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Research Article

Determination of Ascorbic Acid (Vitamin C) Content in Sprouted Brown African Yam Beans (*Sphenostylis stenocarpa*)

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Abstract

Vitamin C is an essential welfare food in human diet, most species of animals including human beings cannot synthesis their own vitamin C from other hexose sugars. And consequently, an experiment was conducted to evaluate the impact of sprouting on the vitamin C (ascorbic acid) content of African yam bean. The ascorbic acid content of brown African yam bean sprouts were determined by titrimetric method in which the acidified aqueous extract was titrated against 2,6-dichlorophenolindophenol. The ascorbic acid content determined daily during sprouting for five consecutive days were:13.2mg/100g,15.4mg/100g, 17.6mg/100g, 20.00mg/100g and 22.00mg/100g respectively. The results showed that the highest amount of ascorbic acid was obtained on the fifth day of sprouting while the lowest amount of ascorbic acid was obtained on the first day of sprouting. It was also observed that the ascorbic acid level was increasing as the sprouting period lasts. From the results, it can be inferred that sprouting enhances the vitamin C (ascorbic acid) content of this bean, as there was an exponential increase in the vitamin C content with number of days of sprouting. Sprouts proffer the safest nutritional advantage of both vegetables and fruits without contamination. In conclusion, sprouting is an inexpensive method of obtaining high amount of vitamin C in seeds.

Introduction

In developing countries, the production of milk and other animal-based foods is highly inadequate to meet the nutritional needs of the teeming population. And humans who do not obtain enough vitamin C develop scurvy, which is characterised by haemorrhage under the skin and tissues, swollen and spongy gums from teeth are easily dislodges, poor wound healing, painful joints and common cold [1]. Human beings and non -human primates cannot synthesis vitamin C from other related hexose sugars and therefore there is need for the dietary intake of this essential welfare food. Furthermore, protein-energy and/or -nutrient malnutrition is the major affront to food and nutrition security in many developing countries like Nigeria [2]. However, legumes are the potential source of protein and calories since their seeds contain high amount of carbohydrate (50-67%) and protein (23-25%) [3]. But, it is worrisome to note that majority of leguminous crops are neglected and grossly underutilized. Meanwhile, studies have shown that combination of lesser –known-legumes with other conventional legumes can be used to combat food and nutrition insecurity particularly in Nigeria and the World at large [4].

African yam bean (*Sphenostylis stenocarpo*) is one of the grossly underutilized legumes with great nutritional potentials [5]. African yam bean is a climbing legume and also an annual crop with an exceptional ability to adapt low land tropical conditions. African yam bean (AYB) is largely cultivated in the Eastern and Southern parts of Nigeria [6]. AYB produces nutritious seeds as well as underground edible tubers. Like a cowpea, African yam bean have its seeds enclosed in pods. Each pod contains ten -thirty seeds of many colours ranging from brown, white, speckled, and marble [4,7].These pods are borne on a climbing stem with wide heart -shape-leaves, one at each node spaced apart long the stem [8]. Studies have shown that African yam bean is grown extensively in West Africa for various dietary preparations due to its potentials for supplementing protein requirement of many families [9]. Processing of African yam bean may bring about value-addition which can alleviate protein -energy malnutrition as well as nutrients deficiency. According to Ajibade *et al.* (2005) [10], African yam bean is a good source of protein, carbohydrate, minerals and vitamins but the major constraints to its utilisation are the presence of anti-nutrient factors and long cooking time. Many literatures highlighted that processing such as soaking or fermentation and heating can reduce the level of anti-nutrient factors to tolerable level [11,12,13]. This study aimed at the effect of sprouting on the ascorbic acid content in African yam bean.

Materials and Methods

Chemical reagents (such as 2, 6-dichlorophenolindophenol, phosphoric acid, tetraoxosulphate (VI) acid) used in this study were of analytical grade.

Preparation of Stock Solutions

0.58g of 2, 6-dichlorophenolindophenol was weighed on analytical balance. The 0.58g of 2, 6-dichlorophenolindophenol powder was dissolved in 2 litres (2000ml) of distilled water and was stored in a brown reagent bottle for future use.

Phosphoric Acid

85 percent of the acid was diluted to ten percent using the dilution formula

$$C_1V_1=C_2V_2 \quad (1)$$

More water was added to 117.7cm^3

Where C_1 is the initial concentration, V_1 is the initial volume, C_2 is the required concentration, and V_2 is the required volume.

Extraction and Determination of Ascorbic Acid

1 gram of sprouted brown African yam bean sample was weighed out on analytical balance and then ground in a mortal with a spatula of acid -washed -sand followed by the addition of 5ml of diluted phosphoric acid and then triturated [14]. After triturating, the macerate was filtered, using cotton wool placed in the plastic funnel into a conical flask. The separation was aided by pressing the residue. The residues returned to the mortar and the extraction was repeated for two more times using the same quantity (i.e. 5ml) of phosphoric acid. The filtrate was poured into 100ml of volumetric flask and then distilled water was added to make up the marks of 100ml.The mixture was shaking for few minutes.10ml of the extract solution was taken into a beaker and was titrated with 2, 6-dichlorophenolindophenol solution from a micro-burette until the last drop that discharges a pink colouration, which persisted for the next 30 seconds. The titration was repeated for two times and the average titres of the two readings were calculated using the formula.

$$Average\,titre = \frac{D1 + D2}{2} \quad (2)$$

Where D1 is the difference between final reading and the initial reading of the first titration;

D2 is the difference between final and initial readings of the second titration for each sprouting day.

Calculation for the Determination of Ascorbic Acid Content in Brown African Yam Bean

The ascorbic acid content of African yam bean for each sprouting day was evaluated according to Stroev and Makarova (1989) [15] using the formula:

$$Y = \frac{Ma\,x\,V\,x100x1000}{10\,x\,b} \quad (3)$$

Where Y = ascorbic acid concentration (mg/kg),

Ma = mass of ascorbic acid (i.e.0.088mg) titrimetrically equivalent to 1ml of 0.001M 2, 6-dichlorophenolindophenol solution,

100 = the dilution ratio of the sample taken,

1000 = the scaling factor for conversion to per kilogram of raw material,

10 = the titre volume in ml

V = titrant volume (i.e. 2, 6- dichlorophenolindophenol solution) in ml, and b = the sample weight in gram.

Statistical Analysis

Data obtained were analysed using analysis of variance and means were separated using Duncan's Multiple Range Test at P =0.05. Statistical Package for Social Science package (version 16) was used.

Results and Discussion

Table 1: *First day titration*

	1st titration	2nd titration
Initial reading in ml	1.60	1.70
Final reading in ml	1.40	1.60
Difference in ml	0.20	0.10

$$Average\,titre = \frac{0.2 + 0.10}{2} = 0.15ml$$

Table 2: *Second day titration*

	1st titration	2nd titration
Initial reading in ml	5.30	5.50
Final reading in ml	5.50	5.65
Difference in ml	0.2	0.15

$$Average\,titre = \frac{0.2 + 0.15}{2} = 0.175ml$$

Table 3: *Third day titration*

	1st titration	2nd titration
Initial reading in ml	2.30	2.50
Final reading in ml	2.50	2.70
Difference in ml	0.20	0.20

$$Average\,titre = \frac{0.2 + 0.2}{2} = 0.2ml$$

Table 4: *Fourth day titration*

	1st titration	2nd titration
Initial reading in ml	8.15	8.30
Final reading in ml	8.30	8.60
Difference in ml	0.15	0.30

$$Average\,titre = \frac{0.15 + 0.30}{2} = 0.225ml$$

Table 5: *Fifth day titration*

	1st titration	2nd titration
Initial reading in ml	14.00	14.20
Final reading in ml	14.20	14.50
Difference in ml	0.2	0.30

$$Average\,titre = \frac{0.2 + 0.30}{2} = 0.25ml$$

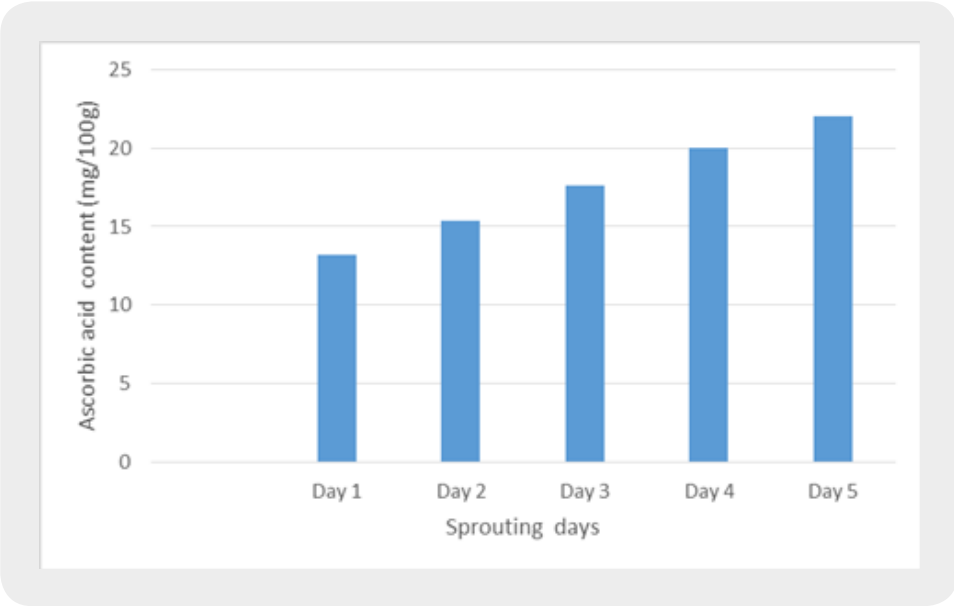

Figure 1: *Ascorbic acid content of African yam beans for five sprouting days*

The ascorbic acid content of sprouted brown African yam bean is presented in Figure 1. From the results, it was observed that sprouting had significantly increased the amount of vitamin C in African yam beans. At day zero, there was no trace of ascorbic acid found in the dry brown African yam beans. The amount of ascorbic acid had increased as the number of sprouting days last. The first sprouting day had the lowest amount of ascorbic acid (13.2mg/100g) while the fifth sprouting day had the highest amount of ascorbic acid (22.0mg/100g). The data obtained from this study were strongly in agreement with the publication report by Colditz *et al.* (1985) [16] that sprouting improves the level of vitamin C in dry seeds. In addition, the observation of this study supported the research findings by Boehringer (1997) [17] which emphasized that sprouting supplies food in pre-digested form as the enzymes would have acted upon the food materials. And as starch is broken down into simple sugars like glucose, fructose, sucrose) and proteins are converted to amino acids and amides as well as fats and oils are converted to free fatty acids. Sprouting is a novel and cheap processing-method that can enhance the nutritional value of beans. In the light of this, Pezacka and Walerych (1991) [18] reported that sprouting makes beans to lose its objectionable gas-producing quality which causes stomach flatulence. The findings of this study were in consonance with Pezacka and Walerych (1991) [18] that sprouting was extremely an inexpensive method of obtaining a high concentration of vitamins, minerals and enzymes. The results of this study was also at par with other literature reports which stated that dried seeds, grains, and legumes do not contain any discernible traces of ascorbic acid yet when sprouted, they revealed quite significant quantities with corresponding decrease in calories and carbohydrate content [19]. Sprouts are the freshest and most nutritious of all vegetables available to human diet. Sprouts were also considered as wonder foods [19,20]. Eating of sprouts was described as the best and safest way of getting the advantage of both fruits and vegetables without the contamination of harmful insecticide [19]. The results of this study vehemently supported the observation from the literature publication bys Onyeike *et al.*(1995) [21] that animal fed on the germinated cooked mash bean gained more weight than those fed on the ungerminated beans.

Bioavailability

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