Original Research Article

Quantitative Analysis of Total Carotenoids in some Vegetables Consumed in Akwa Ibom State, Nigeria

Abstract

The quantitative analysis of total carotenoids in some vegetables consumed in Akwa Ibom State was evaluated using UV-Spectrophotometer and acetone as extraction solvent. Ten different vegetable samples i.e. Carrot (*Daucus carota),*purpleegg plant*(Solanum melongena)*,green egg plant *(Solanum melongena)*, tomato*(Solanum lycopersicum)*, cucumber*(Cucummis sativa)*,fluted pumpkin *(Telfairia occidentalis)*, spinach*(Spinacia oleracea)*, red pepper*(Capsicum annuum*,green pepper(*Capsicum annuum)and* bitterleaf *(Vernonia amygdalina*)were purchased from local markets in Itam and Ikot Akpaden, both in Akwa Ibom State. The fresh samples were washed with tap water, followed by distilled water. The samples were homogenized separately using sterile mortar and pestle with 10ml acetone as extraction solvent. The extract was centrifuged at 10,000rpm for 15 mins in 4^{o}C and the supernatant analysed as soon as possible using spectrophotometer (UV-VIS 2500) at 470nm. The quantification of carotenoids was carried out according to Harbone J B, 1973 andon the basis of Beer Lambert's equation: A=ƹCL in mol/l and converted to mg/ml. From the result obtained, *Capsicum annuum* (red pepper) had the highest amount of carotenoids (2.889mg/ml), *Solanum lycopersicum* showed the lowest amount of carotenoid (0.260mg/ml) the deep green vegetables also had high quantity of carotenoids. Carotenoids are important due to the health benefits associated with the pigment acting as provitamin A, a strong antioxidant and gives colouration to plants. It also helps in preventing photo-damage in plants and has some health benefits like improving sight, reducing cancer risk to animals too. Further studies analyzing the individual carotenoids is recommended and investigation on the effect of time on extraction.

Key words: Carotenoids, vegetables, acetone, vitamin, antioxidant.

1. Introduction

Carotenoids are a class of phyto-nutrients (plant chemicals) found in the cells of a wide varieties of plants, bacteria and algae [1]. As a major plant pigment its distinct structures of biotechnological interest are natural colorants and human diseases' prevention [2]. These pigments play important roles in plant health and in extension, gives protective health benefits to consumers of carotenoids containing foods according to Szalay[1].

The total leaf pigment composition includes the chlorophyll-a, chlorophyll-b and carotenoids which are necessary for photosynthetic processes [3]. According to Sumanta *et al.*[3] some internal factors and environmental conditions can cause variations in the chlorophylls-carotenoids leaf pigment components [3]. The absorbance properties of these pigments facilitate their qualitative and quantitative analysis [3-4].

Carotenoids are usually represented by two carotenes (α- and β-) and five xanthophylls (luteins, zeaxanthin, violaxanthin, antheraxanthin and neoxanthin) which exhibit strong light absorption properties in the blue region of the spectrum and are non-uniformly distributed in photosynthetic and individual pigment protein complexes of chloroplast[5-8]. Carotenoids are synthesized in the plastid of plants and are the most diverse group of pigment found in nature. They are located in chromoplast and they give colouration to vegetables and fruits [3].

In human, carotenoids such as beta-carotene and lycopene are able to participate in free radical reactions where they help quench or prevent the formation of singlet oxygen through an efficient energy transferprocess [1]. A recent study showed lycopene supplementation at the rate of 15 mg per day for 8 weeks to significantly decrease systolic blood pressures from the baseline value of 144mmHg to 134mmHg in mildly hypertensive subjects [9-10]. In another study a significant reduction in plasma lycopene was observed in the hypertensive patients compared to normal subjects [11]. When patients with liver cirrhosis, a condition closely associated with hypertension and disorders of the lymphatic circulation, were compared with matched controls a significant reduction in serum lycopene was observed along with other carotenoid antioxidants, retinol and vitamin E in the cirrhotic group [9,12].

In fact, dietary supplement of carotenoids shows a good relationship in reducing cancer risk by intake of various fruits and vegetables associated with as disease prevention[13]. To date, carotenoids have been associated with a vast range of diseases, especially degenerative diseases. Studies into carotenoids continue to demonstrate the importance of these phytochemicals. These include: oxidative stress, vitamin A deficiency, asthma and chronic obstructive pulmonary disease, Alzheimer's disease[14], cystic fibrosis, human immunodeficiency virus and many more[15].

Several attempts during the last decade have been undertaken to develop nondestructive techniques for carotenoid content assessment at both the leaf and canopy level[16-21]. For senescing leaves, reflectance indices sensitive to the molar concentration of carotenoids and chlorophylls ratio have been reported[16]. Over the years carotenoids have been known just as pro vitamin A with little known also of their quantity in some vegetables (especially those vegetable in Akwa Ibom State).

70 This study therefore was designed to quantify carotenoids in some vegetables consumed in Akwa
71 Ibom State.

2.0 *Materials and Method*

2.1 Sample Collection

Ten different fresh and matured vegetables *(Daucus carota, Solanum melongena (*purple*), Solanum melongena (*green*), Solanum lycopersicum, Cucummis sativa, Telfairia occidentalis, Spinacia oleracea, Capsicum annuum (*red*), Capsicum annuum (*green*)* and *Vernonia amygdalina)*were purchased from local markets in Ikot Akpaden and Itam in Akwa Ibom State, Nigeria. The purchased vegetables were conveyed in sterile black nylon bags to the Genetics and Biotechnology laboratory, Akwa Ibom State University, Ikot Akpaden. The fresh samples were washed thoroughly with flowing tap water, followed by distilled water in the laboratory and further processed for the determination of total carotenoids content.

2.2 Carotenoid Determination

Ten (10) grams of each of the fresh vegetable sample was accurately weighed and homogenized for 10mins, using a sterile mortar and pestle in 10ml (10000µl) of extraction solvent (98% acetone). The homogenized sample was centrifuged at 10,000rpm for 15minutes at $4^{o}C$. The supernatants (the pigment extracts) were separated and stored in ice in appropriately well labeled test tubes. Each extract was analyzed for total carotenoid content in triplicate using UV-VIS spectrophotometer (UV-2500) at a wavelength of 470nm. Averages of triplicate absorbance readings at 470nm were recorded and the quantification of carotenoids carried out according to Harbone JB, 1973 and on the basis of the Beer-Lambert equation according to Lichtenthaler[8]:

$A = \text{Ɛ}CL$

Where;

A is the absorbance

Ɛ is the molar absorption coefficient for carotene (in acetone) at 470nm = $134x10^3$

C is the concentration of the pigment (mol/l)

L is the path length of the light absorbing pigment (=1cm).

2.3 Data Analysis

The triplicate result obtained was analyzed using ANOVA single factor which showed no significant difference, P- value of 0.99.

Table 1 was subjected to T-Test and it showed significant difference among the treatments with a P-value of 0.000097 is less than the *P*-value of the alpha level which is 0.05.

3.0 *Result and Discussion*

Results for the extraction of carotenoids by acetone from different vegetable samples followed the sequence: *Capsicum annuum* (red) >*Vernonia amygdalin* >*Telfairia occidentalis* >*Spinacia oleracea*>*Solanum melongena* (purple) >*Solanum melongena* (green) >*Capsicum annuum* (green) >*Cucumis sativus* >*Daucus carota*>*Solanum lycopersicum*. The highest carotenoids content extraction was noted in *Capsicum annuum* (red) as compared to the nine other samples extracted from. Also, from the result obtained as shown in table 1, coloured or deeply pigmented vegetables contained much more carotenoids when compared to their greenish counterparts as seen in *Solanum melongena* and *Capsicum annuum*. However, some deeply green vegetables like *Telfairia occidentalis and Vernonia amygdalina* with no deeply coloured counterpart contained high quantity of carotenoids too like the coloured vegetables with green counterparts. Results showed that deep green vegetables contains high quantity of carotenoids.

Caroteniod, chemically have high affinity towards polar solvents like the acetone and methanol [4,22], variations in the pigment concentrations is directly influenced by differences in environmental factors, seasons and even the species of the samples[3]. Carotenoid, therefore is important because of both its biotechnological potential[23], and its role in understanding the evolution of secondary metabolism[24].

Costache *et al.*[25] reported that carotenoids group and their derivatives consist of about 70 compounds that are present in most vegetables and fruits. Also, according to Vechetel and Ruppel[26], carotene pigments were the most important photosynthetic pigments and they prevented chlorophyll and thylakoid membrane from the damage of absorbed energy by peroxidation. Previous reports have considered carotenoid in bright coloured vegetables, since carotenoid contribute to their bright colouration. Times food reported that red Capsicum has more carotenoid than green Capsicum which is in line with the result of this research. However, this research has not only considered bright coloured vegetables with their green counterparts alone but has considered carotenoid in deep green vegetables which do not have bright coloured counterpart and the deep green vegetables without bright coloured counterpart has shown to contain high quality of carotenoid.

4.0 *Conclusion*

Having good vision and taking proactive measures in preventing the occurrence of certain diseases like cancer, cardiovascular disease is good for complete wellbeing of humans. Carotenoids which are present in vegetables act as pro-vitamin A and can serve these purposes. The study reveals the high amount of carotenoids in *Capsicum annuum* (red), *Telfairia occidentalis, Vernonia amygdalina*, *Solanum lycopersicum* (purple). These vegetables serve as a good source for high quantity of carotenoids. With health benefits in mind, people should be encouraged to incorporate carotenoids rich vegetables in their daily diets and in line with the result of the studies, *Capsicum annuum* (red), *Vernonia amygdalina*, *Solanum melongenta* (purple), *Telfairia occidentalis* being good sources with

high amount of carotenoids are hereby recommemended. Further studies on carotenoids analysis in vegetables is recommended to check the relationship between extraction and time alongside individual carotenoid concentrations.

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Table 1: Spectrophotometric determination of absorbance and Quantification of carotenoids (mol/l) and (mg/ml)

S/N	Sample Id	Absorbance	Quantity of carotenoids (mol/l)	Quantity of carotenoids (mg/ml) or mgg^{-1} fresh wt
1	*Spinacia oleracea*	0.543±0.010	$4.052x10^{-6}$	2.175
2	*Solanum melongena* (green)	0.304±0.002	$2.269x10^{-6}$	1.218
3	*Solanum melongena* (purple)	0.411±0.017	$3.067x10^{-6}$	1.647
4	*Capsicum annuum* (red)	0.721±0.034	$5.381x10^{-6}$	2.889
5	*Capsicum annuum* (green)	0.276±0.017	$2.060x10^{-6}$	1.106
6	*Vernonia amygdalina*	0.709±0.004	$5.291x10^{-6}$	2.841
7	*Daucus carota*	0.112±0.010	$8.358x10^{-7}$	0.449
8	*Cucumis sativa*	0.146±0.003	$1.089x10^{-6}$	0.585
9	*Solanum lycopersicum*	0.065±0.011	$4.851x10^{-6}$	0.260
10	*Telfairia occidentalis*	0.635±0.018	$4.739x10^{-6}$	2.544

Mean ±SD of mean in 3 Determinants.