Eur J Nutr (2013) 52:583–589
DOI 10.1007/s00394-012-0360-2

ORIGINAL CONTRIBUTION

Sun-dried cowpeas and amaranth leaves recipe improves β-carotene and retinol levels in serum and hemoglobin concentration among preschool children

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Received: 2 December 2011 / Accepted: 6 April 2012 / Published online: 5 May 2012

Abstract
Purpose Vitamin A deficiency (VAD) and anemia are major challenges among children and expecting and lactating mothers in developing countries. Intervention with locally available dark green leafy vegetables (DGLV) is more sustainable to eradicate VAD, being cost-effective and readily adaptable to local communities. DGLV contain high levels of iron and β-carotene (BC) and therefore useful in fighting VAD and anemia. Since DGLVs are season-dependent sun-drying enables their availability during low seasons. However, their contribution to the bioavailability of BC and the improvement of hemoglobin are not well understood. The study therefore investigated the effect of consuming cooked recipe consisting of sun-dried amaranth and cowpea leaves on the levels of BC, retinol, and hemoglobin in preschool children from Machakos District, a semiarid region in Kenya.
Methods Vegetables were purchased from local vegetable market, with some sun-dried in an open shade. Levels of BC and retinol in serum and BC in fresh and processed vegetables were determined by a HPLC method and hemoglobin using a portable Hemocue Analyzer.
Results All-*trans*-BC levels in uncooked fresh cowpea and amaranth leaves were 806.0 μg/g and 599.0 μg/g dry matter, respectively, while the dehydration and cooking processes retained the β-carotene levels at over 60 %. Consumption of the dehydrated vegetables significantly improved both serum BC and retinol levels ($p < 0.05$), while the baseline hemoglobin levels improved by 4.6 %.
Conclusion The study has shown that intervention with locally available sun-dried vegetables improves the bioavailability of BC, retinol, and hemoglobin levels among preschool children.

Keywords β-carotene · Retinol · Hemoglobin · Vitamin A deficiency · Sun-dried vegetables

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Introduction

A significant proportion of deaths in young children in developing countries is attributable to the effects of malnutrition, and therefore making efforts to reduce malnutrition a priority to governments [1]. Although malnutrition is a global issue, the effects of vitamin A deficiency (VAD) and iron deficiency are more prevalent in developing countries, especially among children and women [1–4]. The world health organization has listed Kenya among 72 countries in the world suspected to have low serum retinol levels, with VAD prevalence of 76 % documented in 11 districts [5]. The effects of VAD such as morbidity and mortality of young children, maternal mortality and poor outcomes in pregnancy, and lactation and other diseases such as cancer and heart-related illnesses, can be avoided if the consumption of fruits and vegetable, especially indigenous dark green leafy vegetables (DGLV) is enhanced [1, 6–8]. This nutritional approach for reducing malnutrition is more sustainable in developing countries, although its impact is hampered by seasonal variability of DGLV [9]. Other than being a source of pro-vitamin A carotenoids, DGLVs are also a good source of iron and vitamin C. β-carotene (BC), β-cryptoxanthin, and α-carotene are common carotenoids (pro-vitamin A compounds) in fruits and vegetables that are converted to retinol (vitamin A).

The amount of retinol formed from carotenoid-rich foods depends on the bioavailability (absorption and circulation time in the body) of carotenoids and the selectivity and reactivity of the cleavage enzymes.

Vegetables contain high moisture content and are perishable but they can be made available despite their seasonal variability. Solar dehydration is a technique suitable in many developing countries for the preservation of DGLV to avail them during the seasons of low or no availability [10, 11]. The nutritional impact of dehydrated fruits and vegetables in addressing VAD and anemia needs investigation. Findings from such studies have shown that the consumption of fruits and vegetables improves the bioavailability of BC in serum when various food matrices such as carrots, melons, and oranges were considered [12–19]. Among the various parameters that affect the bioavailability reported are the effects based on the presence of other compounds in the food matrix [12, 13], different food matrices [14–16], and the different pro-vitamin A compounds [17], while the influence of processing food matrices has also been reported [18, 19]. A study on the relationships between the consumption of different types of fruit and vegetable and serum concentrations of antioxidant vitamins indicated that serum BC status was associated with consumption [16]. Different cooking procedures of green leafy vegetables are able to provide high levels of all-*trans*-BC among children [18]. Investigation of the relationship between serum beta-carotene and the consumption of dehydrated green leafy vegetables has not been reported, yet solar and sun-dehydrated vegetables retain BC for about 6 months and thus enabling availability of these foods for use during dry seasons [9]. This study investigated the effect of consuming a recipe consisting of sun-dried cowpea and amaranth leaves on serum BC, retinol, and hemoglobin levels among preschool children aged 2.5–6 years [20]. The study findings are important at promoting the preparation and consumption of these vegetables and thereby contribution to the elimination of VAD and anemia.

Materials and methods

This was an intervention study involving preschool children from schools in Kanzalu Location of Machakos District, Kenya, a semiarid region. One hundred and fifty-two (152) preschool children, aged 2.5–6 years and whose parents consented to the study and were neither ill nor had recently been hospitalized were recruited to the study. The study was approved by the Ethical Review Committee of Kenyatta University and the Ministry of Education, Kenya. The children, in different schools randomly selected from among those with preschool classes were grouped into two study groups and each with a corresponding control group. For easy interventional logics, children in a school belonged to the same study or control group. The intervention was carried out for 13 weeks (a full school academic term), and no child dropped out during the study. Prior to intervention, stool examination for parasitic infection was done under direct light microscopy by smear technique in physiological saline. The subjects who tested positive were subsequently treated with an antihelminthic drug (Albendazole syrup, 10 mls of 400 mg/child; SmithKline Beecham pharmaceuticals).

The first group, the fresh group had 56 children fed on a recipe of mixed fresh cowpea and amaranth leaves (20:80 wt/wt), while 20 children in the second group, the sun-dried group fed on a recipe of the mixed sun-dried cowpea and amaranth leaves (20:80 wt/wt). The children in the control groups ($n = 51$ for the fresh group; $n = 25$ for sun-dried group) fed on a recipe of white cabbage, a low beta-carotene-containing vegetable. There were fewer children in the sun-dried group based on the number of children in the schools selected and on the parental consent. The recipes were prepared at individual schools as per the local community procedure of preparing vegetables. The fresh recipes were prepared by cleaning the amaranth, cowpeas, and white cabbage leaves, chopping into small pieces with a kitchen knife and boiling in an appropriate amount of water for 10–15 min. Then, 80–90 g wet weight of boiled vegetables was fried for 5 min with 8 g of fat and 10 g each of onions and tomatoes, and salted to taste. The sun-dried vegetables were rehydrated by leaving in water for 30 min before boiling and preparing as in fresh vegetable recipe. The children were served with an average of 80 g (wet weight) of the recipe accompanied by a paste made from maize flour (*ugali*) once a day, 5 days a week for 13 weeks. The amount served was expected to meet the required daily allowances (RDA) of retinol equivalents (RE) for the subjects.

The fresh vegetables, obtained from Wakulima market in Nairobi daily, were used either to prepare sun-dried vegetables, delivered to study site daily for the preparation of fresh recipes, while a small portion was used for laboratory analysis. Sun-drying of vegetables was done by spreading blanched (in boiling water) fresh vegetables on perforated wire mesh tray and left to dry in the shade for 6–8 h to a moisture content of <10 %. Drying was done in the entire period of the study, and the drying conditions depended on the prevailing weather conditions, which was mainly dry and sunny with ambient day temperatures ranging between 21 and 28 °C and relative humidity of 41–45 %. The sun-dried vegetables were packed and sealed in clean polythene bags and delivered to study site within 1 week of drying.

A clinical laboratory technician drew 3 ml whole blood by venipuncture into no-additive vacutainers and placed in cool boxes before centrifuging at 800 rpm for 10 min to separate serum within an hour. The serum samples were then transported to Kenya Medical Research Institute (KEMRI) laboratories, Nairobi, in cool boxes and kept at −80 °C until analysis for BC and retinol.

In the procedure for the analysis of BC in the vegetables, 25 g of fresh vegetable samples were initially homogenized by blending (Automix, Fisons, England) with 50 ml distilled de-ionized water containing 0.5 % ascorbic acid for 5 min. Five grams of the resultant mixture was extracted with 50 ml of acetone/hexane 3:2 (v:v) containing 0.1 % BHT by ultrasonically agitating (ultrasonic agitator, Model KS 250) at moderate speed for 10 min. Three or four re-extractions were done to make the mixture colorless. For dehydrated samples, 0.5 g of ground samples were rehydrated with 2 ml of distilled water before extracting as above. Extracts were saponified (to remove chlorophyll) with 25 ml of saturated methanolic potassium hydroxide (10 %) before washing twice with 100 ml sodium chloride (10 %v/v) followed by four times with 100 ml water. Excess water was dried over anhydrous sodium sulfate. Hexane was evaporated to near dryness by a rotary evaporator at 30 °C and the vacuum broken with nitrogen. The extract was reconstituted with methanol/dichloromethane (DCM) (9:1 v/v) containing 0.01 % BHT. HPLC system (Shimadzu) was used to isocratically elute 20 μl on a C_{18} HPLC column (250 × 4.6 mm id, 5 μm particle size, Vydac TP-201) using mobile phase consisting of methanol/DCM/water in the ratio 79:18:3 (v:v:v). The flow rate was 0.8 ml/min with a run time of 40 min.

In the analysis of BC in serum, 200 μl double-distilled de-ionized water was used to dilute 200 μl of serum after thawing and de-protonated by vortex mixing (Automatic Labo-mixture, Model NS-8) with 400μl ethanol containing 0.01 % BHT for 30 s. Duplicate extractions with pure hexane were done by centrifuging at 800 rpm (Model, H-2000C Kokusan, Japan), at 5 °C for 15 min. The combined supernatant was evaporated under nitrogen at 30 °C, and the residue re-dissolved in 150 μl (4:1) methanol/dichloromethane, vortex mixed and sonicated for 10 min. HPLC system (Shimadzu) was used to isocratically elute 20 μl on a C_{18} HPLC column (250 × 4.6 mm id, 5 m particle size, Vydac TP-201) using mobile phase consisting of methanol/DCM/water in the ratio 83:15:2 (v:v:v). The flow rate was 0.8 ml/min with a run time of 15 min for the detection of BC at 450 nm. For retinol extraction, a similar procedure as for BC was followed with volumes of 300 μl serum and reconstitution done using ethyl acetate, while detection was done at 325 nm. HPLC determination for BC and retinol was achieved by preparing standard solutions using all-*trans*-BC (Type IV) and all-*trans*-retinol and using them to prepare a calibration curve [9, 19]. The amount of retinol equivalents was calculated based on a 10:1 conversion for BC/Retinol [37].

Hemoglobin measurement was done based on partial total blood count using a portable battery operated hemocue analyzer (Model B-Hemoglobin, Angelholm, Sweden). The student's *t*-test was used to compare mean values obtained, while linear regression analysis was used to correlate pre-intervention and post-intervention data.

Results

The effect of drying and cooking on the all-trans-*β*-carotene content

The mean levels and retentions of *β*-carotene (BC) with sun-drying and further cooking of amaranthus and cowpea vegetables are shown in Table 1. The indigenous green leafy vegetables contain high levels of BC, with cowpea leaves having a mean value of 806.0 μg/g DM (range, 779.1–827.0) and amaranthus had a mean of 599.0 μg/g DM (range, 553.0–639.1), while white cabbage leaves had

Table 1 The concentration (μg/g DM) and retentions (%) of all-trans-BC in fresh, dried, and cooked vegetables

Vegetables	Concentration (μg/g DM)		Retention (%)
	Mean ± SD (CV, %) ($n = 3$)	Range	
Cow peas leaves			
Fresh uncooked	806.0 ± 21 (2.6)	779.1–827.04	100.0
Fresh cooked	612.1 ± 34 (5.6)	583.0–649.13	77.6
Sun-dried uncooked	553.0 ± 29 (5.2)	533.0–601.01	70.1
Sun-dried cooked	474.0 ± 48 (10.1)	391.1–509.16	61.0
Amaranthus leaves			
Fresh uncooked	599.0 ± 34 (5.7)	553.0–639.07	100.0
Fresh cooked	476.0 ± 46 (9.7)	428.1–526.06	76.5
Sun-dried uncooked	402.0 ± 45 (11.2)	350.0–466.01	66.2
Sun-dried cooked	395.0 ± 42 (10.6)	331.0–439.10	59.0

Table 2 Levels of serum beta-carotene (μmo1/L) for pre- and post-intervention periods in study groups

Intervention	Mean concentration (μmo1/L)[a]	
	Fresh vegetable group ($n = 56$)	Control group ($n = 51$)
Pre-intervention	0.1 ± 0.1 (0.0–0.9)	0.1 ± 0.1 (0.1–0.5)
Post-intervention	0.5 ± 0.2 (0.1–0.9)	0.2 ± 0.1 (0.1–0.6)
Change (%)	253.0	9.4
p value	0.000	0.069
	Sun-dried group ($n = 20$)	Control group ($n = 25$)
Pre-intervention	0.2 ± 0.1 (0.1–0.5)	0.1 ± 0.1 (0.1–0.3)
Post-intervention	0.4 ± 0.2 (0.1–0.8)	0.1 ± 0.0 (0.1–0.2)
Change (%)	(+)133.1	(−)18.98
p value	0.000	0.087

[a] Ranges in parenthesis

Table 3 Levels of serum retinol (μmo1/L) for pre- and post-intervention periods in study groups

Intervention	Mean serum retinol concentration (μmo1/L)[a]	
	Fresh vegetable group ($n = 56$)	Control group ($n = 51$)
Pre-intervention	0.6 ± 0.1 (0.5–0.8)	0.6 ± 0.1 (0.3–1.0)
Post-intervention	0.8 ± 0.1 (0.5–1.1)	0.7 ± 0.1 (0.5–1.0)
Change (%)	(+)28.9	(+)5.9
p value	0.000	0.086
	Sun-dried group ($n = 20$)	Control group ($n = 25$)
Pre-intervention	0.6 ± 0.1 (0.4–0.9)	0.6 ± 0.1 (0.5–0.8)
Post-intervention	0.8 ± 0.1 (0.6–1.0)	0.6 ± 0.1 (0.5–0.8)
Change (%)	(+)25.9	(+)0.2
p value	0.000	0.145

[a] Ranges in parenthesis

a mean of 105.0 μg/g DM (range, 94.0–127.1). Cooking of fresh vegetables reduced the levels of BC, retaining between 77.6 and 76 % in cowpeas and amaranthus, respectively. While sun-drying retained 70.1 and 66 % BC in cowpeas and amaranthus leaves, respectively, cooking resulted in further loss to retain 61 and 59 % BC, respectively. Cowpea leaves have high fiber content and therefore experiences lower reduction in BC content as a result of sun-drying and cooking [11].

The changes of serum β-carotene levels on intervention

Recipes consisting of cooked fresh and sun-dried vegetables were used for the intervention studies and the levels of serum BC monitored. The results of pre- and post-intervention serum BC levels are given in Table 2. The pre-intervention and post-intervention mean levels of serum BC for the study group were 0.1 ± 0.1 and 0.5 ± 0.2 μmo1/L, respectively, for the fresh group, and 0.2 ± 0.1 and 0.4 ± 0.2 μmo1/L for the sun-dried groups, respectively. The student's *t*-test indicates that the mean levels between the pre-intervention and post-intervention were significantly different in the two groups ($p < 0.05$). The control groups for the corresponding study groups, all using white cabbage recipe had mean levels of 0.138 ± 0.1 μmo1/L and 0.1 ± 0.1 μmo1/L, respectively, and were not significantly different from those of the study groups. The mean values were within the normal levels of 0.093–0.465 μmo1/L [21]. However, levels of some subjects (about 30 %) were below the normal range, while 20 % had higher levels than normal range in the study group.

The changes of serum retinol levels on intervention

Table 3 represents the results of the mean serum retinol for the pre- and post-intervention periods. The table indicates the pre-intervention mean levels of retinol for the fresh and sun-dried groups of 0.6 ± 0.1 and 0.6 ± 0.1 μmo1/L, respectively, and those of the post-intervention of 0.8 ± 0.1 and 0.8 ± 0.1 μmo1/L, respectively, gave changes that were statistically different ($p = 0.000$). The corresponding control groups had changes in mean levels between pre-intervention and post-intervention not significant. The pre-intervention levels were less than the minimum recommended level of 0.7μmo1/L for children aged 2–6 years and thus indicating VAD [21], although the individual levels indicated that about 40 % of the subjects had higher levels than the minimum recommended level.

Table 4 Levels of hemoglobin (mg/L) for pre- and post-intervention periods in study groups

Intervention	Hemoglobin concentration (mg/L)[a]	
	Fresh vegetable group ($n = 56$)	Control group ($n = 51$)
Pre-intervention	11.5 ± 1.1 (7.9–13.3)	12.4 ± 1.2 (9.7–14.8)
Post-intervention	12.2 ± 1.2 (7.5–13.9)	12.3 ± 1.2 (9.4–14.7)
Change (%)	(+)5.9	(−)0.4
p value	0.060	0.102
	Sun-dried group ($n = 20$)	Control group ($n = 25$)
Pre-intervention	11.6 ± 1.1 (8.9–13.0)	12.3 ± 1.1 (10.7–14.2)
Post-intervention	12.2 ± 1.2 (9.8–14.2)	12.2 ± 1.1 (10.3–14.0)
Change (%)	(+)4.6	(−)0.3
p value	0.068	0.090

[a] Ranges in parenthesis

The changes of hemoglobin levels on intervention

The levels of the hemoglobin before and after intervention are shown in Table 4. The results for the pre- and post-intervention periods for the fresh and sun-dried study groups were 11.5 ± 1.1 and 11.6 ± 1.0 mg/L, respectively. The pre-intervention mean levels in the fresh and sun-dried study groups were below the recommended minimum level of 12.0 mg/L, although from the individual levels about 30 % of the subjects had levels higher than recommended level. After intervention, these levels improved with 5.9 % in the fresh group, while the sun-dried group showed a 4.6 % but these changes were not significant ($p > 0.05$). However, the number of subjects who had hemoglobin levels below the recommended levels decreased from 82 to 25 % and from 55 to 35 % in the fresh and sun-dried groups, respectively.

Discussion

The BC levels decreased in the vegetables after sun-drying, a process that results from the effect of heat during blanching and drying, and cooking [13]. Carotenoids are destroyed or altered by high temperature, low pH, treatment with acids, irradiation with light in the presence of a catalyst such as iodine, oxygen, and enzymes in the processes of isomerization and oxidation [22, 23]. However, after sun-drying and cooking, the vegetables retained 61 % in cowpea leaves and 59 % in amaranth leaves. Other studies have reported an increase in BC with cooking, attributing this to increased tissue breakdown and accessibility of carotenes to the extracting solvent, possibly as a result of disruption of carotenoid–protein complexes [18, 24, 25]. Similarly, the content of carotenes remaining unchanged under mild cooking conditions has been reported [24].

Consumption of about 80 g of the fresh or dehydrated recipe containing a mixture of cowpea and amaranth leaves provided 546.7 or 437.6 RE per day, respectively; meeting the 500µg RDA for children aged 2–6 years [3]. Some studies have indicated that dehydrated vegetables contain sufficient amounts of beta-carotene when consumed in the required amounts [19, 26]. However, various processing conditions influence the bioavailability of pro-vitamin compounds [18, 19, 24, 29, 33, 34]. Cooking assists in releasing the compounds from the food matrix by disrupting cellular membranes and liberating the nutrients [29]. The effect of heat as a result of sun-drying and cooking potentially increases the bioavailability of BC through disruption/softening of plant cell wall and breaking carotenoid–protein complexes [18, 24]. Addition of fat to the cooking increases carotenoid bioavailability by providing a soluble media for these hydrophobic compounds released from the food matrix and stimulates secretion of bile salts and pancreatic lipases required for micelle formation, thereby increasing absorption [19, 33].

After intervention with the vegetable recipes for 13 weeks, the levels of BC significantly improved in both study groups ($p = 0.00$). The change after intervention in the fresh group indicated an increase of 253 % as compared to an increase of 9.4 % in the corresponding control group, while the sun-dried group showed a 133 % increase as compared to 19 % in its corresponding control group. The correlation analysis between the pre-intervention BC levels and the changes in the BC levels following intervention gave negative correlation coefficient values ($r = -0.6$, fresh group; $r = -0.3$, fresh control; $r = -0.2$, sun-dried group; and $r = -0.5$, sun-dried control). While this indicated that subjects who had low levels of BC at pre-intervention had a higher increase post-intervention for both study groups, the intensity though was higher for subjects in the fresh group.

Similarly, after intervention, the levels of retinol improved significantly in the study groups. The change in the fresh group indicated a significant increase of 29 % ($p = 0.00$) as compared to an increase of 5.9 % in the corresponding control group, while the sun-dried group showed a significant increase of 25.9 % ($p = 0.00$) as compared to 0.2 % in the corresponding control group. The post-intervention levels for subjects in both study groups indicated that the subjects had attained the minimum recommended levels.

While the levels of retinol improved significantly in both study groups, the change (29.0 % for the fresh group and of 25.9 % for the sun-dried group) was not as high as the corresponding change for BC. The correlation analysis for the pre-intervention retinol levels and the changes in the levels of retinol following intervention for the two study groups indicated negative correlation coefficient values, implying that subjects with low levels of retinol at pre-intervention period had higher increase post-intervention for study group subjects, while those with low levels of retinol in the control groups decreased post-intervention. While vegetable consumption was shown to be predictive for serum BC from high correlation values, the serum concentrations, however, correlate only moderately with estimated dietary intake of vegetables and should therefore be used with caution as biomarkers of vegetable intake [7]. The low retinol change may be explained from the fact that bioconversion of BC to retinol is complex. For dietary carotenoids to be absorbed intestinally, they must be released from the food matrix and incorporated into mixed micelle, a process that can be interfered with by several factors such as species of carotenoids, linkages at molecular level, amount of carotenoids, food matrix, effectors of absorption, nutrient status of the host, genetics, host related factors, and interactions of the carotenoids [27–35].

The increase in the levels of hemoglobin between pre-intervention and post-intervention levels was not significant, although there was an increase in the number of subjects with levels above the recommended figure of 12.0 mg/L. This is explained by the fact that iron was mobilized as a result of the increase in serum retinol (Table 3) and as well DGLVs provide iron in a diet [1, 15].

Conclusion

The indigenous dark green leafy vegetables such as amaranth and cowpeas that contain high levels of BC are effective at increasing serum levels of beta-carotene and retinol. Although heat processing by sun-drying and cooking of the fresh vegetables reduces the levels of beta-carotene by between 39 and 41 %, the amount remaining meets the daily required amounts. The intervention with fresh and sun-dried vegetables increased the serum mean levels of BC substantially, by over 100 %, implying that the consumption of these vegetables bioavails BC. The increased post-intervention levels of beta-carotene for subjects in study groups resulted in the subjects attaining the minimum recommended levels of retinol. The results here support the documentation on the effectiveness of DGLVs in improving serum BC and retinol levels in children [35, 36]. However, while serum BC and other carotenoids concentrations have been positively correlated with consumption of both fruit and vegetables in many studies [17, 19–21, 30], other studies have reported that fruits were more effective in improving vitamin A status than DGLVs [12, 37]. With proper awareness and consumption of the right amount of sun-dried dark green leafy vegetables by children in semi-arid and arid lands, VAD will be eradicated. The findings support the realization that one of the most effective and sustainable way to curb VAD is through food-based strategies, which are able with time to build into and become part of the existing dietary systems.

Acknowledgments The authors wish to acknowledge DAAD Germany Academic Exchange Service for full sponsorship, Kenyatta University Deans Research Grant, International Plant Genetic Resource Institute (IPGRI), and Sigma-X_i for partial financial support; Kenya Medical Research Institute (KEMRI) for serum sample storage, Department of Nutritional Science and Technology, Jomo Kenyatta University of Agriculture and Technology (JKUAT) and Department of Chemistry, University of Siegen, Germany for attachment and HPLC method development.

Conflict of interest The financial relationship with the organizations only assisted in enabling carrying of the study and apart from acknowledging the assistance the authors declare that there is no conflict of interest.

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