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RESEARCH ARTICLES

Some phytochemical and functional properties of Pawpaw (*Carica papaya* L.) leaf protein concentrates obtained from three locations in Benin City, Edo State, Nigeria

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Abstract

This research was conducted to determine some phytochemicals and functional properties of pawpaw (*Carica papaya* L.) leaf protein concentrates obtained from three locations in Edo State, Nigeria. Pawpaw leaves were obtained from the University of Benin (location A), Oluku (location B) and Ekenwan (location C), and they were processed into leaf protein concentrates in triplicates using heat coagulation method. The resultant pawpaw leaf protein concentrates (PLPC) were analyzed for their phytochemical and functional properties. The results from the phytochemical analysis showed that tannin was absent in PLPC in all three locations. Saponin was high in location A, only present in location C, but absent in location B. Flavonoids and phytates were present in samples from all locations. In contrast, alkaloids and oxalates were absent in all locations. The results from the analysis of functional properties showed that protein solubility was highest in location B, having a value of $0.82 \pm 0.07\%$ and lowest in location A, having a value of $0.5 \pm 0.02\%$. Water absorption capacity and oil absorption capacity were highest in location B, with values of 1.33 ± 0.15 g/g and 1.9 ± 0 g/g, respectively and lowest in location C, with values of 0.63 ± 0.07 g/g and 1.16 ± 0.38 g/g, respectively. The forming capacity and forming stability were highest in location A, having values of $22.49 \pm 1.28\%$ and $98.78 \pm 0.62\%$, respectively and lowest in location B, with values of $8.77 \pm 0.31\%$ and $89.39 \pm 9.37\%$, respectively. Fat emulsion capacity was highest in location C ($45.32 \pm 0.32\%$) and lowest in location A ($37.47 \pm 0.43\%$). Fat emulsion stability for all locations was 100%. The least gelation capacity for locations B and C had the same value of 16%, and that of location A had a value of 14.7%. The results obtained in this study suggest that pawpaw leaf protein concentrate has the potential as an alternative protein source for livestock and man.

Keywords Leaf protein concentrate · phytochemical properties · Functional properties · Pawpaw leaf · Nigeria

Introduction

Phytochemicals can be simply be defined as plant's chemicals. They are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred by diets high in vegetables, fruits, beans, cereals and plant-based beverages such as tea and wine (Agbugba and Shelaby 2018; Plamada and Vodnar 2022). Phytochemicals have several properties and therapeutic uses. For example, flavonoids have anti-allergic, anti-inflammatory and antioxidant properties. Tannins have wound-healing properties (Anjum et al. 2013; Martel et al. 2020). Some phytochemicals are anti-nutritional factors. Anti-nutritional factors are substances generated in natural food and/or feedstuffs by the normal metabolism of species and several mechanisms. They interfere with the absorption of nutrients

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and digestive processes (Salehi et al. 2018). Although phytochemicals have beneficial effects, it is worth noting that excessive consumption can have detrimental effects on humans and animals. (Aravind et al. 2021).

Functional properties have been defined as the intrinsic physico-chemical characteristics that affect protein behaviour in the food system during processing, storage, and preparation (Adachukwu et al. 2013). They interact with other food components directly or indirectly, affecting processing applications, food quality and ultimate acceptance. Proteins used in foods must possess critical functional properties such as water and oil absorption capacities, nitrogen solubility, bulk density, and emulsifying and foaming properties. These properties govern the suitability of novel proteins as food supplements as well as ingredients for developing new food products (Egwue et al. 2020).

Due to its nutritional and medicinal value, Pawpaw has attracted much attention. Pawpaw leaves contain active compounds such as papain, chymopapain and flavonoids that increase the total antioxidant power in the blood and lowers lipid peroxidation level (Singh et al. 2020).

Leaves have been processed into leaf protein concentrates and offered to man and animals. Leaf protein concentrates are cheap and in ample supply. They can serve as a source of proteins for the production of animal feed and other foods because of their capacity to synthesize amino acids from a wide range of available primary nutrients. Agbonghae (2016) incorporated Pawpaw leaf protein concentrate into rabbit feed. Agbonghae and Nwokoro (2019) reported a yield of 7.12 ± 1.75 for pawpaw leaf protein concentrates obtained through heat coagulation method. However, studies on phytochemical and functional properties of pawpaw leaf protein concentrates have not been carried out. Therefore, if PLPCs are going to find widespread application as an ingredient in the food/feed industry, it is imperative that knowledge of the presence of certain phytochemicals (including anti-nutritional factors) are known to certify it as a suitable replacement and/or supplement in man and livestock diet. It is also important to know the functional properties of this protein concentrate because they govern the behavior of food/feed systems during storage, processing, preparation and consumption (Norahmad et al. 2019).

Location of study

This research was carried out at the Faculty of Agriculture main laboratory, Ugbowo Campus, Benin City, Nigeria, which is located between Latitude $6^{\circ}30'N$ of the Equator and Longitude $5^{\circ}40'$ to $6^{\circ}E$ of the Greenwich Meridian (Google Earth 2021) in the rain forest zone, with an average temperature of $27.6^{\circ}C$, and Benin City has an average

annual rainfall and relative humidity of 2162 mm and 72.5%, respectively (NAA 2018).

Experimental study

The research was carried out in two phases. Firstly, it was to calibrate and produce the Pawpaw Leaf Protein Concentrates (PLPC) from pawpaw leaves obtained from random locations (trials) as well as the production of PLPC from pawpaw leaves obtained from plants in three locations in Edo State, Nigeria. The heat coagulation method, modified by Nwokoro (2015), was used to extract the protein from the pawpaw leaf juice. Secondly, the phytochemical and functional properties of the PLPCs extracted from the pawpaw leaves obtained from three locations in Edo State, Nigeria, were analyzed (Fig. 1).

Phytochemical analysis of pawpaw leaf protein concentrates

Preparation of PLPC extract The sample powder of pawpaw leaf protein concentrate was weighed and extracted in triplicate with 40 mg of dry weight (DW). The extraction was performed using 1.5 ml of a hydro-methanolic solution

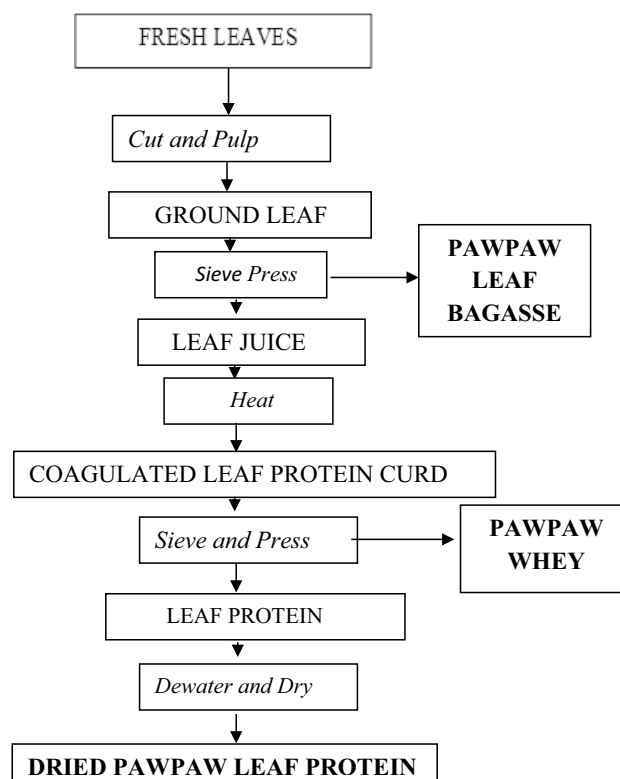


Fig. 1 Flow Chart of the production of pawpaw leaf protein concentrates. Source: Nwokoro (2015); Agbonghae and Nwokoro (2019)

(methanol: H₂O, 70:30, v/v). The crude extract was obtained thereafter, followed by centrifugation at 4000 rpm for 30 min. The supernatants were collected in a 5 ml volumetric flask, and the solid residues were then extracted twice via the same procedure. All the three supernatants from successive extractions were kept together to obtain a final volume with the above-mentioned extraction solvent. The crude extract was stored at 4°C for phytochemical determination. The presence of some phytochemicals was performed following the procedures described by Shubham et al. (2019). Briefly, 2 ml of 5% ferric chloride was added to 1 ml of PLPC extract for tannin. Formation of dark blue or greenish colour indicates the presence of tannin. For saponin, 5–10 ml of distilled water was added to 1 ml of PLPC extract and shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicated the presence of saponin. For alkaloids, 2 ml of concentrated hydrochloric acid was added to 2 ml of PLPC extract. Thereafter, 3 drops of Mayer's reagent were added. The presence of green colour or white precipitate indicates the presence of alkaloids. For flavonoids, 2 ml of plant extract was treated with 4 drops of concentrated sulphuric acid and observed. The formation of orange colour indicates the presence of flavonoids. For oxalate, a small amount of PLPC extract was heated with resorcinol (2–3 flakes) and water (1 ml) in a test tube, 2 ml of concentrated sulphuric acid was added along the sides of the test tube. The appearance of a blue ring at the junction of two layers confirms the presence of oxalic acid. The determination of phytates was based on the analysis of phosphorus or iron in an isolated ferric phytate. Phytates form an insoluble complex (precipitate) with ferric ions in dilute acid solutions.

Determination of functional properties of pawpaw leaf protein concentrates

Some functional properties such as protein solubility, water/oil absorption capacity, foam capacity (FC) and foam stability, emulsification capacity (EC), gelation capacity, of the pawpaw leaf protein concentrate were determined based on

standard methods in the Association of Official Analytical Chemist (AOAC 2012; Ohizua et al. 2017).

Statistical analysis

Data collected from the experiment were subjected to Analysis of Variance at 0.05 level of significance using the GENSTAT Computer Statistical Package, 8th edition for windows. Significant means were separated using Duncan multiple range test in the same statistical package (Tables 1 and 2).

Results and discussion

Phytochemical properties

Results of phytochemical screening of the various PLPC samples revealed the presence of phytochemicals, as shown in Table 1.

Table 2 Functional properties of PLPC samples collected from three location

Functional properties	Location		
	A	B	C
Protein solubility (%)	0.5 ± 0.02	0.82 ± 0.07	0.81 ± 0.01
Water absorption capacity (g/g)	1.07 ± 0.07	1.33 ± 0.15	0.63 ± 0.07
Oil absorption capacity (g/g)	1.73 ± 0.03	1.9 ± 0	1.16 ± 0.38
Fat emulsion capacity (%)	37.47 ± 0.43	41.82 ± 0.43	45.32 ± 0.32
Fat emulsion stability (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00
Least gelation concentration (%)	14.7 ± 1.35	16 ± 0	16 ± 0
Foaming capacity (%)	22.49 ± 1.28	8.77 ± 0.31	14.05 ± 1.18
Foaming stability (%)	98.78 ± 0.62	89.39 ± 9.37	91.11 ± 4.84

Table 1 Phytochemical properties of PLPC samples collected from three locations

Phytochemical	Locations								
	A			B			C		
	1	2	3	1	2	3	1	2	3
Tannin	–	–	–	–	–	–	–	–	–
Saponin	+++	+++	+++	–	–	–	–	+	+
Alkaloid	–	–	–	–	–	–	–	–	–
Flavonoid	+	+	+	+	+	+	+	+	+
Oxalate	–	–	–	–	–	–	–	–	–
Phytate	+	+	+	+	+	+	+	+	+

+++ Highly present, ++ Moderately present, + Present, – Absent

Tannins

Tannin was absent in all samples of PLPC from all three locations but reported to be present in the leaf protein concentrate of *Cnidoscoluma conitifolius* (Tan 2019). The absence of tannin corroborated the results of Ukpo et al. (2017), who reported the absence of tannin in pawpaw leaf extracts. However, tannin is present in *Carica papaya* leaf as reported by Dwivedi et al. (2020).

Flavonoids

It has been reported that flavonoids have anti-inflammatory effects (Choy et al. 2019). Flavonoids were found to be present in all samples of PLPC from all three locations, and this corroborated the results of Ukpo et al. (2017) and Singh et al. (2020), who found flavonoids to be present in the leaves of *C. papaya*. Therefore, this phytochemical is not only present in the leaves of *C. papaya* but also in PLPC.

Alkaloids

Phytochemical screening for alkaloid in all PLPC samples tested negative for all three locations; however, alkaloid has been reported to be present in the leaf protein concentrate of *Cnidoscoluma conitifolius* (Aye 2012). Moreover, in studies carried out on *C. papaya* leaves by Martel et al. (2020) and Aravind et al. (2021), alkaloid was also found to be present. The absence of alkaloids in PLPC requires further investigation.

Saponins

Saponins were present in PLPC samples from Location A and C, just as it was found to be present in *Shorea robusta* leaf protein concentrates (Singh et al. 2014). The presence of saponin in the PLPC samples also corroborated the results of Ukpo et al. (2017), Salehi et al. (2018), and Singh (2020), who showed that saponins were present in the leaves of *C. papaya*, indicating that saponin is still present even after processing the leaves to leaf protein concentrates. The absence of saponins in location B remains obscure and should be a subject of further research. It has been reported that saponins have anti-diarrheal and anthelmintic properties (Singh et al. 2020).

Oxalate

Oxalate was absent in all PLPC samples of locations A, B, and C. However, it was reported in the leaf protein concentrate of *Cnidoscoluma aconitifolius* (Tan, 2019).

Phytate

Phytate was present in all PLPC samples from all three locations. Phytate is an anti-nutrient compound known to complex with proteins and minerals, hence interfering with their diet absorption. There are several methods of decreasing the inhibitory effect of phytate mineral absorption: cooking, fermentation, soaking, and autolysis (Plamada and Vodnar 2022).

Phytochemicals can serve as a valuable source of information and provide appropriate standards to establish the quality of pawpaw leaf protein concentrate in future studies or applications.

Functional properties

Protein solubility

The averages of the protein solubility for locations A, B and C were 0.5%, 0.82% and 0.81%, respectively. The results showed that protein solubility for all PLPC samples was lower than that for walnut protein concentrate at 1.31% (Dwivedi et al. (2020)). The solubility of a protein is the most important functional property since the protein needs to be soluble in order to be applicable in food systems. Other functional properties like emulsification, foaming, and gelation are dependent on the solubility of proteins (Danielsen et al. 2020).

Water/fat absorption capacity

The averages of the water absorption capacity for locations A, B, and C were 1.07 g/g, 1.33 g/g and 0.63 g/g, respectively. Water absorption capacities of all PLPC samples for all locations were lower than that (2.3 g/g) of the leaf protein concentrate (LPC) of *Amaranthus hybridus*. (Nissen et al. 2021). However, location A and B values were higher than that (0.93 g/g) of *Telfairia occidentalis* LPC. (Emmanuel and Folasade 2011). Water absorption capacity is a critical property of proteins in food like soups, gravies dough, and baked products (Shubham et al. 2019), and oil absorption capacity is a critical determinant of flavor retention (Gnanwa et al. 2021).

Fat emulsion capacity and stability

Results showed that averages of the fat emulsion capacity for PLPC samples from the three locations were 37.47%, 41.82%, and 45.32% for locations A, B, and C, respectively, and they were a little bit lower than that of *Amaranthus hybridus* and *Telfairia occidentalis* LPCs with values of 47.5% and 48.7%, respectively (Jasour et al. 2017). However, the value for location C (45.32%) was similar to that of the LPC of *Cnidioscolusa conitifolius* with a value of 45.83% (Tan 2019).

Fat emulsion stability of PLPC samples for the three locations had a value of 100% and is higher than that of *Manihot esculenta* (Singh et al. 2020). This suggests that PLPC may be a good emulsifying agent and useful as a meat additive in sausage production, salad dressing preparation and pie fillings. The formation and stability of emulsion are very important in food systems such as salad dressing. Proteins are made up of charged amino acids (charged, non-charged polar and non-polar), which provide suitability as an emulsifier. In addition, the surfactant has both hydrophilic and hydrophobic properties, making it possible to interact with both water and oil in food systems (Nissen et al. 2021).

Least gelation concentration

The values of least gelation for samples from Location A, B, and C were 14.7%, 16% and 16%, respectively, and these values were higher than that of *Amaranthus hybridus* (0.93 g/g) and *Telfairia occidentalis* (6%), respectively (Nissen et al. 2021). These values were also higher than that of *Manihot esculenta* which had a value of 15% (Salehi et al. 2018). The protein gels provide a structural matrix for holding water, flavours, sugars, and food ingredients, and this is very useful in food application and new product development. However, the high least gelation observed in the PLPC samples may be a disadvantage for its use in the production of curd and cheese (Egharevba 2019).

Foaming capacity and stability

Results obtained showed that PLPC samples for Location A, B, and C had a foaming capacity of 22.49%, 8.77%, and 14.05%, respectively and were all higher than the 5% and 6% obtained for *Amaranthus hybridus* and *Telfairia occidentalis*, respectively (Jasour et al. 2017). However, these values were lower than that (32.1%) obtained for *Manihot esculenta* (Plamada and Vodnar, 2022). The foaming stabilities after 5 min were 98.78%, 89.39% and 91.11% for the PLPC samples of Location A, B and C, respectively: these values are very high. Foaming stability is an important determinant of

the solubility for a whipping agent in food systems. PLPC can thus be utilized because of its good foaming properties for products like whipped toppings, frozen desserts and sponge cakes where foaming is important.

Conclusion

In this study, pawpaw leaf protein concentrate (PLPC) was extracted from pawpaw leaves obtained from three locations. The result showed that PLPC contains saponins, flavonoids, and phytate; however, oxalate, tannins, and alkaloid were absent in phytochemical screening. Pawpaw leaf protein concentrate had desirable functional properties such as protein solubility, water/fat absorption capacity, fat emulsion capacity, fat emulsion stability, least gelation capacity, foaming capacity and stability. These results suggest that pawpaw leaf protein concentrate has strong potential as an alternative source of protein resources and may find important use as a food/feed material in human food and animal feed and serve as binders and extenders in food systems. However, further studies on the consumption safety of pawpaw leaf protein concentrate should be performed to ascertain its suitability in human diets.

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