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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 has 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 µL of 3 mg/mL magnesium carbonate ($MgCO_3$) suspension and 300 µL 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 µL 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 µL THF and 150 µL methanol) were pippeted 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 µL 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 µL petroleum ether and 150 µL (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 µL petroleum ether before the upper phase being carefuuly 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 µL ethyl acetate was added then incubated at room temperature for 15 minutes followed by vortexing and filtering the suspension filtered through 0.45 µm nylon syringe filter (Cameo 3 N syringe filter, GE Water & Process Technologies, USA).

2.3. HPLC Analysis Using YMC C_{30} Column

Carotenoid analysis was carried out in adefined YMC C_{30} 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% methytert-butyl ether; 26-35 ramp to 100% methanol:ammonium acetate) through a guard cartridge (YMC carotenoid S-5, 4.0 mm x 20 mm DC guard, Waters), C_{30} 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 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 ± standard error of the mean. Analysis of variance (ANOVA) followed by Duncans' 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.

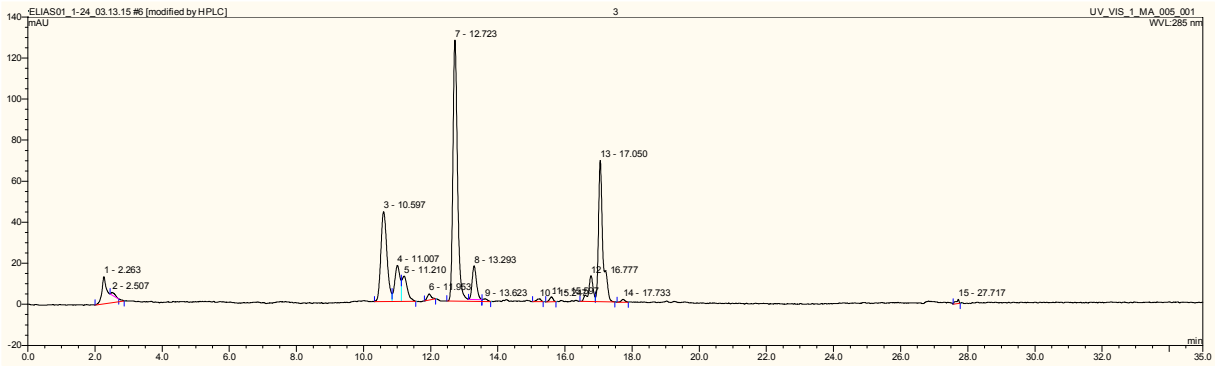

Figure 1. *HPLC chromatogram of one sample showing different carotenoids eluted at different retention.*

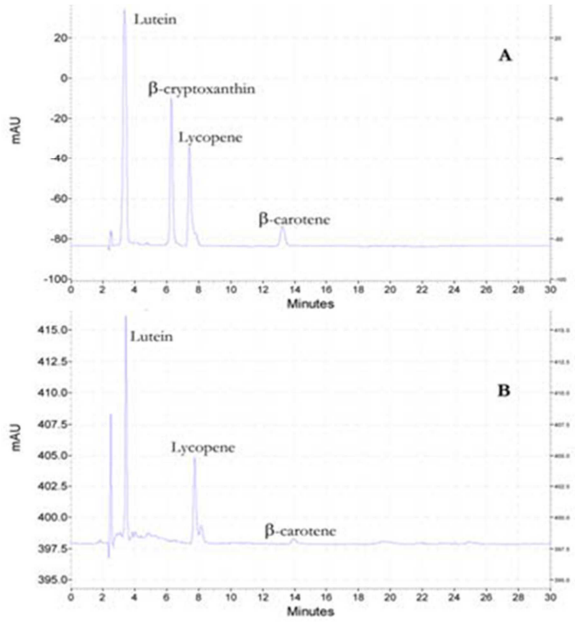

Figure 2. *HPLC chromatograms for carotenoids. Carotenoid standards (A); Annona squamosa (B).*

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 (µg/100 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±0.03 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.

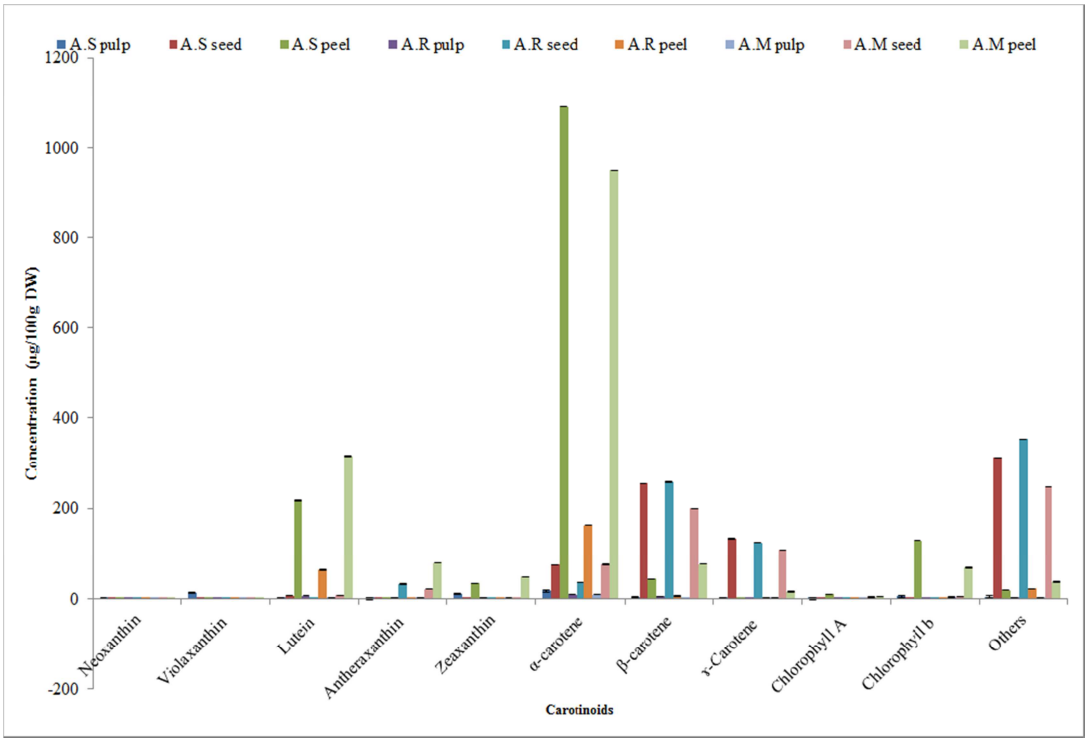

Figure 3. *The concentration carotenoids identified in the different parts of the three Annonaceae fruits analyzed.*

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

Carotinoids	**Concentration (µg/100 g DW)**								
	Annona squamosa			***Annona muricata***			***Annona reticulata***		
	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	315.55b±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\pm^{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
ɤ-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 ± SEM. Means with different superscript letters within a row are significantly different ($P < 0.05$).

4. Discussion

The benefits acrewwed fron inclusion of fruits in the diet cannot be overlooked. Although fruits has provided a delicate balance interms 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. Movreover, 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 supplents 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. *Annanoceae* fruits grown in Kenya have been ignored interms of research in the nutrional and health benefits acrewed from their consumption leading to their underutilization. The results from the current study indicates the presence of 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] suggests 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] influences fruit intake. Family origin affect 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 presence. 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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