Comparative Study of the Proximate Composition and Antioxidant Activity of Ripe and Unripe *Carica papaya* Seeds

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ABSTRACT

This study compared the proximate composition and antioxidant activity of ripe and unripe *Carica papaya* seeds. The proximate analyses (moisture, ash, lipid, protein, fibre, carbohydrate, calorific value) was determined by standard methods and antioxidant activity were evaluated using 2,2-diphenyl-1-picrylhrydrazyl (DPPH) radical scavenging ability. The seeds in unripe *C. papaya* fruit contained higher levels of moisture (9.12%), ash (4.88%), and crude fiber (6.51%), whereas the seeds of ripe *C. papaya* fruit were higher in lipids (6.28%), proteins (5.01%), and carbohydrates (76.72%). Both seeds exhibited a concentration dependent activity. The unripe *C. papaya* seeds extract of 100-500 µg/mL has a percentage free radical scavenging activity of 36.02%, 52.54%, 60.59%, 74.15% and 86.44% and they were significantly ($p \leq 0.05$) more potent source of antioxidants compared to ripe *C. papaya* seed extracts of 100-500 µg/mL with percentage free radical scavenging activity of 20.76%, 47.88%, 56.78%, 68.22% and 82.62%. Based on the result of this study, it can be concluded that major nutrients such as lipid, protein and carbohydrates occur more in seeds of ripe *C. papaya* than in the unripe fruits but the antioxidant activity of seeds in unripe *C. papaya* fruit where higher for the different concentrations than in the unripe fruit.

Keywords: Antioxidant activity, *Carica papaya*, proximate composition, seeds

INTRODUCTION

Pawpaw (*Carica papaya*) belongs to the family Caricaceae with over 22 species while only one member of the genus *Carica* is cultivated as fruit tree [1]. They are found primarily in tropical regions of Central and South America and Africa. The plants are typically short-lived evergreen shrubs or small trees growing to 5–10 m tall [2]. It is distributed throughout tropics and subtropics where it is extensively cultivated. It is a tropical fruit and is available throughout the year. It is much favoured and prized fruit all over the world for its flavour and nutritional properties. The fruit of *Carica papaya* is eaten both ripe and unripe but its seeds are thrown away [3]. The various parts of the papaya including the leaves, seed, bark, fruit, root, and latex have been used in traditional folklore medicine and as a resource in industrial products [4,5].

The fruit is an excellent source of minerals and nutrients which are widely used in diet [6]. For instance, the fruit is a rich source of three antioxidants: vitamin C, vitamin A and vitamin E; the minerals: magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. In addition, it contains a digestive enzyme-papaintha, which treats causes of trauma, allergies and sports injuries [7]. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is a source of β-carotene that prevents damage caused by free radicals that may cause some forms of cancer. It has also been reported to prevent diabetic heart disease. *C. papaya* lowers high cholesterol levels as it is a good source of fiber and can treat or improve all types of digestive and abdominal disorders [7]. Papaya fruit is also rich in carbohydrate (42.28% starch and 15.5% sugar in pulp), but is deficient in protein and fat [8, 9].

Despite the predominant focus on the fruit's flesh, recent attention has turned to the seeds of *Carica papaya*, owing to its potential health-promoting attributes, particularly their antioxidant prowess [10]. A study conducted on seed of ripe *C. papaya* focused on the nutritional composition and revealed that it contains moisture (11.02%), protein (27.41%), fat (28.61%), crude fibre (8.02%), ash (5.21%) and carbohydrate (19.70%) [11]. In another study, papaya seed demonstrated a potent antioxidant activity against 2,2-diphenyl-1-picrylhrydrazyl [12], yet the comparison of seeds of the ripe and unripe fruit remained under-explored.

Therefore, this study assessed the proximate/nutritional composition of ripe and unripe seed of *C. papaya*. A stable free radical molecule (DPPH) was also tested against both seed extracts to evaluate the antioxidant potentials of the ripe and unripe *Carica papaya* seed.

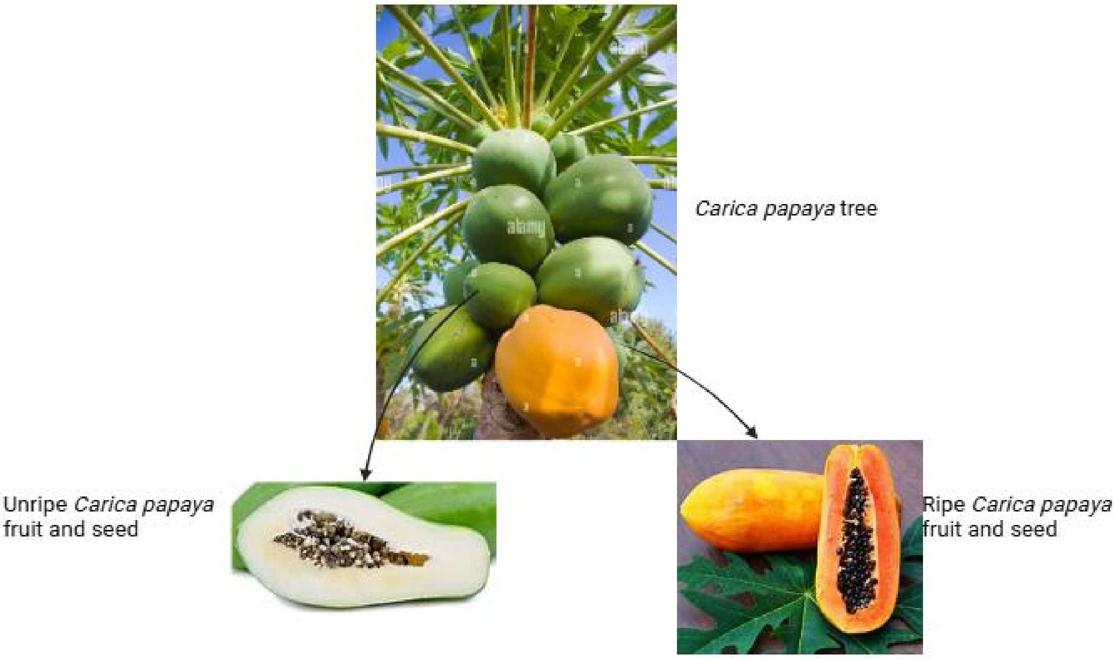

Figure 1: *Carica papaya* tree, ripe and unripe fruits with seed

MATERIALS AND METHODS

Source of materials

Ripe and unripe pawpaw fruit which were visually defined according to skin colour [10], were purchased from a local market in Kashere, Gombe State. The fruits were identified in the herbarium unit of Federal University Kashere. Subsequently, the fruits were transported to the laboratory of the Department of Biological Sciences, Gombe State University for processing and analyses. All chemicals and reagents used were of analytical grades.

Sample preparation

The fruits were washed under running tap water and separated into flesh and seeds. Each of the samples were air-dried at room temperature for 14 days. The air-dried samples were ground into fine powder using a dry grinder [11]. Pulverized samples were then sieved to get uniform particle size, kept in air tight containers and stored in a freezer until further analysis.

Proximate analysis

The study examined the following nutritional content of both seed types: Moisture was measured according to the method described by Umeaku et al [13]. Crude protein was determined by the Kjeldahl method, according to Association of Official Analytical Chemists (AOAC) 984.13 [14]. The ash content was determined by calcination at 50 °C, according to the method described by Umeaku et al [13]. Lipids were extracted in Soxhlet apparatus with petroleum ether at 40 °C, and other procedures described by Umeaku et al [13] were adopted. Crude fibre was also determined according to the method of Umeaku et al [13]. The carbohydrate content of the samples was estimated as the difference obtained after subtracting the values of organic protein, ash content, fat or oil, crude fibre, and moisture content from 100 [15].

$$\text{Carbohydrate content} = 100 - (\text{protein} + \text{ash} + \text{oil} + \text{crude fibre} + \text{moisture content}) \quad (1)$$

Energy values were calculated by multiplying the data of each macronutrient (total protein, carbohydrates) in gram by their respective energy (4, 4, and 9 kcal/g) [16]. The assay was replicated thrice, and the result was taken as mean ± standard error of mean (SEM)

Antioxidant analysis

Antioxidant activity of the seeds was assessed using 2,2-diphenyl-1-picrylhrydrazyl free radical [17, 18]. DPPH solution (0.2 mM) was prepared by dissolving 7.8 mg of DPPH in 100 mL methanol. Different concentrations (100, 200, 300, 400 and 500 µg/mL) of methanolic solutions of seed extract were prepared by serial dilution of the stock solution of the respective seed extract. To each 0.5 mL extract solution, 2.5 mL DPPH (0.2 mM) solution was added. A standard to be compared with the seed was prepared by mixing 0.5 mL of ascorbic acid concentrates (100, 200, 300, 400 and 500 µg/mL) and 2.5 mL DPPH (0.2 mM) methanolic solution. A control was prepared by mixing 0.5 mL distilled water and 2.5 mL DPPH (0.2 mM) methanolic solution. These samples were shaken well, incubated at 37 °C for half an hour, and the absorbance was recorded at 517 nm by UV spectrophotometer (SP-VG722, China). The radical scavenging activity was expressed as the radical scavenging percentage using the following formula [19, 20].

$$\text{DPPH Inhibition (\%)} = [1 - A1/A0] \times 100 \quad (2)$$

Where A1 = Absorbance of the sample, and A0 = Absorbance of control. The assay was replicated thrice, and the result was taken as mean ± standard error of mean (SEM).

RESULTS AND DISCUSSION

Proximate composition of ripe and unripe *Carica papaya* seed

The comparative proximate composition and energy content of ripe and unripe *Carica papaya* seeds, highlighting differences in moisture, ash, lipid, crude protein, crude fiber, carbohydrate content, and caloric energy is presented in Table 1. Unripe seeds of *C. papaya* have a higher moisture content compared to ripe seeds. This could be attributed to the fact that ripening generally involves the loss of water as the fruit matures. Lower moisture content in ripe seeds can also suggest a longer shelf life due to reduced susceptibility to microbial growth [21]. The moisture content of the seed of matured *C. papaya* recorded in this study, 6.11% is lower than the 7.34% recorded in a similar study [22]. The ash content is higher in unripe seeds (4.88 %) than ripe seeds (2.87 %), indicating a greater presence of mineral content. This could be attributed to the physiological differences in the development stages of the seeds. As seeds mature, some of the mineral content might be redistributed or diluted by the increased organic content. The ash content of the seed of matured *C. papaya* recorded in this study, 2.87%, is lower than the 5.21% recorded in a study conducted by Makanjuola et al [11]. The lipid content of ripe seeds contains almost double the lipid content (6.28 %) compared to unripe seeds (3.19 %). Lipid accumulation is common as seeds mature, which enhances their energy density and potential use as a source of oils. This increased lipid content in ripe seeds could make them more suitable for applications requiring oil extraction [21].

The crude protein content of the ripe seeds also exhibits a higher crude protein content (5.01%) compared to unripe seeds (3.22 %). Protein synthesis and accumulation often increase during seed maturation, contributing to the higher protein content observed in ripe seeds. This suggests that ripe seeds might offer better nutritional value in terms of protein.

The crude fiber content of the unripe seeds recorded a significantly higher crude fiber content (6.51 %) than the ripe seeds (3.03 %). This could be due to the more rigid and less developed cellular structure in unripe seeds, which often contains more fibrous material. As seeds ripen, the fiber content might decrease due to the breakdown of some of these fibrous components.

The lipid (6.28 %), protein (5.01 %) and fibre (3.03 %) recorded in seeds of ripe *Carica papaya* is highly lower than that recorded in a study that accessed the proximate composition of aqueous seed extract (lipid: 28.63%, protein:31.26%, and fibre: 25.23%) of ripe *Carica papaya* [23].

The carbohydrates (CHO) content of the ripe seeds showed a slightly higher carbohydrate content (76.72 %). This is expected as the process of ripening typically involves the conversion of complex carbohydrates into simpler sugars, which might contribute to the observed increase in carbohydrate content.

The energy content is higher in ripe seeds (383.36 Kcal/100g > 333.91 Kcal/100g), reflecting the greater lipid and carbohydrate content. This higher caloric value is indicative of the increased energy density of the ripe seeds, which could make them more suitable for high-energy dietary applications or as a bioenergy source [24].

Comparatively, ripe *Carica papaya* seeds were richer in lipids, proteins, and carbohydrates, and possess a higher energy content compared to unripe seeds. In contrast, unripe seeds contain more moisture, ash, and fiber. These differences are likely due to the biochemical changes that occur during the ripening process, making ripe seeds potentially more valuable in applications requiring higher energy and nutrient content, while unripe seeds might be preferred in scenarios where higher fiber content is desired [25].

Table 1: Proximate composition of ripe and unripe *Carica papaya* seed

Parameters	Ripe *C. papaya* seed	Unripe *C. papaya* seed
Moisture (%)	6.11 ± 0.14	9.12 ± 0.21
Ash (%)	2.87 ± 0.17	4.88 ± 0.41
Lipid (%)	5.01 ± 0.44	3.22 ± 0.27
Protein (%)	5.01 ± 0.44	3.22 ± 0.27
Fibre (%)	3.03 ± 0.58	6.51 ± 0.45
Carbohydrate (%)	76.72 ± 0.48	73.10 ± 0.47
Calorific value (Kcal/100g)	383.36 ± 5.1	333.91 ± 5.87

Antioxidant activity of ripe and unripe *Carica papaya* seed

The DPPH antioxidant activity of ripe and unripe *Carica papaya* seeds across different concentrations (100 – 500 µg/ml) and with ascorbic acid serving as the standard reference is demonstrated in Figure 2. The percentage inhibition values indicate the effectiveness of the extracts in scavenging free radicals, thereby reflecting their antioxidant potential. At the lowest concentration (100 µg/ml), unripe *C. papaya* seeds exhibit higher antioxidant activity than ripe seeds (36.02 % > 20.76 %), though both are significantly lower ($p \leq 0.05$) than ascorbic acid (71.19 %). This suggests that at lower concentrations, unripe seeds may have a relatively higher concentration of active antioxidant compounds compared to ripe seeds. With an increase in concentration to 200 µg/ml, the antioxidant activity of both ripe (47.88%) and unripe seeds (52.54%) improves. The increase in inhibition percentage indicates a dose-dependent antioxidant response. At 300 µg/ml, the inhibition percentage of unripe seeds (60.59%) is marginally higher than that of ripe seeds (56.78%). Both are still lower than ascorbic acid (81.78%) but show substantial increases compared to lower concentrations, suggesting effective radical scavenging capability at this concentration. At 400 µg/ml, the antioxidant activity of both ripe (68.22%) and unripe seeds (74.15%) continues to rise, with unripe seeds demonstrating more potent activity than ripe seeds. The gap between the antioxidant activity of the seeds and ascorbic acid decreases, showing enhanced efficacy at this higher concentration. At the highest concentration of 500 µg/ml, both ripe (82.62%) and unripe (86.44%) *C. papaya* seeds exhibit their maximum antioxidant activity, with unripe seeds showing slightly higher ($p \leq 0.05$) inhibition than ripe seeds. However, both remain lower than ascorbic acid (92.80%), which nearly reaches full inhibition. This suggests that while both types of seeds have strong antioxidant properties at high concentrations, unripe seeds may possess a slightly greater concentration of antioxidant compounds. In support, Ang et al [26], also reported a concentration dependent activity of extract from the seed of matured *C. papaya* against DPPH free radicals. The high antioxidant activity could be due to the increased in hydroxyl groups or the acidic nature of unripe fruit [27].

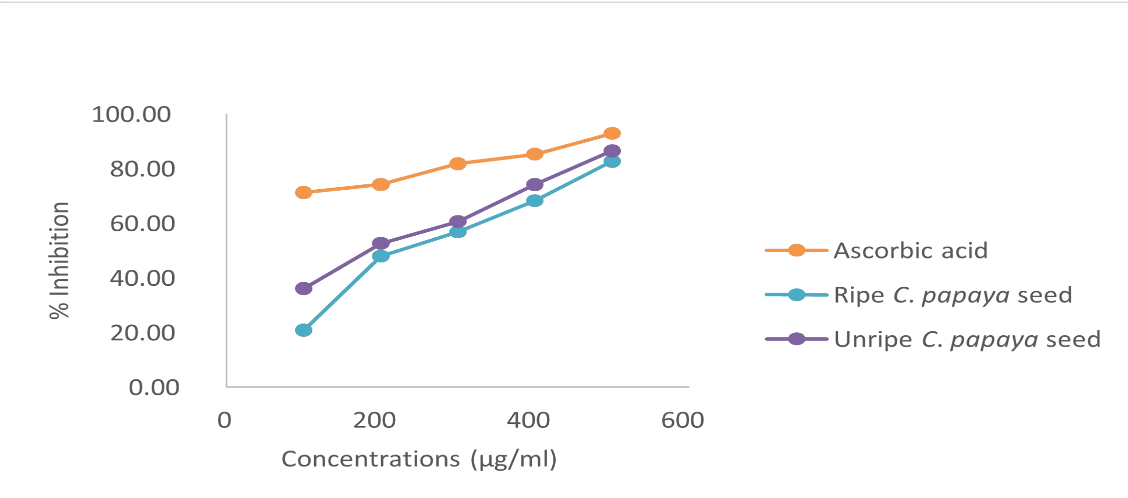

Figure 2: DPPH radical scavenging activity (%) of ripe and unripe *Carica papaya* seed

CONCLUSION

The findings from this study suggest that the seeds in unripe *C. papaya* fruit contain higher levels of moisture, ash, and crude fiber, while seeds of ripe *C. papaya* fruit are significantly higher in lipids, proteins, and carbohydrates. Both seeds exhibited a concentration dependent activity and unripe *C. papaya* seeds are a more potent source of antioxidants compared to ripe seeds, as indicated by their higher DPPH radical scavenging activity across all tested concentrations. Further studies should be conducted to identify and characterize the specific bioactive components present in both ripe and unripe *Carica papaya* seeds. In addition, investigations into the mechanism of antioxidant action are recommended to elucidate how these bioactive compounds exert their free radical scavenging effects at the molecular level.

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