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Determination of β-carotene and α-tocopherol Content in Selected Fresh and Dry Vegetables in Butula in Busia County

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Authors' contributions

This work was carried out in collaboration among all authors. Author RN performed the lab and statistical analyses, wrote the protocol, and wrote the first draft of the manuscript. Authors HN and SS designed the study and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The study involved the determination of β-carotene and α-tocopherol content in selected fresh and dry vegetables (amaranthus, cowpea leaves, nightshade, slender leaf, pumpkin leaves and frying spider) using High Performance Liquid Chromatography (HPLC) procedure. Fresh blanched vegetables contained high levels of β-carotene; 4000-9700µg/100g and α-tocopherol levels; 3000-7360µg/100g (WW). The solar dried vegetables contained β- carotene levels ranging from 572 to 854µg g^{-1} dry weight (DW) and α-tocopherol levels ranging from 281 to 673µg g^{-1} (DW). Solar dried vegetables contained significantly lower ($P<0.05$) amounts of β-carotene and α-tocopherol which were moderately bioavailable when mixed in good proportion to meet Recommended Dietary Allowance (RDA) of vitamins A and E; which are 750µg retinol equivalent/day and 8mg/day respectively. The results will provide nutritional information on the indigenous vegetables grown in Butula in Busia County.

Keywords: Indigenous vegetables; β-carotene; and α-tocopherol.

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1. INTRODUCTION

Vegetables form an important part of the diet in just about every household in Africa [1,2]. Various types of vegetables are cultivated, mostly in small back or front yard gardens, but also increasingly in medium to large-scale commercial enterprises [3]. The types of vegetables grown vary with agro-ecology and consumption preferences. Consumer preferences are influenced to an extent by culture, traditions and income available to the household [1]. In almost all countries the vegetables grown can be divided into two categories:

(a) Introduced vegetables
(b) Indigenous vegetables

In East Africa, i.e. Kenya, Tanzania and Uganda, introduced vegetables may include kale, white and red cabbage, tomatoes, French beans, carrots, spinach, some onions, green peas, some egg plants, and green pepper [2]. These vegetables are more popular in urban areas where many households cultivate their own small gardens to meet some of the household requirements. For many urban households however, a good proportion of vegetables consumed are purchased from markets. Some common indigenous vegetables include: spider weed, amaranth, pigweed, black nightshade, pumpkin leaves, cowpeas and black jack, as well as the less common sun hemp, jute plant, stinging nettle, African eggplant and okra [4-6].

Indigenous Green Leafy Vegetables (GLVs) occupy an important place among the food crops as these provide adequate amounts of vitamins and minerals for humans. The nutritive value of greens remains underutilized due to lack of awareness and promotion of appropriate technologies for their effective utilization. Green leafy vegetables are a very good source of minerals and vitamins and when consumed regularly they can substantially improve micronutrient status of the Kenyan population [5,6]. Several of these are used for medicinal purposes [1]. These health promoting properties along with their rich nutrient profile make these GLVs an important nominee for their use in the food-based approach to combat several public health problems of Kenya [2,6].

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Busia district which is geographically located between 34 º 54' 32'' E and 34 º 25' 24'' longitude and 0 º 1' 36'' s and 0 º 1' 33'' N latitude. The district covers an area of 1,261.3 km^2. The district has a varied topography with low altitude of 1,130 m above sea level and the highest altitude of 1,375 m above sea level. The area experiences two main rainfall seasons. Long rains fall between March to May whereas the short rainfalls between August to October. The average temperature of the area is 26 º C [3].

Busia district has a population density of 321 people/ km^2 [3]. Crops grown in Busia district include; maize, sorghum, sweet potatoes, soya beans, cowpeas, green gram, kales, simsim, sunflowers, avocadoes, oranges, bananas, sugarcane and indigenous vegetables [3].

2.2 Research Design

The baseline information involved collection of demographic and food consumption pattern using a questionnaire, collection of vegetable samples. Levels of vitamin A, and E in selected indigenous foods known to be rich in the vitamins were determined by HPLC technique.

2.3 Field Work

Field work involved determination of availability of indigenous vegetables, by use of a questionnaire. Purchase of food samples for laboratory analysis. Pre-testing of working of the questionnaire was done by giving to potential subjects who were not included in the study.

2.4 Apparatus and Instruments

2.4.1 Glassware

All the glassware used was cleaned with chromic acid followed by a washing detergent. They were then rinsed with distilled water.

2.4.2 HPLC instrument and operating system

The HPLC chromatograph used was model L-6000 with dual plunger reciprocating pump (Hitachi instrument inc., model L-6000).

2.4.3 Chemical reagents and solvents

All the analytical standards were purchased from Sigma Aldrich (United States). All other chemical reagents used were purchased from Kobian (Kenya) chemical stores. The chemicals used included anhydrous sodium sulphate, sodium sulphite, potassium hydroxide, α-tocopherol (purity 95%) and β-carotene (purity: 97%) and

carotene (purity: 97%), Methanol, Acetonitrile, Ethanol, Dichloromethane (DCM), Hexane, Ethyl acetate, Butylated Hydroxytoluene (BHT). All the solvents were of HPLC grade. All the reagents were used without further purification. Deionized water, purified by milli Q system (Millipore, Milford, MA, USA) was used throughout the study.

3. EXPERIMENTAL

3.1 Sampling and Pre-treatment of Food Sample

The food samples were obtained from Butula market and household gardens. The samples were washed and trimmed to remove the fibrous materials in the laboratory. The trimmed samples were blanched by dipping into boiling water for 1-3 minutes. The blanched samples were cut with a knife into small pieces then solar dried. The solar dried samples were packed into clean polythene bags and sealed awaiting analysis. Samples which were to be analysed while fresh were washed, trimmed, blanched and stored in clean polythene bags in a refrigerator awaiting analysis.

3.2 Food Sample Pre-treatment

Fresh raw vegetables samples (cowpea leaves, frying spider, pumpkin leaves, slender leaf and nightshade) collected from Butula market and household gardens were thoroughly washed under tap water and then destalked and all inedible parts removed before shredding according to common household practice. The samples were then blanched for 1-3 minutes in boiling water and divided into two portions, one for solar drying and another for analysis when fresh. Fresh samples that were not analyzed on the same day were kept frozen until use. The vegetables were solar dried using an indirect solar dryer [5]. The model is shown in plate 1.

Samples were spread in a wire mesh tray before inserting into dryer. The dryer was made of wood and covered on top with a black polythene bag. The inside was painted black to concentrate the heat and ensure that air inside was heated appropriately. The dryer measured 1.5m in length, 1.2m in width, and the front height was 0.9m and a back height of 0.6m making an angle

Plate 1. Solar drier used in this study

of 30^{o} towards the incident light. It was raised 1.5m from the ground. A small opening of 1inch was left beneath the tray and chimney was inserted in the front of the tray to allow free circulation of air into and out of the dryer. The vegetable samples were spread onto the tray forming a uniform layer. After inserting the tray containing the samples into the dryer, the dryer was kept in the open and positioned in such a way that the sunrays fell directly on top of the dryer. The dryer was rotated appropriately with the change in light direction throughout the day. It took 6 to 8 hours when the temperature ranged from 25ºC to 27ºC for the vegetables to dry. The solar dried samples were stored in polythene bags, nitrogen flashed and then sealed tightly to prevent any oxygen getting in [5].

3.3 Determination of Moisture Content

1 gram of dried/fresh vegetable material was weighed and placed in a dry pre-weighed crucible. This sample was placed in an oven at 105ºC for at least 3hours. The dry weight of the vegetables was then taken. The moisture content in the vegetables was calculated using the initial weight before drying and final weight after drying. As shown below in Equation 1.

$$100\left(\frac{initial\ weight - final\ weight}{initial\ weight}\right) \quad (1)$$

3.4 Procedures for Analysis

3.4.1 Preparation of β-carotene and α-tocopherol standards

β-Carotene and α-tocopherol containing 100µg/ml were prepared by weighing 10mg standard reagents and dissolving each in n-hexane and making up the solution to 100ml. The stock solutions were kept under refrigeration conditions to be used for 2 weeks. The working solutions of different concentrations were prepared daily by serial dilution of the standard.

3.4.2 Method validation

The reliability of the method was validated through its linearity, reproducibility and recovery. Samples were quantified using peak areas of α-tocopherol and β-carotene standards. Limit of quantification was based on the lower concentration validated by the methods.

3.4.3 Food sample preparation, extraction and analysis

All extraction steps were performed in glass apparatus covered with aluminum foil. Extractions were completed on the same day and extracts injected into the HPLC column to reduce exposure time of the sample extracts.

3.4.4 Extraction of β–carotene and α–tocopherol in vegetables

Using a procedure by Nyambaka and Nyaga [7] twenty-five grams of vegetable samples were blended for 5 minutes with 0.3 g ascorbic acid to form a puree. Five grams of the pretreated sample was weighed and transferred into 150 ml round bottomed flask. 30 ml of hexane: dichloromethane mixture in the ratio of 3:2 was added to the flask and the mixture shaken for 2 minutes and allowed to separate. The hexane layer was then decanted into a 250 ml separating funnel which was then corked. The residue was similarly re-extracted with 50 ml of n-hexane three times each time decanting the hexane layer into the separating funnel. The combined hexane layer was then washed with 50 ml of saturated potassium hydroxide in methanol followed by portions of 50ml distilled water until there was no colouration on phenolphthalein indicator. The hexane layer was then dried by filtering over anhydrous sodium sulphate and evaporated to dryness under a stream of nitrogen. The residue was immediately dissolved in 10ml of methanol. An aliquot of the solution was filtered with 0.45 µm millipore filter and injected into HPLC system for analysis

3.4.5 Preparation of food supplement

Locally available vegetables confirmed to be rich in vitamin A, and E were used to prepare locally acceptable food products. The samples constituted raw foods, which include indigenous vegetables and fruits. This study used frying spider and cowpea leaves in the preparation of the food supplement. Dried samples were ground into powder, weighed, mixed and packed in ratios which would meet the RDA.

4. RESULTS AND DISCUSSIONS

4.1 Moisture Content in Some Selected Vegetables and Fruits

The percent moisture content of some fresh and dried vegetables and fresh fruits was determined and the results are given in Table 1.

The moisture content ranged from 73.50 to 96.14 % for the fresh vegetables. The moisture content

of most fresh tissue foods like vegetables and fruits is usually very high above 70% which makes them very susceptible to microbial spoilage and hence limiting their storage stability [5]. The moisture content for the dry vegetable sampled ranged from 5.18% to 6.4%. The total moisture content for vegetables is taken as the sum of free water that is loosely held outside the tissue matrix and the bound water held within the tissue matrix [8]. Usually, the free unbound water is lost during the dehydration process. The bound water constitutes the moisture content of the dry samples while free unbound water constitutes the moisture content of the fresh sample to ensure safe storage the final moisture content of the food should be less than 20% for fruits and less than 10% for vegetables [9,10]. Since dried fruits are generally eaten without being rehydrated, they should not be dehydrated to the point of brittleness [5].

Most foods are preserved through canning, sun drying, freezing and refrigeration, use of chemical additives and packaging [8]. Some of these techniques such as canning, freezing and refrigeration require sophisticated equipment; their cost is high and need electricity to provide the energy for running them. In most rural areas in Kenya, electricity is unavailable therefore less sophisticated methods such as solar drying; smoking; curing and fermentation are commonly used. In this study, solar drying was advocated for to be used in Butula Division. A number of studies carried out to examine the loss of β-carotene on drying have shown that there are lower loss of β-carotene and α-tocopherol while using the solar drier [5,7,9,11,12]. Nyambaka et al. [9] reported β-carotene losses of 16.41% in cowpea leaves, 31.93% for nightshade and 31.93% for amaranth for solar dried vegetables. Manuche [11] reported retentions of β-carotene of between 55% and 90% for solar dried green leafy vegetables as compared to the sun-dried vegetables whose retention was between 20% and 70%. The vegetables which were preserved in the study area included the cowpeas, spider herb, black night shade and pumpkin leaves. Preservation of these vegetables ensures that they are available during the dry season when they are scarce [5,9].

4.2 Vegetable Availability

Information obtained from field work, Busia district development plan 2002 and government of Kenya nutritional micronutrients survey 1988 indicates that production of food crops in Butula location is mainly done on a small-scale basis [13]. The crops grown include; maize, sorghum, sweet potatoes, soya beans, cowpeas, green grams, kales, ‘‘simsim’’, sunflower, cassava, avocadoes, oranges, watermelons, bananas, sugarcane, slender leaf, amaranth, pumpkins, frying spider, night shade, and other local vegetables. The foods act as a source of food and income for the inhabitants of this region [13]. However, in many households, maize, millet, sorghum and indigenous vegetables are dried and stored for use in times of scarcity. Most of the indigenous vegetables are available throughout the year such as pumpkin leaves and sun hemp (Table 2).

4.3 Method Validation

The β-Carotene and α-tocopherol standards were prepared as above. The solutions were injected into the HPLC column and eluted using a mobile phase consisting of methanol - acetonitrile-chloroform-water (46:30:18:6). The β-carotene and α-tocopherol were eluted at relatively sharp peaks at a retention time of 5 minutes for α-tocopherol and 10 minutes for β-carotene. Calibration curves of peak areas against the concentrations of β-carotene and α-tocopherol standard solutions were plotted Figs. 1 and 2.

Table 1. Moisture content in some selected vegetables

Vegetable	Moisture content %	Moisture content %
	Fresh	Dry
Pumpkin leaves	89.00 ± 2.19	5.98 ± 0.83
Slender leaf	94.00 ± 0.75	5.20 ± 0.61
Night shade	96.14 ± 0.31	6.40 ± 0.96
Cow pea leaves	94.14 ± 0.71	5.18 ± 0.60

Table 2. Vegetables availability calendar

Month	High	Medium	Low
January		A,H	C,F,G, D, B, I
February		A,H	C,F,G, D, B, I
March	A	B,F,G,H	C,D,E,I
April	A,B,E,F,G,H	C,D,I	
May	A,B,E,F,G,H	C,D,I	
June	B,C,D,F,G,H,I	A,E	
July	C,D,F,G,H,I	A,B,E	E
August	C,D,F,G,I	A,B,E,H	
September	B	A,C,D,F,G,H,I	F,G,I
October	C,H	A	F,G,H,B,C,D,E
November		A,B,C,D,E,H	I
December		A	

Key: A - Pumpkin Leaves; B - Sun Hemp; C - Spider Flower; D - Black Night Shade; E - Jute Plant; F - Pigweed; G - Amaranthus; H - Cowpea leaves; I - Kale (sukuma wiki)

Table 3. β-carotene and α-tocopherol mean content in μgg^{-1} (± Standard deviation) of some selected vegetables (dry matter) and μg/100g wet weight

Vegetable	β-carotene Dry matter	α-tocopherol Dry matter	β-carotene wet weight	α-tocopherol wet weight
Cow pea leaves	680.00 ± 4.35	513.60 ± 13.95	7437 ± 391.38	6400 ± 582.54
Pumpkin leaves	548.00 ± 54.68	693.55 ± 66.53	8000 ± 604.18	7350 ± 941.60
Amaranthus LL	650.00 ± 9.065	653.63 ± 48.67	7400 ± 337.40	6750 ± 714.83
Slender leaf	572.60 ± 43.68	281.60 ± 117.70	7000 ± 226.21	2800 ± 778.13
Frying spider	854.00 ± 82.17	445.75 ± 44.29	9700 ± 1246.72	3000 ± 702.54
Night shade	717.00 ± 20.90	680.60 ± 60.74	7625 ± 462.44	7500 ± 998.30

Table 4. Preparation of food supplement

Vegetables	β-carotene μgg^{-1} dry weight	α-tocopherol μgg^{-1} dry weight
Cowpea leaves (CL)	580	514
Frying spider (FS)	680	446
Total	1260	960
RDA	750 μg RE/day	8 mg/day

The β-carotene curve was linear within the concentration range determined (0 to 100mg/ml). This calibration line gave a correlation coefficient with r^2 = 0.9975. The correlation coefficient obtained in this study was comparable to those obtained in other studies [5,7,14]. Nyambaka and Nyaga [7] obtained r^2= 0.9970 using a HPLC system consisting of μ Bondak C_{18} reversed phase column and a mobile phase of methanol-acetonitrile-chloroform-water in ratio of 46:30:18:6 and detection limit of 297nm for both β-carotene and α-tocopherol. Nyambaka et al. [5] reported r^2 = 0.9987 using a HPLC system and a mobile phase of methanol-dichloromethane-water (79:18:3) and detection limit of 450nm. Nawiri [9] reported r^2=0.9981 using a HPLC system and a mobile phase methanol: DCM: water (83:15:2) and a detection limit of 450 nm. This value indicates that there was a linear relationship between the chromatographic peak area and the β-carotene concentration. The linearity also indicates that the detectors of the HPLC equipment were responding positively to different concentrations of the analyte [15]. The regression equation was therefore y = 6459.2 x + 2066.9. The calibration curve was used to determine the concentration of β-carotene in the vegetable samples Table 3.

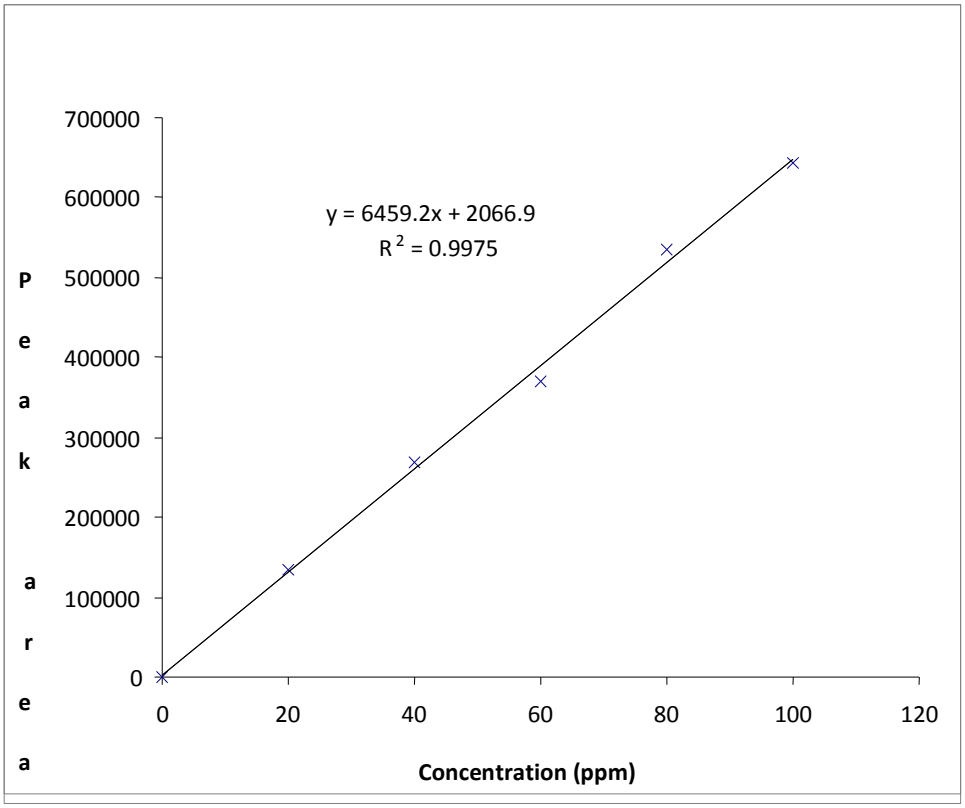

Fig. 1. The calibration curve for β-carotene standard solution

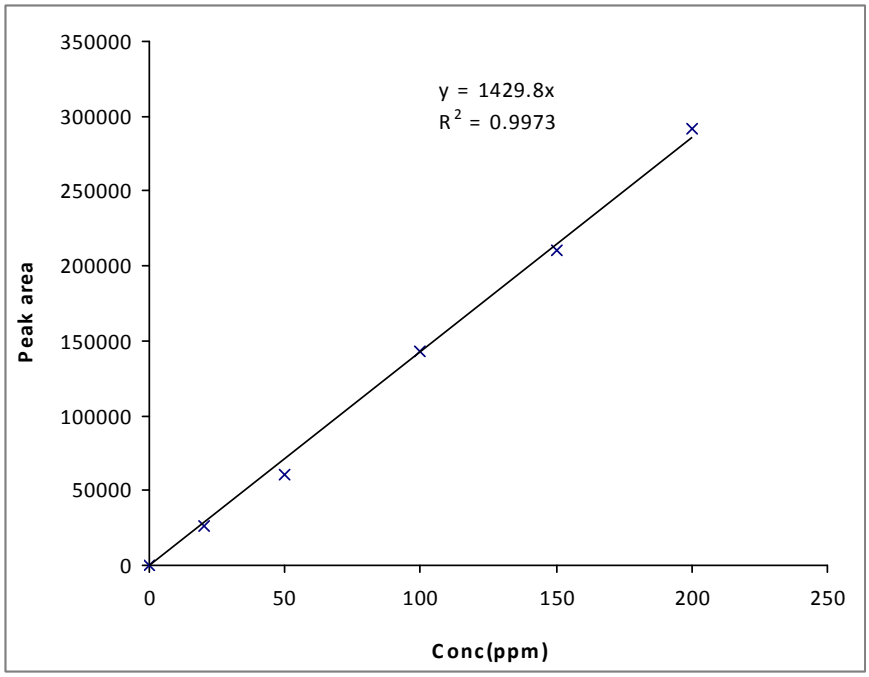

Fig. 2. The calibration curve for α-tocopherol standard solution

4.4 Preparation of Food Supplement

The food supplement consisted of cowpea leaves and frying spider in the ratio of 1:1. One gram of cowpea leaves produces 580 µg while one gram of frying spider produces 680 µg making up a total of 1260 µg in 2 grams. Therefore 2 g of CL+FS mixture provide 1260 µg β-carotene which is equivalent to 210 µg RE vitamin value obtained by dividing by 1260 µg by 6. The RDA is based on the assumption of 100% bioconversion set at 750 µg RE/day for adults. Required mixture to meet RDA is 7.14g of CL and FS mixture in the ratio of 1:1.α- tocopherol RDA is set at 8 mg/day for adults. Two grams of CL+FS mixture provide 960µg g^{-1} with cowpea leaves contributing 514 µgg^{-1} while frying spider contributing 446 µgg^{-1} to the mixture. Required mixture to meet the RDA is 16.67 g CL+FS mixture in the ratio of 1:1. If 18 g of the CL+FS mixture was to be taken by each of the PLWHA on daily basis the mixture would provide 2.5xRDA β- carotene and 1xRDA α-tocopherol Table 4.

5. CONCLUSION

The study indicated that the fresh vegetables have high β-carotene (4000 to 9700 µg/100g) and α-tocopherol (2800 to7500 µg/100g) wet weight; 548 to 854 µgg^{-1} and 281 to693 µgg^{-1} dry weight repectively. Information from the questionnaire indicated that most of the indigenous vegetables are available throughout the year. The moisture content of the fresh vegetables ranged from 73.5% to 96.4% while the dry vegetable samples were in the range of 5.18% to 6.4%. Solar drying was the method used to dry vegetables and advocated for as a method of preservation in this study since it has been shown in other studies to result to lower loses of β-carotene .

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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