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Assessment of Antioxidant Properties of Tamarind Fruit Pulp and its Effect on Storage Stability of African Bread Fruit Seed dhal and Flour

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ABSTRACT

This study evaluated the antioxidant activities of tamarind fruit pulp in scavenging 1, 1-diphenyl-2-picryl hydroxyl radical (DPPH) and in suppressing thiobarbituric acid reactive substances (TBARS) formation, as a marker of lipid oxidation, in African breadfruit seed dhal and flour. Water and ethanol extracts of tamarind fruit pulp at different concentrations were used to scavenge DPPH radical. Parboiled (100°C; 15 min) breadfruit seeds were dehulled to seed dhal, oven-dried (50°C; 72 h), and half of the dhal milled into flour. Samples (100 g each) of the seed dhal and flour added and mixed together with aqueous suspensions (0, 1.0, 2.0, 3.0 or 5.0 g per 5 ml water) of tamarind fruit pulp were analysed for TBA values within 4 months of storage at 26 ± 2°C. The water and ethanol extracts scavenged DPPH in a dose-dependent manner. The ethanol extract had IC50 of 38.17 while the water extract had IC50 of 7.32, indicating much higher antioxidant activity of water extract. Tamarind fruit pulp inhibited lipid oxidation in breadfruit seed dhal and flour as evident from the mean thiobarbituric acid (TBA) value which decreased with increasing concentrations of the fruit pulp. Antioxidant activity of the fruit pulp was higher in the flour than in the dhal within 4 months of storage. Both seed dhal and flour treated with tamarind fruit pulp had lower mean TBA values ranging from 2.80 to 4.12 ppm Malonaldehyde as against 4.55 to 4.91 ppm for untreated samples. Tamarind fruit can thus be further studied for possible exploitation as a natural antioxidant for use in food, drug and cosmetic products.

Keywords: Tamarind, antioxidant activity, breadfruit.

Introduction

African bread fruit (*Treculia africana*) is native to the tropical region and produces abundant fruits from which the edible seeds are harvested. The seeds are of high nutritional value and are currently used as components of the daily diets of many Nigerians (Iwe and Ngoddy, 2001). The flour is a good complement to wheat flour for bakery products (Giami *et al.*, 2004). Unfortunately, the seeds are oil seeds and when processed (dhal and flour) easily become rancid on storage due to lipid oxidation. Lipid oxidation results in decreased nutritional and physiological values of lipids, deterioration of fat-soluble vitamins and essential fatty acids (Hudson, 1990). It has been reported that products of lipid oxidation can cause pathological changes in the nucleus membrane of the alimentary tract, inhibit the activity of enzymes and increase the contents of cholesterols and peroxide in blood serum, thus activating the process of arteriosclerosis and aging (Christen, 2000; Karpinska *et al.*, 2001). Products of lipid oxidation can also initiate carcinogenic activities and cause many chronic diseases in human body (Jacob, 1995; Reosler *et al.*, 2005).

Application of antioxidants is one of the simplest means of reducing lipid oxidation in foods (Karpinska *et al.*, 2001). Antioxidants delay or inhibit oxidative damage in living cells, including the

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human body, even when present in small amounts. Antioxidants can be of synthetic or of natural origin. Natural antioxidants are more ecologically friendly, less costly and safer than synthetic ones (Dorlo, 1994). Both epidemiological and clinical studies have shown that phenolic antioxidants present in plant materials, including some food grains, fruits, vegetables and spices are principal contributing factors accounting for reduced incidence of many chronic diseases encountered by population whose diets are composed of high intake of many of these plant products (Maillard *et al.*, 1996; Shahidi and Naczk, 1996; Halvorsen *et al.*, 2002). Some well-known spices, including rosemary (*Rosemarinus officinalis*), majoram (*Origanum majoram*), garden thyme (*Thymus vulgaris*) and sage (*Salvia officinalis*), have long exhibited antioxidant activity in food and biological systems (Al-Jalay *et al.*, 1987; Oktay *et al.*, 2003; Dwivedi *et al.*, 2005). However, many indigenous spices in the tropics, particularly in Nigeria, have not been fully exploited for their antioxidant potential. Tamarind (*Tamarindus indica*) is one of such spices that have not been fully investigated for their antioxidant properties. Tamarind fruit pulp is used locally in spicing many beverages such as "kunu zaki" or prepared into fruit drinks in Northern Nigeria.

The objective of this study was to investigate the antioxidant properties of tamarind fruit pulp in extending the shelf life of *Treculia africana* seed dhal and flour.

Materials and Methods

Materials

Dry seeds of African breadfruit (*Treculia africana Decne*) seeds were purchased from peasant farmers at Ogbede-Aku market of Igbo-Etiti Local Government Area (LGA), Enugu State, while tamarind fruits (*Tamarindus indica Linn*) were obtained from the Kwasaka village farm outreach of the Faculty of Agriculture, Nasarawa State University, Keffi, all in Nigeria.

Extraction and preparation of tamarind fruit pulp

The fruits pods were dehulled after which the sticky pulps were scraped from the seeds manually and milled into a smooth paste in an attrition mill. The paste was oven-dried (65°C) for 72 h, re-milled and packed in airtight container. Part of the milled fruit pulp powder (10 g) mixed with 200 ml of distilled water or ethanol was extracted for 10 min, using a kitchen blender; rested for 24 h, and extracted for another 20 min before filtering through a double fold Whatman No. 5 filter paper into a holding flask for use for antioxidant study.

Determination of radical scavenging activity

Radical scavenging analysis was determined using the radical 1, 1-diphenyl-2-picryl hydrazyl (DPPH) (Sgma Chemical Co., St Louis, Mo, USA). The analysis was carried out as described by Van der Sluis *et al.* (2000). From the aqueous and ethanol extracts, ethanol solutions in different concentrations (1.0 μL to 200 μL) were prepared by adding 1000 μL of DPPH (0.004% w/v), and the final volume brought to 1200μL with ethanol in test tubes. The tubes were incubated for 30 min at room temperature (26 ± 2°C) in the dark and then transferred to cuvettes for absorbance readings at 517 nm for the DPPH solution and the resulting mixture, using ethanol as a blank Absorbance Radical scavenging, was expressed as antioxidant activity (AA%) which is a percentage inhibition relative to the control, and was calculated using the formula (Yen and duh, 1994):

$$\% \text{ Inhibition} = [(A_{DPPH} - A_{EXTR}) / A_{DPPH}] \times 100$$

Where ADPPH is the absorbance of the DPPH blank sample, and AEXTR is the absorbance value of the test solution. AEXTR was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank.

Sample preparation

Seeds (1.25 kg) of African breadfruit (*Treculia africana Decne*) were cleaned and parboiled in excess boiling water for 15 min, drained out of water and cracked in a hand-operated (kitchen) colloid mill to

remove the hulls from seeds. The dehulled seeds (dhal) were oven-dried at 50°C for 48 h, and half of the dried dhal milled into flour. Duplicate samples, each of 100 g, of the seed dhal and flour were separately mixed with different concentrations (0 g [control], 1 g, 2 g, 3 g or 5 g) of tamarind fruit pulp using a kitchen (Kenwood) mixer. Samples were bagged in nylon sacks and stored at 26 ± 2°C for 6 months in the laboratory for oxidation study.

TBA test

Thiobarbituric acid values (TBA) for thiobarbituric acid reactive substances (TBARS) as an indicator of oxidation were determined (Buege and Aust, 1978) on the 3rd, 5th, 10th, 17th, 25th and 31st day, and again on the 2nd, 3rd and 4th month of the storage period. Five grams of each sample was mixed with 2.5 ml of the stock solution containing 0.375% TBA (Sigma Chemical Co., St. Louis Mo, USA), 15% Trichloro-acetic acid (TCA) (Mallinkrodt Beker Inc., Paris Ky, USA) and 0.25 N HCL. The mixtures were heated for 10 min in a boiling water bath (100°C) to develop a pink colour, cooled in tap water and centrifuged (Beckman Coulter Ltd Palo, Alto, California, USA) at 3000 rpm for 20 min before taking the absorbance of the supernatants at 532 nm (Spectronic 21d, Multon Roy, Rochester Ny, USA) against a blank that had no fruit pulp. Absorbance values were multiplied by a factor of 7.8 to give the TBARS values (Van der Sluis *et al.*, 2000).

Data analysis

The data were analysed by ANOVA and Fisher's least significant difference was used to ascertain significant effects at $P < 0.05$, using SAS 2005 (Version 9.1, SAS Inst. Inc., Cary; N.C, U.S.A.).

Results and Discussions

DPPH radical scavenging activity

A radical scavenging activity towards the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical was used in this study because it was a very simple, sensitive and very rapid method; as already noted to be very convenient and easily reproducible in screening antioxidant activities of many samples with different constituents of different polarities (Koleva *et al.*, 2002). Free radical scavenging potential of ethanol and water extracts of tamarind fruit pulp at different concentrations were tested with the DPPH method; and the results expressed as final concentrations of test material are shown in Figure 1. Antioxidants react with DPPH, a stable free radical, and convert it to 2, 2-diphenyl-1-picryl hydrazine. The degree of discolouration of DPPH in solution indicates the scavenging potential of antioxidant extracts. Both the aqueous and ethanol extracts of tamarind fruit pulp had exceptionally high scavenging activity. A stronger radical quenching agent generally resulted in a lower IC_{50} value. The aqueous extract (IC_{50} = 7.32) had a higher antioxidant activity than the ethanol extract (IC_{50} = 38.17). Regression analysis of scavenging activity of both aqueous and ethanol extracts of the fruit pulp is shown in Figure 1. Scavenging activity of both extracts was highly linear dependent on concentrations of the spice pulp. The slopes for aqueous and ethanol extracts were 5.123 and 1.230 respectively. Comparison of the slopes of linear regressions of both extracts showed significantly ($P < 0.05$) higher scavenging activity of the aqueous extract than the ethanol extract.

Figure 1 shows the mean TBA values of *Treculia africana* seed dhal treated with tamarind spice fruit pulp under storage for 25 days. Mean TBA values decreased with increased tamarind fruit pulp concentration in the samples. Also mean TBA values increased linearly with storage days. The control, ST00, had higher mean TBA value than samples treated with tamarind fruit pulp. The suppressive effect of 3% and 5% concentration of tamarind fruit pulp against oxidative spoilage of treculia seed dhal were approximately twice those of 1% and 2% fruit pulp used respectively. It is evident that tamarind fruit pulp proffers antioxidant effect against fat rancidity in *Treculia africana* seed dhal. The antioxidant effect was dose-dependent but loses potency with prolonged storage time. The radical scavenging ability of these spice extracts seem to indicate that they could act as

functional foods, promoting good health with their therapeutic properties (Geil and Anderson, 1994). Plant extracts with radical scavenging activities are likely to show low glycemic indices (Foster Powell and Miller, 1995), hypocholestolaemic effects (Anderson *et al.*, 1999), breast cancer prevention (Adebunawo *et al.*, 2005) and health benefits with respect to cardiovascular diseases (Kushi *et al.*, 1999; Huang *et al.*, 2005) and bone health (Alekel *et al.*, 2000). Tamarind fruit pulp is likely to exhibit low glycemic index, hypocholestolaemic effect and could prevent breast cancer as is the case with many (Foster Powell and Miller, 1995; Anderson *et al.*, 1999; Adebunawo *et al.*, 2005).

Antioxidant effect of tamarind fruit pulp on Treculia africana seed dhal and flour during storage

Treatment (fruit pulp extract), pulp concentrations (0%, 1%, 3%, 4%, 5%) and days (3, 5, 10, 17, 25) of storage (26 ± 2°C) significantly ($p < 0.05$) affected mean TBA values of *Treculia africana* seed dhal and flour (Table 1). Interactions of spice pulp concentration X day, and treatment X spice concentration X day of storage also affected ($p < 0.05$) TBA values.

Figure 2 shows the mean TBA values *Treculia africana* seed dhal treatment with tamarind spice fruit pulp under storage for 25 days. Mean TBA values decreased with increased tamarind fruit pulp concentration in the samples. Also, mean TBA values increased linearly with storage days. The control, ST00, had higher mean TBA value than samples treated with tamarind fruit pulp. The suppressive effects at 3% and 5% concentration of tamarind fruit pulp against oxidative spoilage of treculia seed dhal were approximately twice those of 1% and 2% fruit pulp used respectively. It is evident that tamarind fruit pulp proffers antioxidant effect against fat rancidity in *Treculia africana* seed dhal. The antioxidant effect was dose-dependent but loses potency with prolonged storage time.

Figure 3 shows the mean TBA values of *Treculia africana* seed flour treated with aqueous fruit pulp of tamarind stored at 26 ± 2°C for 25 days. Mean TBA values of flour treated with tamarind fruit pulp decreased significantly ($p < 0.05$) with increasing concentrations of the pulp. On the 25th day of storage, FT2.0 (5% fruit pulp) had mean TBA value of 0.78 while the control (no fruit pulp) had mean TBA value of 1.34. Mean TBA value increased progressively with storage time but was dose-dependent with fruit pulp concentration in the samples.

Table 1: Summary of significance ($p < 0.05$) as determined by analysis of variance (ANOVA) for *Treculia africana* seed dhal and flour stored at 26 ± 2°C

Seed dhal	Df	TBA	P level
Treatment	1	*	0.001*
Spice pulp concentration	4	*	0.001*
Day of storage	4	*	0.001*
Spice pulp conc. x day	16	*	0.001*
Treatment x spice pulp conc. x day	24	*	0.001*
Seed flour			
Treatment	1	*	0.001*
Spice pulp conc.	4	*	0.001*
Day of storage	4	*	0.001*
Spice pulp conc. x day	16	*	0.001*
Treatment x spice pulp conc. x day	24	*	0.001*

Table 2 shows the mean TBA values of both *Treculia africana* seed dhal and flour stored at 26 ± 2°C for 4 months. Mean TBA values of flours were significantly lower ($p < 0.05$) than those of dhal. On the 4th month, mean TBA values for 1% fruit pulp addition was 1.46 in dhal (ST1) and 1.17 in flour (FT1). Also for 3% pulp addition, mean TBA values were 1.13 in dhal (ST3) and 1.07 in flour (FT3). However, with 5% pulp addition on the same 4th month, dhal sample conversely had lower TBA value ($p < 0.05$) than flour (Table 2). Tamarind fruit pulp furnishes protection against fat rancidity in both *Treculia africana* seed dhal and flour; and its antioxidant effect is dose-dependent, increasing with fruit pulp concentration. Antioxidant activities of plant materials, including spices, is attributed to their polyphenol contents (Oboh, 2006). Natural polyphenols exert beneficial health effects by their antioxidant activity. Polyphenols are capable of removing free radicals, chelae metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and oxidants (Yamaguchi *et al.*, 1998, Oboh, 2006). Their antioxidant properties are principally based on the redox potential of their hydroxyl groups and the structural relationship between different parts of their chemical structures. Polyphenols inhibit auto-oxidation of unsaturated lipids, thus preventing the formation of oxidised low density lipoprotein (LDL) which is considered to induce cardiovascular diseases in man (Steinbrecher, 1987; Huang *et al.*, 2005).

Table 2: Changes in TBA values (n = 4) of *Treculia africana* seed dhal and flour treated with different concentrations of tamarind fruit pulp

Sample code	Fruit pulp (%v/w)	Storage time (Months) 0	1	2	3	4
ST0	0.0	0.78 ± 0.02^{d}	0.89 ± 0.04c	1.02 ± 0.02^{b}	1.31 ± 0.02^{c}	1.62 ± 0.02^{b}
ST1	0.5	1.05 ± 0.02^{a}	0.95 ± 0.02^{d}	0.96 ± 0.01^{c}	1.46 ± 0.09^{b}	1.46 ± 0.01^{c}
ST2	1.0	0.86 ± 0.01^{c}	0.93 ± 0.01^{d}	0.95 ± 0.02^{c}	1.07 ± 0.01^{d}	1.35 ± 0.01^{d}
ST3	1.5	0.77 ± 0.02^{d}	0.90 ± 0.02^{e}	0.80 ± 0.02^{d}	0.95 ± 0.01^{e}	1.13 ± 0.02^{f}
ST4	2.0	0.74 ± 0.01^{e}	0.78 ± 0.01^{f}	0.77 ± 0.02^{e}	0.84 ± 0.01^{g}	0.99 ± 0.02^{i}
FT0	0.0	0.84 ± 0.05^{bc}	1.32 ± 0.04^{a}	1.47 ± 0.04^{a}	1.73 ± 0.03^{a}	1.74 ± 0.00^{a}
FT1	0.5	0.85 ± 0.00^{b}	0.96 ± 0.03^{b}	0.82 ± 0.02^{d}	1.07 ± 0.00^{d}	$1.170.04^{e}$
FT2	1.0	0.71 ± 0.01^{f}	0.73 ± 0.01^{g}	0.74 ± 0.02^{f}	0.87 ± 0.03^{f}	1.11 ± 0.01^{f}
FT3	1.5	0.68 ± 0.01^{g}	0.71 ± 0.03^{g}	0.74 ± 0.01^{f}	0.87 ± 0.04^{f}	1.07 ± 0.03^{g}
FT4	2.0	0.68 ± 0.01	0.69 ± 0.03^{h}	0.72 ± 0.03^{f}	0.84 ± 0.02^{g}	1.00 ± 0.02^{h}
L S D		0.04	0.04	0.04	0.06	0.04

Data are TBA values expressed as mean of 4 determinations ± standard deviation. Values within each month in the same column with the same superscripts are not ($p > 0.05$) significantly different. ST0 to ST 4 and FT0 to FT4 are 100 g samples of *Treculia africana* seed dhal (ST) and flour (FT) treated with 0.00, 0.50, 1.0, 1.50 and 2.0 ml of tamarind fruit pulp respectively.

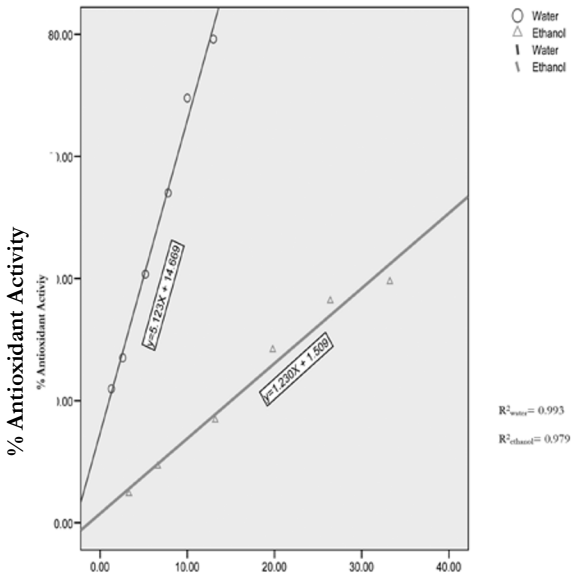

Fig. 1: DPPH radical scavenging activity of aqueous and ethanol extracts from *Tamarindus indica* fruit pod

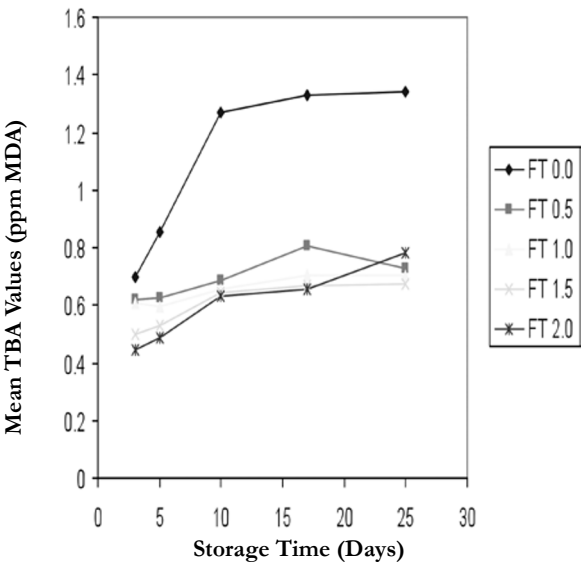

Fig. 2: Effect of *Tamarind fruit* pulp concentration (0.0, 0.5%, 1.0%, 1.5%, 2% w/w basis) on thiobarbituric acid (TBA) value of *Treculia africana* seed flour during storage at 26 ± 2°C

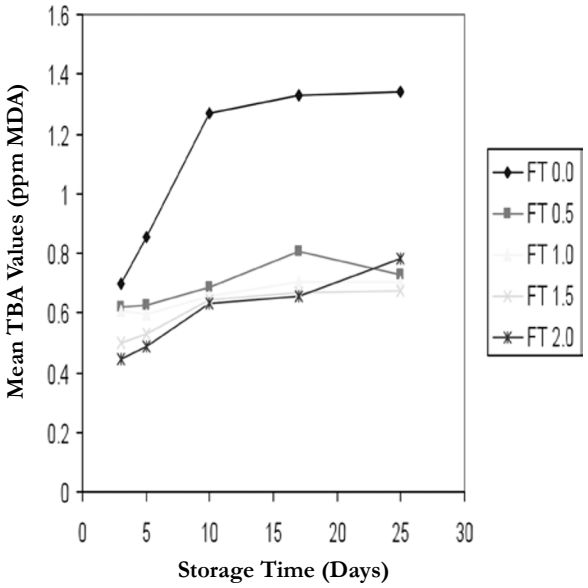

Fig 3: Effect of *Tamarind fruit* pulp concentration (0.0, 0.5%, 1.0%, 1.5%, 2% w/w basis) on thiobarbituric acid (TBA) value of *Treculia africana* seed flour during storage at 26 ± 2°C

Conclusions

Both aqueous and ethanol extracts of tamarind fruit pulp exhibited antioxidant effects in scavenging DPPH radical; and in suppressing malonaldehyde formation, expressed in suppressed TBA values in treated *Treculia africana* seed dhal and flour stored at 26 ± 2°C for 4 months. The aqueous extract conferred a higher antioxidant effect than the ethanol extract; and the antioxidant activity with DPPH in the seed dhal and flour increased with increasing concentrations of both extracts. Tamarind fruit pulp therefore has natural antioxidant constituents. The fruit pulp should be further extracted with many other food grade solvents and re-evaluated for antioxidant and even antimicrobial properties in different model food systems for broader applications.

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