

## Fortification of sunflower and palm oils using beta carotene extracted from *Asystasia mysorensis* and *Solanum nigrum*

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### Abstract

In Kenya, indigenous vegetables such as *Solanum nigrum* (*S. nigrum*) and *Asystasia mysorensis* (*A. mysorensis*) are locally available but grossly underutilized. While the vegetables are a rich source of beta carotene (BC), a provitamin A carotenoid essential for mitigating vitamin A deficiency, they are highly perishable. Extractable BC quickly degrades in the presence of oxygen, light, and heat and therefore cannot be stored by itself. To address this antagonism, BC was extracted from *S. nigrum* and *A. mysorensis* and fortified in edible oils, sunflower and palm oils for 180 days. The peroxide and acid values of the oils were titrimetrically determined while RP-HPLC was employed for BC analysis. Peroxide values increased significantly ( $p < 0.001$ ) by 74.05% and 20%, while acid values increase by 88.23% and 345% in sunflower and palm oils respectively over a 1 month period. Although the mean levels of BC reduced significantly ( $p < 0.001$ ) by over 65% for the entire storage period, the levels of BC remaining were within the recommended daily allowance (RDA), implying that BC can be fortified in sunflower and palm oils to address the antagonism of its storage and degradation.

## 1. Introduction

Dark green leafy vegetables are rich sources of the fat-soluble pro-vitamin A carotenoid, beta carotene (BC) (Nyambaka and Ryley, 2001; Rodriguez-Amaya and Kimura, 2004; Stahl and Sies, 2005; Nawiri *et al.*, 2013). Vitamin A is an essential nutrient needed in small amounts for the normal functioning of the visual system, growth, and development and is an antioxidant that would protect against the growth of cancerous cells (Young and Lowe, 2001; Stahl and Sies, 2005). Over the years, Vitamin A deficiency (VAD) is reported as the single most important cause of childhood blindness in developing countries (West Jr. and Darnton-Hill, 2008). Consumption of fruits and vegetables has been associated therefore with reduced cases of VAD and related illnesses (Codjia, 2001).

Kenya is home to more than 210 species of African Indigenous Vegetables (AIVs), most of which are underutilized such as black nightshade (*Solanum nigrum*), cowpeas (*vigna unguiculata*), spider plant (*cleome gynandra*) and *Asystasia mysorensis* (Mbugua *et al.*, 2006). The AIVs are considered superior for the source of both human nutrition and for medicinal value. However, their nutritional and medicinal contribution depends on factors such as variety, climate, maturity,

harvesting and postharvest handling and the plant part (Rodriguez-Amaya and Kimura, 2004). Further, the availability of AIVs is season dependent, being plenty during the rainy season and hence posing a challenge to their perishability due to limited preservation techniques and consequently the loss of BC. Preservation methods such as refrigeration, canning and drying that are employed to address vegetable perishability are not reached by the poor in developing counties. Preservation procedures create an additional challenge for BC since it degrades in the presence of oxygen, light, and heat (Moura *et al.*, 2015). We visualized extraction of the carotenoid and its fortification in various matrices as mitigation to its degradation. In a similar study, Mungai *et al.* (2017) reported a 10% retention of beta carotene extracted from *Amaranthus spp* and preserved in virgin coconut oil and unadulterated honey for 180 days. The study recommended an increase in the amounts of BC in the fortification matrices for higher retentions. Similarly, BC content in oven-dried cassava reduced from 72% to 32% after four weeks of storage at room temperature although high retentions of 70% were recorded when BC was encapsulated in phospholipids and stored in vacuum at normal room temperatures (Moura *et al.*, 2015)

The edible vegetable oils, sunflower and palm oils

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are triglycerides, natural products of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acids. Their degree of unsaturation is higher in sunflower than in palm oil, as indicated by their iodine value (IV) (Dauqan *et al.*, 2011). The polyunsaturated fatty acids in vegetable oils are prone to oxidative and hydrolytic rancidity giving peroxides and other compounds that give oils objectionable odour (Kamau and Nanua, 2008). Light, heat, the composition of fatty acids, contact with oxygen and minor compounds (metals, pigments, phospholipids, free fatty acids, mono- and diacylglycerols, thermally oxidized compounds, and antioxidants) determine the extent of oxidation. Peroxide values of up to 10 mEq/kg of active oxygen/kg and acid values of 0.6 mg KOH/g are commended (Codex Alimentarius Commission, 1999). Choe and Min (2006) showed that oxidation of one gram of soybean oil which dissolved 55 µg oxygen at room temperature gave a peroxide value of approximately 10 mEq/kg in the dark. Other than peroxide values, acid values and thiobarbituric acid values are useful parameters in assessing the extent of spoilage or deterioration in vegetable oils. Semi-refined sunflower oil packed in containers with a headspace increase their peroxide values from 0.75 - 6.66 mEq/kg and full 0.75 - 2.92 mEq/kg respectively for one month Kamau and Nanua (2008). Although peroxidation encourages oxidation of BC, edible oils eventually stabilize BC and consequently slow down its degradation (Choe and Min, 2006). It was envisioned that BC extracted from *S. nigrum* and *A. mysorensis* would be fortified in sunflower and palm oils to contribute to the scientific investigations on fortification of this valuable carotenoid.

## 2. Materials and methods

### 2.1 Chemicals and reagents

Glacial acetic acid, chloroform, potassium iodide, soluble starch, sodium thiosulfate, phenolphthalein, and potassium hydroxide were purchased from Kobian Kenya limited. All the other chemicals used including beta carotene standard, acetonitrile, methanol, dichloromethane and other solvents were of analytical grade purchased from Sigma Chemicals Company (USA).

### 2.2 HPLC specifications

Separation was performed using HPLC (Shimadzu Prominence LC-20A) with a LC-20AD pump and UV-VIS SPD-20VA deuterium lamp detector. Samples were injected on to a Gemini-NX 18110A column (5µm; 250 × 4.6 mm) at 30°C. 1 µL volumes of standard and samples were injected and allowed 20 minutes elution time. A mobile phase consisting of Acetonitrile:

Methanol: Dichloromethane (70:10:20) was isocratically eluted at a flow rate of 0.8mL/min.

### 2.3 Sampling and pretreatment

The AIVs *A. mysorensis* and *S. nigrum* were randomly sampled from five vendors (to make approximately 600 g of a homogenized sample) at Githurai market, Nairobi County and transported to Kenyatta University Department of Chemistry Laboratory. The vegetables were washed thoroughly using distilled before weighing 500 g sample for extraction. Sunflower and palm oils that had been manufactured two weeks prior were purchased from stores in Nairobi County.

### 2.4 Peroxide and acid value determination

Sunflower and palm oils were stored at room temperature while a control experiment was done in limited atmospheric oxygen. For a period of 28 days, oil containers were opened daily for five seconds simulating the daily activity in the kitchen. AOCS Cd 8-53 method was followed for peroxide value determination (Steltzer, 2012). Five (5) g of vegetable oil was dissolved in 30 mL of glacial acetic acid: chloroform solution (3:2). Exactly 0.5 mL of saturated potassium iodide solution was added and the flask stoppered with occasional shaking of the mixture. After exactly 1 minute, 30 mL of distilled water was added and the headspace flushed with nitrogen. Three drops of 1% starch solution were added to produce a bluish purple colour. The solution was then titrated with 0.01 N sodium thiosulfate until the blue colour disappears. Peroxide values were calculated using Equation 1.

$$PV = \frac{1000 \times V_s \times N_s}{g} \quad (1)$$

Where

PV is peroxide value in mEq/kg of vegetable oil

V<sub>s</sub> is the volume of Na<sub>2</sub>SO<sub>3</sub> used in the vegetable oil sample until yellow colour fades (mL)

N<sub>s</sub> is the normality of Na<sub>2</sub>SO<sub>3</sub> (mEq/mL used for titration)

g is the mass of vegetable oil sample used in grams

To measure acid value, exactly 2.5 g of oil was dissolved in 25 mL of petroleum ether in a flask and heated on a steam bath for 2 minutes before 2 drops of phenolphthalein indicator were added. The solution was titrated with 0.1 N potassium hydroxide until a pink colour appeared. Acid values were calculated using Equation 2

$$AV = \frac{V_s \times N_s \times 56.1}{g} \quad (2)$$

Where

AV is acid value in mg KOH/g in vegetable oil sample.

Vs is the volume of potassium hydroxide (KOH) used in the vegetable oil sample until pink colour appears (mL).

Ns is the normality of KOH

56.1 is the molecular weight of KOH.

g is the mass of vegetable oil sample in grams.

### 2.5 Preparation of calibration curve

Stock solution for beta carotene was prepared by dissolving 1 mg of pure beta carotene standard in exactly 2 mL of methanol to make 0.5 mg/mL (500ppm) of solution. Five serial working standard solutions of 250, 125, 62.5 and 31.25 ppm were prepared through dilution of stock solution and used to prepare calibration on HPLC. The series of standards were injected in triplicates to evaluate the reproducibility of the detector.

### 2.6 Extraction of beta carotene

The method by Rodriguez-Amaya and Kimura (2004) was adopted with slight modifications. Vegetable leaves were blanched in hot water at 90°C for 3 minutes then blended with celite powder to a smooth paste. Exactly 20 g of the homogeneous paste was placed in a conical flask and 50 mL cold acetone added. The mixture was sonicated for 10 minutes and filtered using a Buchner funnel. This procedure was repeated until there was no more colour change to the residue. Exactly 25 mL of petroleum ether (PE) was placed in a separating funnel and 10 g of butylated hydroxytoluene (BHT) added. Small portions of the acetone extract were added at a time until all the extract was transferred to PE. Distilled water was added slowly along the walls of the funnel and the two phases allowed to separate. The lower aqueous-acetone phase was discarded and washed repeatedly 5 times with distilled water to remove any residual acetone.

The PE extract was washed with 0.1% potassium hydroxide in ethanol and allowed to stand for 15 minutes to allow further separation before washing 5 more times with distilled water to remove impurities. The carotenoid layer collected was dried using anhydrous sodium sulphate and the solvent removed with a rotary evaporator at 30°C. Solid beta carotene obtained was

weighed accurately and then preserved in sunflower and palm oils.

### 2.7 Fortification and analysis of beta carotene

Exactly 425 mg of the beta carotene extracted from *A. mysorens* and 448 mg were fortified in 15 cm<sup>3</sup> sunflower and palm oils individually and separately. A homogenized mixture was made using a magnetic stirrer in a stream of nitrogen gas. Using a syringe the mixture was placed into eight 1.5 cm<sup>3</sup> amber air-tight vial and kept at room temperature. The procedure was done in an environment with subdued light to prevent degradation. The amount of beta carotene was determined on the first day (day 1), and after 14, 30, 60, 90, 120, 150 and 180 days. For control, dry nitrogen was blown into 8 empty 1.5 cm<sup>3</sup> dark bottles to drive out air and packed with extracted of beta carotene dissolved in petroleum ether under nitrogen. The amount of beta carotene in fortified samples was determined using HPLC conditions given in above.

### 2.8 Data analysis

SPSS package was used for data analysis. One way ANOVA was employed for significant differences between means of concentration and separation of means using multiple range tests (Student Newman Keul Test) was employed. The level of confidence ( $\alpha$ ) for all the values analyzed was all performed at 95%.

## 3. Results and discussion

### 3.1 Peroxide and acid values of oils

The peroxide and acid values which indicate oxidative rancidity and hydrolytic rancidity respectively for the sunflower and palm oils are presented in Table 1.

Peroxide values obtained indicated lipid oxidation onset with values falling within the FAO recommended limits (Codex Alimentarius Commission, 1999). The values increased significantly ( $p < 0.001$ ) from 3.93±0.03 to 6.84±0.04 and 2.00±0.00 to 2.40±0.00 mEq/kg oil in sunflower and palm oils respectively over 28 days. This increase, 74.05% and 20% in sunflower and palm oils

Table 1. Peroxide and acid values for sunflower and palm oils over 28 days

Days	Peroxide values (mEq/Kg oil) (n=3)		Acid value (mg in KOH/ g) (n=3)	
	Sunflower oil	Palm oil	Sunflower oil	Palm oil
1	3.93±0.03 <sup>a</sup>	2.00±0.00 <sup>a</sup>	0.34±0.00 <sup>a</sup>	0.11±0.00 <sup>a</sup>
2	4.80±0.00 <sup>b</sup>	2.10±0.00 <sup>b</sup>	0.41±0.04 <sup>b</sup>	0.19±0.04 <sup>a</sup>
14	5.40±0.06 <sup>c</sup>	2.17±0.03 <sup>c</sup>	0.49±0.04 <sup>c</sup>	0.30±0.04 <sup>b</sup>
21	6.03±0.03 <sup>d</sup>	2.27±0.03 <sup>d</sup>	0.56±0.00 <sup>d</sup>	0.37±0.04 <sup>b</sup>
28	6.84±0.04 <sup>c</sup>	2.40±0.00 <sup>c</sup>	0.64±0.04 <sup>c</sup>	0.49±0.04 <sup>c</sup>
p-value	<0.001	<0.001	<0.001	<0.001

Values are mean±SD. Mean values followed by the same letter(s) within the same column do not differ significantly from one another. (One-way ANOVA, SNK-test,  $\alpha = 0.05$ )

Table 2. The mean levels of beta carotene fortified in sunflower and palm oils.

Days	Concentration mg/100g DM (n=3)					
	<i>A. mysorensis</i>			<i>S. nigrum</i>		
	Nitrogen	Sunflower oil	Palm oil	Nitrogen	Sunflower oil	Palm oil
1	24.85±0.002	24.83±0.002 <sup>a</sup>	24.82±0.001 <sup>a</sup>	41.49±0.02	41.48±0.003 <sup>a</sup>	41.46±0.003 <sup>a</sup>
14	24.82±0.007	23.58±0.008 <sup>b</sup>	23.49±0.001 <sup>b</sup>	41.44±0.07	35.55±0.003 <sup>b</sup>	40.42±0.003 <sup>a</sup>
30	24.78±0.003	18.61±0.002 <sup>c</sup>	20.58±0.002 <sup>c</sup>	41.38±0.03	27.62±0.002 <sup>c</sup>	35.20±0.002 <sup>b</sup>
60	24.61±0.001	15.38±0.001 <sup>d</sup>	18.89±0.001 <sup>d</sup>	41.21±0.01	21.82±0.003 <sup>d</sup>	30.38±0.001 <sup>c</sup>
90	24.50±0.003	12.89±0.001 <sup>e</sup>	15.24±0.002 <sup>e</sup>	41.10±0.03	16.81±0.002 <sup>e</sup>	26.09±0.002 <sup>d</sup>
120	24.38±0.002	10.16±0.002 <sup>f</sup>	12.38±0.002 <sup>f</sup>	40.98±0.02	13.07±0.003 <sup>f</sup>	19.65±0.001 <sup>e</sup>
150	24.25±0.004	7.42±0.002 <sup>g</sup>	9.74±0.002 <sup>g</sup>	40.65±0.20	8.91±0.003 <sup>g</sup>	17.51±0.002 <sup>f</sup>
180	24.05±0.008	6.67±0.002 <sup>h</sup>	8.45±0.001 <sup>h</sup>	40.14±0.08	7.65±0.003 <sup>g</sup>	14.28±0.001 <sup>g</sup>
p-value	> 0.05	< 0.001	<0.001	> 0.05	< 0.001	<0.001

Values are mean±SD. Mean values followed by different letters are significantly different ( $\alpha=0.05$ ), One-way ANOVA, SNK-test

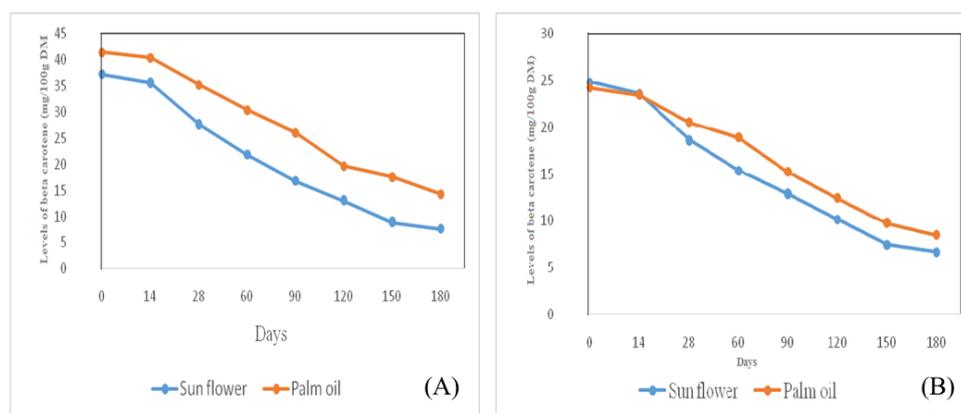


Figure 1. Trend of mean levels of beta carotene stored in sunflower and palm oils over 180 days. *S. nigrum* (A); *A. mysorensis* (B)

respectively are attributed to oxidation of unsaturation lipids in the presence of oxygen and light, although the initiation effect of autoxidation and photosensitized oxidation during processing may not be ignored (Choe and Min, 2006). Peroxide values increased more in sunflower than palm oil possibly due to the differences in lipids. Oils such as sunflower with IV>130 have higher unsaturation are more oxidized than palm oil with IV< 20 (Tan *et al.*, 2002; Packer *et al.*, 2005; Kamau and Nanua, 2008). The nature of packing, transparent packs against opaque plastic containers has shown to influence levels of peroxide values (Velasco and Dobarganes, 2002).

The acid values increased from 0.34±0.00 to 0.64±0.04 (88.23%) and 0.11±0.00 to 0.49±0.04 (345.45%) mg in KOH/g for in sunflower and Palm oils respectively. This increase in hydrolytic rancidity was found to be significant ( $p<0.001$ ) although values were within the FAO recommended limits (Codex Alimentarius Commission, 1999). Moisture and lipolytic enzymes in oils influence increase in acid values (Kamau and Nanua, 2008).

### 3.2 Beta carotene retention in vegetable oils

The levels of beta-carotene retained in sunflower and

palm oils over a period of 180 days and the trend is presented in Table 2 and Figure 1 respectively.

The AIVs, *A. mysorensis*, and *S. nigrum* contained 24.83±0.02 and 24.82±0.001 for sunflower and palm oils while 41.48±0.003 and 41.46±0.003 mg/100g DM of BC before fortification. These levels translate to 1.91±0.001 and 1.863±0.0001, 2.860±0.0002 and 3.19±0.001 retinol activity equivalent (RAE) respectively. On fortification in sunflower oil, the mean levels of BC extracted from *A. mysorensis* decreased to a level of 6.67±0.002 mg/100g DM (73.14%). In palm oil, a 65.95% reduction was observed to a level of 8.45±0.001mg/100g DM by the 180<sup>th</sup> day. Similarly, mean levels of BC extracted from *S. nigrum* reduced significantly ( $P<0.001$ ) when fortified in sunflower (18.44% reduction) and palm oils (34.44%). After 180 days, the RAE in sunflower and palm oils stood at 0.513±0.0002 and 0.650±0.0001, for *A. mysorensis* and 0.588±0.0002 and 1.098±0.0001 for *S. nigrum*. Recommended daily allowance (RDA) for vitamin A falls within the range of 400 µg for infants to 1300 µg for adults (Canada Health, 2014).

The trends showed lowest reductions ( $p<0.001$ ) during the first 14 days (Figure 1). During this period the peroxide and acid values of the oils were also low (Table

1). Lower reductions of the carotenoid, however, have been shown in oven-dried cassava storage at room temperature (Moura *et al.*, 2015) Due to conjugation nature in BC molecule, degradation is inevitable with oxygen, heat, acids, free radicals and other components in the preservation matrices (Rodriguez-Amaya, 2001; Moura *et al.*, 2015). Sunflower and palm oils are known to contain palmitic, stearic, oleic, linoleic and traces of free fatty acids (Choe and Min, 2006). There was a significantly higher level of BC in the fortified palm oil than in sunflower oil ( $p < 0.001$ ), which was attributed to the higher peroxide and acid values. Increased peroxide and acid values of the oils with time affected the beta carotene retained. This is evident from the correlation coefficients of peroxide and acid values of sunflower and palm oils whose values were  $R^2 = 0.854, 0.908$  and  $0.839, 0.850$  for BC extracted from *A. mysorensis* and  $R^2 = 0.837, 0.856$  and  $0.966, 0.873$  for BC extracted from *S. nigrum* respectively.

#### 4. Conclusion

The peroxide and acid values of sunflower and palm oils increased significantly over a period of 28 days indicating that the oils rapidly deteriorate. This contributed to the low retention of beta carotene extracted from *A. mysorensis* and *S. nigrum* indigenous vegetables when fortified in the oils. The retained amounts of BC, however, were still sufficient to provide recommended daily allowance (RDA). These findings reveal may suggest a potential of using the edible oils sunflower and palm oils as matrices for fortifying BC, although further studies on the control of oxidative rancidity and hydrolytic rancidity of the oils is required.

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