INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 11, p. 154-163, 2013

RESEARCH PAPER OPEN ACCESS

Effect of drying methods on the phytochemicals composition and antioxidant activities of *Carica papaya* seed

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Key words: *C. papaya* seeds, medicinal plants, drying methods,phytochemicals, antioxidant activities.

doi: http://dx.doi.org/10.12692/ijb/3.11.154-163 Article published on November 17, 2013

Abstract

The effect of drying methods, namely, air-drying (AD), freeze-drying (FD), oven-drying (OD) and sun-drying (SD) on the phytochemicals composition and antioxidant activities of *Carica papaya* seeds, a medicinal plant, was investigated. Drying methods had significant ($P < 0.05$) effect on the antioxidant phytochemicals, that is, total phenol, tannin, total flavonoid, total carotenoid, and vitamin C levels of the seeds, with freeze-drying resulting in the highest levels of these phytochemicals followed by AD, OD and SD. Oven-drying retained the highest ($P < 0.05$) level of total saponin; while the total saponin levels due to AD, FD, and SD were comparable ($P > 0.05$). Sun-drying led to the highest ($P < 0.05$) level of total alkaloid, followed by FD and OD which were comparable ($P > 0.05$), and then AD. Consistent with the trend of the effect of the drying methods on the antioxidant phytochemicals, FD displayed the highest ($P < 0.05$) levels of antioxidant activities - 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging ability, trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP), followed by AD, OD and SD. The DPPH scavenging ability of the seeds due to drying methods increased in a dose-dependent manner, having IC_{50} in the order of FD (14.19 mg/ml) < AD (15.7 mg/ml) < OD (16.56 mg/ml) < SD (17.85 mg/ml). Freeze-drying could be the preferred drying method for *C. papaya* seeds for a higher retention of its antioxidant properties.

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Introduction

Carica papaya Linn. (pawpaw) is a plant that belongs to the family of *Caricaceae*. It is an herbaceous succulent plant with self-supporting stems (Dick, 2003) which grows in all tropical countries and many sub-tropical regions of the world. The ripe fruit is edible and unripe (which is a rich source of vitamin A) can be eaten cooked (Lohiya *et al.,* 2002). C. papaya is largely used in tropical folk medicines (Jaime *et al.,* 2007), and several reports exist on its medicinal and therapeutic activities including anti-diabetic (Gbolade, 2009), antihyperlipidemic (Banerjee *et al.,* 2006), hypoglycemic (Adeneye and Olagunju, 2009), nephroprotective (Olagunju *et al.,* 2009), bactericidal (Emeruwa, 1982) anti-oxidant (Majdi and Luciana, 2010), and anti-inflammatory activities (Anaga and Onehi, 2010).

Different parts of the plant are attributed with different medicinal values. For example, its fresh leaves are efficacious in the treatment of gonorrhea, syphilis and amoebic dysentery (Gill, 1992); the mature (ripe) fruit treats ringworm; the green fruits treat high blood pressure, and are used as an aphrodisiac (Singh *et al.,* 1980). The seeds are effective to control diabetes mellitus, hypertension and hypercholesterolemia (Gill, 1992). Extract of the seed is used to treat bleeding piles and enlarged liver and spleen, while the seed decoction is beneficial to cure liver and renal disorders (Adeneye *et al.,* 2009).
Several researchers have shown that medicinal plants contain phytochemicals with antioxidation potential which are responsible for their therapeutic effects (Iwu *et al.,* 1984). The major plant-derived chemical groups now recognized as having potential health promoting effects, at least under some circumstances, are the flavonoids, alkaloids, carotenoids, pre- and pro-biotics, phytosterols, tannins, fatty acids, terpenoids, saponins and soluble and insoluble dietary fibres (Basu *et al.,* 2007).

Medicinal plants can be used in fresh or dried form. Drying is the most common method for post-harvest preservation of medicinal plants, and must be accomplished as soon as possible after harvesting, to increase the quality of plants and to prevent the expected contamination and losses (Garg and Kumar, 2001). Factors such as scale of production, availability of new technologies and pharmaceutical quality standards must be considered for the drying of medicinal plants. It is a common practice to dry small quantities of medicinal plants without auxiliary energy in domestic drying procedures. However, for mass production, the use of technical drying applications is indispensable. Drying method plays an important role in the processing of medicinal plants as it could either lead to the conservation or loss of the bioactive compounds and their concomitant antioxidant capacity. Hence, this study was designed to assess the effect of four drying methods, namely, air-drying, freeze-drying, oven-drying and sun-drying on the phytochemicals composition and antioxidant activities *C. papaya* seeds.

Materials and methods

Sample collection and preparation

Fresh *C. papaya* seed samples were collected from the mature fruit in Idi-Ose village in Ibadan, Nigeria, in June 2012. The samples were authenticated at Department of Botany, University of Ibadan, Nigeria. Thereafter, the seeds were sorted and divided into four portions for drying.

Drying of C. papaya seeds

Fresh C. papaya seeds were dried to a constant weight by four different methods: air-drying at room temperature (AD), freeze-drying (FD), oven-drying and sun-drying (SD). Air-drying at room temperature was conducted in a dark, well-ventilated room for a period of two weeks. Freeze-drying was carried out in Edwards Freeze-dryer, Modulyo (England) under reduced pressure for 72 hours. Oven-drying was conducted in a hot-air oven (Gallenkamp, UK) at 50°C for 48 hours, while sun-drying was carried out by exposing the seeds to sun light for three days. After drying, all the dried samples were milled into a fine particle size (0.5 mm), put in air-tight bottles and stored at -4°C for subsequent analyses.

Preparation of methanolic extract

Methanolic extract of the seed was prepared following the method of Chan *et al.,* (2007), by adding 25 ml of methanol to 0.5 g of sample contained in a covered 50 ml centrifuge tube, and shaking continuously for 1 h at room temperature. The mixture was centrifuged at 3,000 rpm for 10 min, and then the supernatant (subsequently referred to as methanolic extract) was collected and store at -4°C until analysis.

All the chemicals used for analysis were of analytical grade.

Determination of total phenol content

The total phenol content of samples methanolic extracts was determined according to the Folin–Ciocalteu method reported by Chan *et al.,* (2007). Briefly, 300 µl of extract was dispensed into test tube (in triplicates). To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na_2CO_3 solution (7.5% w/v). The reaction mixture was shaken, and then allowed to stand for 30 min at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 µl of distilled instead of sample extract. Total phenol content was expressed as gallic acid equivalent (GAE) in mg/g material.

Determination of total flavonoid content

Total flavonoid content was determined using aluminum chloride method as reported by Kale *et al.,* (2010). 0.5 ml of methanolic extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was shaken, and then allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm. Total flavonoid content was expressed as quercetin equivalent (QE) in mg/g material.

Determination total saponin content

Total saponin was determined by the method described by Makkar *et al.,* (2007). 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 hours, after which contents of the tubes were centrifuged for 10 min at 3,000 rpm. In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 min. Then tubes were cooled in ice for 4 min and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g dry matter of sample.

Determination total alkaloid content

The total alkaloid content of the samples was measured using 1,10-phenanthroline method described by Singh *et al.,* (2004) with slight modifications. 100mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000 rpm for 10 min. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M $FeCl_3$ in 0.5 M HCl and 1ml of 0.05 M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2°C. The absorbance of red coloured complex was measured at 510 nm against reagent blank. Alkaloid contents were calculated with the help of standard curve of quinine. The values were expressed as mg/g of dry weight.

Determination of total carotenoid content

Total carotenoid content of samples was determined according the method of Rodriguez-Amaya (1999). Briefly, 0.2 – 0.3 g of sample was transferred into a mortar and a small amount (0.5 g) of celite was added. The mixture was ground with 20 ml of cold acetone and filtered with suction through a Buckner funnel with filter paper. The residue was further macerated with cold acetone, filtered into the same

suction flask repeatedly until the residue became colourless. After extraction, the acetone extract was partitioned with 10 ml of petroleum ether in a separatory funnel. The mixture was washed repeatedly with distilled water until acetone was completely washed and drained off, and then the total carotenoid was eluted with ether to a final volume of 25 ml. The absorbance of the extract was read at 450 nm. To avoid the degradation of the total carotenoid, analysis was carried out under subdued light and the extract was collected into a vial wrapped with aluminum foil.

Determination of vitamin C content

The vitamin C content of the aqueous extract was determined using the method reported by Benderitter *et al.,* (1998). Briefly, 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 ml of 5 M H_2SO_4) was added to 500 µl reaction mixture (300 µl appropriate dilution of hydrophilic extract with 100 µl of 13.3% trichloroacetic acid and distilled water). The reaction mixture was subsequently incubated for 3 hours at 37^oC, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the vitamin C content of the sample was subsequently calculated from the calibration curve prepared with ascorbic acid standard.

Estimation of DPPH free-radical-scavenging ability

The free radical-scavenging ability of the methanolic extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Cervato *et al.,* (2000) with slight modification. Briefly, appropriate dilution of the extracts (1 ml) was mixed with 3 ml of 60µM methanolic solution of DPPH radicals; the mixture was left in the dark for 30 min before the absorbance was taken at 517 nm. The decrease in absorbance of DPPH· on addition of test samples in relation to the control was used to calculate the percentage inhibition (% Inh.) following the equation:

$$\%Inh. = [(A517_{control} - A517_{sample}) \div A517_{control}] \times 100.$$

The IC_{50}, which stands for the concentration of extract required for 50% scavenging activity, was calculated from the dose-inhibition linear regression equation of each extract.

Estimation of ABTS radical-scavenging ability*

The ABTS* [2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)] radical-scavenging ability of both extracts were determined according to the method described by Sellappan and Akoh (2002). The ABTS* radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 h at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 ml appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance was measured at 734 nm after 15 min. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated.

Determination of ferric reducing antioxidant power (FRAP)

The reducing property of the methanolic extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50^oC for 20 min. and then 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. Then 5 ml supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated.

Statistical analysis

Experimental results were reported as Mean ± standard deviation of triplicate parallel measurements. Analysis of variance (ANOVA) and least significance difference (LSD) were carried out on the result data using SAS (2001) at 95% confidence level.

Results and discussion

Table 1 presents the effects of drying methods on the phytochemicals composition of *C. papaya* seeds. The results revealed that the four drying methods used

AD, FD, OD and SD affected the antioxidant phytochemicals, namely, total phenol, tannin, total flavonoid, total carotenoids and vitamin C in a similar manner, although to different extent.

Freeze-drying (FD) proved to be the drying method that preserved the highest levels of total phenol, tannin and total flavonoid, followed by AD, OD and SD. This finding is in accordance with that of Zhou *et al.,* (2011), who also reported that flavonoids and total phenolics were better conserved in loquat flowers by freeze-drying than other drying methods. Freeze-drying is one of the most sophisticated dehydration methods and can maintain many bioactive components, especially various heat-sensitive biological compounds, although the drying rate is relatively slow (Krokida and Philippopoulos, 2006). The development of ice crystal within the tissue matrix resulting from freezing may lead to a higher extraction efficiency of phenolics compounds. Ice crystals in turn could result in a greater rupturing of cell structure, which may lead to better solvent access and extraction (Shih *et al.,* 2009).

Table 1. Effect of drying method on the phytochemicals composition of *C. papaya* seeds on dry weight basis.

Phytochemical	AD	FD	OV	SD
Total phenol (mg/g)	1.77 ± 0.01^{b}	2.61 ± 0.04^{a}	1.65 ± 0.01^{c}	1.25 ± 0.02^{d}
Tannin (mg/g)	2.61 ± 0.02^{b}	3.1 ± 0.07^{a}	1.97 ± 0.01^{c}	1.26 ± 0.02^{d}
Total flavonoid (mg/g)	0.43 ± 0.01^{b}	0.52 ± 0.02^{a}	0.37 ± 0.01^{c}	0.26 ± 0.01^{d}
Total carotenoid (µg/g)	5.23 ± 0.04^{b}	8.12 ± 0.07^{a}	4.52 ± 0.01^{c}	3.49 ± 0.03^{d}
Vitamin C (mg/100g)	12.42 ± 0.24^{b}	14.69 ± 0.13^{a}	8.91 ± 0.08^{c}	6.71 ± 0.15^{d}
Total saponin (mg/g)	6.25 ± 0.06^{b}	6.28 ± 0.02^{b}	6.45 ± 0.02^{a}	6.3 ± 0.01^{b}
Total alkaloid (mg/g)	15.65 ± 0.07^{c}	15.87 ± 0.03^{b}	15.85 ± 0.03^{b}	16.91 ± 0.01^{a}

Data represent the mean ± standard deviation of triplicate readings; values with the same lowercase superscript letter along the same row are not significantly different ($P > 0.05$).

Reduction in total phenol tannin and total flavonoid levels resulting from air-drying could be attributed to enzymatic degradation due to the longer period it took to dry at room temperature (Chan *et al.,* 2009). High temperatures lead to oxidation of bioactive compound that are associated to antioxidant capacity (Yoshioka *et al.,* 1990); this could account for the losses in total phenol, tannin and total flavonoids of the oven-dried and sun-dried seeds.

Polyphenols are well known to exhibit antioxidant activity through a variety of mechanisms, including free radical scavenging, lipid peroxidation and chelating of metal ions (Shahidi *et al.,* 1997), in addition to having many other biological activities, such as anti-histamine (Nitta *et al.,* 2007), anti-inflammatory and anticarcinogenic (Srikanth *et al.,* 2010), and antibacterial activities (Romero *et al.,* 2007). They have also been reported to inhibit α-amylase, sucrase, as well as the action of sodium glucose-transporter 1 (SGLUT-1) of the intestinal brush border, hence their antidiabetic action (Tiwari and Rao, 2002).

The total carotenoid and vitamin C levels of *C. papaya* seeds were affected in a similar trend as the total phenol, tannin and total flavonoid (i.e. FD > AD > OD > SD). This is in agreement with the findings of Nawirska *et al.,* (2009) who reported that freeze-drying method resulted in the highest average carotenoid content in dried pumpkin. Chen *et al.,* (2007) also reported that lyophilization of mango fruit led to more carotenoids content than oven drying. In contrast, relative to other drying methods, sun-drying displayed the least level of total carotenoid. Rodriguez-Amaya and Kimura (2004) had earlier reported that exposure to light especially sun-light or UV light may induce trans-cis photomerisation and photodegradation of carotenoids. Carotenoids are lipid-soluble

antioxidant, which have been reported to have health benefits such as pro-vitamin A activity (Rodriguez-Amaya, 1997), antioxidant activity (Tian, 2007), anticancer effect (Nishino, 1998), and antiobesity effect (Jaswir, 2011). On the other hand, vitamin C is a water-soluble antioxidant which can scavenge reactive oxygen species and has anticarcinogenic effects (Kim and Lee, 2004; Lee *et al.,* 2001).

Oven-drying led to the highest (P >0.05) level of total saponin followed by AD, FD and SD which were all comparable (P > 0.05). A possible reason for a higher conservation of total saponin by OD relative to AD, FD and SD could be as a result of its faster drying process, thereby forestalling any possible degradation with time. Clinical studies have suggested that saponins have health-benefitting effects such as protection against cancers, lowering of cholesterol levels and reduction in blood glucose response (Shi *et al.,* 2004), and possess antioxidant properties (Blumert and Liu, 2003), although they also have some deleterious effects.

Table 2. Effect of drying method on the DPPH IC_{50}, trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) of methanolic extracts of *C. papaya* seeds.

Antioxidant activity	AD	FD	OD	SD
DPPH IC_{50} (mg/ml)	15.7	14.19	16.56	17.85
TEAC (µM TE/g)	51.37 ± 0.57^{b}	56.54 ± 0.24^{a}	47.42 ± 0.26^{c}	43.72 ± 0.16^{d}
FRAP (µg GAE/g)	113.33 ± 3.33^{b}	150.01 ± 1.45^{a}	100.02 ± 1.97^{c}	73.33 ± 2.61^{d}

Data for FRAP and TEAC represent the mean ± standard deviation of triplicate readings; TE is Trolox equivalent; values with the same lowercase superscript letter along the same row are not significantly different (P >0.05).

The highest (P >0.05) total alkaloid level was observed in SD followed by FD and OV which were both comparable (P > 0.05), and then AD. Beier (1990) reported that exposure of potatoes to light in the field or marketplace led to increased synthesis of the glycoalkaloids. Similarly, Balick *et al.,* (1982) reported that sun or herbarium drying was necessary to ensure that non-volatile alkaloids were retained within leaf material of *Erythroxylum*. Alkaloids are the most efficient therapeutically significant plant substances. Plant-derived alkaloids have such clinical uses as anticancer agents, gout suppressant, muscle relaxant, antiarrythmic, antibiotic, and sedative agents (Facchini, 2001). Sharma *et al.,* (2012) also reported that alkaloids are preventive or therapeutic agents against human free radical associated diseases. The antioxidant activities of the methanolic extracts of *C. papaya* seeds after the different drying methods are presented in table 2. In consonant with the total phenol and the other antioxidant phytochemicals results, drying methods had significant effects (P >0.05) on the antioxidant activities of *C. papaya* seeds. FD displayed the highest antioxidant activities followed by AD, OD and SD. The DPPH free radical scavenging ability of the methanolic extracts, tested at different concentrations, showed a dose-dependent scavenging ability as depicted by their concentration-response curves (Figure 1). Antioxidant reacts with DPPH, which is a stable free radical, and convert it to α,α-diphenyl-β-picryl hydrazine. As antioxidants donate protons to this radical, the absorption decreases. So the degree of discoloration indicates the scavenging potentials of the antioxidant extract. The half maximal inhibitory concentration (IC_{50}) of the extracts against DPPH were in the order of FD (14.19 mg/ml) < AD (15.7 mg/ml) < OD (16.56 mg/ml) < SD (17.85 mg/ml). Since IC_{50}, defined as the concentration of extract causing 50 per cent inhibition of DPPH absorbance, is a measure of inhibitory concentration, a lower IC_{50} value is a reflection of greater antioxidant activity of the sample (Irondi *et al.,* 2012). Hence, FD proved to retain the highest free radical scavenging ability whereas SD retained the lowest.

The ABTS* scavenging ability of *C. papaya* seeds due to the different drying method reported as the trolox equivalent antioxidant capacity (TEAC) (table 2)

revealed ABTS* scavenging ability in the order of FD > AD > OD > SD. ABTS radicals are known to be more reactive than DPPH radicals, and unlike the reactions with DPPH radical which involve H atom transfer; the reactions with ABTS radicals involve electron transfer process (Srikanth *et al.,* 2010).

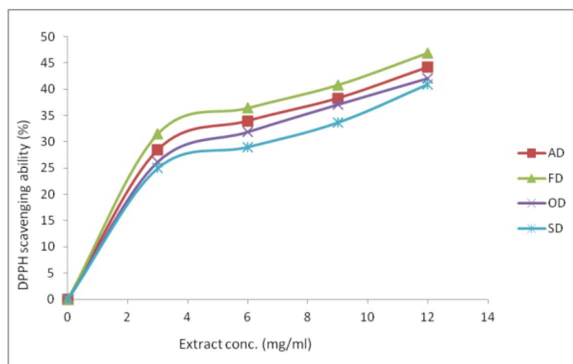

Fig. 1. Concentration-response curve of methanolic extracts of *C. papaya* seed from different drying methods against DPPH free-radical.

The reducing power (from Fe^{3+} to Fe^{2+}) of *C. papaya* seeds due to the drying method reported as gallic acid equivalents, GAE/mg dry weight (table 2) was in the same order of FD > AD > OD > SD. Decrease in antioxidant properties are often accompanied by the loss of other bioactive phytochemicals (Roy *et al.,* 2007) and these losses have been attributed to thermal degradation of phenolic compounds, to degradative enzymes, and to loss of antioxidant enzyme activities (Lim and Murtijaya, 2007; Larrauri *et al.,* 1997).

Conclusion

The results of this study show that drying methods namely air-drying, freeze-drying, oven-drying and sun-drying have effects on the phytochemicals composition and antioxidant activities of *C. papaya* seeds. Freeze-drying method resulted in the highest levels of antioxidant phytochemicals, (that is, total phenol, tannin, total flavonoid, total carotenoid, and vitamin C) than the air-drying, oven-drying and sun-drying methods. This consequently conferred the freeze-dried seeds with higher antioxidant activities than the other drying methods used in this study. It is therefore concluded that for optimal retention of antioxidant properties in *C. papaya* seeds for use in phytomedicine, freeze-drying might be the preferred drying method, although it is more sophisticated.

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