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Biochemical effect of some food processing methods on the health promoting properties of under-utilized *Carica papaya* seed

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ABSTRACT

Papaya seed is a waste product of *Carica papaya Linn.* fruits that is highly abundant in Nigeria. This present study deals with the effect of some potential food processing methods (drying at 45-50^{o}C for 48.0 hours, and fermentation for 72.0 hours) on the seeds. Products from both processing methods, and the unripe seeds were examined for their biochemical properties compared to fresh sample. Significant reductions (P<0.05) were observed for pH of the unripe (5.541), and dried seeds (5.560); titratable acidity, tannin (0.935mg/gm) and acid phosphatase activity of fermented seed; oil level of unripe seed (5.271%). Significant increases (P<0.05) were observed for pH (6.437), oil level (25.600%), and alkaline phosphatase activity of fermented seed; polyphenol oxidase activities of dried and fermented seeds; tannin of unripe (1.265mg/gm); titratable acidity of unripe and dried seeds; phytin levels of all the products. In conclusion**,** the seed, and the fermentation product may be useful for bio-fuel, medicinal and industrial purposes.

Keywords: *Carica papaya* seed; Processing; Waste reduction; Phytonutrients.

INTRODUCTION

Carica papaya belongs to the fruits and vegetable class, it is highly abundant and is commonly known as pawpaw in Nigeria. About 30-100% of the fruits and vegetables are been wasted in Nigeria (Oluwalana, 2006). It is an invaluable plant that is prevalent throughout tropical Africa. Nigeria continues to be the third largest producer of papaya globally, and its level of production in Nigeria has been estimated to be 765,000 metric tonnes (FAO, 2007). Practically every part of the papaya plant is of economic value. Its uses range from nutritional to medicinal. Both fruit and seed of the ripe papaya are edible. The usefulness of fruits, roots and several parts of this plant has been largely reported with some minor negative effects (Onibon, 2007). However its seeds are currently undervalued in this part of the world and so there are no standardized processing methods available at present.

The papaya seed is currently a waste product as it is often discarded after eaten the papaya fruits due to its very limited uses at the moment. In Hawaii for example the seeds constitute 22% of the waste from papaya puree plants, and oil extraction has been examined as a possible method of utilization (FAO, 1992, Marfo, et al., 1986). Papaya seed are recently gaining importance due to its medicinal value. The seed had recently been linked to curing sickle cell dieseases (Imaga, et al., 2009), poisoning related renal disorder (Olagunju, et al., 2009), and as an anti-helminthes (Okeniyi, et al., 2007). There are scarce informations on this relatively underutilized seed despite its importance. The phytochemical screening of papaya seed had been reported (Imaga, et al., 2009), but had not yet been quantified. This study was designed to examine the effect of some potential processing methods of the papaya seed on the physicochemical, phytochemical, and biochemical properties of the seed.

MATERIALS AND METHODS

Chemicals: The following chemicals Ninhydrin and tin chloride dihydrate were obtained from Merck Chemicals, while Sodium deoxycholate, Methylcellulose powder, Folin Cioucalteus reagent, cathecol, Starch, Bovine albumin were obtained from BDH Laboratory Chemical division (Poole, England), Johnson metthey Company, SISCO Research Laboratory PVT Ltd (Mumbai India), Sigma Adrich Incorporation (U.S.A), Mallinkrodt Baker Incorporation (U.S.A) and Biotec Laboratories Ltd (U.K) respectively. All other chemicals were of analytical grade.

Sources of papaya seed: The unripe papaya seeds were collected from papaya fruits that was harvested within the Covenant University environment, Nigeria. The *Carica papaya* was identified by scientist in the Applied Biology and Biotechnology Unit of the Biological Sciences Department, Covenant University, Nigeria. The mature ripe seeds were collected as waste products from fruits salad sellers in a Covenant University cafeteria, Nigeria.

Processing of papaya seed: The fruits were cut open and the seeds were removed and placed in a bowl. The seeds were coated with a slimy sac-like substance. The seed coat was removed by bursting with the aid of a mortar and pestle, followed by vigorous washing to remove debris producing a clean seeds.

Method of drying: the cleaned seeds where placed on a foil paper and put in the oven at a temperature of 45-50°C for 48 hours to dry. The dried seeds were stored in an air tight container at ambient temperature prior to their analysis.

Method of fermentation: the cleaned seed were soaked in a plastic container for 72.0 hours following the usual practice of processing sorghum, maize, and millet into pap in Nigeria. The fermented seeds were dehulled using mortar and pestle, and the endosperm were obtained unbruised using decantation method. The final products obtained from both the drying and fermentation methods, and the unripe seeds were assessed for physicochemical, phytochemical, and biochemical properties. Three replications were used for the analysis and the results obtained were subjected to analysis using ANOVA statistical software package.

Methods of analysis: pH of extract was determined by the method of Thompson, (1984). Titratable acidity and oil content were determined by the method of A.O.A.C, (1980). Phytate and Tannin were determined by the method of Reddy and Love, (1999), modified by Nkamiya, et al., (2007). Oxalate was determined by the method of Agbede, (2007); Alkaloids was determined by the method of Haborne, (2007) modified by Adeniyi, et al., (2009); Alkaline and acid phosphatase activities were determined by the method of Mishra and Dubey, (2008). α-Amylase activity was determined by the method of Okolie and Ugochuckwu, (1988). The extract of all the enzymes assayed was analysed for protein using Biuret methods of Gornall, et al., (1949).

RESULTS AND DISCUSSION

Physicochemical properties

pH: The pH of both the unripe, and dried seeds (Table 1) were significantly lowered (P<0.05), while that of the fermented seeds were significantly increased (P<0.05). The low pH value of the unripe papaya seeds, and that obtained from drying is an indication of increased organic acids. This is evidence in our result (Table 1) where the pH values were inversely related to that of the titratable acidity. Increase in organic acids had been linked to pH reduction in fruits and vegetables (Ke, et al., 1994; Osuji, 1985; Steinkraus, 1996). Also, the unripe papaya seeds, and products obtained from drying may be of longer shelf-life due to the presence of more organic acid. The high pH value of fermented seed may be responsible for its high and low alkaline and acid phosphatase activities respectively; and vice versa for the unripe and dried seeds.

Titratable acidity: Titratable acidity is an indication of the level of sourness of fruits (Rangana, et al., 1981). The titratable acidity (Table 1) of fermented and dried seed were significantly (P<0.05) reduced, and increased respectively. The unripe and dried seeds were higher in titratable acidity compared to the mature fresh seed, while the product obtained from the fermented seeds (endosperm) possessed low titratable acidity level. Thus, the fermented products will be less sour in taste, unlike that obtained from drying that showed possibilities of been highly sour in taste. However, the seed obtained from the mature-unripe seed may be useful for the same purposes as that obtained from the ripe fruits putting into consideration its level of sourness, since its titratable acidity was similar to that of the seed obtained from the ripe fruits.

Table-1: Physicochemical Properties of Ripe and Unripe Papaya Seeds.

Physicochemical Parameters	Mature ripe	Unripe	Dried	Fermented
pH of extract	5.670± 0.017	5.541 ± 0.101	5.560 ± 0.046	6.437 ± 0.058
Titratable acidity (ml/100ml extract)	3.750 ± 0.500	7.000 ± 3.160	9.000 ± 3.610	1.500 ± 1.000
Oil content (%)	12.700± 3.253	5.271 ± 0.783	9.948 ± 0.498	25.600 ± 0.849
Phytate (mg/gm)	3.334 ± 0.152	4.484 ± 0.294	10.452 ±0.366	6.496 ± 0.042
Tannin (mg/gm)	1.465± 0.065	1.265 ± 0.020	1.310 ± 0.065	0.935 ± 0.035
Oxalate (mg/gm)	0.495 ± 0.090	0.562 ± 0.045	0.585 ± 0.116	0.450 ± 0.074
Alkaloids (%)	4.019 ± 1.050	2.874 ± 0.009	5.198 ± 1.031	6.693 ± 0.504
Protein (mg/gm)	0.183 ± 0.095	0.347 ± 0.184	0.417 ± 0.151	0.693 ± 0.134

- **1 Value is a mean of three determinations ± Standard Deviation.**
- **2 P = 0.05**

Oil content: The fermentation method increased significantly (P<0.05) the oil value of the papaya seed (Table 1). However, the oil value of the seed was significantly reduced (P<0.05) at the unripe stage. Edible vegetable oils are foodstuffs that are composed primarily of glycerides of fatty acids, and small amounts of other lipids such as phosphatase, unsaponifiable constituents and free fatty acids (CODEX, 2001). The fermentation methods was able to appreciate the level of the oil yield by 50.4% over that obtained from the fresh fruits. The oil yield was however, low in the unripe seed. The 25.600% oil value of the fermented papaya seed product is comparable to the 32.303%, and 33.490% reported for groundnut and palmkernel respectively (Afolabi, 2008). This value may be of commercial importance to Nigeria and the world at large especially for biofuel, and medicinal purposes in the future. The oil value reported in this work is similar to that reported for the papaya seed in Florida varieties (25.3%), and Senegal varieties (28.8%) (FAO, 1992). The oil is pale yellow and almost odourless and flavorless (FAO, 1992). The seed obtained from the mature unripe papaya fruits is not recommended for the purpose of oil generation since the oil yield is very low.

The endosperm from papaya seed had been reported to contain 60% fat with very high levels of oleic (79.1%) and palmitic acids (16.6%) (Passera and Spettoli, 1981; Puangsri, et al., 2005), and to some extent stearic (4–5%) and linoleic (2.5–3.5%) acids. The main triacylglycerols (TAGs) were sn-glycerol-oleate-oleate-oleate (OOO) (43.5–45.5%) and 1-palmitoyl-dioleoyl glycerol (POO) +stearoyl-oleoyl-linoleoyl glycerol (SOL) (29.5–30.5%) (Puangsri, et al., 2005). The papaya seed oil contains mainly about 70.7% unsaturated fatty acids (FAO, 1992). Reports from rat feeding trials that caused enlarged livers and kidneys indicated that the oil may contain toxic components that would make it unsuitable for use in human foods (FAO, 1992). It is our believe that these toxic component could have been eliminated by fermentation process. Fermentation is often used as a value added processing method to improve the quality and safety (e.g. cyanide reduction) of some food crops like sorghum, millet (Ahmed, et al., 1996; Obizoba and Atti, 1991) and cassava (Achi and Akomas, 2006; Onyesom, et al., 2008). It has been suggested that oil extraction from papaya seeds could improve the viability of industry in countries where it is cultivated for papain production and processing (Marfo, et al., 1986).

***Phytonutrients*:** Both the treatments (drying, and fermentation) and the early harvesting at unripened stage significantly increased ($P<0.05$) the phytin content of papaya seeds (Table 1). There was a significant decrease ($P<0.05$) in tannin concentration in both the mature unripe, and the fermented seeds. Drying at 45-50^{o}C however did not affect tannins content in papaya seed (Table 1). Both treatments (drying, and fermentation), and harvesting at the unripe stage showed no significant effect ($P>0.05$) on alkaloids, and oxalate content of papaya seed (Table 1). The 0.495, 0.562, 0.585, and 0.450 mg/gm sample oxalate contents observed in this work for the mature ripe seed, unripe seed, dried seed, and the endosperm obtained from fermentation of *Carica papaya* respectively, is considered very low compared to that reported for the following commonly consumed food products (Adeniyi, et al., 2009). *Solanum tuberosum* L.(32.5 mg/gm fresh wt.), *Ipomea batatas* L. (20.3 mg/gm fresh wt.), *Discorea alta*(24.3 mg/gm fresh wt.), *Discorea rotundata*(33.8mg/gm fresh wt.), *Colocasia esculenta* L. (40.5 mg/gm fresh wt), *Triticum vulgare* (27.0 mg/gm fresh wt), *Soja hispida* (85.10 mg/gm fresh wt.), *Amarathus* sp. (91.90 mg/gm fresh wt). The papaya seeds may therefore be considered safe for livestock and human consumption. Excessive consumption of oxalate rich foods had been linked to the development of kidney stone disease (Adeniyi, et al., 2009).

Also, the 4.019%, 2.874%, 5.198%, and 6.693% alkaloids contents observed in this work for the mature ripe seed, unripe seed, dried seed, and fermented endosperm of *Carica papaya* respectively, are also considered very low compared to that reported for some commonly consumed food products (Adeniyi, et al., 2009) like *Solanum tuberosum* L.(29.50%), *Ipomea batatas* L. (19.4%), *Discorea alta* (11.4%), *Discorea rotundata* (12.8%), *Colocasia esculenta* L. (25.5%), *Triticum vulgare* (14.8%), *Soja hispida* (25.2%), *Amarathus* sp. (28.5%). The *Carica papaya* seeds may therefore be considered safe for livestock and human consumption.

Phytate had been reported to possessed antioxidant activity (Coelho, et al., 2008). The high phytin levels of the unripe seeds (Table 1) can be attributed to its use in preference for the ripe *Carica papaya* seed in medicinal medicine for curing diseases. The reduction of tannin concentrations of fermented papaya seeds is expected, since earlier report indicated that processing methods such as soaking, boiling and fermentation lowered the tannin contents of foods (Jude, et al., 2009). In addition, the reduction was also observed in the unripe sample. The recommended tannin level for whole sorghum grains was max. 0.5% on a dry matter basis (CODEX, 1989). Steroid saponins and other phytonutrients are common in plants used as herbs or for their health-promoting properties and their antimicrobial activity has long been recognized (Zohar, et al., 2005). They have been suggested to constitute part of some plant defense systems.

Biochemical properties

***Phosphatase*:** Only the fermented seeds were significantly increased (*P*<0.05) in alkaline phosphatase activity (Table 2). Conversely, fermented seeds were significantly reduced (*P*<0.05) in acid phosphatase activity (Table 2). The high pH value of fermented seed (Table 1) may be responsible for its high and low alkaline, and acid phosphatise activities (Table 2) respectively; and vice versa for the unripe and dried seeds. The enzyme acid phosphatase has been reported to have an intracellular as well as an extracellular localization. Both enzymes respond dramatically to cellular inorganic phosphate (Pi) status. Acid phosphatase is induced by low Pi status (Mishra and Dubey, 2008). Hence, high phosphate status will suppress acid phophatase activity. It can therefore be implied that the processing method has affected the level of phosphate status of the seed**.** The change in pH reported in this work may also effect a change in conformation of alkaline phosphatase enzyme. Consequently, leading to its change in activity. Alteration in the activity of acid phosphatase in plants has been observed under variety of stressful conditions including under toxicities due to influence of various metals (Mishra and Dubey, 2008).

Table-2: Biochemical Properties of Ripe and Unripe Papaya Seeds.

Biochemical Parameters	Mature ripe	Unripe	Dried	Fermented
Alkaline phosphatase activity (nmol./min./gm)	1073.238 ± 16.785	1043.285 ± 5.180	1091.888 ± 9.590	1999.535 ± 13.705
Acid phosphatase activity (nmol./min./gm sample)	866.623 ± 13.198	824.735 ± 9.808	829.388 ± 29.155	811.703 ± 3.225
α-Amylase activity (μg/min./gm)	7.50 ± 5.17	16.33 ± 3.83	18.00 ± 9.33	15.33 ± 2.33
Polyphenol oxidase activity(μmol./min./gm)	0.00	0.00	12.00 ± 0.00	0.84 ± 0.00

- **1 Value is a mean of three determinations ± Standard Deviation.**
- **2 P = 0.05**

***α-Amylase*:** Both the drying and fermentation methods, and harvesting at unripe stage showed no significant effect (*P*>0.05) on α-amylase activity in papaya seed (Table 2). All the processing methods will not affect the rate of production of soluble sugars in the seed since α-amylase is responsible for breaking down of starch to simple sugars **(**Osuji, 1985). The low α-amylase activity reported in the papaya seeds is an indication of its low starch content. Thus, the processing methods (fermentation, and drying) will not affect the qualities (viscosity, and texture) of the final products obtained from the seed.

***Polyphenol oxidase*:** Both the drying and fermentation methods significantly increased (P<0.05) polyphenol oxidase activity (Table 2) in papaya seed. The development of polyphenol oxidase activity had been linked to increased level of having enzymatic browning during processing, storage, and increased ability of plants resistance to invading microorganisms (DeEll, et al., 2003; Suliman, et al., 2004; Thipyapong, et al., 2007). The increased polyphenol oxidase activities in papaya seeds subjected to drying and fermentation is an indication of a development of browning in papaya seed products obtained from both processing methods. The heat of drying had earlier been attributed to the increased level of polyphenol oxidase activity in dried tomato (Oloyede and Afolabi, 2001), as observed in the dried papaya seeds.

CONCLUSIONS

This work reveals that fermentation method can be use to facilitate increase oil yield from papaya seed. Harvesting at the unripe stage is not encouraged, if the seed is required for oil production. The seed, and the oil rich products obtained through fermentation may be useful for both biofuel and industrial purposes. The reasonable level of phytonutrients in the seed, and its products may account for its medicinal value. *Carica papaya* seeds possessed very low value of tannins, and oxalate. But, the phytate values were generally on the higher side that may be responsible for its medicinal value. Also, drying at 45-50^{o}C can be recommended as an appropriate processing method for papaya seed to reduce its level of wastage because it does not negatively affect its phytochemical and biochemical properties.

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