Determination of vitamin A content from selected Nigerian fruits using spectrophotometric method

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Abstract

The vitamin A content of the fresh fruits was determined by the extraction and quantitative determination of the pro-vitamin A carotenoid-β-carotene. The Retinol Equivalent (RE) was then obtained using standard conversion formula. The result indicated that the fruit with the minimum vitamin A content and percentage among the six samples was found in mango 301.61 ± 1.03 (7%), which was followed closely by pineapple 306.40 ± 1.67 (7%). The maximum vitamin A content was found in carrot 2054.10 ± 1.28 (46%) in (RE/100 g). Statistical calculations and analysis showed that there was significant difference in vitamin A content among the fruits (p<0.05). Thus, besides major nutrients fruits and vegetables contribute very good amount of micro nutrient-vitamin A from β-carotene.

Keywords: Fruits; Vegetables; β-carotene; Vitamin A; Spectrophotometer

Introduction

Vitamin A is not a single compound but exists in several forms: retinol (an alcohol), retinal (an aldehyde), retinoic acid and other related compounds, all known as retinoids. It helps prevent diseases such as lung and breast cancer (Lacroix and Lippman, 1980; Prakash et al., 2000) and can be used therapeutically in the treatment of retinitis pigmentosa, leukemia (Rego et al., 2000) and skin disorders (Heller and Shiffman, 1985) such as psoriasis and acne. β-carotene which is a pigment-carotenoid is a precursor of vitamin A. Vitamin A is needed in the retina of the eye in the form of a specific metabolite, the light absorbing retinal, that is absolutely necessary for both low light (scotopic vision) and colour vision (Tang et al., 2005).

Vitamin A functions in different roles, as an irreversible oxidized form of retinoic acid, which is an important hormone-like growth factor for epithelial and other cells (Duester, 2008).

Among the various classes of pigments in nature, the carotenoids are among the most widespread and most important ones, especially due to their varied functions. These are fat-soluble pigments found mostly in plants, fruits, flowers, algae and photosynthetic bacteria, but they also occur in some non-photosynthetic bacteria, yeasts and molds. The most abundant carotenoids in natural consumed foods are beta-carotene, alphacarotene, gamma-carotene, lycopene, lutein, beta cryptoxanthin,zeaxanthin and astaxanthin. Carotenoids have extensive applications as anti-oxidants in dietary supplements and as colours in foods and beverages as well as pigments in poultry and fish. The carotenoids used as food ingredients include astaxanthin, beta-apocarotenal, canthaxanthin, beta-carotene, lutein, zeaxanthin and lycopene. Carotenoids are important for human health, but its structure ultimately determines its potential biological function(s). The essential role of beta-carotene and others as the main dietary source of vitamin A has been known for many years (Carlier et al., 1993). More recently, protective effects of carotenoids against serious disorders such as cancer (Donaldson, 2004; Kantoff, 2006), heart disease (Lonn and Yusuf, 1999; Sesso et al., 2003) and degenerative eye disease(Mozaffarieh et al., 2003) have been recognized and have stimulated intensive research into the role of carotenoids as antioxidants and as regulators of the immune response system. Carotenoids which are alpha and beta carotene are the most common and important pro vitamin A carotenoid because it can be represented as two connected retinal groups (Carolyn, 1997), gamma carotene and beta cryptoxanthin.

Despite the availability of fruits and other sources of vitamin A, World Health Organization (WHO, 2005)
database on vitamin A deficiency (1995-2005) showed that approximately one third of children under the age of five around the world is suffering from vitamin A deficiency and it is estimated to claim the lives of 670,000 children under the age of five annually (Black et al., 2008). The highest prevalence is in developing countries of which Southeast Asia and Africa continents are not spared.

Fruit is part of plant which is developed from a fertilized ovary with one or two seeds (Mauseth, 2003). Most fruits have been discovered to be functional foods because they are rich in micronutrients and other phytochemicals.

Tomato - (Solanum lycopersicum) is a savoury, red and edible fruit. It originated from South America and spread around the world following the Spanish colonization of America and its many varieties are now widely grown, often in greenhouses in cooler climates. The tomato fruit is consumed in diverse ways, raw, as an ingredient in many dishes and sauces and in drinks (Burton-Freeman and Sesso, 2014). Tomato is also used in diverse ways including raw in salads and processed into purees, ketchups and tomato soups.

Water melon (Citrullus lanatus) is a member of the Cucurbitaceae family; its close relatives include pumpkin, squash and cantaloupe that also grow on vines on the ground. Their shapes may be roundish, oblonged, or spherical and it has feature of thick green rinds that are often spotted or striped with an internal deep red-pink colour when ripened. Their seeds are black, brown with a few seedless variety. It has extremely high water content, approximately 92%, giving its flesh a crumbly and subtly crunchy texture and making it a favourite thirst-quenching fruit.

Mango (Magnifera indica) is a tropical tree cultivated in many regions of India now distributed widely across the Globe in many continents. Its Outer skin is smooth and is green when unripe but turns into golden yellow or orange red when ripening depending on the cultivar. Internally, its pulp is Juicy and yellowish in colour with numerous soft fibrils radiating from the husk enveloping a single large kidney-shaped seed. The flavour is pleasant and rich and tastes sweet with mild tartness.

Pawpaw (Carica papaya) has an oblong shape, normally greenish-yellow, yellow or orange in colour. It has sweet taste when ripe, but tart or bland when unripe. It grows well in tropical regions because of its higher humidity. It is native to Mexico, countries of Central America, Thailand, Africa and Asia and grows well in Australia. The fruits, seeds, stem and leaves are all useful. One can buy pawpaw in all forms, in a form of fermented papaya, herbal teas and tablets and of course plenty of the fresh fruit, which can be eaten as it is or in a form of juice (Sunita and Attar, 2008).

Guava (Psidium guajava) is a tropical fruit with pear shape and it is of two types the green and pinkish flesh with small seeds inside. It belongs to the family of myrtaceae. And it originates from America. They are usually eaten raw or processed into juice (De boer and Cotingting, 2014).

Carrot (Daucus carota), they belong to the umbrelliferae family named after the umbrella like flower clusters plant. Carrot roots have crunchy texture, sweet, minty aromatic taste and are eaten raw in salads or processed into juice (Ensminger and Esmsinger, 1986). The carrot can trace its ancestry back to thousands of years originally having been cultivated in central Asia and Middle East. Recently there has been renewed interest in examining bio-availability factors of vitamin A by using more quantitative stable isotope techniques for measuring whole-body stores in response to controlled intakes spread around the world following some Spanish research work (Adams and Green, 1994; Furr, 1989; Haskell, 1998; Maron, 2014). Also by following post absorption carotenoids in the triacylglycerol-rich lipoprotein fraction (Van and Van, 1998; Castenmiller and West, 1998).

Thus, the present study aims at determining the pro vitamin A carotenoid (beta carotene) content in selected Nigerian fruits which include tomato, water melon, mango, pawpaw, pink guava and carrots using the spectrophotometric method because it is the precursor of vitamin A (i.e. it can be converted into vitamin A) and it is eaten in both raw and cooked form by humans in daily life (Bendich and Higdon, 2004) and hence determine the vitamin A by getting the retinol equivalent (RE/100g) of the beta carotene content of these fruits by using the standard conversion formulae.

Materials and methods

Sample collection and preparation

The six different fresh ripe fruits were purchased from five different zones called Groups in Keffi local government area of Nasarawa state, Nigeria. Group I: Keffi market; Group II: Nasarawa State University, Keffi (NSUK) fruits shop; Group III: Dadinkowa market; Group IV: Kofa Hausa market; Group V: Karu market. The ripe fruits included tomato (Lycopersum sculentum), pawpaw (Carica papaya), watermelon (Citrullus lanastus), mango (Magnifera indica), guava (Psidium guajava) and carrot (Daucus carota). Each paste of these six different samples was prepared from well washed fruit with tap water in order to remove any dirt or adhering particles. Each fruit was peeled off to remove the bark and the seeds removed. The flesh was then crushed...
using food blender (Binatone product) to get a fine pulp or paste.

\textit{β-carotene extraction and analysis}

The β-carotene was determined by soaking 1 g of the sample (that is the paste or pulp of the fresh fruits) in 5 ml of methanol for 2 h at room temperature under dark condition in order to get a complete extraction. The β-carotene layer was separated using hexane through separating funnel. The volume was made up to 10 ml with hexane and then this layer was again passed through sodium sulphonate through a funnel in order to remove any moisture from the layer. The absorbance of the layer was measured at 436 nm using hexane as a blank (Ranganna, 1999). The beta carotene was calculated using the formula:

\begin{equation}
\text{Beta-carotene (µg/100g)} = \text{Absorbance (436 nm) x V x D x 100 x 100/W x Y}
\end{equation}

where: V = Total volume of extract; D = Dilution factor; W = Sample weight; Y = Percentage dry matter content of the sample.

\textbf{Vitamin A determination}

To express the vitamin A activity of carotenoids in diets on a common basis, a joint FAO/WHO Expert Group (FAO/WHO) in 1967 introduced the concept of the Retinol Equivalent (RE) and established the following relationships among food sources of vitamin A:

1 µg retinol = 1 RE method used. Variation in ecological growth conditions

1 µg β-carotene = 0.167 µg RE like variety and environmental aspects may also be

1 µg other pro-vitamin A carotenoids = 0.084 µg RE contributing factors.

These equivalencies were derived from balance studies to account for the less-efficient absorption of carotenoids (thought to be about one-third that of retinol) and their bioconversion to vitamin A (one-half for β-carotene and one-fourth for other pro-vitamin carotenoids). It was recognized at that time that the recommended conversion factors (i.e., 1:6 for vitamin A: β-carotene and 1:12 for vitamin A: all other pro-vitamin carotenoids) were only average estimates for a mixed diet. β-carotene and 1:12 for vitamin A: all other pro-vitamin carotenoids) were only average estimates for a mixed diet.

\textbf{Results and discussion}

Table I shows that the fruits are of the same species and was grown in similar environmental conditions since the beta carotene content obtained were very close in range. The result in Table II showed that vitamin A content varied from relatively lower amount found in mango and water melon, when compared to the relatively higher amounts found in guava, tomato, pawpaw and carrot which have the maximum content of beta-carotene among the fruits assayed. From the data, it was observed that all the fruits assayed contained good sources of Vitamin A. This agreed with the literature and other work done which showed that all the fruits assayed are rich in beta carotene and hence vitamin A (Williams and Caliendo, 1984; Bereau and Bushway, 1986; Ahamad \textit{et al.}, 2007).

To express the vitamin A deviation or the sample error mean (SEM) of the six fruit ranged from 1.28 to 20.20 while its variance or standard deviation (%) ranged from 0.06 to 5.43. There may be slight variation in the data when compared to other work done which may be due to difference in sources of vitamin A: experimental condition, extraction procedures, method used. Variation in ecological growth conditions like variety and environmental aspects may also be contributing factors. For example; Comparatively lower contents were reported in carrots (11210 µg/100g) and tomato (1610 µg/100g) by Ahmad \textit{et al.} (2007) in the determination of beta carotene and those reported in which beta carotene content in carrot was 10110 µg/100g and tomato 1930 µg/100g. The variation observed in the tomato content of beta carotene may be due to the use of overripe samples in their own study, because content of beta carotene drops by 77% during the ripening process (Rigo \textit{et al.}, 1999) and may also be due to varietal difference among other factors.

Like wise, the beta carotene content of the fruits assayed also corresponds to those reported by Robinson \textit{et al.} (1986). They analyzed summer fruits and vegetables for their micronutrient content, though there was still variation in tomato. Patricia \textit{et al.} (2004) worked on new data of carotenoid in raw salad vegetables and they reported much lower content of beta carotene in carrot (5340 µg/100 g). This may be due to their sample of carrot having higher moisture content as compared to those analyzed in the present study (Ahmad \textit{et al.}, 2007). However, the report about beta carotene content in tomato (3500 µg/100g) was in fair agreement with the result of the present study. But one thing is similar in all the work carried out and worthy of note in the present study which is that carrot is the richest and well known source of beta-carotene and hence vitamin A among the selected fruits in Nigeria (Lieberman and Bruning, 1990; Rigo \textit{et al.}, 1999; Patricia \textit{et al.}, 2004). This was clearly shown in Fig. 1.
Table I. Result of Beta carotene (µg/100 g) in the six fresh tropical fruits

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Scientific name</th>
<th>Group I (µg)</th>
<th>Group II (µg)</th>
<th>Group III (µg)</th>
<th>Group IV (µg)</th>
<th>Group V (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guava</td>
<td>Psidium guajava</td>
<td>3015.27</td>
<td>3012.94</td>
<td>3025.15</td>
<td>3021.86</td>
<td>3017.97</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>Carica papaya</td>
<td>4043.45</td>
<td>4139.96</td>
<td>4072.13</td>
<td>4100.90</td>
<td>4097.01</td>
</tr>
<tr>
<td>Water melon</td>
<td>Citrullis lanastus</td>
<td>2148.05</td>
<td>2090.78</td>
<td>2156.89</td>
<td>2021.84</td>
<td>2156.14</td>
</tr>
<tr>
<td>Tomato</td>
<td>Lycopersum esculentum</td>
<td>3294.91</td>
<td>3317.22</td>
<td>3317.22</td>
<td>2995.03</td>
<td>3321.26</td>
</tr>
<tr>
<td>Mango</td>
<td>Magnifera indica</td>
<td>1797.21</td>
<td>1803.48</td>
<td>1812.84</td>
<td>1807.19</td>
<td>1809.49</td>
</tr>
<tr>
<td>Carrot</td>
<td>Daucus carota</td>
<td>12288.02</td>
<td>12311.98</td>
<td>12300.00</td>
<td>12300.60</td>
<td>12299.40</td>
</tr>
</tbody>
</table>

Table II. Vitamin A content (RE/100g), IU and standard deviation in percentage of the six tropical fresh fruits

<table>
<thead>
<tr>
<th>Fruits</th>
<th>Scientific name</th>
<th>*Vitamin A content (µg or RE)</th>
<th>Standard Deviation (%)</th>
<th>Vitamin A content (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guava</td>
<td>*P. guajava</td>
<td>504.10 ± 0.00.75</td>
<td>0.15</td>
<td>151.23</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>*C. papaya</td>
<td>683.53 ± 0.15</td>
<td>0.75</td>
<td>206.98</td>
</tr>
<tr>
<td>Water melon</td>
<td>*C. lanastus</td>
<td>350.12 ± 19.01</td>
<td>5.43</td>
<td>105.04</td>
</tr>
<tr>
<td>Tomato</td>
<td>*L. esculentum</td>
<td>542.86 ± 20.20</td>
<td>3.72</td>
<td>162.86</td>
</tr>
<tr>
<td>Mango</td>
<td>*M. indica</td>
<td>301.61 ± 01.03</td>
<td>0.34</td>
<td>90.48</td>
</tr>
<tr>
<td>Carrot</td>
<td>*D. carota</td>
<td>2054.10 ± 01.28</td>
<td>0.06</td>
<td>616.20</td>
</tr>
</tbody>
</table>

*Vitamin A content (µg or RE) is in Mean±SD of five determinations

A test of significance (t-test) was carried out on the mean of the vitamin A content of the fruits using the computer software - Statistical Package for Social Sciences version 21.0 (SPSS 21.0) and it showed that there was significant difference (p<0.05 that is the p-value was less than 0.05) in Vitamin A content among the fruits.
Conclusion

The study has revealed that these fruits are good sources of vitamin A; they can alleviate vitamin A deficiency in mass population of the country and even the globe. They are also cost effective and can substitute the animal food sources and even supplements, which are expensive and beyond the purchasing power of the low income groups of the population such as in rural areas.

References


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Pawpaw

Pawpaw is a tropical fruit which is native to Africa but it is grown in many regions of India now distributed widely across the globe in many continents. Its Outer skin is smooth and is red when ripening depending on the cultivar. Internally, its flesh is yellowish colour and it is sweet, juicy and aromatic. The seeds are hard, smooth and oval in shape. The fruit is consumed in diverse ways, raw, as an ingredient in many soups.

Tomato

Carrot roots have crunchy texture, sweet, minty aromatic taste and are eaten raw in salads or processed into juice. Like wise, the beta carotene content of the fruits assayed also differed among food sources of vitamin A: Carrot (11210 μg/100g) and tomato (1610 μg/100g) were the highest and lowest respectively. Highest beta carotene content in carrot (5340 μg/100 g). This may be due to their grown in similar environmental conditions since the beta carotene and those reported in which beta carotene content reported in carrots (11210 μg/100g) and tomato (1610 μg/100g) and may also be due to the difference (p<0.05 that is the p-value was less than 0.05) in the beta carotene content of the fruits assayed also.

The six different fresh ripe fruits were purchased from five shops; Group I: General supermarket; Group II: Mom and Pop shop; Group III: Dadinkowa market; Group IV: Kofa Hausa market; Group V: Warda market. The samples were packed in polythene bags and transported to the laboratory as soon as possible. The samples were washed, peeled and divided into 2 parts. The first part was washed in water and then dried at 68°C for 24 hours. The second part was washed in water and then homogenised and made into a paste. The homogenised paste and the dried samples were used for the study.

Materials and methods

The present study aims at determining the pro vitamin A carotenoids in pawpaw, mango, tomato and carrot. The fruits were purchased from the local market in the state of Kofa, Nigeria. The pro vitamin A carotenoids were determined as described by (Patricia Y, Niizu H, Delia B and Rodriguez A, 2004).

Vitamin A determination

The pro vitamin A carotenoids were converted into vitamin A (retinyl alcohol) and vitamin A (retinal) by acid fractionation and analysis of the fat soluble vitamin A. Vitamin A (retinol) was extracted from the sample with hexane and then this layer was again passed through sodium sulphate through a funnel in order to remove any water content. The vitamin A (retinol) was then measured at 436 nm using hexane as a blank (Ranganna, S., 1999). This was clearly shown in Fig. 1.

Beta-carotene determination

Beta-carotene content of the fruits assayed was determined as described by (Ranganna, S., 1999; Patricia Y., Niizu H, Delia B and Rodriguez A, 2004). This was clearly shown in Fig. 1.


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