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Use of kitchen steel wool as oxygen absorber improves storage retention of beta-carotene in solar-dried vegetables

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Abstract: Vitamin A deficiency remains a major health concern in developing countries whereas the season availability of vegetables could provide for vitamin A. Dehydration is widely used to preserve dark green leafy vegetables (DGLV) but storage in normal atmosphere condition losses beta-carotene by oxidation, therefore requiring use of an oxygen absorber. The study examined use of kitchen steel wool as an oxygen absorber in reducing the loss of beta-carotene content in three indigenous DGLVs that were solar dried and stored for a period of 168 days in four different packing conditions. Fresh vegetables contained between 781.94 to 1047.42 µg/g dry matter (DM) beta-carotene, reducing significantly (p=0.01) to between 653.63 to 712.99 µg/g DM after dehydration. Steel wool oxygen absorber significantly improved (p = 0.02) beta-carotene retention, recording a loss of 19.5 to 37.6% compared to 47 to 72% in normal conditions. Storage of DGLVs under kitchen steel wool oxygen absorber preserves vegetables and retains high levels of beta-carotene.

Key words: Dark green leafy vegetables, beta-carotene, oxygen absorbers, kitchen steel wool, solar drying

Introduction

Vitamin A (VA) is extremely important for normal growth and development especially for young children where it prevents night blindness, drying of the skin and mucus membranes, and enhances immune function in the body[1,2]. In animal tissues, VA is present in preformed VA or retinol while in plants it exists pro-vitamin A carotenoids, with beta-carotene being predominant. Most developing countries experience inadequate VA provision because the main sources is fruits and vegetables that have seasonal variability and animal based sources are limited and expensive for the majority population. VA availability in the body is limited due to such factors as the quality and accessibility of pro-VA rich foods, bio-availability and health status in relation to diseases and/or infection[3,4].

The vitamin A deficiency (VAD) is attributed to over 70% of all cases of children blindness in the world, especially in developing countries[2]. The intervention measures used to alleviate VAD include such strategies as distribution of VA capsules and food fortification[5,6]. However, the best long-term approach is through production and consumption of locally available VA rich foods particularly dark-green leafy vegetables (DGLVs) such as cowpea leaves (*vigna uinguiculata*), amaranthus (*amaranthus hybridus (ll)*) and black nightshade (*solanum nigrum*). Dark green leafy vegetables are important sources of micronutrients (pro-VA, vitamin C, vitamin E, zinc, iron) and dietary fibre[7,8]. Among the pro-VA carotenoids,

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beta-carotene is the most important and abundant carotenoid with high pro-VA activity[9]. The DGLVs are fairly acceptable among the population and although they are season dependent, they are known to be effective in combating VAD, enhancing immunity and combating other diseases such as cancer and coronary diseases[5,10]. Processing and preservation procedures are therefore used to counter seasonal availability in order to avail them all year round[8,11].

Dehydration is the most widely used method of preservation in most developing countries through sun- and solar-drying processes. However, storage of the dried vegetables under normal atmospheric conditions results in nutritive degradation, especially of beta-carotene[8,12]. During processing and storage of the dried vegetables, beta-carotene is degraded through oxidation reactions[8,13]. Methods that focus on removal of oxygen during storage of specific foods or food products such as modified atmosphere Packing (MAP), controlled atmosphere storage (CAS), thin film packaging and use of oxygen absorbers are able to preserve and retain high levels of beta-carotene but initial auto-oxidation process that result in partial reduction is inevitable[11,14,15].

Use of oxygen absorbers is most favored in the preservation of oxygen sensitive foods especially those containing polyunsaturated fats and carotenoids[11,16]. The procedure involves use of sachets like desiccant wrapped in gas permeable materials and placed inside the packaged product. Other models for oxygen removal include use of an ethyl acetate polymer film as the reaction medium to absorb excited oxygen produced by a photosensitized dye in the headspace of a transparent package[12,16]. Use of iron-based oxygen absorbers based on the principle of irreversible iron rust formation are common and when effectively used are capable of reducing oxygen concentrations in packaging containers to levels less than 0.01% and maintaining these levels during the entire storage period[12]. Such methods are cheap and convenient to use, prevent oxidation of food or food products, prevent the growth of aerobic pathogens and spoilage organisms, including moulds, eliminates the need for additives such as BHA, BHT, sulphur dioxide, sorbates and benzoate and are capable of extending the shelf life of foods[17,18]. The purpose of this study was to determine the effectiveness of using iron wool sachets in reducing the degradation of beta-carotene in stored solar dehydrated DGLV.

Results and Discussion

The levels and the percentage retention of beta-carotene in fresh and solar dehydrated vegetables are presented in Table 1.

Table 1: β-Carotene content (μg/g DM) in fresh and solar dried vegetables and % retention

Vegetable	**Concentration (mean**±SD) **(μg/g)**		**% retention**
	Fresh	**Solar-dried**	
Amaranth	1047.42±0.01	712.99±0.01	68.07
Nightshade	1024.18±0.03	693.55±0.02	68.72
Cowpea leaves	781.94±0.01	653.63±0.03	83.59

The content of beta-carotene in fresh vegetables varied from 781.94 μg/g to 1047.42 μg/g dry matter (DM) while that in the solar dried vegetables ranged from 653.63 to 712.99 μg/g. The content of β-carotene in dark green leafy vegetables depends on variety, season, stage of vegetable maturity and losses between harvesting and analysis[19]. The solar dried vegetables retained between 68 and 83% of the beta-carotene in the fresh vegetables, with cowpea leaves

experiencing the lowest degradation of beta-carotene. Cowpea leaves are more fibrous than the other leafy vegetables and thus beta-carotene experiencing less exposure to oxidation during drying[19].

Three types of solar dried vegetables; amaranthus (AMR), nightshade (NTS) and cowpeas (CWP) were stored under an oxygen absorber (STW), a non-oxygen absorber as a control (CTR), nitrogen (N_2) and normal conditions (NOR), and the trends of reduction in beta-carotene content during the storage of 168 days are presented in Figures 1-3.

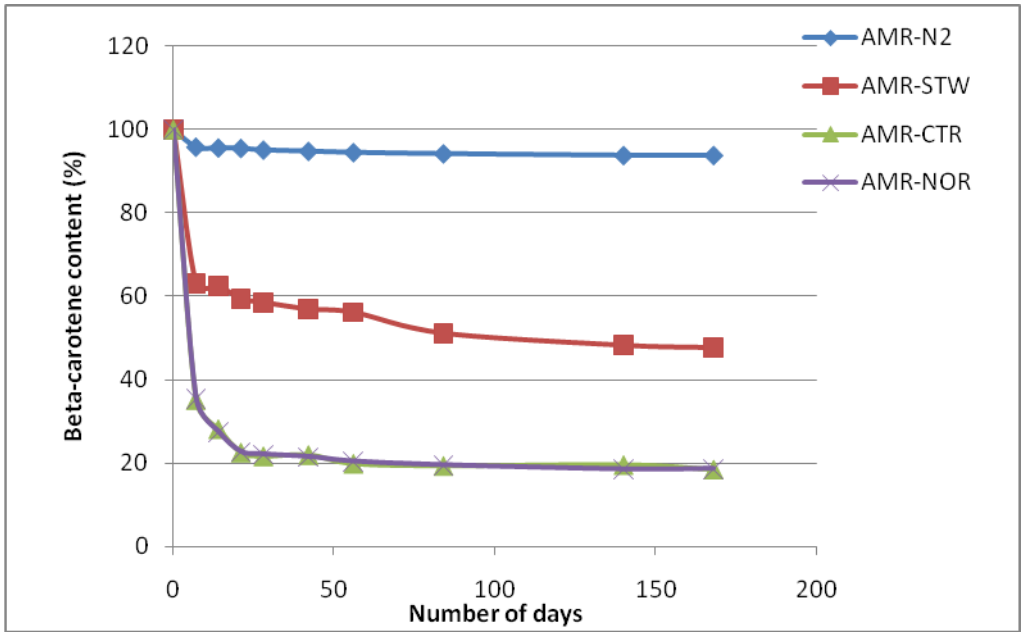

Figure 1: Trends of beta-carotene degradation in solar-dried amaranth leaves stored in four different conditions for 168 days.

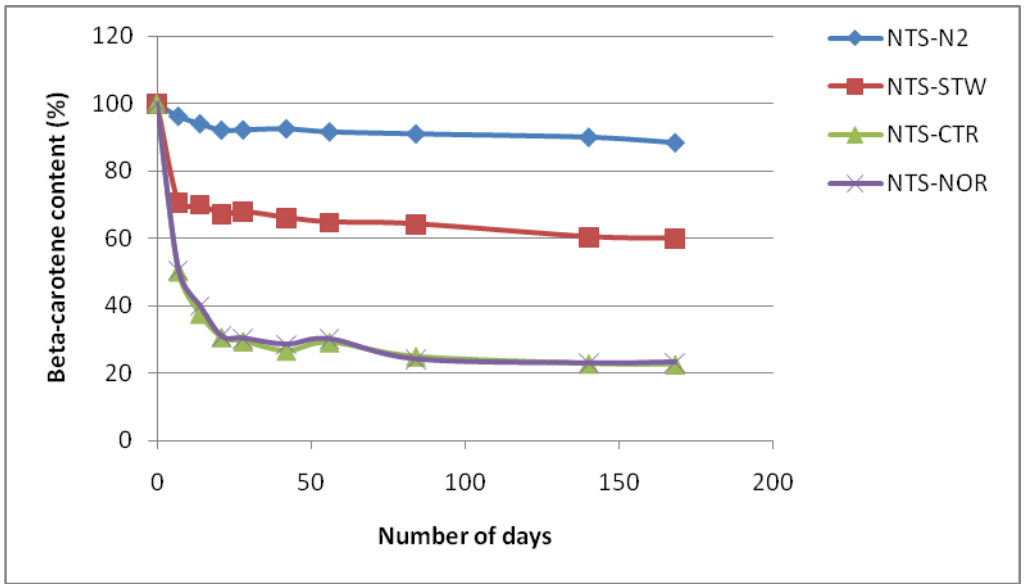

Figure 2: Trends of beta-carotene degradation in solar-dried nightshade leaves stored in four conditions for 168 days.

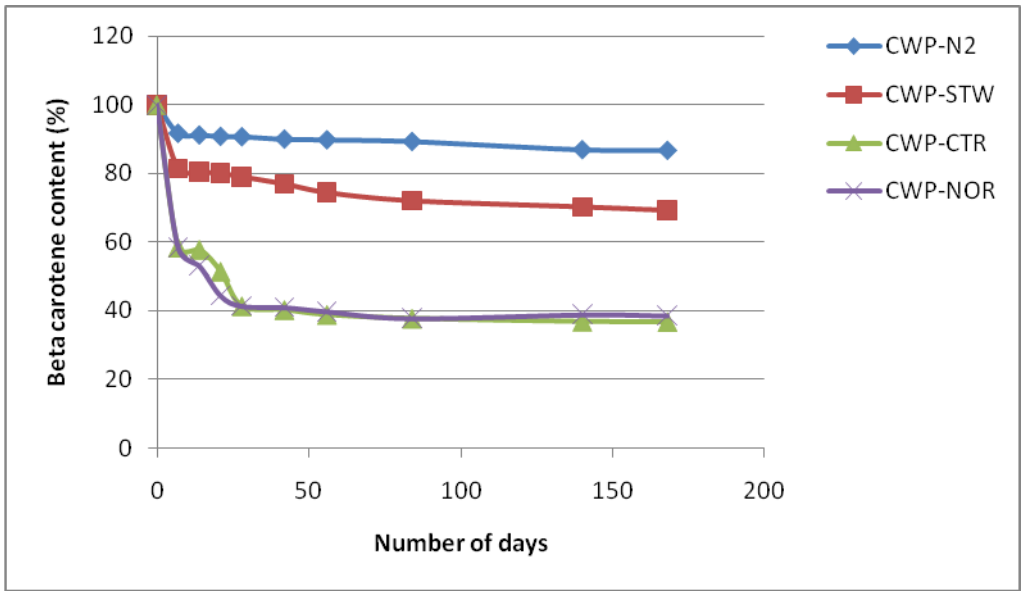

Figure 3: Trends of beta-carotene degradation in solar-dried cowpea leaves stored in four conditions for 168 days.

The degradation trends are identical for the three vegetable varieties, with the rate of degradation being considerable in the first 7 days of storage but slowing thereafter. However, the rate and extent of degradation was affected by the storage condition and the type of vegetable. The beta-carotene content for vegetables stored under oxygen absorber was significantly higher ($p<0.05$) than those stored under normal conditions, where between 46.7 and 69.3% was retained after 168 days of storage (Table 2). Storage under nitrogen retained highest levels in all vegetables while storage under normal conditions and in control conditions gave same effects. Cowpea exhibited higher beta-carotene stability, retaining 69.3% of the total beta-carotene after 168 days storage under oxygen absorber while nightshade and amaranthus retained 59.9% and 47.6% respectively (Figure 1-3).

Table 2: Content and percent loss of β-carotene for dried samples after six months storage

Sample	Content of β-carotene (µg/g) DM			
	After Dehydration	After six months storage (% loss)		
		N_2	STW	NOR
Amaranth	712.99	668.50 (6.2 %)	339.42 (52.4 %)	133.43 (81.3 %)
Nightshade	693.55	613.92 (11.5 %)	415.55 (40.0 %)	161.51 (76.7 %)
Cowpea leaves	653.63	565.69 (13.5 %)	453.02 (30.7 %)	252.30 (61.4%)

The reduction in beta-content in the vegetables during dehydration results in a significant amount of β-carotene lost, with the extent of loss determined by the amount of heat involved in the dehydration process[11]. The degradation of beta-carotene during processing and storage of vegetables may be attributed to the oxidation reactions triggered by metal catalysts present in the food material[9,13,20]. However, some studies have reported that conventional blanching and cooking result in a significant increase in the concentration of carotenoids in the cowpea, peanut and pumpkin leaves, attributed to the inactivation of enzymes responsible for β-carotene degradation [9,21,22]. The extent of reduction of beta-carotene in various food matrices is determined by several factors, including species, variety, presence of enzymes, seasonality, maturity and losses between harvesting and analysis periods[9,21,22].

The reduction in the content of beta-carotene was comparatively low for samples stored under nitrogen gas, resulting in a loss of between 6.2 and 13.5 % after 168 days storage period (Table 2). The presence of nitrogen gas in the package headspace drastically reduces the oxidation process of beta-carotene, thereby improving its stability and maintaining it throughout the storage period. The loss of beta-carotene that occurred during the first 7 days of the storage may be attributed to the initial oxidation process encouraged by residual oxygen probably trapped within the vegetable samples and the effect of free radicals; however, the reaction is not propagated due to lack of oxygen[13].In the presence of oxygen, the initiated oxidation reaction process is propagated in the entire storage period, resulting in the continuous loss of beta-carotene. This explains the steady decrease in beta-carotene content in samples stored under normal condition with and without a non-oxygen absorber, the cotton wool sachet.

Table 3: Rate constant and correlation coefficients for β-carotene using initial and amounts after seven days in dried samples stored for six months

Sample	Rate constants (1/day)		R^2	
	Used at t = 0	Used at t = 7 days	Used at t = 0	Used at t = 7 days
Oxygen absorber				
Amaranth	0.002	0.033	0.541	0.956
Nightshade	0.001	0.019	0.445	0.981
Cowpea leaves	0.001	0.020	0.539	0.966
Nitrogen				
Amaranth	0.004	0.002	0.650	0.924
Nightshade	0.008	0.006	0.792	0.808
Cowpea leaves	0.009	0.006	0.714	0.843
Normal condition				
Amaranth	0.112	0.058	0.572	0.775
Nightshade	0.115	0.079	0.737	0.840
Cowpea leaves	0.068	0.040	0.621	0.720

The use of kitchen steel wool as an oxygen absorber significantly improved the storage stability of beta-carotene, recording a loss of between 30.7% and 52.4% as compared to 61.4 and 81.6% loss under normal conditions (Table 2). The loss of beta-carotene was considerable in the first 7 days of storage as compared to storage in inert conditions (under nitrogen) but thereafter the rate of degradation slowed down. This indicates that steel wool takes time to absorb all the oxygen available to inhibit degradation.

The results indicate that the oxidative degradation reaction of the beta-carotene follows a first-order kinetic model, that is, $\ln C = \ln C0 - (k)(t)$. A plot of natural-log beta-carotene amounts vs. time was used to best fit a kinetic model and to obtain reaction rate constants. A first-order kinetic model has been previously reported for β-carotene degradation[22-24]. The correlation coefficients and reaction rate constants measured with initial amounts (t = 0) and without initial amounts (t = 7 days) the storage conditions are listed in Table 3.

The rate constant cowpea leaves under oxygen absorber (0.001) was half rate to that of amaranth (0.002), indicating that cowpea leaves being more fibrous than other vegetables experience reduced oxidation. When the rate constants are determined after reduction on nitrogen in the package (7 days) the correlation coefficients rose, implying that oxygen levels

are greatly reduced resulting in reduced oxidation of beta-carotene. The rate constants under normal and nitrogen conditions showed a reverse process where beta-carotene degraded faster in amaranth and nightshade than in cowpea leaves. The efficiency of oxygen absorber lies in its ability to effectively absorb all headspace oxygen within a short time to stop the propagation of the oxidation reaction[12]. The absorptivity of steel wool may be improves by increasing the absorption sites though increase of the surface area and using a sachet that has high gas permeability.

Conclusion

Drying technologies are the main food preservation processes in developing countries and the need to improve enhanced nutritive retentions on storage has popularized the packing technique based on oxygen absorption. The study has indicated that storage of dehydrated DGLVs under ordinary kitchen steel wool as an oxygen absorber significantly improves the retention of beta-carotene as compared to when stored under normal conditions. Since kitchen steel wool is readily available and is easy to use and handle in developing countries where preservation methods such as freezing and heat treatment are not available or are expensive to use, this technique can be adopted for preservation of dehydrated fruits and vegetables. However, the oxygen absorption capacity of the absorber may be improved by increasing the surface areas of the steel wool through grinding into fine particles and packing in a sachet material that allows adequate gas permeability. These conditions are likely to increase rate of oxygen absorption, thereby enhancing the stability of beta-carotene by encouraging faster oxygen absorption. Areas that require work include influence of level of moisture contents of dehydrated vegetables on bet-carotene stability and levels of microbial loads with storage; which we are currently studying. The risk of steel wool contaminating the food products and the extent of sanitary risks require investigation.

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We acknowledge Jomo Kenyatta University of Agriculture and Technology for use of the HPLC equipment in the analysis of beta-carotene.

Experimental Section

Sampling and sample Preparation

The varieties of indigenous dark-green leafy vegetables (DGLVs); cowpea leaves (*vigna uinguiculata*), amaranth leaves (*amaranthus hybridus (ll)*) and black nightshade (*solanum nigrum*) were bought fresh from a local market (Githurai) in Nairobi Kenya, trimmed to remove non-edible parts in accordance with the household practice and blanched in boiling water for two minutes. The samples were then spread on a wire mesh tray and dried in an indirect solar dryer covered with black polyethene paper for 6 to 8 hours until moisture content was less than 10%. The samples were then packed in 6cm x 9cm polythene bags (300 gauge) for storage at four different conditions: with an oxygen absorber (STW), with a non-oxygen absorber as a control (CTR), under nitrogen (N2) and in normal conditions (NOR). Several bags were used to cater for the different analysis times.

The oxygen absorber was prepared by packing 1.0g of fine steel wool in sachets made of air permeable material and sealed hanging at the top of the storage polythene bag. The sachets

were wetted with a few drops of distilled water before sealing. A non-oxygen absorber sachet containing same weight of cotton wool was similarly packed in the storage bags as a control condition. Samples stored under nitrogen were flushed with the nitrogen gas and immediately sealed while those stored on normal conditions were sealed after packing. All samples were stored at room temperature for a period of 168 days (6 months).

Glassware and Reagents

All glassware were thoroughly cleaned with a detergent and rinsed with distilled water. Crystalline β-carotene type (IV) standard was obtained from Sigma Chemical Company, England. All other chemical and reagents including potassium hydroxide, anhydrous sodium sulphate, sodium chloride, butylated hydroxytoluene (BHT), ascorbic acid were Analar grade. Dichloromethane (DCM) and methanol for HPLC analysis were of HPLC grade while other solvents from local laboratory suppliers like acetone, hexane, methanol and diethyl ether were double distilled before use. The extraction solvent was prepared by mixing acetone and hexane in the ratio of 3:2 in 0.1% BHT (w/v).

Extraction and analysis

0.5 g of dry and finely ground samples, 1.0 ml of distilled water was added to wet the sample followed by 50 ml of the extracting solvent consisting of acetone-hexane mixture (3:2 v/v) containing 0.1% BHT. The mixture was shaken at moderate speed with a mechanical shaker for ten minutes, decanted into a separating funnel and the residue re-extracted until it became clear (three extractions were found sufficient). The combined extracts were saponified by adding 25 ml of saturated potassium hydroxide in methanol, shaken lightly and decanted after settling for 30 minutes[25]. The mixture was washed with 100 ml of 10% sodium chloride solution followed by three 100 ml portions of distilled water to remove acetone while discarding the lower aqueous layer each time. The extract was then dried over anhydrous sodium sulphate and concentrated in a rotatory evaporator at 30°C to near dryness before reconstituting in methanol-dichloromethane, DCM (9:1 v/v) and made to volume in a 50 ml volumetric flask. For the fresh samples 25.0 g of vegetable samples were blended for five minutes with 50 ml of water containing 0.5% ascorbic acid and 5.0 g of the resultant puree extracted as per the above procedure. The extracts were filtered using a 0.45μm millipore filter before injecting into the HPLC column. All extractions were done in duplicate in subdued light and/or containers covered with aluminum foil to minimize destruction of carotene.

Beta-carotene was separated on a reversed phase HPLC column with the mobile phase of methanol-DCM-water (79:18:3) at a flow rate of 1.0 ml/min. Fresh mobile phase was prepared each time and always degassed using a ultrasonic agitator before use. Samples and standards were introduced into the HPLC column through a 10 μl syringe and detected at 450 nm using a UV-visible detector. The detector sensitivity was set at 0.05-absorbance units full scale (aufs). The beta-carotene peak was identified by comparing the retention time to that of the standard and by spiking the samples with a known concentration of the standard. Quantification was done based on the calibration curves using peak areas.

Equipment specification

The HPLC instrument consisted of the pump (LC–10AS), stainless steel reversed phase column (Vydack TP–201, 250 x 4.6 mm id and 5µm particle size) from Chrompack (Middleburg, The Netherlands), UV–visible detector (SPD-10AV) from Chromatopac, Shimadzu Corporation, Japan and a recorder (C-R6A) with chart speed of 12 cm/min.

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