ISOLATION AND DETERMINATION OF TOTAL CONCENTRATION OF β-CAROTENE FROM THE JUICES OF SOME FRUITS AND VEGETABLES

A PROJECT REPORT

BY

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A DISSERTATION SUBMITTED TO THE INSTITUTE OF SCIENCE TECHNOLOGY, LONDON IN PARTIAL FULFILMENTOF THE REQUIREMENT FOR THE AWARD OF HIGHER DIPLOMA IN BIOCHEMICAL LABORATORY TECHNIQUES

JUNE 2003

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DECLARATION

I declare that the experimental work reported in this dissertation was carried out by me at the Food Research Institute, Accra, under the supervision of Dr. Pearl Adu-Amankwa of the Processing & Engineering Division.

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ACKNOWLEDGEMENT

This work would not have been possible without the supervision of Dr.Pearl Adu-Amankwa of the Processing & Engineering Division of the Food Research Institute.

I wish to express my deep gratitude to the Management of Food Research Institute for having given me the opportunity to take part in this course. My thanks to Joshua A. Amfo, the Managing Director of Dor's bakery and Dr. P.N-T Johnson of Food Research Institute for the role they played in completing the course.

I am sincerely grateful to the lecturers in the Biochemistry Department of the University of Ghana, Legon especially to Drs J. Adjimani, Y. Osei, and L. Okine.

My heartfelt gratitude to Miss Joyce Osei for typing the manuscripts and also Mr. Elvis Baidoo for the role he played in putting this manuscript in place.

Finally my thanks go to Mr. F.O.K. Seiku of Botany Department of the University of Ghana, Legon for his patience.

DEDICATION

This piece of work is dedicated to my dear mother, Mrs. Florence Nyarko for her great effort in bringing me up and seeing me through my education.

ABSTRACT

The determination of concentration of β-carotene in fruits and vegetables is an essential tool for the recommendation of relevant dietary intake of those fruits and vegetables for Vitamin A.

β-carotene, a carotenoid found in nature and a precursor of Vitamin A known in all visual pigments was isolated from the juices of tomato, carrot, red pepper, mango, avocado pear and orange and the concentration in each was determined by the phase separation method using n-hexane as the solvent.

The results revealed that red pepper had the highest concentration of β-carotene (77.2μg/2ml of extract) and avocado pear the least (0.9 μg/2ml of extract). The other fruits and vegetables had intermediate concentrations of β-carotene.

For a person suffering from night- blindness, vegetables and fruits such as red pepper, carrot and mango could be added to the diet for a quicker synthesis of Vitamin A.

1.0 INTRODUCTION

Carotenoids are a group of yellow–orange and orange-red fat-soluble pigments widely distributed in nature. These pigments are isoprenoid compounds. In green plants they occur in the chloroplast and are present in the lipid material along with the chlorophylls. The bright fresh yellow-green colour of the spring leaves is the result of carotenoids and small amount of chlorophylls. These pigments are also present in a wide variety of fruits e.g. banana skins, tomatoes, red pepper, parika, squash etc. as well as other parts of plants-- in carrots, sweet potatoes and in most yellow-orange red flowers. When they are consumed by animals they tend to concentrate in lipids and hence are found in blood, milk, egg-yolk and depot fat. The name carotenoid is applied to all pigments chemically related to carotenes which were first isolated. In 1831, Wackenroder extracted a pigment from carrots and called the fraction carotene, this "carotene" is a mixture of three isomers- α, ß, and γ-carotene. (Meyer, 1964)

Fig.1

ß-carotene

α-Carotene

γ-Carotene

Source: [Meyers, 1964]

The difference between the - α, ß-carotene is a replacement of the double bond in ring 2. Alpha and γ-carotene occur in nature associated with ß-carotene, though usually in smaller amounts. Alpha-carotene predominates in a very few plant products. For example, in red palm oil it accounts for approximately 30-40% of the carotenes present. Carotenoids which contain hydroxyl groups are called xanthophylls.

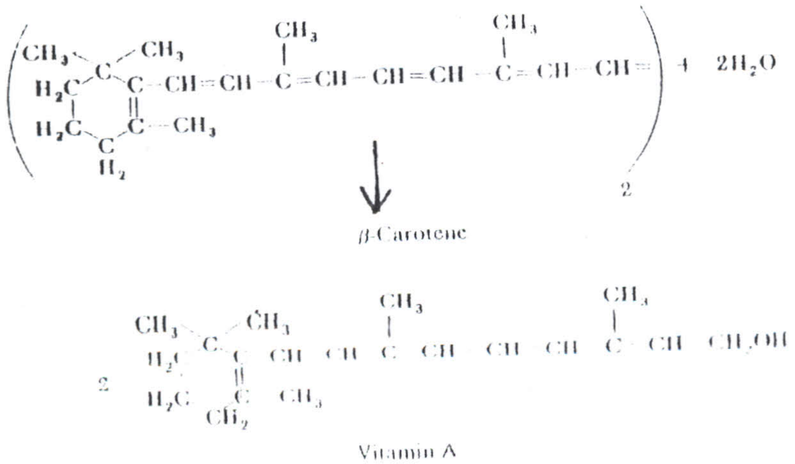
Table 1: The distribution of carotenoids in some foods.

Food item	Distribution of carotenoid
Carrot (*Daucus Carota*)	α-carotene, β-carotene, γ-carotene, Xanthophyll, two hydrocarbons of unknown composition
Wheat Germ	Xanthophyll, Carotene
Corn *(Zea Mays*)	Zeaxanthin, Cryptoxanthin, Xanthophyll, α-Carotene.
Apricot (*Prunus armerriaca*)	β- carotene, γ- carotene, lycopene
Peach (***Prunus persica)***	β- carotene, crytoxanthin, Xanthophyll, Zeaxanthin and unknown carotenoid.
Soya Bean (*Glycinemax)*	α- carotene, Xanthophyll
Orange *(Citrus aurantium*)	β- carotene, lycopene, cryptoxanthin, Xanthophyll, violaxanthin, Zeaxanthin, β-citraurin
Cowpea *(Vigna sinensis*)	β- carotene, xanthophylls
Grape Fruit *(Citrus grandis*)	β- carotene, lycopene
Squash (*Cucurbita maxima*)	α- carotene, β- carotene, Xanthophyll, violaxanthin.
Red Pepper or Chilli (*Capsicum fructescens*	Capsanthin, α-carotene, β- carotene

Source: Meyer, 1964.

Plant pigments impart beauty to fruits and vegetables, however, some of the carotenoids are important nutritionally as precursors for the synthesis of vitamin A in the body. Vitamin A is identified with one half of a β-carotene molecule plus a hydroxyl group, and in animals one molecule of β-carotene is converted to two molecules of vitamin A by hydrolysis.

Fig. 2



[Moyers 1964]

Vitamin A itself does not occur in plants but many plants contain isoprenoids compounds (Carotenoids) which can be enzymatically converted into vitamin A by most animals.

There are two natural forms of vitamins A, i.e. Vitamin A_1 or retinol obtained from marine fish livers and vitamins A_2 from livers of fresh water fish. These are 20 carbon alcohols formed of isoprene units.

Fig 3 shows how vitamin A is formed by cleavage of β-carotene, which gives carrots, sweet potatoes and other yellow vegetables their characteristic colour. The colour of tomatoes and carrots comes basically from lycopene and β-carotene. (Stryer, 1988) These compounds absorb lights because they contain polyenes which is an extended network of single and double bonds. Their C_{40} carbon skeletons are built by successive addition of C_5 units to form geranyl- geranyl pyrophosphate, a carbon intermediate which then condenses tail to tail with

Fig. 3

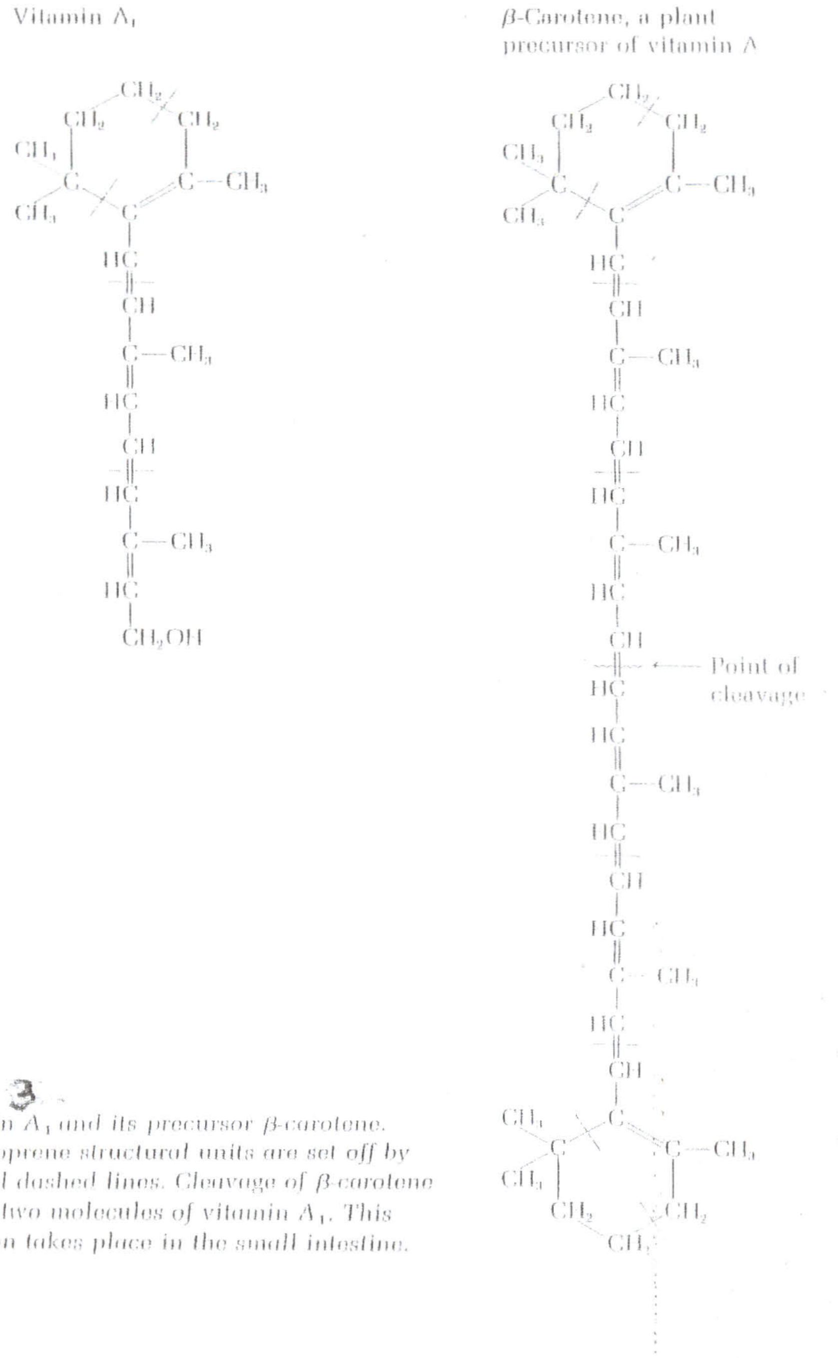


Figure 3.
Vitamin A_1 and its precursor β-carotene. The isoprene structural units are set off by colored dashed lines. Cleavage of β-carotene yields two molecules of vitamin A_1. This reaction takes place in the small intestine.

source: [Lehninger, 1982]

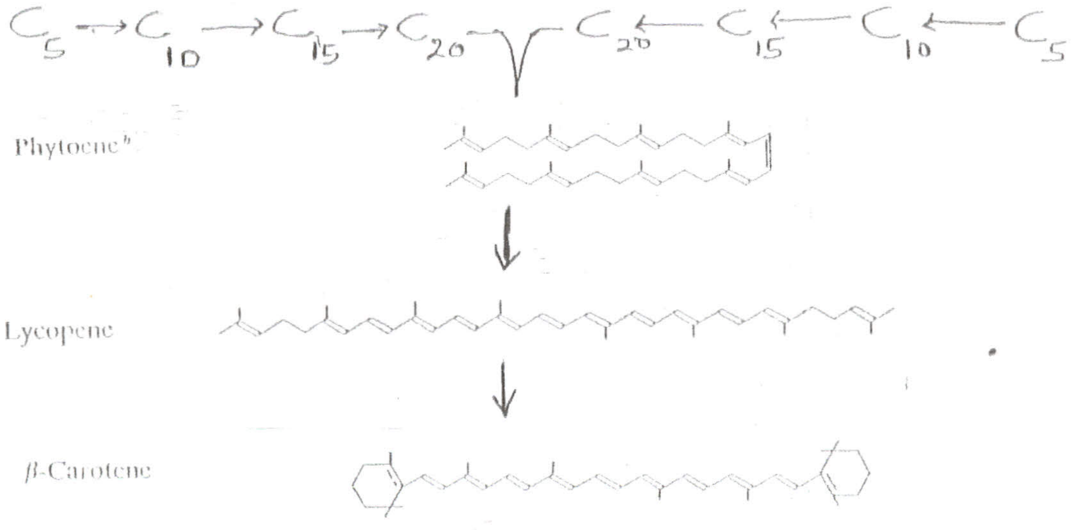
another molecule to geranyl- geranyl pyrophosphate. This biosynthetic pathway is like that of squalene except that C_{20} rather then C_{15} units are assembled and condensed.

$C_5 \rightarrow C_{10} \rightarrow C_{15} \rightarrow C_{30}$ (Squalene)

$C_5 \rightarrow C_{10} \rightarrow C_{15} \rightarrow C_{20} \rightarrow C_{40}$ (Phyteone)

Phyteone, the C_{40} condensation product is dehydrated to yield lycopene. Cyclization of both ends of lycopene gives β-carotene. As shown in Fig 4.

Fig 4



Source [HULME 1970]

Carotenoids serves as light harvesting molecules in photosynthetic assemblies by absorbing light at wavelength other than those absorbed by chlorophyll. The carotenoid pigments consist of two major classes, the hydrocarbon carotenes and the oxygenated xanthophylls. (Nielsen, 1998). The two yellow plant pigments lycopene and carotene belong to the unsaturated hydrocarbon with a terpene-like character. The resemble the oxygen-containing pigment in chemical structure and physical behaviour. This characteristic group of yellow plant pigment is known under the name carotenoids proposed by Tswett after the pigment of carrot and carotene. Their solubility in fats and their occurrence in animals and plants has earned them the name lypochrome pigments. Lycopene and β-carotene found in fruits and some vegetables are isomers with empirical formula $C_{40}H_{56}$. It contains 13 double bonds which can be catalytically hydrogenated to give rise to a saturated hydrocarbon $C_{40}H_{82}$ (Karer, 1938)

The relative amounts of the different carotenoids vary characteristically from one species of plant to another. Variations in the proportion of these pigments are responsible for the characteristic colour of photo-synthetic cells which vary from a deep blue-green as in spruce noodles to a greener green as in maple leaves to red brown or even purple colour of different species of multicellular algae and leaves of some decorative plants.

They also play a role in protecting prokaryotes from deleterious effects of light and are also essential for vision. B-carotene is the precursor of retinal, the chromophore in all known visual pigment.

FUNCTIONS OF VITAMIN A

Deficiency of vitamin A has a multiple effects. It causes the nutritional diseases xeropthalmia ("dry eye") and Keratomalacia (Excessive keratin formation in the skin and the cornea of the eye), retarded development and growth, sterility in male animals and night blindness an early symptom commonly used for diagnosis of vitamin A deficiency in people.

In the United States it occurs in people with intestinal or pancreatic diseases in which there is defective fat absorption, such people fail to absorb dietary vitamin A or carotene which are fat-soluble. In its early stages xeropthalmia is manifested as night blindness to deficient synthesis of visual pigment- rhodopsin which contains as its active group retinal for which vitamin A is the precursor.

Through intensive biochemical and biophysical studies first pioneered by George Wald at the Harvard University, we now have comprehensive information on the function of vitamin A in vision. Lehninger, 1982(Fig 5) outlines the visual cycle in rod cells of the retina.

These cells function in seeing low intensity of light but do not sense colours. In the visual cycle an oxidized form of retinol, retinal or vitamin A aldehyde is the active component bound to a protein called opsin. The retinal opsin complex called rhodopsin is present stacked intracellular membranes in the rod cells. When rhodopsin is excited by visible light, the retinal which the double bonds at the 11th position is cis from (the rest of the double bond are trans) undergoes a number of a very complex, rapid molecular changes and finally isomerized to form all-trans-retinal. These alternations which changes the geometrical configuration of the retinal in Fig 5 are believed to accompany a change in the shape of the entire rhodopsin molecule.

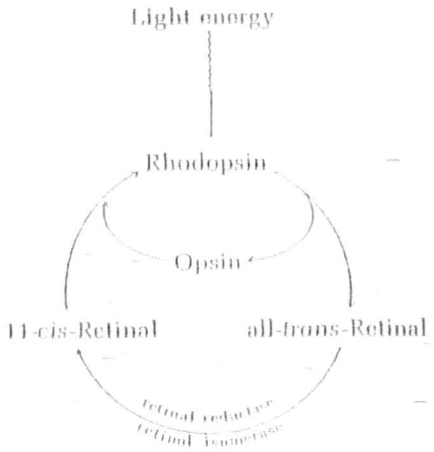
This event is a molecule trigger producing in the brain. The all-trans-retinal formed during illumination is then enzymatically converted back into 11-cis- retinal in "dark" reaction.

It has also been observed that repeated great over dosage with vitamin A can cause fragility of the bone or swelling in rats. Doses of 500,000 units daily for extended periods to infants have produced moderate symptoms. Illness and even death have been reported in Arctic-exploring parties as a result of the consumption of polar bear liver, an extremely concentrated source of Vitamin A .In recent Swedish study, researchers analysed blood samples from 2,047 men for 30years.Those with the highest levels of Vitamin A in the blood were1.6 times more likely to break a bone than men with low levels of the vitamin and hip fractures were 2.5 times common. (The Mirror)

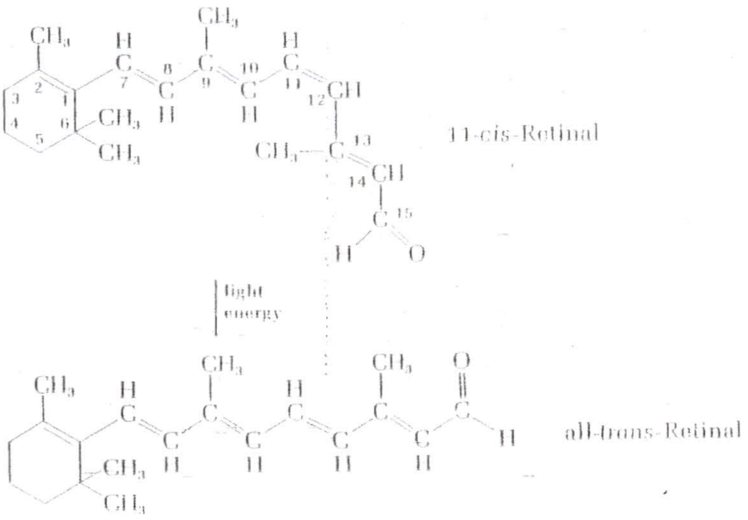
Fig 5

Visual cycle rod cells of the Retina

Figure 5
The visual cycle. When a rhodopsin molecule is excited by visible light its 11-cis-retinal prosthetic group, which absorbs light energy, is isomerized in several steps to all-trans-retinal, a process that triggers the nerve impulse. The all-trans-retinal no longer fits the active site on the opsin protein and leaves it. Through two subsequent enzymatic reactions the all-trans-retinal is converted back into 11-cis-retinal, which binds to the opsin again to reconstitute rhodopsin.



Cis-trans isomerization of 11-cis-retinal



Source : [Lehninger, 1982]

Table 2: **Daily recommended dietary allowance of vitamin A (FAO/WHO, 1974)**

AGE (Years)	BODY WEIGHT (Kg)	Vit. A (µg)
Males and females		
0 – 1	7.3	300
1 – 3	13.4	250
4 – 6	20.2	300
7 – 9	28.1	400
Males		
10 – 12	36.9	575
13 – 15	51.3	725
16 – 19	62.9	750
Adult	65.0	750
Females		
10 – 12	38.0	575
13 – 15	49.9	725
16 – 19	54.4	750
Adult	55.0	750
Pregnant		750
Lactating		1200

1.1 OBJECTIVES

The objectives of this work are:

1. Isolate and identify carotenoids from the juices of tomatoes, carrots, orange avocado pear, mango and red pepper.
2. Determine the concentration of β-carotene in the juices of tomatoes, carrots, orange avocado pear, mango and red pepper.

The results will lead to a recommendation relevant to dietary intake of the mentioned fruits and vegetables.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 CONSUMMABLES

- Tomato
- Carrot
- Red pepper
- Mango
- Avocado Pear
- Orange

2.2 CHEMICAL USED

- n-hexane

2.3 EQUIPMENTS

- Cotton wool
- Funnels (6)
- 125ml separating funnels (6)
- 10ml measuring cylinder (1)
- 250ml beaker (6)
- 2ml pipette (6)
- pH meter model (model PHM 92)
- UV – Visible Spectrophotometer (model PERKIN-ELMER 295E)
- Glass Sample cuvettes (1cm)
- Blender (Model HR-2815i- Philips)
- Colour meter (Model CR- 310)

2.4 β-CAROTENE DETERMINATION

In determining the concentration of β- carotene in the juices of Tomato, Carrot, Red pepper, Mango, Avocado pear and Orange the methods in the AOAC, fifteenth edition volume II 1998 and Experimental Methods in Organic Chemistry by More and Dalymple (1976) was modified and used.

β- carotene was isolated from the juices by the phase separation method using n-Hexane and their absorbances were read using a spectrophotometer. Their pH and colour were also determined using the pH meter and colour meter (chromameter) respectively.

2.5 EXRACTION OF β-CAROTENE

The fruits and vegetables obtained were ripe and fresh. All the fruits and vegetables were washed clean.

2.5.1 ORANGE

The oranges were peeled, cut transversely and squeezed to obtain the juice. The juice was separated from the seeds by filtration using a cotton wool and a funnel into a 250ml beaker.

2.5.2 CARROTS

The carrots were blended and the juice was also filtered.

2.5.3 AVOCADO PEAR

The fruits were peeled and the seeds removed. The fruits were then blended and the juice filtered.

2.5.4 TOMATOES

The tomatoes were cut into two. The seeds were removed and flesh blended. The juice was then filtered.

2.5.5 MANGO

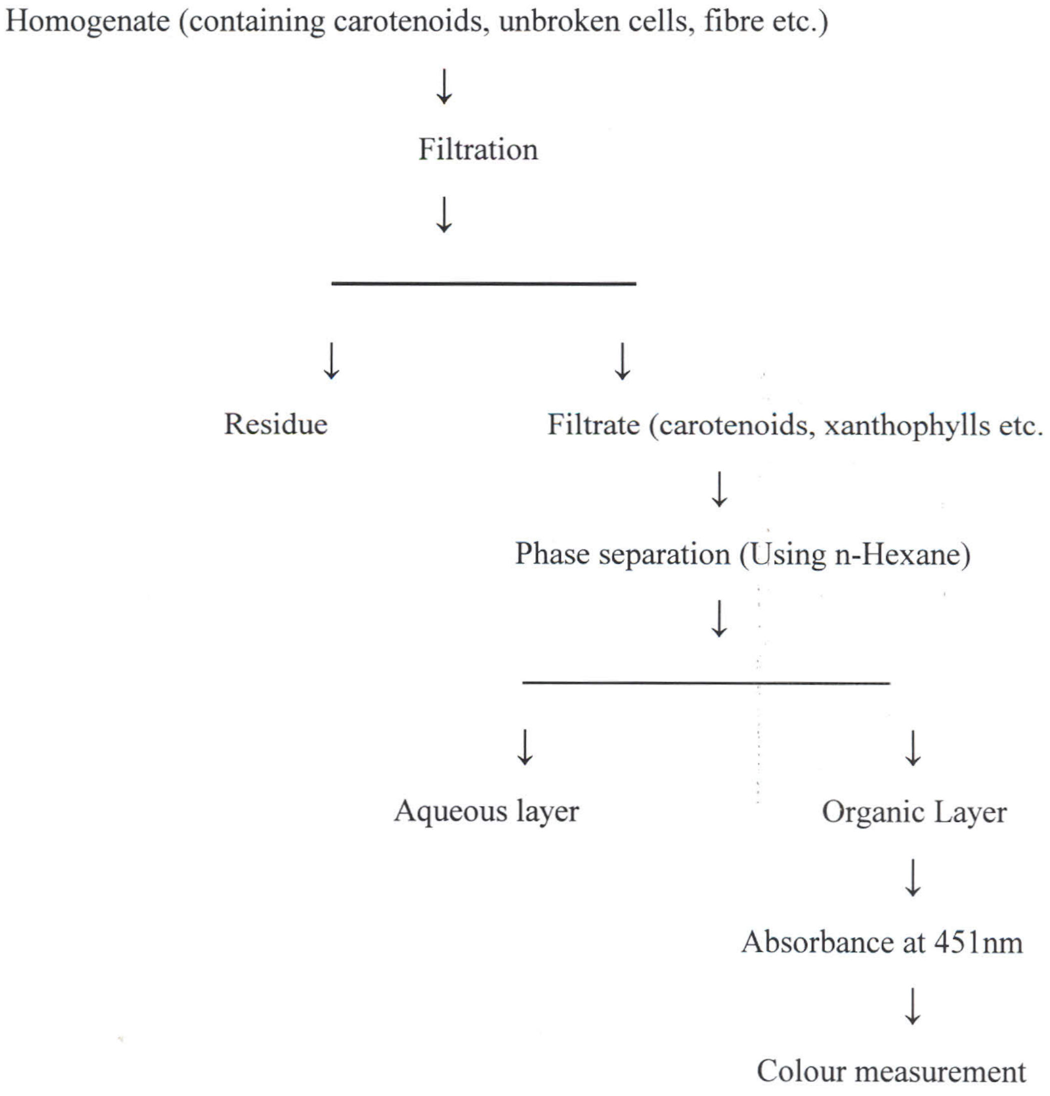
The fruits were peeled, cut to remove seeds and then blended. The juice was also obtained by filtration.

2.5.6 RED PEPPER

The pepper was cut and seeds removed. It was then blended and the juice was obtained by filtration.

The experimental procedure can be described in a flow diagram as shown below.

FLOW CHART FOR THE DETERMINATION OF β-CAROTENE



2.6 PREPARATION OF SAMPLE FOR ANALYSIS

The extracted juices were stirred to obtain a uniform mixture and their pH's were determined and recorded.

- 2mls of each of the juices were pipetted into each of the 125ml separating funnels and 10ml of n-Hexane was added to each.

- The separating funnels were corked and hand shaken for 5minutes.

- They were allowed to stand for 1 minute and the aqueous phase was separated from the organic layer (containing β- carotene extract) i.e. organic at the top and aqueous down.

- The organic layers were separated into a 25ml volumetric flasks and the levels were topped to the 25ml mark with n-Hexane. They were corked and shaken to obtain a uniform mixture.

- The absorbances were determined at 451nm using the single beam spectrophotometer.

- The n-Hexane was used as a blank.

- The colours of the organic layers were also determined and recorded using the colour meter (Chromameter)

2.7 DETERMINATION OF ABSORBANCE

The single beam spectrophotometer was used to determine the absorbance of the organic layer extract. The major components are a light source, a monochrometer, a sample chamber, a detector and a recorder.

The principle is based on the Bear-Lambert law which is a combination of two laws each dealing separately with the absorption of light related to the concentration of the absorber (the substance responsible for absorbing the light) and the path length or thickness of the layer (related to the absolute amount of the absorber) which states that, Absorbance is proportional to both the concentration of absorbent and thickness of the layer.

$A = \varepsilon_{\lambda}CL$

Where ε_{λ} is the Molar absorbance coefficient (or Molar extinction coefficient) for the absorber at a wavelength λ. C is the concentration of the absorbing solution and L is the path length through the solution.

A has no units.

ε depends on the units of C and L. It is used in the form of molar extinction coefficient, which is defined as 1 Molar solution of pure absorbing substance in 1 cm cell under specified conditions of wavelength of solvent.

If the molar extinction Coefficient (ε) of an absorbing substance is known, the concentration of that substance can be calculated from the relation.

$A = \varepsilon_{\lambda}CL$

Thus,

$C= A/ \varepsilon_{\lambda}L$

According to htt://omlc.ogi.edu/spectra/photochem CAD/abs html/beta-carotene.html, the molar extinction coefficient of Beta – carotene dissolved in hexane based on the absorbance measurement made by R.A. Fuh on summer 1995 using a cary 3 [H. Du; R.A. Fuh, J. Li, A. Corkan, J.S. Lindsey, "Photochem CAD: A computer- aided design and research tool in photochemistry" Photochemistry and photobiology 68, 141 – 142, 1998],the absorbance data was normalized to have an extinction coefficient of 139500 at 451nm. [L. Zechmelster, Cis-trans Isomeric carotenoids vitamin A and aryl-polyenes, springer-verlag, 1962]

2.8 COLOUR DETERMINATION

The colour of the organic layer extract was determined using the colour meter. (L,a,b) (chromameter)

The L, a, b, measurements are based on the following:

The L axis indicates lightness/ darkness on a scale of 100/0
The a axis indicates Red (+ values) / Green (-values)
The b axis indicates Yellow (+values) / blue (-values)

The CIE LAB colour parameter L*, a*, b* for the organic layers of the extracted juices were measured with a chromameter (Minolta CR310). The instrument was standardised each time with a white ceramic plate. The angles h, representing the degree of yellowness and the chroma (i.e. the brightness) were calculated from the equation (MacDougall, 1988).

$$h = \tan^{-1}(b/a)$$

$$\text{Chroma} = (a^2 + b^2)^{1/2}$$

Where L, a, b, are colour parameters of the organic layers of the fruits and vegetables.

The values used are the means of the three measurements.

CHAPTER THREE

3.0 RESULTS

3.1 ABSORBANCE AND pH READINGS

Table 3:

FRUIT /VEGETABLE	ABSOR-BANCE	ABSOR-BANCE	ABSOR-BANCE	ABSOR-BANCE	pH
	1	2	3	MEAN	
MANGO	0.235	0.236	0.235	0.235	4.70
ORANGE	0.020	0.020	0.020	0.020	3.82
AVOCADO PEAR	0.010	0.010	0.010	0.010	6.72
PEPPER	0.800	0.810	0.800	0.810	5.03
CARROT	0.285	0.284	0.285	0.285	5.99
TOMATO	0.155	0.154	0.155	0.155	4.11

3.2 COLOUR READINGS

Table 4:

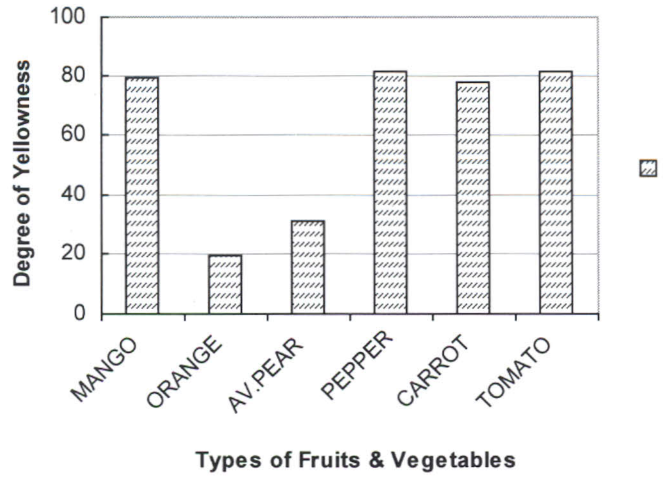
FRUIT /VEGETABLES	L	Mean	a	Mean	b	Mean
MANGO	76.85		-1.16		+5.86	
	76.10	76.79	-1.18	-1.16	+5.98	+5.93
	76.79		-1.14		+5.96	
ORANGE	76.15		+2.00		-0.75	
	76.29	76.22	+2.06	+2.05	-0.74	-0.72
	76.25		+2.08		-0.68	
AVOCADO PEAR	77.18		+2.05		-1.28	
	77.17	77.16	+2.04	+2.05	-1.21	-1.25
	77.12		+2.05		-1.27	
PEPPER	75.81		-2.55		+16.28	
	75.63	75.70	-2.51	-2.53	+16.25	+16.27
	75.65		-2.54		+16.28	
CARROT	76.87		-1.68		+7.70	
	76.71	76.79	-1.71	-1.70	+7.87	+7.85
	76.79		-1.72		+7.99	
TOMATO	76.71		+0.80		+4.93	
	76.47	76.58	+0.80	+0.78	+4.97	+4.96
	76.55		+0.78		+4.99	

Table 5: TABULAR REPRESENTATION OF ANALYSIS

FRUIT/ VEGETABLE	pH	ABSORBANCE (at 451 nm)	Conc. (M x 10^{-6})	Conc. of β-carotene extract (µg/2ml)	COLOUR PARAMETERS OF THE ORGANIC EXTRACT				
					L	*a*	*b*	Hue angle (h)	Chroma
MANGO	4.70	0.235	1.60	21.40	76.79	-1.16	+5.93	78.93	6.04
ORANGE	3.82	0.020	0.14	1.88	76.22	+2.05	-0.72	19.35	2.17
AVOCADO PEAR	6.72	0.010	0.07	0.96	77.16	+2.05	-1.25	31.37	2.40
PEPPER	5.03	0.810	5.80	77.72	75.70	-2.53	+16.27	81.16	16.46
CARROT	5.99	0.285	2.00	26.80	76.79	-1.70	+7.85	77.78	8.03
TOMATO	4.11	0.155	1.10	14.74	76.58	+0.78	+4.96	81.06	4.96

Hue angle (degree of yellowness) of the organic layer (carotenoids) of Tomatoes, Carrots, Red pepper, Mangoes, Oranges and Avocado pear, juices extracted.

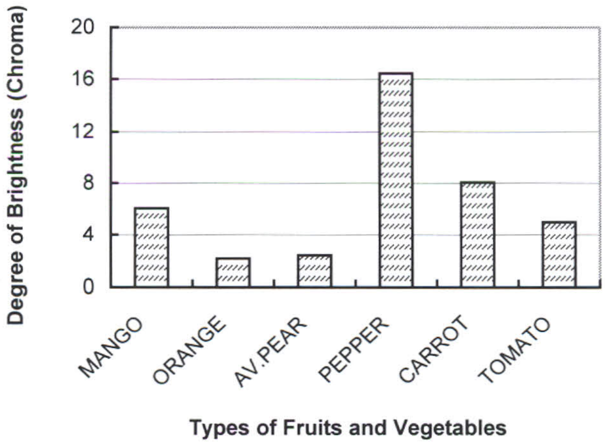
Fig. 6 Hue angle (degree of yellowness) of the organic layer (carotenoids) of juice extracted from some fruits and vegetables



(Chroma degree of brightness of the organic layer of Carotenoid.

Tomatoes, Carrots, Red pepper, Mangoes, Oranges and Avocado Pear juices.

Fig. 7 Degree of brightness of the organic layers of juices extracted from some fruits and vegetables.



CHAPTER 4

4.1 DISCUSSION

The results obtained from the work show that pepper has the highest β-carotene concentration (77.2μg) , carrot (26.8μg), mango (21.40μg), tomato(14.74μg), orange (1.88μg) and the least is avocado pear (0.9μg).

Avocado fruit being a high fat containing fruit was expected to be rich in vitamin A, especially the lipid soluble ones, but rather poor in carotenoid (β- carotene). This corresponds to the report in J.AM. Dietet. Assoc. [Polansky and Morphy, 1996] where vitamin A data were collected from several sources and were compared.

In the b column on the L, a, b, scale pepper had the highest b value +16.27 followed by carrot +7.85, mango (+5.93) tomato (+4.96), orange (-0.72) and the least avocado pear (-1.25) suggest the highest level of β- carotene in pepper since the highest positive value on the b scale implies more yellowish colour and also carotenoids being yellow, orange and orange-red fat soluble pigments.

The low level of carotenoid concentration in orange is due to the fact that about 50 – 75% of the total carotenoids of orange exist in the peels (Gonzalez-silita, 1949, Curland- Bailey 1954) but it was the juice which was analysed.

Also Fig.6 shows the hue angle or the degree of yellowness of the organic layer of the juices extracted. The graph indicates that Pepper, Tomato, Mango and carrot show high degrees of yellowness which may be due to the presence of high carotenoid concentrations while orange and avocado pear showed low degree of yellowness.

Fig.7 shows the chroma or the degree of brightness of the fruits. Pepper, carrot, mango and tomato are brightly coloured while others like orange and avocado are dark colours. The colour of the organic layer ranges from yellow, orange-yellow, and light green.

Table 3 revealed that avocado fruit recorded the highest pH value of 6.72 but had the lowest carotenoid (β-carotene) concentration. Orange recorded the lowest pH of 3.82 and also had a lower carotenoid concentration. Carrot which has a high pH of 5.99 compared to the others is lower in carotenoids than pepper whose pH is 5.03, which recorded the highest carotenoid (β-carotene) concentration. It can therefore be suggested that carotenoid concentration is not affected by pH.

4.2 CONCLUSION AND RECOMMENDATIONS

The results of this experiment show that the concentration of β-carotene was very high in pepper, carrot, mango and tomato. Pepper had the highest concentration of beta-carotene and avocado had the least. Though vitamin A is not found in pepper, carrot, mango and tomato they are important and rich source of vitamin A because they contain the provitamins beta-gamma-carotene and crytoxanthin which are transferred in the human liver into vitamin A. It should therefore be suggested that for a person suffering from night blindness, red pepper, carrot and tomato should be included in the diet. Also a fruit like mango should also be eaten in addition for a faster rate in the synthesis of vitamin A.

The daily vitamin requirement is met by the consumption of 3 – 4g (1/2 table spoon ground red pepper (Lantz, 1943) (Bosland and Votania)

It is suggested that further work be done on carotenoids in specific local fruits and vegetables like magnifera spp and Capsicum spp. This should include the concentration of lycopene and carotene during harvesting and storage since prolong storage of fruits and vegetables with red or red violet pigments is accompanied by bleaching of some pigments and development of red brown and finally brown colour (Meyer,1964)

APPENDIX1

CALCULATIONS

CONCENTRATION OF β- CAROTENE IN MOLARITY

From the relation $A = \varepsilon_{\lambda} CL$

$$C = \frac{A}{\varepsilon L}$$

For Mango $C = \frac{0.235}{139500M^{-1}cm^{-1} \times 1cm}$

$= \mathbf{1.68 \times 10^{-6}M}$

Orange $C = \frac{0.02}{139500M^{-1}cm^{-1} \times 1\ cm}$

$= \mathbf{1.4 \times 10^{-7}\ M}$

Avocado Pear $C = \frac{0.01}{139500M^{-1}cm^{-1} \times 1\ cm}$

$= \mathbf{7.2 \times 10^{-8}M}$

Pepper $C = \mathbf{5.8 \times 10^{-6}M}$

Carrot $$C = \frac{0.285}{139500M^{-1}cm^{-1} \times 1\ cm}$$

$$= \mathbf{2.0 \times 10^{-6}M}$$

Tomatoes $$C = \frac{0.155}{139500M^{-1}cm^{-1} \times 1\ cm}$$

$$= \mathbf{1.1 \times 10^{-6}M}$$

Molarity $$= \frac{\text{No. of Moles}}{\text{Volume in } dm^3} \qquad \text{———} \qquad (1)$$

No. of Moles $$= \frac{\text{Mass}}{\text{Molar mass}} \qquad \text{———} \qquad (2)$$

Putting (2) into (1)

Molarity $$= \frac{\text{Mass}}{\text{Molar mass}} \times \frac{1}{\text{Vol. } dm^3}$$

Mass(g) $$= \text{Molarity} \times \text{Molar mass} \times \text{Vol. } dm^3$$

The molar mass of β – carotene is 536g

For Mango: Mass = 1.6 x10^{-6} X 536 X 25 x 10^{-3} g
= 1.6 x 10^{-9} X 536 X 25g
= 1.6 x 10^{-9} X 536 X 25 x 10^{6}μg
= 21440 x 10^{-3}μg
= 21.40μg

Orange: Mass = 1.4 x 10^{-7} X 536 X 25 x10^{-3} X 10^{6}
= 18760 x 10^{-4}
= 1.88μg

Avocado Pear: Mass = 7.2 x 10^{-8} X 536 X 25 x10^{-3} X10^{6}
= 96480 x 10^{-4}
= **0.96μg**

Pepper: Mass = 5.8 x 10^{-6} X 536 X 25 x 10^{-3} X 10^{6}
= 77720 x10^{-3}
= **77.72μg**

Carrots: Mass = 2.0 x 10^{-6} X 536 X 25 x 10^{-3} X 10^{6}
= 34840 x 10^{-3}
= **34.84μg**

Tomatoes: Mass = 1.1 X 10^{-6} X 536 X 25 x 10^{-3} X 10^{6}
= 14740 X 10^{-3}
= **14.74μg**

For the Degree of Yellowness

From $h = \tan^{-1}(b/a)$

Mango: $h = \tan^{-1}\left(5.93/-1.16\right)$

$= \tan^{-1}\left(5.112\right)$

$= 78.93^0$

Orange: $h = \tan^{-1}\left(-0.72/2.05\right)$

$= \tan^{-1}\left(0.3512\right)$

$= 19.35^0$

Avocado Pear: $h = \tan^{-1}\left(-1.25/2.05\right)$

$= \tan^{-1}\left(-0.6098\right)$

$= 31.37^0$

Pepper: $h = \tan^{-1}\left(16.27/-2.53\right)$

$= \tan^{-1}\left(6.4308\right)$

$= 81.16^0$

Carrots: $h = \tan^{-1}\left(7.85/-1.70\right)$

$$= \tan^{-1}\left(4.6176\right)$$

$$= 77.78^{0}$$

Tomatoes: $h = \tan^{-1}\left(4.96/0.78\right)$

$$= \tan^{-1}\left(6.3590\right)$$

$$= 81.06^{0}$$

Chroma from the relation: $\acute{C} = (a^2 + b^2)^{1/2}$

For Mango: $\acute{C} = (a^2 + b^2)^{1/2}$

$$= \left\{(5.93)^2 + (1.16)^2\right\}^{1/2}$$

$$= \left\{35.165 + 1.34\right\}^{1/2}$$

$$= 6.04$$

Orange: $\acute{C} = \left\{(2.05)^2 + (0.72)^2\right\}^{1/2}$

$$= \left\{4.20 + 0.518\right\}^{1/2}$$

$$= 2.17$$

Avocado pear: $\acute{C} = \left\{(2.05)^2 + (-1.25)^2\right\}^{1/}$

$= \left\{4.20 + 1.56\right\}^{1/2}$

$= 2.4$

Pepper: $\acute{C} = \left\{(-2.53)^2 + (16.27)^2\right\}^{1/2}$

$= \left\{6.4 + 264.7\right\}^{1/2}$

$= 16.46$

Carrot: $\acute{C} = \left\{(-1.76)^2 + (7.85)^2\right\}^{1/2}$

$= \left\{2.89 + 61.62\right\}^{1/2}$

$= 8.03$

Tomatoes: $\acute{C} = \left\{(0.75)^2 + (4.90)^2\right\}^{1/2}$

$= \left\{0.56 + 24.01\right\}^{1/2}$

$= 4.96$

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