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Determination of Amino Acid Constituents of *Carica papaya* Seeds

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ABSTRACT
This study evaluated the levels of amino acid constituents in *Carica papaya* seeds. These seeds were purchased from Dutsin-Ma central market in Katsina state and identified at the Department of Biological Science Herbarium with voucher number: 0035. The seeds were cleaned to remove impurities, air-dried to 10% moisture content, dehulled and ground into fine powder and then analysed using high-performance liquid chromatography (HPLC). The results revealed that the seeds contain essential amino acids, with relatively high concentrations of methionine (201.29×10^{-6} mg/g), cysteine (184.20×10^{-6} mg/g), and lysine (39.37×10^{-6} mg/g). The amino acid profile showed a balance of polar and non-polar amino acids, indicating potential nutritional and pharmacological benefits. The seeds also showed relatively high levels of cysteine, a precursor to glutathione, an antioxidant with immune-boosting properties. Comparative analysis with flaxseeds and chia seeds demonstrated that papaya seeds possess comparable nutritional value. The study highlighted the potential of *Carica papaya* seeds as a potential source of amino acids for human nutrition and pharmaceutical applications, thereby contributing to food waste reduction and sustainable agricultural practices.

Keywords: *Carica papaya*, amino acids, HPLC, essential amino acids, nutritional value

INTRODUCTION
Carica papaya (Linn.) belonging to the family Caricaceae, is commonly known as papaya in English and by various local names including Gwanda (Hausa), Ibepe (Yoruba), and Okwuru-bekee (Igbo). This tropical fruit tree is cultivated extensively across Nigeria and many parts of the world primarily for human consumption. While ripe papaya fruits are typically consumed fresh and unripe or green fruits are used as vegetables, the plant has found numerous industrial and medicinal applications beyond its use as food (Danbaba *et al*., 2012).

Various parts of the papaya plant have been extensively studied for their bioactive compounds and therapeutic properties. The latex from unripe papaya fruits contains papain, a proteolytic enzyme widely utilized in pharmaceutical, food, and beverage industries, as well as in the manufacture of soap and cosmetics (Archbold *et al*., 2018). Several proteins and alkaloids have been isolated from papaya leaves and fruits with far-reaching relevance in medicine and industry (Kruger *et al*., 2011). Papaya stands out as one of the cheap and highly nutritional fruits cultivated and consumed in Nigeria and globally.

However, papaya seeds, which constitute approximately 15% of the fruit weight, are predominantly discarded as agricultural waste during fruit processing and consumption. This represents a significant loss of potentially valuable nutrients and bioactive compounds. In recent years, these seeds have gained increasing attention in scientific research due to their potential health benefits, including antioxidant, anti-inflammatory, antimicrobial, and anthelminthic properties (Aravind *et al.*, 2013; Moshi *et al.*, 2019). Given their value to folk medicine enthusiasts and preliminary phytochemical studies, papaya seeds are potentially an important source of nutraceuticals that have been largely overlooked.

Amino acids are fundamental biological molecules that serve as the building blocks of proteins and play crucial roles in virtually all physiological processes within the human body. They are essential for protein synthesis, enzyme function, neurotransmitter production, hormone regulation, immune system support, and numerous metabolic pathways (Wu, 2013). Amino acids are classified into two main categories: essential amino acids, which cannot be synthesized by the human body and must be obtained through dietary sources, and non-essential amino acids, which can be produced internally but are equally important for optimal health (Young & Pellett, 1994).

Previous research has indicated that *Carica papaya* seeds contain significant amounts of protein, ranging from 20 to 25% on a dry weight basis, along with essential fatty acids, particularly linoleic acid (Siddhuraju *et al.*, 2019). Early studies have shown that these seeds are rich in essential amino acids such as lysine, leucine, and valine, as well as non-essential amino acids like glutamic acid and aspartic acid (Oboh *et al.*, 2015). These amino acids are vital for various physiological processes including muscle growth and repair, cognitive function, immune system enhancement, and metabolic regulation.

Despite the potential nutritional benefits of *Carica papaya* seeds and their traditional use in folk medicine, there remains a significant gap in comprehensive scientific studies on their detailed amino acid profile. While previous research has focused primarily on the phytochemical and pharmacological properties of papaya seeds, including their antimicrobial and anti-inflammatory activities, a th orough analysis of their amino acid composition using modern analytical techniques is essential to fully understand and quantify their nutritional value. This knowledge gap limits the effective utilization of papaya seeds as a protein source and hinders the development of value-added products from this agricultural waste.

Investigating the nutritional value of *Carica papaya* seeds through detailed amino acid analysis will not only establish their value as an additional food source for humans and animals but also provide scientific evidence for their traditional medicinal uses. Identifying and quantifying the amino acid constituents responsible for the seeds' efficacy will add substantial value to their pharmaceutical potential and support the development of dietary supplements and functional foods (Archbold *et al.*, 2018). Furthermore, the utilization of these often-discarded seeds could contribute to food waste reduction, sustainable agricultural practices, and enhanced food security, particularly in regions where protein deficiency and malnutrition remain significant public health challenges.

MATERIALS AND METHODS

Sample Collection and preparation

Carica papaya seeds were purchased from Dutsin-Ma Central Market, Dutsin-Ma Local Government, Katsina State, Nigeria. The seeds were identified at the Department of Biological Science Herbarium, Federal University Dutsin-Ma (Voucher Number: 0035). Mature seeds were cleaned to remove impurities, air-dried to approximately 10% moisture content, dehulled to remove the outer seed coat, and ground into fine powder using a mortar and pestle.

Amino Acid Extraction

Amino acids were extracted using acid hydrolysis. Approximately 1 gram of papaya seed powder was placed in a hydrolysis tube with 10 mL of 6M hydrochloric acid (HCl), sealed, and heated at 110°C for 24 hours (Moore & Stein, 1954). After hydrolysis, the mixture was cooled, filtered through Whatman No. 1 filter paper, and neutralized with 6M sodium hydroxide (NaOH) to pH 7.0.

Chromatographic Analysis

High-performance liquid chromatography (HPLC) was used to separate and quantify amino acids following the method of Bidlingmeyer *et al.* (1984). The mobile phase consisted of 0.1M sodium acetate buffer (pH 5.8) and acetonitrile. O-phthaldialdehyde (OPA) was used for derivatization. A C18 reverse-phase column (250 mm × 4.6 mm, 5 µm) was used with a fluorescence detector (excitation: 340 nm; emission: 450 nm). The flow rate was 1.0 mL/min at 25°C.

Data Analysis

Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

RESULTS

Amino Acid Composition

The amino acid analysis revealed the presence of both essential and non-essential amino acids in *Carica papaya* seeds (Tables 1 and 2).

Table 1: Essential Amino Acid Content of *Carica papaya* Seeds

Essential Amino Acid	Concentration (mg/g) × 10^{-6}
Isoleucine	1.44 ± 0.12
Leucine	11.84 ± 0.89
Lysine	39.37 ± 2.15
Methionine	201.29 ± 15.47
Phenylalanine	8.90 ± 0.67
Valine	ND

ND = Not detected.

Table 2: Non-Essential Amino Acid Content of *Carica papaya* Seeds

Non-Essential Amino Acid	Concentration (mg/g) × 10^{-6}
Arginine	1.95 ± 0.15
Aspartic Acid	ND
Cysteine	184.20 ± 12.34
Proline	20.37 ± 1.58
Serine	27.26 ± 2.03
Tyrosine	0.24 ± 0.02

ND = Not detected.

DISCUSSION

The amino acid analysis of *Carica papaya* seeds revealed a distinctive nutritional profile with relatively high concentrations of sulfur-containing amino acids methionine (201.29 ± 15.47 × 10^{-6} mg/g) and cysteine (184.20 ± 12.34 × 10^{-6} mg/g). These concentrations substantially exceed those typically found in conventional plant protein sources, positioning papaya seeds as a unique nutritional resource. Methionine, an essential amino acid, plays critical roles in protein synthesis, liver detoxification, and fat metabolism through phospholipid synthesis and choline production (Martínez *et al.*, 2017; Brosnan & Brosnan, 2020). Additionally, methionine serves as a methyl donor in numerous biochemical reactions and acts as a precursor for S-adenosylmethionine (SAM), which participates in epigenetic regulation and neurotransmitter synthesis (Martínez *et al.*, 2017; Vargas-Mendoza *et al.*, 2019).

Cysteine's prominence in papaya seeds is particularly significant given its role as the rate-limiting precursor for glutathione biosynthesis, one of the body's most potent endogenous antioxidants (Gu *et al.*, 2015; Forman *et al.*, 2021). The high cysteine content suggests potential applications in functional foods targeting oxidative stress reduction, immune enhancement, and detoxification support (Paul *et al.*, 2018; Zafarullah *et al.*, 2023). This finding is particularly relevant for populations experiencing high oxidative stress due to environmental pollutants, chronic diseases, or aging.

The presence of relatively high concentrations of essential amino acids, including lysine (39.37 ± 2.15 × 10^{-6} mg/g), leucine (11.84 ± 0.89 × 10^{-6} mg/g), and phenylalanine (8.90 ± 0.67 × 10^{-6} mg/g), indicates that papaya seeds can contribute to dietary protein requirements. Lysine, often limiting in cereal-based diets prevalent in developing regions, plays crucial roles in calcium absorption, collagen formation, and immune function (Committee on Military Nutrition Research, 2015; van Vliet *et al.*, 2015). The adequate lysine content in papaya seeds could help address nutritional gaps in populations heavily dependent on cereal grains, thereby improving overall protein quality when incorporated into mixed diets.

Leucine, a branched-chain amino acid (BCAA), stimulates muscle protein synthesis through mTOR pathway activation and regulates glucose metabolism (Yoon, 2016; Liang *et al.*, 2020). Its presence suggests potential applications in sports nutrition, geriatric nutrition for sarcopenia prevention, and clinical nutrition for patients requiring enhanced protein anabolism. The detection of isoleucine (1.44 ± 0.12 × 10^{-6} mg/g) further enhances nutritional value, as this BCAA contributes to hemoglobin formation and energy regulation (Neinast *et al.*, 2019; Pimentel *et al.*, 2020). However, the absence of detectable valine represents a nutritional limitation requiring complementation with other protein sources.

Among non-essential amino acids, serine ($27.26 \pm 2.03 \times 10^{-6}$ mg/g) and proline ($20.37 \pm 1.58 \times 10^{-6}$ mg/g) were present in appreciable amounts. Proline's role in collagen synthesis makes it essential for skin health, wound healing, and maintaining connective tissue integrity (Li & Wu, 2018; Wu *et al.*, 2021). Serine participates in cell proliferation, nervous system function, and serves as a precursor for glycine, cysteine, and phospholipids (Yang & Vousden, 2016; Gupta *et al.*, 2017). The conditionally essential amino acid arginine ($1.95 \pm 0.15 \times 10^{-6}$ mg/g) supports nitric oxide synthesis, immune function, and wound healing (Rath *et al.*, 2016; Gambardella *et al.*, 2020).

The absence of detectable aspartic acid and valine warrants further investigation using more sensitive analytical methods, as these amino acids typically participate in important metabolic pathways. This absence may reflect analytical limitations, biological variation, or degradation during processing.

Comparative analysis with nutritionally recognized seeds such as flaxseed and chia revealed that some amino acid concentrations in papaya seeds may be lower generally, the relatively high levels of methionine and cysteine content distinguished them as a complementary protein source (Ullah *et al.*, 2016; De Falco *et al.*, 2017). Legumes, despite being rich in lysine, are typically deficient in sulfur amino acids (Nosworthy *et al.*, 2017; Day, 2021). The amino acid profile of papaya seeds suggests they could complement legume-based diets, providing a more balanced amino acid composition that aligns with WHO/FAO recommended ratios for optimal human nutrition (FAO, 2013; Phillips, 2017).

CONCLUSION

This study revealed that *Carica papaya* seeds contain only trace amounts of amino acids with limited concentrations of both essential and non-essential amino acids. The trace levels of essential amino acids detected along with the complete absence of detectable valine and aspartic acid, suggest nutritional limitations. These extremely low concentrations of amino acids indicate that papaya seeds would not serve as an efficient dietary protein source. Future research should investigate the bioavailability of these amino acids, explore long-term health benefits through clinical trials, and evaluate the effects of different processing methods on nutritional quality.

CONFLICT OF INTEREST

The authors declared no conflicts of interest

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