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Annonaceae Fruits Growing in Coast Region of Kenya as an Alternative Source of Dietary Carotenoids

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Abstract: Fruit and vegetables provides most of the carotenoids in the human diet, and their consumption is always an essential requirement for human nutrition due to the presence of bioactive compounds. The study investigated the presence of carotenoids in different parts (pulp, peel and seeds) of the underutilized, exotic fruits *Annonaceae* family species (*Annona squamosa*, *Annona muricata* and *Annona reticulata*) growing in the coast region of Kenya. HPLC profiling of the pulp extracts revealed the presence of neoxanthin, violaxanthin and zeaxanthin, α -carotene, β -carotene, γ -carotene, chlorophyll a and chlorophyll b. *A. squamosa* had higher levels of α -carotene (16.95 ± 0.01), Zeaxanthin (10.45 ± 0.04) and Violaxanthin (12.44 ± 0.17). Lutein (6.62 ± 0.18) and β -carotene (5.77 ± 0.02) were in *A. reticulata* pulp while the pulp of *A. muricata* had the lowest levels of all the identified carotenoids. There was no significant difference in levels of the identified carotenoids in the pulp of the three species of *Annonaceae* fruits ($p > 0.05$). Results in this study demonstrate that the *Annonaceae* fruits are good source of antioxidant carotenoids and can be incorporated in the diet to promote human nutritional requirements and health.

Keywords: *Annonaceae*, Carotenoids, Nutritional and Health, HPLC Profiling

1. Introduction

Provision of modern healthcare to many rural areas in Kenya and other developing countries is still a far-reaching goal due to economic constraints. This has led to high dependence on locally available plant materials for provision of various essential nutrients and cure health disorders [1]. Various health benefits of plants are due to

the presence of bioactive compounds [2]. Among these are carotenoids naturally synthesized by plants, algae, and photosynthetic bacteria responsible [3], [4]. Broadly, carotenoids can be classified into two classes; carotenes and xanthophylls [5]. Carotenes include α -carotene, β -carotene, γ -carotene and lycopene while xanthophylls include β -

cryptoxanthin, lutein, and zeaxanthin. The α -Carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, and zeaxanthin are the most common dietary carotenoids. The α -carotene, β -carotene, γ -carotene and β -cryptoxanthin are provitamin A thus can be converted to retinol by the body [6], [7] while Lutein, zeaxanthin, and lycopene cannot be converted to retinol by the body thus has no vitamin A activities [8]. The biological effects of carotenoids in humans are related to either their antioxidant activity or other non-antioxidant activities [9], [10], [11], [12].

Several epidemiological studies have been conducted and the roles of carotenoids in human health documented [6]. It has been shown that carotenoid-rich fruit and vegetables diets are associated with reduced risk of cardiovascular disease and some cancers than high-dose β -carotene supplements [13], [14], [15]. Further, studies have also established that high-dose β -carotene supplements increases the risk of lung cancer in smokers and former asbestos workers [16], [17]. Although there are about 20 to 30 carotenoids [18], only Lutein and zeaxanthin are the only carotenoids associated with retina and lens of the eye [19], [20] (Bone et al., 1995; Yeum et al., 1999) and diets rich in lutein and zeaxanthin have been shown to slow the development of age related eye diseases such as macular degeneration and cataracts [21], [22], [23], [24], [25]. Further, a high lycopene diet has also been associated with reduced risk of developing prostate cancer [26], [27], [28]. However, it's not clear whether these carotenoids supplements are able to slow the development of these degenerative diseases.

Fruit and vegetables provide most of the carotenoids in the human diet, and their consumption is always an essential requirement in human nutrition. The information on the nutrient composition of locally available fruits is of immense importance because of the need to supply foods according to their nutritive values to the people. The family *Annonaceae* are groups of fruit producing plants that grow in tropical region [29]. The most widely grown of all the species in Kenya are *A. muricata*, *A. reticulata*, and *A. squamosa*. The fruits are popularly consumed by other parts of the world [30] with Kenyans having similar gastronomic habits [31]. Although carotenoids have been extensively studied in different matrices to analyze their distribution and levels in plants due to their importance in diet and health [30], the Kenyan *Annonaceae* fruits are neglected and under-utilized for reasons that are not clear. High Performance Liquid Chromatography (HPLC) has proved to be important equipment for carotenoid analysis [32]. Based on this, it's crucial to analyze carotenoids which may highlight pools of carotenoids that play important roles in metabolism and physiology of a pre-requisite to human health due to their nutritional and medicinal properties. Therefore the study aimed to evaluate, identify and determine the levels of carotenoids in *Annonaceae* fruit species growing in the coast region of Kenya as an alternative source of carotenoids.

2. Materials and Methods

2.1. Collection Plant Material

Ripe fresh *Annonaceae* fruits (*A. muricata*, *A. squamosa* and *A. reticulata*) were harvested from selected farms in Kilifi and Kwale Counties in the Coast region of Kenya. They were washed with clean running chlorinated water and transported to the laboratory. In the laboratory, the fruits peel, pulp and seeds were separated and dried separately at 37°C and 95% relative humidity (Tokyo Thermo Tech CO. Ltd, Japan). Later, they were ground into fine powder using a grinding machine (Mitamura Riken, Kogyo Inc. Tokyo, Japan). The powder for each part was weighed using a top-loading balance, transferred into polythene bags, sealed, and stored at -80°C until further analysis of carotenoid by HPLC.

2.2. Extraction of Carotenoids from Different Parts of *Annonaceae* Fruits

The extraction of carotenoids for HPLC analysis was performed as describe by Samuagam and co-workers [33] with modifications. In briefly, about 200 mg fruit tissue was weighed and tranfered into 2 mL Eppendorf tubes containing 2 beads. In each tube, 50 μ L of 3 mg/mL magnesium carbonate ($MgCO_3$) suspension and 300 μ L of tetrahydrofuran (THF) were added. Homogenization was done in FastPrep machine set at 5.0 speed for 45 seconds followed by incubation at 4°C for 20 minutes in dark. Also added to the two tubes was 300 μ L of methanol before homogenization and incubation at 4°C for 10 minutes. The homogenate was then transferred to Spin-X filter, centrifuged for 1 minute at a speed of 4,000 rpm (4°C). Two equal volume (150 μ L THF and 150 μ L methanol) were pipetted into original extraction tube then followed by vortexing. All THF/methanol/debris was pipetted into spin-X filter and centrifuged again. The filtrate was then transferred to an empty clean 2 mL tube before 450 μ L of THF was added to debris pellet in spin-X filter and incubated on ice for 15 minutes in the dark and centrifuged at maximum speed for 5 minutes. The filtrates were combined and 375 μ L petroleum ether and 150 μ L (25% NaCl) added to each combined extract and vortexed vigorously before being centrifuged at a maximum speed for 3 minutes at 4°C for phase separation. The upper phase was then transferred to new 2 mL tube. Second extraction was done with 500 μ L petroleum ether before the upper phase being carefully removed and mixed with the initial filtrate. To oncentrate the petroleum ether extract to near dryness, the extract was rotor evaporated at 45°C for 20. The dried extracts were stored in the Nitrogen (N_2) refrigerator at -80°C (dark) whenever samples were not analysed by HPLC immediately after extraction. To resuspend carotenoids; 500 μ L ethyl acetate was added then incubated at room temperature for 15 minutes followed by vortexing and filtering the suspension filtered through 0.45 μ m nylon syringe filter (Cameo 3 N syringe filter, GE Water & Process Technologies, USA).

2.3. HPLC Analysis Using YMC C₃₀ Column

Carotenoid analysis was carried out in a defined YMC C₃₀ column using a Dionex HPLC machine (P680 HPLC pump, ASI-100 Automated Sample Injector; PDA-100 Photo Array Detector) and Chromeleon (v6.40 software package). Carotenoids separation was based on polarity gradient (0-5 min 100% methanol: 0.1% ammonium acetate; 6-25 min 4% methanol: ammonium acetate and 96% methyl-tert-butyl ether; 26-35 min ramp to 100% methanol: ammonium acetate) through a guard cartridge (YMC carotenoid S-5, 4.0 mm x 20 mm DC guard, Waters), C₃₀ column (YMC carotenoid S-5, 4.6 mm x 250 mm, Waters) assembly.

2.4. Identification and Quantification of Carotenoids

Peak identification was performed as described in [34], where peak areas of the standards were determined at the respective wavelengths providing maximum absorbance. The carotenoids identification was based on retention time and spectra comparison using respective standards analyzed under identical analytical conditions. Standard Calibration curves for each standard was

generated automatically and used to quantify carotenoids.

2.5. Data Analysis

Three independent biological replicates were used per analysis and the results were expressed as mean values \pm standard error of the mean. Analysis of variance (ANOVA) followed by Duncan's test ($P \leq 0.05$) was used for comparison of means. All statistical analyses were carried out by GenStat discovery 14th Edition [35].

3. Results

This study reports a detailed analysis of carotenoid profiles of three *Annonaceae* fruits was conducted to find out the types and concentration of the carotenoids present in different parts of the fruits. The different parts (pulp, peel (kennel) and seeds) of three *Annonaceae* fruits (*A. squamosa*, *A. muricata* and *A. reticulata*) were evaluated for carotenoid profile. The HPLC profiles of carotenoids are given in Figure 1 and 2, while their carotenoid content is given in Figure 3 and Table 2.

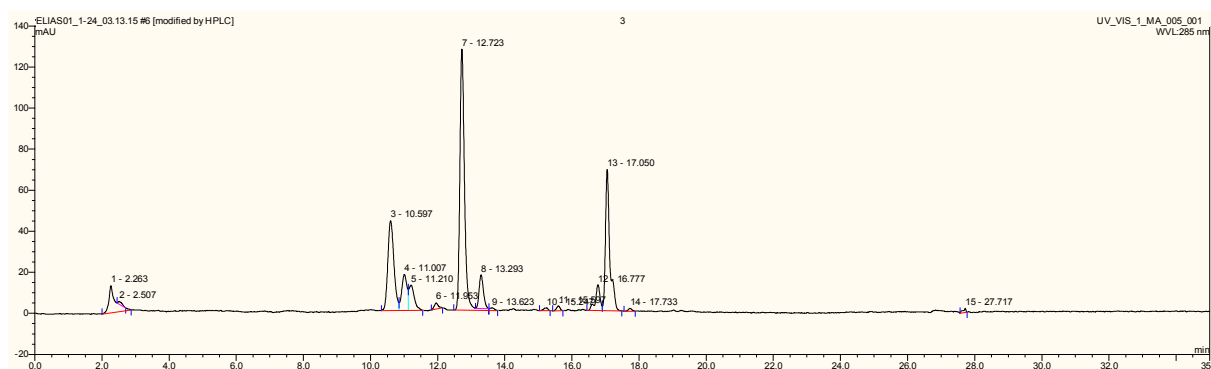


Figure 1. HPLC chromatogram of one sample showing different carotenoids eluted at different retention.

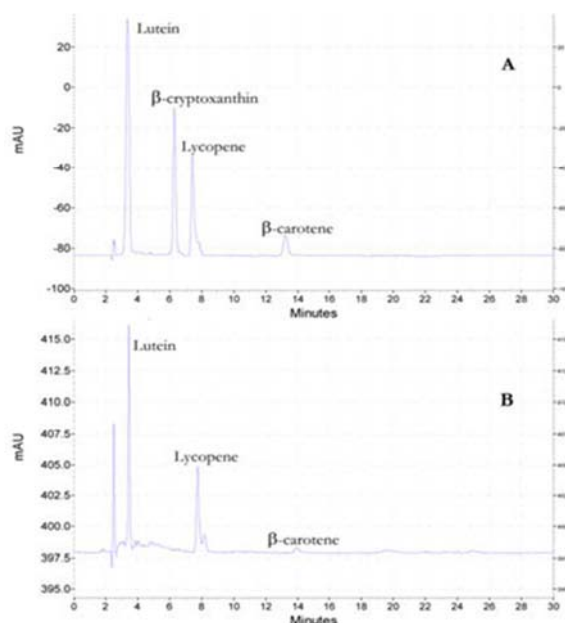


Figure 2. HPLC chromatograms for carotenoids. Carotenoid standards (A); *Annona squamosa* (B).

The HPLC fingerprinting of the different parts of *Annonaceae* fruits extracts revealed the presence of neoxanthin, violaxanthin and zeaxanthin, α -carotene, β -carotene γ -carotene, chlorophyll a, chlorophyll b and other unknowns (Table 2). These carotenoids were identified by comparisons to the retention times and UV spectra of authentic standards analyzed under identical analytical conditions (Table 1).

Table 1. Carotenoids separation on a reverse-phase C_{30} HPLC system and spectral characteristics used in identification from photodiode array detection.

Carotenoid	Spectral characteristics (nm λ_{max})	Retention time (min)
Neoxanthin	466	6.99
Violaxanthin	433	7.6
Lutein	442	11
Antheraxanthin	442	14.93
Zeaxanthin	430	13.23
α -carotene	408	17.06
β -carotene	450	17.2
γ -Carotene	444	17.6
Chlorophyll a	430	12.7
Chlorophyll b	465	10.6
Others	446, 441, 443	11.8, 15.9, 16.3

Among the carotenoids studied in the three fruit species, *A. squamosa* had higher levels ($\mu\text{g}/100\text{ g}$ dry weight) of α -carotene (16.95 ± 0.01), Zeaxanthin (10.45 ± 0.04) and Violaxanthin (12.44 ± 0.17). The pulp of *A. reticulata* had higher levels of Lutein (6.62 ± 0.18) and β -carotene (5.77 ± 0.02) while the pulp of *A. muricata* had the lowest levels of all the identified carotenoids. There was no significant difference in concentration of the identified

carotenoids in the pulp of the three species of *Annonaceae* fruits. However, a significant difference ($p<0.05$) in the levels of identified carotenoids was observed between the different parts of the three analyzed species of *Annonaceae* fruits.

The peel (kennel) had the highest levels of α -carotene (163.10 ± 0.04 and 1089.76 ± 0.001 in *A. reticulata* and *A. squamosa* respectively) and Lutein (217.96 ± 0.66 and 315.55 ± 0.89 in *A. reticulata* and *A. muricata* respectively). However neoxanthin was almost equally distributed throughout different parts and species of the analyzed *Annonaceae* fruits. High levels of violaxanthin (12.44 ± 0.17) were present in the pulp of *A. squamosa* while antheraxanthin levels (78.53 ± 0.50) were highest in *A. muricata* peel although the seed had also good amounts (20.57 ± 0.06 and 32.913 ± 0.68 in *A. muricata* and *A. reticulata* respectively). The seeds had higher levels of β -carotene (200.00 ± 0.39 and 257.76 ± 0.96 in *A. muricata* and *A. reticulata* respectively) and γ -carotene (108.12 ± 0.03 and 124.40 ± 0.33 in *A. muricata* and *A. reticulata* respectively). The peel were also rich in chlorophyll a (3.83 ± 0.02 and 10.17 ± 0.04 in *A. squamosa* and *A. muricata* respectively), chlorophyll b (68.31 ± 0.10 and 129.11 ± 0.01 in *A. muricata* and *A. squamosa* respectively) and unknown carotenoids ($2.74.90\pm0.03$ and 353.64 ± 0.01 in *A. muricata* and *A. squamosa* respectively). Although higher levels of the identified carotenoids were observed in the peel and the seeds, the levels in the pulps could be deemed to be sufficient enough to cater for the needs in human diet only if the bioavailability, synergisms and/or toxicity of the identified carotenoids are investigated. The results demonstrate that among the *Annonaceae* fruits evaluated for carotenoids are good source of antioxidant carotenoids.

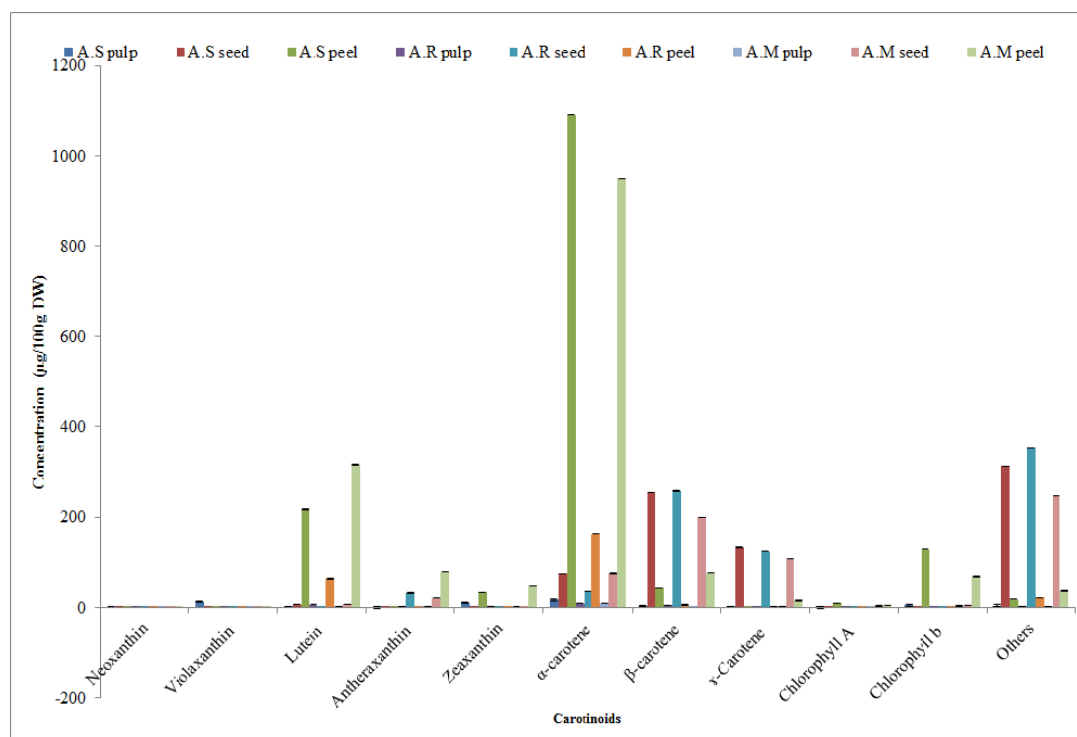


Figure 3. The concentration carotenoids identified in the different parts of the three *Annonaceae* fruits analyzed.

Table 2. The total carotenoid concentration identified in different parts of *Annonaceae* fruits.

Carotinoids	Concentration ($\mu\text{g}/100 \text{ g DW}$)								
	<i>Annona squamosa</i>			<i>Annona muricata</i>			<i>Annona reticulata</i>		
	Pulp	Peel	Seed	Pulp	Peel	Seeds	Pulp	Peel	Seeds
Neoxanthin	0.68 ^c ±0.01	0.44 ^a ±0.02	0.50 ^b ±0.03	0.677 ^c ±0.03	0.32 ^a ±0.02	0.56 ^b ±0.03	0.59 ^c ±0.02	0.03 ^a ±0.01	0.388 ^b ±0.003
Violaxanthin	12.44 ^a ±0.17	0.842 ^a ±0.01	0.31 ^a ±0.02	0.326 ^a ±0.05	0.04 ^a ±0.02	0.18 ^a ±0.05	0.09 ^a ±0.02	0.25 ^a ±0.01	0.232 ^a ±0.002
Lutein	1.140 ^a ±0.01	217.96 ^b ±0.66	6.34 ^a ±0.04	2.864 ^a ±0.51	315.55 ^b ±0.89	7.67 ^a ±0.01	6.62 ^a ±0.18	63.38 ^b ±0.34	0.030 ^a ±0.000
Antheraxanthin	0.20 ^a ±0.001	0.03 ^b ±0.001	0.09 ^{ab} ±0.01	2.511 ^a ±0.34	78.53 ^b ±0.50	20.57 ^{ab} ±0.06	1.06 ^a ±0.007	0.28 ^b ±0.003	32.913 ^{ab} ±0.68
Zeaxanthin	10.45 ^a ±0.04	33.57 ^b ±0.37	0.28 ^a ±0.01	1.088 ^a ±0.12	47.927 ^b ±0.32	0.08 ^a ±0.01	1.37 ^a ±0.03	0.1708 ^b ±0.00	0.578 ^a ±0.005
α -Carotene	16.95 ^a ±0.01	1089.76 ^b ±0.01	74.60 ^a ±0.12	8.493 ^a ±0.05	949.60 ^b ±0.03	75.47 ^a ±0.15	8.76 ^a ±0.01	163.10 ^b ±0.04	36.76 ^a ±0.01
β -Carotene	3.82 ^a ±0.01	43.939 ^b ±0.19	255.36 ^c ±0.20	0.187 ^a ±0.01	75.97 ^b ±0.39	200.00 ^c ±0.39	5.77 ^a ±0.02	5.68 ^b ±0.17	257.76 ^a ±0.96
γ -Carotene	1.74 ^a ±0.03	0.06 ^a ±0.01	132.54 ^b ±0.84	1.304 ^a ±0.17	15.51 ^a ±0.05	108.12 ^b ±0.03	0.93 ^a ±0.04	2.11 ^a ±0.03	124.40 ^b ±0.33
Chlorophyll a	0.16 ^a ±0.01	10.17 ^b ±0.04	0.03 ^a ±0.01	0.285 ^a ±0.02	3.83 ^b ±0.02	3.42 ^a ±0.07	0.06 ^a ±0.00	0.83 ^b ±0.01	0.020 ^a ±0.002
Chlorophyll b	4.75 ^a ±0.12	129.11 ^b ±0.01	0.03 ^a ±0.01	2.973 ^a ±0.53	68.31 ^b ±0.10	5.30 ^a ±0.02	0.18 ^a ±0.01	0.45 ^b ±0.01	0.06 ^a ±0.004
Others	4.20 ^a ±0.01	18.05 ^b ±0.12	312.42 ^c ±0.51	1.692 ^a ±0.13	37.13 ^b ±0.35	247.90 ^c ±0.03	2.22 ^a ±0.05	21.79 ^b ±0.01	353.64 ^c ±0.01

Values are given as means of three replicates \pm SEM. Means with different superscript letters within a row are significantly different ($P < 0.05$).

4. Discussion

The benefits accrued from inclusion of fruits in the diet cannot be overlooked. Although fruits have provided a delicate balance in terms of nutrition and health, a shift from using fruits to nutritional supplement has always been experienced. Consumer interest in the relationship between diet and health has increased the demand for information on functional foods. Moreover, the rapid advances in science and technology, increasing health-care costs, changes in food laws affecting label and product claims, an aging population, and a rising interest in attaining wellness through diet are among the factors fueling interest in functional foods.

Credible scientific research indicates many potential nutritional and health benefits from natural occurring food components and supplement. However, toxicity arising from these supplements is an issue. Further, the retention of carotenoids and other functional foods is a major concern. Alteration or loss of carotenoids during processing and storage of foods through physical removal (e.g. peeling), geometric isomerisation, and enzymatic or non-enzymatic oxidation has been reported [36]. Since good eating habits and healthy lifestyle is crucial, supplements, natural and chemically produced products are used in provision of nutrition. This has led to ignorance on the importance of dietary fruits which has been exacerbated by the presence of a suitable substitute for these essential components in the diet [37]. However, carotenoids cannot be synthesized in animals or humans, they are found in fruits and vegetables therefore need to be part of the dietary intake.

Although *Annonaceae* fruits are underutilized exotic fruit not only in Kenya but also in other countries such as India, preliminary screening has revealed the nutraceutical potential of these fruits and their uses as valuable source in functional foods [38] which has been confirmed by the results of this study. *Annonaceae* fruits grown in Kenya have been ignored in terms of research in the nutritional and health benefits accrued from their consumption leading to their

underutilization. The results from the current study indicate the presence of neoxanthin, violaxanthin and zeaxanthin, α -carotene, β -carotene γ -carotene, chlorophyll a, and chlorophyll b. Further, the results of this study showed that under the conditions used, *Annonaceae* fruits contained several unidentified components, eluted at 11.8, 15.9, and 16.3 minutes (refer to chromatogram). On the basis of their retention times and spectral characteristics, these are most likely to be xanthophyll esters, exhibiting identical UV-vis spectra, but are less polar and elute differently. Similar results under isocratic elution in mango fruits reported [39] suggest that they could be xanthophylls based on their spectral characteristics.

Adequate intake of fruits forms an important part of a healthy diet [40], [41] and can help to prevent diet-related chronic diseases [42]. However, gender, age and income [43], family origin and socioeconomic status [44], and education and nutritional knowledge [45] influence fruit intake. Family origin affects the purchasing power of food, food choice, food preparation and food availability which in turn affects consumption [46], [47]. In Kenya, studies have not been undertaken to generate data on the low popularity of *Annonaceae* fruits in diets. The study therefore, reveals the importance of Kenyan *Annonaceae* fruits in supplementing dietary nutrition due to the high levels of carotenoids present. In this way, *Annonaceae* fruits can provide a source of bioactive compounds with nutritional and functional properties beneficial to health, which should stimulate the pharmaceutical and food industries to develop new products.

5. Conclusion

In conclusion, this is the first report on carotenoid composition of Kenyan *Annonaceae* fruit species. The study showed that the fruits contain significantly higher levels of carotenoids. The data generated on the composition of carotenoids in the present study could be exploited for nutritional purpose which may be helpful to suggest better

sources of carotenoids as a part of the daily meal to consumers, to overcome health disorders such as age-related diseases, including eye diseases as cataract, diabetic retinopathy, glaucoma, and age-related macular degeneration (AMD) [48].

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References

- [1] Grover JK, Yadav S, Vats V. Medicinal plants of India with hypoglycemic potentials. *J Ethnopharmacology*. 2002;81:81–100.
- [2] Tanabe H, Yoshida M, Tomita N. Comparison of the antioxidant activities of 22 commonly used culinary herbs and spices on the lipid oxidation of pork meat. *J. Anim. Sci.* 2002; 73:389-393.
- [3] International Agency for Research on Cancer (IARC). *Handbooks of Cancer Prevention: Carotenoids*. Lyon. 1998.
- [4] Stipanuk MH. *Biochemical and physiological aspects of human nutrition*. Philadelphia: WB Saunders Company. 2000.
- [5] Higdo B. The α -Carotene, β -Carotene, β -Cryptoxanthin, Lycopene, Lutein, and Zeaxanthin. The Micronutrient Information Center is a public service of the Linus Pauling Institute. 2004.
- [6] Institute of Medicine, Food and Nutrition Board (IMFNB). *Beta-carotene and other carotenoids. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids..* Washington, D. C. National Academy Press. 2000; 325-400.
- [7] Krinsky NI, and Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med.* 2005; 26:459–516.
- [8] Johnson EJ. The role of carotenoids in human health. *Nutr Clin Care.* 2002; 5:56–65.
- [9] Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. Third ed. New York, NY: Oxford University Press. 1999.
- [10] Fiedor J, Burda K. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. *Nutrients*. 2014; (6): 466-488.
- [11] Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys.* 1989; 274 (2):532-538.
- [12] Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys.* 2001; 385 (1):20-27.
- [13] Omenn GS, Goodman GE, Thornquist MD, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. *J Natl Cancer Inst.* 1996; 88 (21):1550-1559.
- [14] Morris CD, Carson S. Routine vitamin supplementation to prevent cardiovascular disease: a summary of the evidence for the US Preventive Services Task Force. *Ann Intern Med.* 2003; 139 (1):56-70.
- [15] Voutilainen, S, Nurmi T, Mursu J, Rissanen TH. Carotenoids and cardiovascular health. *Am J Clin Nutr.* 2006; 83 (6):1265-1271.
- [16] Holick CN, Michaud DS, Stolzenberg-Solomon R, et al. Dietary carotenoids, serum beta-carotene, and retinol and risk of lung cancer in the alpha-tocopherol, beta-carotene cohort study. *Am J Epidemiol.* 2002; 15 (6):536-547.
- [17] Albanes D, Heinonen OP, Taylor PR, et al. Alpha-Tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst.* 1996; 88 (21):1560-1570.
- [18] Parker RS. Carotenoids in human blood and tissues. *J Nutr.* 1989; 119: 101–102.
- [19] Bone RA, Landrum JT, S. L. Tarsis Preliminary identification of the human macular pigment. *Vision Res.* 1995; 25:1531–1535.
- [20] Yeum KJ, Shang F, Schalch W, Russell RM, Taylor A. Fat-soluble nutrient concentrations in different layers of human cataractous lens. *Curr Eye Res.* 1999; 19:502–505.
- [21] Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *Eye Disease Case-Control Study Group. JAMA.* 1994; 272 (18):1413-1420.
- [22] Mares-Perlman JA, Fisher A, Klein R, Palta M, Block G, Millen AE, Wright JD. Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am J Epidemiol.* 2001; 153 (5):424-432.
- [23] Snellen EL, Verbeek AL, Van Den Hoogen GW, Cruysberg JR, Hoyng CB. Neovascular age-related macular degeneration and its relationship to antioxidant intake. *Acta Ophthalmol Scand.* 2002; 80 (4):368-371.
- [24] Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. *J Nutr.* 2002; 132 (3):518 S-524 S.
- [25] Gale CR, Hall NF, Phillips DI, Martyn, CN. Lutein and zeaxanthin status and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2003; 44 (6):2461-2465.
- [26] Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J Natl Cancer Inst.* 1995; 87 (23):1767-1776.
- [27] Giovannucci E. A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Exp Biol Med (Maywood).* 2002; 227 (10):852-859.

- [28] Giovannucci E. Lycopene and prostate cancer risk. Methodological considerations in the epidemiologic literature. *Pure and Applied Chemistry*. 2002; 74, 1427–1434.
- [29] Hotta M, Ogata K, Nitta A, Hoshikawa K, Yanagi M, Yamazaki K. *Useful plants of the world*, Heibonsha LTD, Tokyo. 1989.
- [30] Pinto AC, de Cordeiro Q, de Andrade SRM, Ferreira FR, Filgueiras HA, de C, Alves RE, Kinpara DI. *Annona species*. International Centre for Underutilised Crops, University of Southampton, Southampton, UK. 2005; ISBN 0854327851.
- [31] Maunda PM, Ngugi GW, Kabuye CHS. *Traditional food plants of Kenya*. National Museum of Kenya. 1999.
- [32] Dachtler M, Glaser T, Kohler K, Albert K. Combined HPLC-MS and HPLC-NMR on-line coupling for the separation and determination of lutein and zeaxanthin stereoisomers in spinach and in retina. *Analytical chemistry*. 2001; 73 (3), 667–67.
- [33] Samuagam L, Khoo HE, Akowuah GA, Okechukwu PN, Yim HS. HPLC analysis of antioxidant compounds in some selected tropical fruits' peel. *Innovative Romanian Food Biotechnology*. 2014; 14, 61–68.
- [34] Alba R, Payton P, Fei ZJ, Mc Quinn R, Debbie P, Martin GB, et al. Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. *Plant Cell*. 2005; 17:2954–2965.
- [35] Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. *An introduction to GenStat for Windows*, 14th ed. VSN International, Hemel Hempstead, UK. 2011.
- [36] Rodriguez-Amaya DB, Rodriguez EB, Amaya-Farfan J. Advances in food carotenoids research: Chemical and technological aspects, implication in human health. *Mal. J. Nutr*. 2006; 12 (1): 101–121.
- [37] van Rooyen J, Esterhuyse AJ, Engelbrecht AM, Du Toit EF. Health benefits of a natural carotenoid rich oil: a proposed mechanism of protection against ischaemia/reperfusion injury. *Asia Pacific Journal of Clinical Nutrition*. 2008; 17 (1):316–319.
- [38] Bhardwaj A, Satpathy G, Gupta RK. Preliminary screening of nutraceutical potential of *Annona squamosa*, an underutilized exotic fruit of India and its use as a valuable source in functional foods. *Journal of Pharmacognosy and Phytochemistry*. 2014; 3 (2): 172–180.
- [39] Burns J, Fraser PD, Bramley PM. Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. *Phytochemistry, Plant Metabolomics*. 2003; 62 (6): 939–947.
- [40] Van Duyn MA, Pivonka E. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional. *J Am Diet Assoc*. 2000; 99 (10): 1241–1248.
- [41] During A, Harrison EH. Intestinal absorption and metabolism of carotenoids: insights from cell culture. *Arch Biochem Biophys*. 2004; 430 (1):77–88.
- [42] WHO. Population nutrient intake goals for preventing diet-related chronic diseases. 2008. www.who.int/nutrition.
- [43] Wang Y, Bentley ME, Zhai F, Popkin BM. Tracking dietary intake patterns of Chinese from childhood to adolescence over a six-year follow-up period. *J Nutr*. 2002; 132 (93):430–438.
- [44] Giskes K, Turrell G, Patterson C, Newman B. Socio-economic differences in fruit and vegetable consumption among Australian adolescents and adults. *Publ Health Nutr*. 2002; 5 (5): 663–669.
- [45] Wardle J, Permenter K, Waller J. Nutrition knowledge and food intake. *Appet*. 2000; 34 (3): 269–275.
- [46] Obuntibejua OO, Truter EJ, Esterhuyse AJ. The Role of Fruit and Vegetable Consumption in Human Health and Disease Prevention. *Diabetes Mellitus-Insights and Perspectives*. 2013; 3 (2): 172–180.
- [47] van Het Hof KH, West CE, Weststrate JA, Hautvast JG. Dietary factors that affect the bioavailability of carotenoids. *J Nutr*. 2000; 130 (3):503–506.
- [48] Johnson EJ, Chung HY, Caldarella SM, Snodderly DM. The influence of supplemental lutein and docosahexaenoic acid on serum, lipoproteins, and macular pigmentation. *Am. J. Clin. Nutr*. 2008; 87, 1521–1529.