# QUANTITATIVE DETERMINATION OF VITAMINS A, B<sub>1</sub>, B<sub>2</sub> AND B<sub>6</sub> IN PALMYRA FRUITS MARKETED IN GOMBE METROPOLIS

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### Abstract

The study was carried out to determine the amount of vitamin A (retinol),  $B_1$  (thiamine),  $B_2$  (riboflavin) and  $B_6$  (pyridoxine) in Palmyra fruit. The fruits were purchased from Gombe market. One (1) g in 100ml of each of the fruit extract was freshly prepared and used for the analysis. UV/Visible spectrophotometer (model 5400/5405) was used to quantify these vitamins. The results obtained for vitamins A,  $B_1$ ,  $B_2$  and  $B_6$  were 8.89±0.03 µg/100g, 0.0 mg/100g, 0.03±0.006 mg/100g and 3.50±0.20 mg/100g at  $\lambda_{max}$  335,747,445 and 465nm respectively. It could be concluded that the consumption of palmyra fruits should be encouraged as it is rich in vitamins.

Keywords: Palmyra fruits, Spectrophotometry, and Vitamins

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### Introduction

The term vitamin was derived from "vitamine", a compound word coined in 1912 by the Polish biochemist Kazimierz Fonk (Maciej, 1981) when working at the Lister Institute of Preventive Medicine. The name is from vital and amine, meaning amine of life, because it was suggested in 1912 that the organic micronutrient are food factors that prevent beriberi and perhaps other similar dietary-deficiency diseases might be chemical amines. This was true of thiamine (vitamin  $B_1$ ), but after it was found that other such micronutrients were not amines the word was shortened to vitamin in English.

Thirteen vitamins are universally recognized at present and classified by their biological and chemical activity, not their structure. (Wikipedia, 2016) Vitamins are classified as either water soluble or fat soluble. In humans there are 13 vitamins: 4 fat soluble (A, D, E, and K) and 9 water-soluble (8 B vitamins and vitamin C). Water-soluble vitamins are readily excreted from the body, to the degree that urinary output is a predictor vitamin consumption strong of (Fukuwatiri and Shibata, 2008). Many types of water-soluble vitamins are synthesized by bacteria and Mohammed, 2006). Fat-soluble (Said

vitamins are absorbed through the intestinal track with the help of lipids (fats). Because they are more likely to accumulate in the body, they are more likely to lead to hypervitaminesis than are water-soluble vitamins. Fat-soluble vitamins regulation is of particular significance in cystic fibrosis (Maqbool and Stallings, 2008).

The Palmyra plant (Borassus aethiopum) has been described as a palm tree with huge fan shaped leaves (Ahmed et al., 2010). In Nigeria, the Hausa call it Giginva, the Yoruba call it Agbonolodu, and the Igbo call it Ubiri. The plant is a dioecious plant and can reach up to 20 m high on average and 1 m in diameter (Muller, 1988). The fruits have a large fibrous pulp (around 500 g each) that smells strongly of therbenthine. They are consumed raw or cooked, preferably with rice. The kernels contain albumen, which before ripening is sweet and refreshing. The immature seeds can be eaten and contain a sweet jelly that has a refreshing taste. The mature seeds can be buried in pits and allowed to germinate, and the shoots are said to be a delicacy. Fresh sap is used as yeast or made into vinegar.

Ozgur and Koyunco (2000), Nethaji *et al.* (2010), Ruengsitagoon and Hallanat (2012)

carried out the spectrophotometric determination of vitamins in fruits and pharmaceutical products using various reagents at different wavelengths. Pei *et al.* (2010) used a developed HPLC – UV spectrophotometry for the determination of riboflavin in capsules while Ekinci and Kadakal (2005) developed a reversed – phase HPLC for the determination of water soluble vitamins in tar – hana samples, a traditional Turkish cereal food.

The aim of this research is to quantify the amount of vitamins A,  $B_1$ ,  $B_2$ , and  $B_6$  present in Palmyra fruits locally consumed in Gombe State using UV- Visible spectrophotometer.

### **Materials and Method**

### **Sample Collection and Preparation**

Palmyra fruits were purchased from Gombe Market (*Kasuwar mata*) A 0.01 g/ml of the fruit extract was prepared by dissolving 1 g of the fruit gel in 100 ml of deionized water and kept ready for analysis.

## Methods

## **Determination of vitamin A**

The method described by Maciej et al. (2007) was used and adopted where 1ml of the extract (Palmyra fruit extract) was measured into the centrifugal test tube with a tight stopper, and 1ml of the KOH solution (1M solution of KOH in 90% ethanol) was added. The test tube was plugged followed by vigorous shaking for 1 minute. It was then heated in a water bath at 60°C for 20 minutes, cooled in cold water and added 1 ml of xylene which is also followed by vigorous shaking for 1 minute. The test tube was centrifuged at 1500 revolutions per 10 minutes. The upper layer (extract) was collected and transferred to a sodium borosilicate glass. The absorbance  $A_1$  of the obtained content was measured at 335 nm against xylene with a UV/ visible spectrometer. The extract in the test tube was exposed to sunlight for 45 minutes and the absorbance A<sub>2</sub> was measured.

The concentration of vitamin A  $C_x$  (µm) in the analyzed liquid was calculated using the formula.

$$C_x = (A_1 - A_2) \times 22.23$$

Where:

 $A_1$  = Absorbance of the obtained extract after centrifugation

 $A_2$  = Absorbance of the obtained extract after exposure to sunlight

22.23 = Multiplier received on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335nm in a measuring cuvette about thickness = 1 cm

 $C_x$  = concentration of vitamin A in the analyzed liquid.

The above procedure was carried out in triplicate form

## **Determination of Vitamin B<sub>1</sub> (Thiamine)**

A modified method of Nethaji *et al.* (2010) was adopted. A 50 mg of thiamine hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask. It was dissolved using distilled water to the mark to give a stock solution 100  $\mu$ gml<sup>-1</sup>. Different aliquots of the stock solution were taken in seven different test tubes and serially diluted to cover the range of calibration curve (0.2 – 14  $\mu$ gml<sup>-1</sup>). A 0.19 ml of 0.1M ferric sulphate was added forming a soluble Prussian blue colour. After addition of the ferric sulphate solution, the

solution was shaken well and 0.6 ml of 0.1M of potassium hexacyanoferate (III) was added and then deionized water was added to the solution to make it up to 25 ml and the mixture was allowed to stand for 20 minutes in a water bath at 40°C. The absorbances were measured at  $\lambda_{max}$  747 nm against a blank (deionized water). The sample solution was also prepared in the same way but containing no thiamine hydrochloride and the absorbance obtained at 747 nm with a UV/visible spectrophotometer.

# Determination of Vitamin B<sub>2</sub> (riboflavin)

A 50.0 µg/cm<sup>3</sup> riboflavin standard solution in 10% methanol solution was made by dissolving 0.0133 g of riboflavin in 100 cm<sup>3</sup> of 10% methanol solution. Five solutions of riboflavin in the interval 5 µg/cm<sup>3</sup> were made from the standard solution in a 50 ml volumetric flask. The sample solution was also prepared in the same way but containing no riboflavin. The absorbances of the solutions were determined at  $\lambda_{max}$  445nm with a UV/visible spectrophotometer with deionized water as the flask.

# Determination of Vitamin B<sub>6</sub> (pyridoxine)

An accurately weight 50mg of pyridoxine hydrochloride was transferred to a 100 ml volumetric flasks, distilled water was added and made to the mark. Different aliquots of the standard pyridoxine HCl solution equivalent 50-700 $\mu$ g (i.e. 0.5 – 7ml) were transferred into a

series of eight 50ml volumetric flasks. A 0.5ml of buffer solutions pH = 3 and 7ml of ferric ammonium sulphate solutions were added. The contents were mixed and allowed to stand for 5 minutes with occasional shaking. Approximately 10 ml of distilled water was added to make it up to 25 ml and the solutions were mixed up well. The sample solution was also prepared in the same way but containing no pyridoxine HCl solution and the absorbances of the solutions were measured at  $\lambda_{max}$  465 nm with a UV/visible spectrophotometer against distilled water as the blank (Ahmad, 2012).

### Results

The Table below shows the results for Vitamin A (retinol), Vitamin  $B_1$  (Thiamine), Vitamin  $B_2$  (Riboflavin) and vitamin  $B_6$  (pyridoxine) determinations with the recommended dietary daily allowance and results from literature.

Table 1: Results for Vitamin A (retinol),	Vitamin B <sub>1</sub> , (thiamine).	, Vitamin B <sub>2</sub> , (riboflavin) a	and Vitamin B <sub>6</sub>
(pyridoxine) determinations			

Vitamin	Wavelength $\lambda_{max}$ (nm)	Recommended Daily Allowance	Results (per 100g)	Results from other studies (per 100g)
Vitamin A (Retinol)	335	700 µg	8.89±0.03 µg	9.80 µg
· /	747	1.1 mg	0.00	0.00
Vitamin B <sub>2</sub> (Riboflavin)	445	1.2 mg	0.03±0.006 mg	0.02 mg
Vitamin B <sub>6</sub> (Pyridoxine)	465	1.3 mg	3.50±0.20 mg	2.89 mg

# Discussions

The recommended daily allowance (RDA) for vitamin A in adults is 700  $\mu$ g per day (Food and Nutrition Board, 2015). The retinol content of Palmyra fruit obtained in this research is 8.89  $\mu$ g/100g which is close to the value (9.80  $\mu$ g/100g) as reported by Pemberton (2005) as given in the Table 1. This indicates that more of the Palmyra fruits would have to be consumed alongside other sources to meet up the RDA.

The recommended daily allowance for vitamin  $B_1$  (thiamine) in adults is 1.1 mg, (Food and Nutrition Board, 2015). In this research, it was found that no amount of thiamine is present in the fruit. This result is in agreement to that reported by Davis and Johnson (1987) who reported that Palmyra jaggery contains no thiamine. This means that Palmyra fruit is not a source of vitamin  $B_1$ . However, according to Mission Clean and Lean (2015), the fruit contains about 0.04 mg/100g of thiamine. These variations may be due to the geographical locations, climatic factors, effect of temperature and time (Danbature *et al.*, 2014).

The recommended daily allowance for vitamin  $B_2$  (Riboflavin) in adults is 1.2 mg, (Food and Nutrition Board, 2015). The riboflavin content of Palmyra obtained in this research was 0.03 mg/100g which is higher than that from Mission Clean and Lean (2015), which is 0.02 mg per 100 g of the palm sugar. This shows that Palmyra fruit alone is not a good source of vitamin  $B_2$  but if consumed with other sources of vitamin  $B_2$  could be a good supplement. One kilogramme if consumed daily is capable of supplying 25% of the body need of this vitamin.

The recommended daily allowance for vitamin  $B_6$  (pyridoxine) in adults is 1.3 mg (Food and Nutrition Board, 2015). The pyridoxine content of Palmyra obtained in this research is 3.5 mg/100g. This means that 100 g of the fruit alone is capable of supplying the body with about three times the body requirement. Mission Clean and Lean (2015) report was that a table spoon of Palmyra jaggery contains 222% of the adult's RDA for vitamin  $B_6$  which is 2.886 mg/100g. Bailey (2015) also reported that the jaggery being much less processed than table sugar contains a wealth of vitamins and minerals including iron, vitamin  $B_6$ , calcium, potassium and vitamin  $B_{12}$ .

# Conclusion

The study revealed that Palmyra fruits contain vitamin A (retinol), vitamin B<sub>2</sub> (riboflavin) and vitamin  $B_6$  (pyridoxine) with the following quantities:  $8.89 \pm 0.03$ μg/100g,  $0.03 \pm 0.003$ mg/100g and 3.50±0.20 mg/100g respectively. It contains no vitamin  $B_1$ . The Palmyra fruit is very rich in vitamin  $B_6$ , 100 g alone can supply the daily requirement with about 3 times the body need whereas 1 kg of vitamin B<sub>2</sub> will be able to provide 25% of the body need. From the results obtained, the consumption of Palmyra fruits should be encouraged since it is capable of supplying the body with some of the vitamins the body need.

It is recommended that some of the vitamins not determined in this research should be determined so as to reveal the full potentials of Palmyra fruit as a source of vitamin.

# References

- Ahmed A., Djibrilla A., Clerge, T. and Clement,
  S. (2010). Physico-chemical properties of Palmyrah palm (*Borassus aethiopum*) fruits from Northern Cameroon." *Afr. J.Fd. Sci.* 4(3):115 – 119.
- Bailey, S. W. and Ayling, J. E. (2009). The extremely slow and variable activity of dihydrofolatereductase in human liver and its implications for high folic acid intake. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(36):15424-15429.
- Danbature, W. L., Fai, F.Y., Usman, A. A. and Patrick, A. (2014). Nutritional Evaluation of Baobab Seed. *International Journal of Research in Agriculture and Food*, **2**(2): 44-48
- Ekinci, R. and Kadakal, Ç. (2005). Determination of Seven Water-Soluble Vitamins In Tarhana, A Traditional Turkish Cereal Food, By High-Performance Liquid Chromatography. Acta chromatographica.**15**:289-295.
- Fatima, I., Farah, N. T. and Memon, A.N. (2013). Determination of Water Soluble Vitamin in Fruits and Vegetables Marketed in Sindh Pakistan. *Pak. J. Nutr.***12** (2): 197-199
- Food And Nutrition Board Institute of Medicine (2001) *Dietary Reference Intakes:Vitamins.* Washington D. C.: National Academy Press. Pp. 230-241.
- Fukuwatiri, T. and Shibata, K. (2008). Urinary water-soluble vitamins and their metabolite contents as nutritional markers for evaluating vitamin intakes in young Japanese women, J. nutr.Sci. vitaminol, 54 (3): 223-229.

http://missioncleanlean.com/blog/2015/6/15/super simple-2-ingredient-pancakes. http://theindianvegan.blogspot.com.

Mahmure, U.O. and Ikbal, K. (2000). Determination of Ternary Mixtures of Vitamins  $(B_1, B_6, B_{12})$  by Zero-Crossing Derivative Spectrophotometry. *Turk J. Chem*, **26**:385-391.

- Maqbool, A. and Stallings, VA. (2008). Update on fat-soluble vitamins in cystic fibrosis. *Curr.Opin.Pulm. Med*, **14**(6):574-581.
- Maton, A., Jean, H., Charles, W., McLaughlin, S. J., MaryannaQuon, W., David, L. and Jill, D. W. (1993).Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall. https://en.m.wikipedia.org/wiki/Vitamin.
- Muller, H.G. (1988). An Introduction to Tropical Food Science. Cambridge, Cambridge University Press. pp. 59-118.
- Nethaji, S., Sivasamy, A., Thennarasu, G. and Saravanan, S. (2010). Adsorption of Malachite Green dye onto activated carbon derived from *Borassus aethiopum* flower biomass. *J Hazard Mater*, 181:271-280.
- Nief, R. A. (2012). Spectrophotometric Determination Of Pyridoxine Hydrochloride Via Complexation With Fe (III) In Pharmaceutical and Environmental Wastewater Samples. *Iraqi Nat. J. Chem.*, 48:413-423.
- Pei, C., Wayne, R. W., Isabel, C. and Ana, S. (2012). A LC/UV/Vis Method For Determination Of Cyanocobalamin (VB<sub>12</sub>) In Multi-Vitamin Dietary Supplements With Online Sample Clean-Up. *Royal Soc. Chem. Anal.Met.*, 2:1171-1175.
- Pemberton, J. (2006). Medical experiments carried out in Sheffield on conscientious objectors to military service during the 1939-45 war. *Intl. j. Epidem.*, 35 (3):556-558.
- Ruengsitagoon W., Hattanat, N. (2012). Simple Spectrophotometric Method for Determination of Folic Acid. The 4th Annual Northeast Pharmacy Research Conference of 2012 Pharmacy Profession in Harmony, Faculty of Pharmaceutical Sciences, KhonKaen University, Thailand. pp. 281

Said, H. M. and Mohammed, Z. M. (2006). Intestinal absorption of water soluble vitamins: An update. *Curr.Opin.Gastroentero*, .22 (2):140-146.

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Wikipedia(2016).<u>https://en.m.wikipedia.org/wiki/</u> <u>Vitamin</u>