of vitamin A.

However, in Cameroon like in many developing countries, foods rich in preformed vitamin A are not within the reach of all social groups. This makes poor populations especially those living in rural area to depend on plant foods containing carotenoïds to meet their vitamin A requirements (Van den Berg *et al*., 2000). From the food potential of Cameroon, several sources of provitamin A can be identified (Gouado *et al*., 2007). The presence of VAD may be due to the fact that these foods are not well known and are underutilized. Thus, the promotion of carotenoïds rich foods such as yellow to orange-red flesh fruits or non leafy vegetables is important.

Squashes are species of cucurbitaceae with yellow to orange-red flesh fruits. The flesh of squashes is known to be amongst the richest sources of carotenoïds and vitamin C (Arima and Rodríguez-Amaya, 1990). Thus, squashes can be included in the health-promoting properties of fruits and vegetables. Furthermore, the sweetness of their flesh would make them acceptable by children, who are the most exposed to vitamin A deficiency.

The objective of this study was to determine the beta-carotene, vitamin C and total lipids contents of three species of steamed-dried squashes from Cameroon.

MATERIALS AND METHODS

Collection and Handling

The three squashes species cultivated in Cameroon were collected during the harvest period (June and July 2007) from Dschang in the West Province, situated in altitude with tropical and temperate climate. The varieties studied were: Dickinson (*C. moshata*), Hungarian Blue (*C. maxima*) and Sacred Indian Rattle (*C. pepo*). These squashes were placed in polyethylene bags with holes and transported to the laboratory for processing. They were washed with tap water and wiped with dry clothes. Each variety of squashes was splitted into two parts. The seeds were removed with a spoon by scratching the inner part until all the soft part was taken away.

Preparation of Steamed-Dried Squashes

The flesh was sliced into 5 cm thickness and peeled. Sliced squashes were steamed in a heating bath (Büchi B-490) at 85°C for 30 min. The steamed squashes were sliced again into 5 mm thickness, dried in an oven (Binder FDL 115) at 80°C for 4 h and cooled in a desiccator's. Each of the squashes' samples was divided into two parts. One part was left in dark airtight bottles and stored at -16°C for beta-carotene analysis. The other part was used immediately for the assessment of vitamin C, moisture and lipid contents. Samples of crude and steamed squashes were also analyzed. Preliminary assays were done to determine conditions (temperature and duration) of steaming and drying.

Steaming

Sliced squashes flesh of 5 cm was steamed at four different temperatures (80, 85, 90 and 95°C). For each temperature the flesh was steamed for 30 and 60 min. For each of the steaming conditions, slices were removed and the texture judged by 10 panellists accustomed to eating squashes.

Drying

The steamed squashes were sliced again into 5 mm thickness and dried in an oven (Binder FDL 115) at 80°C. After every 30 min, slices were removed and their moisture content determined. For a good preservation, the slices were considered dried when the moisture content was between 8 and 12%.

Chemical Analysis

Moisture Content Determination

Five grams of each of the samples was dried in an oven (Binder FDL 115) at 105°C until constant weight, cooled in a desiccator's and weighed. All samples were analyzed in triplicate.

Beta-Carotene Determination

The beta- carotene content was determined according to the method of Epler *et al*. (1993). Briefly, samples were thawed the day of analysis and homogenized. 0.5 g were introduced into a 15 mL test tube and ethanol (1 Ml), hexane (4 Ml) and NaCl 3M (1 Ml) were added. The mixture was homogenized for 1 min using a vortex mixer and left for 15 h (overnight) at 4°C in the dark. After centrifugation at 3000 rpm for 10 min at 5°C, the hexane phase was removed using a micropipette and introduced into a new test tube. The extraction was repeated one time and the hexane phase was pooled together and evaporated under nitrogen flow. The residue obtained was collected into 1 mL of mobile phase (acetonitrile/methanol/dichloromethane (70/20/10; v/v/v)) and homogenized by a vortex mixer. By using a micro syringe, 60 µL of the extract was injected into the column of HPLC with the following characteristics: column: Supelcosil ™LC- 18; 25 cm length; 4.6 cm diameter and 5 µm particles size; flow rate of the mobile phase: 2 Ml min^{-1}; pressure: 79 to 500 kg cm^{-2}; wavelength of detection: 450 nm. Data were integrated with a Borwin type programme (JMBS, France). All analysis were carried out in duplicate under dim light and the samples were protected by aluminium foil. Identification and evaluation were possible using the retention time and the peak area of the mixture of Carotenoïds standard of known concentration.

Vitamin C Content

The vitamin C content was determined by the method of Harris and Ray (1935). A triplicate of 3 g of sample was homogenized in 5 mL of glacial acetic acid and filtered. The extract was transferred to a volumetric flask of 50 mL and the remaining volume was completed with distilled water. This extract was titrated with 2 mL of 2, 6 dichlorophenol indophenol.

Total Lipids Content

Three grams of sample previously dried at 45°C was extracted continuously in a Soxhlet apparatus for 6 h using hexane as solvent. The determination was made in triplicate. Statistical analysis was done using one way ANOVA, Fisher Snedecor test and Pearson correlation.

RESULTS

Steaming and Drying Conditions

The vitamin C content and the aspect of the squashes according to the temperature and time of steaming presented in Table 1, shows that vitamin C contents decreased significantly ($p<0.05$) with the increasing steaming temperature until 85°C. On the contrary, flesh softening increased with the increase of the steaming temperature and from 90°C the texture was soft and crumbled easily or when slicing. The steaming time influenced neither the vitamin C contents nor the aspect (softness) of the squashes at the same temperature. The residual moisture content of squashes according to drying time (Table 2) shows that the water content decreased significantly with the drying time and after 240 min of drying, the residual moisture of *C. maxima* was 12.25±0.23 g/100 g serving. *Cucurbita pepo* and *C. moshata* seemed to dry faster because the same water content was reached after 150 min of drying.

Beta-Carotene, Vitamin C and Total Lipids Content of Steamed-Dried, Steamed and Raw Squashes Analyzed

The beta-carotene content of steamed-dried squashes (Table 3) ranged from 2834.75±11.22 (*C. pepo*) to 5917.83±720.49 µg/100 g (*C. maxima*) serving. These contents were significantly higher

Table 1: Vitamin C contents and aspect of the flesh squashes at various temperatures and steaming time

Steaming conditions			Squashes species		
Temperature (°C)	Time (min)	Aspect of the flesh	*C. moshata*	*C. maxima*	*C. pepo*
0	0	Raw, solid	9.31±0.26^{a}	17.51±0.76^{a}	19.13±1.67^{a}
80	30	Not cooked, solid	7.50±0.41^{b}	15.42±0.04^{b}	13.09±0.92^{b}
80	60	Not cooked, solid	6.77±0.31^{c}	15.21±0.22^{b}	12.50±0.75^{b}
85	30	Cooked, lightly soft	5.54±0.05^{d}	13.06±1.09^{c}	11.21±1.51^{b,c}
85	60	Cooked, lightly soft	5.30±0.07^{d}	12.30±1.21^{c}	9.01±0.39^{c,d}
90	30	Soft, good to eat, crumble when slicing	3.38±0.04^{e}	11.80±1.19^{c}	9.57±0.76^{c,d}
90	60	Soft, good to eat, crumble when slicing	3.09±0.02^{e}	9.66±0.18^{d}	9.10±0.83$^{c, d}$
95	30	Too soft, crumble easily	3.36±0.05^{e}	8.92±0.07^{d}	8.72±1.46^{d}
95	60	Too soft, crumble easily	3.30±0.05^{e}	8.80±0.09^{d}	8.61±0.18^{d}

Data is expressed as Mean±SD in mg/100 g serving. Values that do not share the same superscript letter(s) are significantly different, Analysis of variance, $p<0.05$

Table 2: Water content in flesh squashes at various drying time

	Squashes species		
Times (min)	*C. moshata*	*C. maxima*	*C. pepo*
0	85.89±0.02^{a}	96.58 ±0.22^{a}	84.51±0.23^{a}
30	76.24±0.18^{b}	93.20±0.20^{b}	80.40±0.15^{b}
60	64.70±0.11^{c}	74.03±0.15^{c}	60.21±0.50^{c}
90	38.64±0.34^{d}	63.23±0.16^{d}	32.31±0.08^{d}
120	23.46±0.24^{e}	34.01±0.00^{e}	14.39±0.37^{e}
150	11.71±0.29^{f}	16.67±0.03^{f}	11.78±0.01^{f}
180	10.40±0.17^{g}	15.68±0.44$^{f, g}$	9.90±0.12^{g}
210	9.99±0.04$^{g, h}$	16.34±0.19^{g}	9.83±0.03^{g}
240	9.22±0.72^{h}	12.25±0.23^{g}	9.23±0.14^{g}

Data is expressed as Mean±SD (g/100 g serving). Values that do not share the same superscript letter(s) are significantly different, Analysis of variance, $p<0.05$

Table 3: Beta-carotene contents of squashes flesh after steaming-drying and steaming

	Squashes species (µg/100 g serving)		
Forms of squashes	*C. moshata*	*C. maxima*	*C. pepo*
Steamed-dried	3043.91±1.65^{a}	5917.83±720.49^{a}	2834.75±11.22^{a}
Steamed	42.76±0.00^{b}	82.86±0.00^{b}	31.85±6.31^{b}
Crude	207.12±0.81^{b}	320.27±13.46^{c}	324.69±6.99^{c}

Data is expressed as Mean±SD. Values that do not share the same superscript letter(s) are significantly different, Analysis of variance, $p<0.05$

Table 4: Vitamin C contents of squashes flesh after steaming-drying and steaming

	Squashes species (mg/100 g serving)		
Forms of squashes	*C. moshata*	*C. maxima*	*C. pepo*
Steamed-dried	5.70±0.32^{cd}	11.81±0.19^{a}	9.82±0.7^{b}
Steamed	3.59±0.02^{e}	6.75±0.27^{c}	4.80±0.38^{de}
Crude	6.73±0.27^{cd}	12.05±1.68^{a}	7.08±0.31^{c}

Data is expressed as Mean±SD. Values that do not share the same superscript letter(s) are significantly different, Analysis of variance, $p<0.05$

($p<0.01$) than those of steamed and crude squashes whatever the species. The vitamin C contents expressed in mg/100 g serving (Table 4) were about 5.70±0.32 (*C. moshata*), 9.82±0.07 (*C. pepo*) and 11.81±0.19 (*C. maxima*). These contents were also significantly higher than those of steamed squashes ($p<0.01$). Table 5 shows that the total lipid content of dried steamed squashes significantly increased ($p<0.01$) as compared with those of steamed squashes and varied from 6.22±0.00 (*C. pepo*) to 7.09±0.11 g/100 g (*C. maxima*) serving. The moisture contents (Table 6) expressed in g/100 g serving of steamed-dried squashes decreased significantly ($p<0.01$) as compared with those of steamed squashes and at the end of drying the water remaining ranged from 6.39±1.18 (*C. maxima*) to 8.19±0.71 (*C. pepo*).

Table 5: Lipid contents of squashes flesh after steaming-drying and steaming

	Squashes species (g/100 g serving)		
Forms of squashes	*C. moshata*	*C. maxima*	*C. pepo*
Steamed-dried	7.09±0.11^{a}	6.74±0.27^{b}	6.22±0.00^{c}
Steamed	0.54±0.02^{fg}	1.04±0.18^{d}	0.67±0.01^{ef}
Crude	0.82±0.07^{de}	0.74±0.02^{ef}	0.39±0.04^{g}

Data is expressed as Mean±SD. Values that do not share the same superscript letters are significantly different, Analysis of variance, $p<0.05$

Table 6: Water contents of squashes flesh after steaming-drying and steaming

	Squashes species (g/100 g serving)		
Forms of squashes	*C. moshata*	*C. maxima*	*C. pepo*
Steamed-dried	6.80±0.49^{e}	6.39±1.18^{e}	8.19±0.71^{c}
Steamed	86.45±0.20^{b}	87.47±2.00^{b}	92.46±0.61^{a}
Crude	87.18±0.86^{b}	90.72±0.56^{a}	92.54±0.87^{a}

Data is expressed as Mean±SD. Values that do not share the same superscript letters are significantly different, Analysis of variance, $p<0.05$

Table 7: Pearson correlation coefficients between parameters

	Squashes species		
Parameters	*C. moshata*	*C. pepo*	*C. maxima*
Water/Lipids	-0.99*	-0.99*	-0.99**
Water/Beta-carotene	-0.99*	-0.99*	-0.99*
Water/Vitamin C	-0.19	-0.89	-0.43
Lipids/Vitamin C	+0.24	+0.87	+0.42
Lipids/Beta-carotene	+0.99**	+0.99*	+0.99*
Vitamin C/Beta-carotene	+0.24	+0.93	+0.50

**,*Significant at 1 and 5%, respectively

Correlations Between Parameters

Significant correlations ($p< 0.05$) existed between the following parameters: water/ beta carotene, water/ total lipids and total lipids/beta carotene (Table 7).

DISCUSSION

The preliminary investigation to choose the temperature and duration of steaming showed that vitamin C contents decreased with the elevation of temperature. This may be due to the diffusion of heat inside squashes with the softening of the pulp. In fact, the softening of the pulp increased with the increase in temperature. This situation would facilitate the diffusion of the heat in squashes and the higher the temperature, the higher the degree of destruction of vitamin C. It is a known fact that vitamin C is easily destroyed by heat (McCay, 1945; Naidu, 2003). This phenomenon was already observed in our earlier research during frying of plantain chips (Demasse Mawamba *et al*., 2007). Seeing that the vitamin C contents of squashes flesh cooked at 85°C were higher than those steamed at 95 and 90°C and because of the crumbling texture at these latter temperatures, the steaming condition chosen was 85°C during 30 min since the steaming time did not influenced significantly the vitamin C losses. To limit micro organisms' development, the drying time where water content was round 12% of serving was chosen. It was 240 min for *C. maxima* species. To have the same conditions of drying this time was chosen. However, the faster drying of *C. pepo* and *C. moshata* lets insinuate that the drying time depended on the initial moisture content as in the steamed-dried squashes, *C. pepo* species had the highest moisture content.

The beta-carotene contents of steamed-dried squashes ranged from 2834.75 (*C. pepo*) to 5917.83 µg/100 g (*C. maxima*) serving. The same values (µg/100 g serving) were found in dried

Cucumis colossus; Khachri leaves (5340±29.00) and Khachri fruit (2450±32.00) from India (Chaturvedi and Nagar, 2001). These values were also similar to those of cooked spinach (6000±1000) and some cultivars of carrot (3400±1500-6100±900) all from Sao Paulo. At the contrary, Sao Paulo mangoes had the beta- carotene contents lower (2500 µg/100 g) than those of steamed-dried squashes (Niizu and Rodriguez-Amaya, 2005). The beta-carotene content of steamed-dried squashes was significantly higher ($p<0.01$) than those of steamed and raw squashes. This higher content may be due to its concentration due to loss of water. In fact, during air drying operation, water moves from food by evaporation and nutrients are not drained. The same phenomenon was observed when the Kachri and Kachri fruit were dried (Chaturvedi and Nagar, 2001). The consumption of 50 g of dried steamed squashed would supply between 54 to 65% of the daily vitamin A requirement of pregnant women and children under 12 years on the basis of conversion factor 1/12.

The total lipid contents of dried steamed squashes varied from 6.22 (*C. pepo*) to 7.09 g/100 g (*C. maxima*) serving. These values are sufficient for a good absorption of carotenoïds. According to earlier researches, 3 to 5 g of lipids is the optimal quantity of lipids for a better absorption of carotenoïds (Roodenburg *et al*., 2000; Van Het Hof *et al*., 2000). This total lipid content was also significantly higher than those of steamed squashes. The situation would be equally due to the concentration of the nutrients due to loss of water through evaporation. The importance of lipids for a good intestinal absorption of carotenoïds had been already proved (Nestle et Ritu, 2003), hence, the concentration of lipids in dried steamed squashes would increase the bioavailability of beta-carotene in this form of consumption of squashes as compared with steamed squashes.

Concerning the vitamin C content, this parameter varied from 5.70 (*C. moshata*) to 11.81 mg/100 g (*C. maxima*) serving. On the contrary, in another study where dry fruits were analyzed, vitamin C was absent (Agte *et al*., 2002). This situation may be due to the drying conditions (temperature and duration) or to the shelf life between packaging and analysis. In fact, those fruits were collected directly from market and the drying conditions were not determined. This content was significantly higher ($p<0.05$) in dried steamed squashes than in steamed squashes. However, the absence of correlation between water and vitamin C allow us to think that although the water losses led to the increase in concentration of vitamin C, it was also destroyed by the heat of drying. Drying of squashes resulted in water loss of about 91% as compared with those of steamed squashes. The reduction in moisture content limits micro organisms' development and nutrients deterioration due to the low water activity (Guiraud, 1998) and consequently allow longer storage.

CONCLUSION

Steamed-dried squashes resulted in a good retention of beta-carotene, total lipids and vitamin C. In view of this, steamed-dried squashes would be an effective tool to contribute to fight against vitamin A deficiency related diseases. It is therefore important to implement and develop small industries of steaming-drying of squashes flesh in order to prolong their shelf life and consequently their availability.

ACKNOWLEDGMENTS

This study was funded by The International Foundation for Science (IFS) (Grant E -3584-2) and FOSNNA research Grant 2006 allocated, respectively to Gouado Innocent and Demasse Mawamba A.

REFERENCES

Agte, V., K. Tarwadi, S. Mengale, A. Hinge and S. Chiplonkar, 2002. Vitamin profile of cooked foods: How healthy is the practice of ready-to-eat foods? Int. J. Food. Sci. Nutr., 53: 197-208.

Arima, H.K. and D.B. Rodriguez-Amaya, 1990. Carotenoid composition and vitamin A value of a squash and a pumpkin from Northeastern Brazil. Arch Latinoam Nutr., 40: 284-292.

Chaturvedi, Y. and R. Nagar, 2001. Levels of beta-carotene and effects of processing on selected fruits and vegetables of the arid zone of India. Plant Foods Hum. Nutr., 56: 127-132.

Demasse-Mawamba, A., I. Gouado, M. Leng, R. Ejoh Aba, J.M. Njinkoue and F. Tchouanguep-Mbiapo, 2007. Losses in beta-carotene and vitamin C due to frying of plantain (*Musa paradisiacal*) chips. Afr. J. Biotechnol., 6: 280-284.

Epler, K.S., R.G. Ziegler and N.E. Craft, 1993. Liquid chromatogrphy methods for the determination of carotenoïds, retinoids and tocopherols in human serum and in food. J. Chromatogr., 619: 37-48.

Gouado, I., T.F. Mbiapo, F.P. Moundipa and M.C. Teugwa, 1998. Vitamin A and E status of some rural populations in the North of Cameroon. Int. J. Vitam. Nutr. Res., 68: 21-25.

Gouado, I., R.A. Ejoh, M. Kenne, F. Ndifor and T.F. Mbiapo, 2005. Serum concentration of vitamins A and E and lipid in rural population of North Cameroon. Ann. Nutr. Metab., 49: 26-32.

Gouado, I., E.R. Aba, I.T. Some, J.S. Florian and M.F. Tchouanguep, 2007. Carotenoids content of some locally consumed fruits and yams in Cameroon. Pak. J. Nutr., 6: 497-501.

Guiraud, J.P., 1998. Conditions du Developpement Microbien. In: Microbiologie Alimentaire, Dunod, R.I.A. (Ed.). MARC, Paris, ISBN: 9782100072590, pp: 1-16.

Harris, L.J. and S.N. Ray, 1935. Diagnosis of vitamin C sub nutrition by urine analysis: With a note on the antiscorbutic value of human milk. The Lancet, 225: 71-77.

Humphrey, J.H., T. Agoestina, L. Wu, A. Usman and M. Nurachim *et al*., 1996. Impact of neonatal vitamin A supplementation and infant morbidity and mortality. J. Pediatrics, 128: 489-496.

Hussey, G.D. and M. Klein, 1990. A randomized controlled trial of vitamin A in children with severe measles. N. Engl. J. Med., 323: 160-464.

McCay, C.M., 1945. Nutritional problems that arise in large scale cookery. Am. J. Public Health, 35: 143-152.

Naidu, A.K., 2003. Vitamin C in human health and disease is still a mystery? An overview. Nutr. J, 2: 7-7.

Nestle, P. and N. Ritu, 2003. Food Preparation Practices can Affect Provitamin a Carotenods Content and Bioavailability. Human Nutrition Institute, USA., pp: 3.

Niizu, P.Y. and D.B. Rodriguez-Amaya, 2005. New data on the carotenoid composition of raw salad vegetables. J. Food Composit. Anal., 18: 739-749.

Roodenburg, A.J.C., R. Leenen, K.H. Van-Het-Hof, J.A. Weststrate and L.B.M. Tijburg, 2000. Amount of fat in the diet affects bioavailability of lutein esters but not of α-carotene, β-carotene and vitamin E in humans. Am. J. Clin. Nutr., 71: 1187-1193.

Van-Het-Hof, K.H., C.E. West, J.A. Weststrate and J.G. Hautvast, 2000. Dietary factors that affect the bioavailability of carotenods. J. Nutr., 130: 503-506.

Van-den-Berg, H., R. Faulks, H.F. Granado, J. Hirschberg and B. Olmedilla *et al*., 2000. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. J. Sci. Food Agric., 80: 880-912.

West, K.P., 2002. Extent of vitamin a deficiency among preschool children and women of reproductive age. J. Nutr., 132: 2857S-2866S.