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Research Article

African Nutmeg (*Monodora myristica*) Possesses Antioxidant Properties and Can Preserve African Breadfruit (*Treculia africana*) Seed and Flour

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Abstract

In this study, the antioxidant activity of oil extracted from African nutmeg (*Monodora myristica*) in suppressing the reactive free radicals found in African breadfruit was evaluated. Ethanol and aqueous extracts of African nutmeg are used to treat scavenging 2, 2-diphenyl-1-picryl hydroxyl (DPPH) radical, a method of evaluating antioxidant activity. The oil extracts from *Monodora myristica* are also used treat African breadfruit flour and seed dhal on storage (which usually forms thiobarbituric acid reactive substances (TBARS). Parboiled (100^0C 15 min.) breadfruit seeds (1.5kg) are de-hulled and processed into seed dhal which is oven-dried (50^0C 72h) and half of it milled into flour. The seed dhal and flour are treated with varying levels of oil extracted from nutmeg and monitored (4 months, at 26 ± 2^0C) for suppressing TBARS formation. The ethanol and aqueous extracts scavenged DPPH radical, with ethanol extract exhibiting higher antioxidant activity than water extract. The oil from African nutmeg suppressed TBARS in the seed dhal and flour in a dose-dependent order but was more effective in the dhal. African nutmeg possesses natural antioxidants and could extend the shelf life of food.

Keywords: Free Radical-Scavenging-Activity; Antioxidant Effect; African Nutmeg (*Monodora myristic*a); African Breadfruit (*Treculia africana*); Storage.

Introduction

The members of the family Leguminosae are very important crops that are relatively cheap in terms of production. The seed grains are rich in lysine and tryptophan but poor in sulphur-containing amino acids, namely methionine and cysteine [1,2]. They are cheaper than animal products-meat, fish and egg. They are therefore more frequently consumed globally as major source of protein, in developing countries where the consumption of animal protein may be limited due to socio-cultural and economic factors. However, while some legumes such as cowpeas and groundnuts, are commonly consumed, there are other underutilized legumes, which are nutritious and healthy. One of such legumes is African breadfruit (*Treculia africana*) which is currently a source of nutrients in the diet of many Nigerians. Unfortunately African breadfruit, unlike many other tropical legumes that have antioxidant properties [2,3], is an oil seed which becomes rancid and deteriorates easily on storage, especially when processed into seed dhal and flour.

Oxidation of lipid which occur during storage, processing, preservation and heat treatment is one of the basic processes causing rancidity in food products [4], leading to their being rejected by consumers. Oxidative deterioration leads to changes in flavor, colour, texture and nutritive value; and the production of some toxic compounds [5]. Many products of lipid oxidation exert toxic effects in both living animals and cellular systems [6-9]. It has been shown that products of lipid oxidation can cause pathological changes in the mucus membrane of the alimentary tract, inhibit the activity of enzymes, and increase the content of cholesterol and peroxides in blood stream, thus activating the process of atherosclerosis [8-10].

Application of antioxidants is one of the technically simple ways of reducing oxidation of lipids [5,11]. The antioxidant can be of synthetic or natural origin. Some synthetic antioxidants, such as Butylated Hydroxy Toluene (BHT), Butylated Hydroxy Anisole (BHA) and Gallic Acid (GA) might be dangerous for living organisms and must be used only at the safe levels [12]. Some well known herbs and spices, which have antioxidant substances, are rosemary (*Rosemarinus officinalis*), sage (*Salinia officianlis*), garden thyme (*Thymus vulgaris*), oregano (*Oragenum vulgare*), majoram (*Origanum majorana*) and many others [14-16]. Many such spices and their extracts have been reported to exert protective effect against cancer, heart diseases and cataracts [17,18]. Unfortunately many Nigerian spices used in indigenous dishes have not been evaluated for their antioxidant properties.

The aim of this study is to evaluate the antioxidant properties of African nutmeg (*Monodora myristica*) in scavenging 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) and antioxidant effect of *Monodora myristica* oil in inhibiting lipid oxidation in Treculia africana seed dhal and flour during storage.

Figure 1: African nutmeg.

Figure 2: The pod of *Treculia africana* (African breadfruit).

Materials and Methods

Materials

Dry wholesome undehulled African breadfruit (*Treculia africana* Decne) seeds were purchased from peasant farmers at Ogbede-Aku market of Igbo-Etiti Local Government Area (LGA), Enugu State, Nigeria. Dry fruits of African nutmeg (*Monodora myristica* Gaertn) were obtained from commercial stockers at the Ogige Main Market, Nsukka, in Enugu State, Nigeria. All laboratory reagents used were of analytical grade.

Experimental design

The experiment was designed as a completely randomized block [19]. Water extracts and ethanol extracts were used at four different levels. *Monodora myristica* oil was administered at four different levels. The controls were the untreated samples.

Preparation of aqueous and ethanol extracts and scavenging activity against DPPH radical

Oven-dried (500C for 48h) fruits (20g) of African nutmeg (*Monodora myristica*) were de-hulled and ground into powder, using a hand-operated kitchen (colloid) mill. Ten grammes (10g) of the powder were mixed and extracted with 200 ml of ethanol or distilled water by swirling for 10 minutes before resting for 24 hours. Extraction was repeated for another 20 minutes before filtering through a double fold Whatman no 5 filter paper to get ethanol and aqueous extracts. Each extract was stoppered in a holding tube for use.

Radical scavenging analysis was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical [20-22]. From ethanol and aqueous extracts of the spice, solutions in different concentrations were prepared by adding 1000 µl of DPPH (0.004% weight/volume), and the final volumes made up 1200µL with ethanol. Final concentrations of spice extracts in the cuvettes ranged from 0.03mg/ml to 27.5mg/ml. These were pipetted into 15-ml test tubes and incubated in the dark for 30 min. at room temperature (26 ± 2^0C). Ethanol was used in place of extracts as blank while absorbances of the resulting mixtures were measured at 517nm with a UV/Visible spectrophotometer and converted to antioxidant activity (AA%). Antioxidant activity (AA%) was calculated as percentage inhibition relative to control.

Extraction of African nutmeg oil

The seeds (200g) were cracked manually and the hard dry hull winnowed away. The intact seeds were milled into flour in an attrition mill. The flour was mixed with 500ml of n-hexane and shaken for 30 minutes on a rotary shaker. The suspension was rested for 48 h, filtered off the solid residue through nylon sieve and evaporated on a water bath maintained at 60^0C for 30min (to evaporate the hexane) [23,24]. The fruit oil was then stored in a Bijou bottle for use. A water extract of the residue was also prepared.

Thiobarbituric acid (TBA) value of stored *Treculia africana* seed dhal and flour

Dried seeds (2kg) of *Treculia africana* were parboiled in excess boiling water for 15 minutes, drained out of water and cracked in

a hand-operated (kitchen) colloid mill to remove the hulls from seeds. The dehulled seeds (dhals) were oven-dried at 500C for 48 h, and half of the dried dhal milled into flour. Five duplicated samples (100g each) of the seed dhal and flour were separately mixed with different concentrations (0, 0.5, 1.0, 1.5, 2.0ml) of *Monodora myristica* oil, using a kitchen (Kenwood) mixer. Each sample was bagged in nylon sack and stored at ambient temperature (26 ± 2°C) for 4 months.

Thiobarbituric acid (TBA) reactive substances (TBARS) formed in the seed dhal and flour samples were determined on the 3rd, 5th, 10th, 17th, 25th and 31st day, and again on the 2nd, 3rd and 4th month of the storage time as described by Buege and Aust [25]. Five grammes, each of ground seed dhal or flour, was mixed with 2.5ml of the stock solution containing 0.375% TBA (Sigma chemical Co., St. Louis Mo, U.S.A.), 15% Trichloro-acetic acid (TCA) (Mallinkrodt Beker Inc., Paris KY, USA) and 0.25 N HCl. The mixtures were heated for 10min in a boiling water bath (100°C) to develop a pink colour, cooled in tap water and centrifuged (Beckman coulter Ltd Palo, Alto, Califonia, U.S.A) at 3000 rpm for 20min. The absorbance of the supernatants were measured spectrophotometrically using Spectronic 21D, (Multon Roy, Rochester NY, U.S.A) at 532 nm against a blank that contained all the reagents except the *Treculia* seed or flour. The absorbance values were multiplied by a factor of 7.8 to obtain the TBARS values [26].

Data analysis

Data were analyzed with SAS computer software [27], using analysis of variance (ANOVA) mixed procedure (mixed) and Fisher's least significant difference (LSD) was used to ascertain significant effects at $P<0.05$ level of significance among treatments.

Results and Discussion

Free Radical scavenging activity

Figure 3 shows the scavenging activities of aqueous extract of fruits and of *Monodora myristica* (African nutmeg) against 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) l. There was an increase in antioxidant activity as the concentration of spice increased.

Figure 4 shows the antioxidant effect of the ethanol extract of *M. myristica* on the DPPH radical. The trend was similar to that observed with the water extract. As the concentration of the extract increased, antioxidant activity also increased.

Figure 5 shows the antioxidant effect of water extract from fruit residue (after extraction of oil with n-hexane). The same trend similar to those observed with water extract and ethanol extract was also observed.

Figure 3: Antioxidant activity of water extract from *Monodora myristica* on DPPH radical

Figure 4: Antioxidant effect of ethanol extract of *Monodora myristica* on DPPH radical.

Figure 5: Antioxidant effect of *Monodora myristica* residue on DPPH.

Comparison of the antioxidant effects of the water extract, ethanol extract and water extract of the residue after hexane extraction showed the scavenging activities were in the order: water extract of fruit > ethanol extract of residue > water extract of fruit residue after hexane extraction. It is generally recognized that free radicals produced in the body are partly associated with the etiology of cancer and other chronic diseases. Dietary antioxidants capable of scavenging free radicals are capable of reducing the risks of these degenerative diseases. It is therefore very important to understand the radical scavenging activity of African nutmeg commonly consumed in most Nigerian homes for its therapeutic and antioxidant applications. DPPH is a free radical stable at room temperature and produces a violet solution in ethanol. Reduction of DPPH by extracts of African nutmeg results in loss of absorbance, an indication of discoloration which indicates scavenging efficiency and antioxidant potency of the spice.

Inhibition of lipid oxidation in *Treculia africana* (Decne) seed dhal and flour

Different concentrations (0%, 0.5%, 1%, 1.5%, 20%) of *Monodora myristica* oil in *Treculia africana* seed dhal and flour stored for different days (3, 5, 10, 17, 25 days) under ambient condition (26 ± 2^0C) significantly ($P < 0.05$) affected mean TBA values of the seed dhal (Figure 6).

Figure 6: TBA values of Treculia africana seed dhal stored for 25 days.

SMO.O= Seed dhal without treatment.

TBA values of *Treculia africana* seed dhals treated with *Monodora myristica* oil extract

Monodora myristica oil significantly ($p<0.05$) suppressed oxidation of *Treculia africana* seed dhal. The antioxidant effect of the spice oil was dose-dependent and increased with increasing storage time at 26 ± 2^0C (Figure 6.). Within 25 days of storage, the control, FM00 (untreated flour or flour without *Monodora myristica* oil), had mean TBA value of 1.35, which was approximately twice the mean TBA value (0.70) of the sample treated with 2% *Monodora myristica* oil (FM2.0). From 5^{th} to 17^{th} day of storage, the sample treated with 0.05% of *Monodora myristica* oil (FM.5) had mean TBA value that did not differ ($p>0.01$) significantly from that of the control. However, after 17 days of storage, all the treated samples had mean TBA values significantly lower ($p<0.05$ than that of the control throughout the rest of the storage period.

Figure 7 shows the TBA values of *Treculia africana* flour after treatment with *Monodora myristica* oil.

Figure 7: Effect of concentration of M. myristica oil on the TBA values of stored Treculia africana flour.

FHO=four sample without treatment.

Table 1 shows the changes in TBA values of *Treculia africana* seed dhals and flour stored for four months. The 2-way interaction (spice oil level x day) and 3-way interaction (treatment x spice oil level x day) also affected TBA values ($p<0.05$). The mean TBA values of dehulled *Treculia africana* seed dhal for the 3-way interaction of spice oil treatment x spice oil level x day of storage for four months are shown in Table 1.

Data are TBA values expressed as mean of 4 determinations ± standard deviation. Values within each month in the same column with the same superscript are not ($p>0.05$) significantly different SM00 to SM2.0 and FM00 to FM2.0. Samples are 100g samples of

Sample code	Oil conc. (%v/w)	Storage Time (months)				
		0	1	2	3	4
SM00	0.00	0.79 ± 0.00^{b}	0.89 ± 0.02^{c}	1.02 ± 0.02^{c}	1.32 ± 0.02^{d}	1.62 ± 0.02^{a}
SM0.5	0.5	1.09 ± 0.01^{a}	0.96 ± 0.02^{c}	1.17 ± 0.22^{bc}	1.03 ± 0.02^{h}	1.20 ± 0.01^{ab}
SM1.0	1.0	$0.68 \pm 0.ds00^{bc}$	0.73 ± 0.02^{cd}	0.76 ± 0.00^{d}	1.07 ± 0.02^{f}	1.10 ± 0.01^{bc}
SM1.5	1.5	0.59 ± 0.03^{c}	0.66 ± 0.00^{d}	0.71 ± 0.00^{d}	1.03 ± 0.00^{g}	0.89 ± 0.00^{c}
SM2.0	2.0	0.32 ± 0.03^{d}	0.52 ± 0.01^{e}	0.64 ± 0.00^{d}	1.01 ± 0.00^{g}	1.02 ± 0.00^{bc}
FM00	0.0	1.17 ± 0.02^{a}	1.32 ± 0.01^{a}	1.47 ± 0.03^{ab}	1.73 ± 0.02^{c}	1.71 ± 0.00^{ab}
FM0.5	0.5	1.16 ± 0.14^{a}	1.30 ± 0.04^{a}	1.32 ± 0.04^{a}	1.35 ± 0.03^{a}	1.34 ± 0.03^{a}
FM1.0	1.0	1.17 ± 0.02^{a}	1.14 ± 0.5^{b}	1.26 ± 0.04^{b}	1.52 ± 0.01^{b}	1.09 ± 0.05^{bc}
FM1.5	1.5	1.14 ± 0.01^{a}	1.20 ± 0.02^{b}	1.25 ± 0.00^{b}	1.29 ± 0.02^{e}	1.15 ± 0.02^{b}
FM2.0	2.0	0.61 ± 0.00^{c}	0.92 ± 0.02	1.31 ± 003^{ab}	1.01 ± 0.00^{i}	1.05 ± 0.02^{bc}
L S D		0.35	0.28^{c}	0.56	0.03	0.83

Table 1: Changes in meanTBA values of *Treculia africana* seed dhal and flour treated with *Monodora myristica* oil during storage.

Treculia africana seed dhals and flour treated with 0.00, 0.5, 1.0, 1.5 and 2.0ml *Monodora myristica* oil respectively. SMOO= Seed dhal without treatment; FMOO=four sample without treatment.

The control with no oil addition (SM00) had significantly higher mean TBA values ($p<0.05$) than treated samples. Mean TBA values decreased linearly with increase in the spice oil concentration in the samples and was in the order SM0 > SM.50>SM1.0>SM1.5>SM2; and increased in that order with increasing days of storage. The mean TBA values were significantly ($p<0.05$) and inversely dose-dependent confirming high antioxidant activity of the spice oil in the *Treculia* flour. The suppressive effect of *Monodora* oil against oxidation was higher in the seed dhal than in flour.

The mean TBA values after 4 months of storage of *Treculia* seed dhal (SM00 to SM2.0) and flour (FM00 to FM2.0) with 0, 0.5, 1, 1.5 and 2% oil concentration at 26 ± 20C are shown in Table 1. *Monodora* spice oil dose-dependently and significantly ($p<0.05$) suppressed oxidative reaction in both seed dhal and flour.

Treculia africana seed dhal and flour treated with 2% *Monodora* spice oil had mean TBA values of 1.02 and 1.05 respectively on the 4th month of storage. The mean TBA values also increased with time but inversely with spice oil concentrations. *Monodora* spice oil had antioxidant effect both in the seed dhal and flour. Similar results had been obtained with oils and water extracts from many spices in many model food systems. Cinnamon essential oil and rosemary extracts were reported to suppress rancidity development in chicken frankfurters [28].

The mean TBA values of the treated *Treculia africana* seed dhal and flour samples stored for 4 months did not differ significantly from the TBA values of those stored for one month. Apparently, the malonaldehyde which is the oxidation product detectable with thiobarbituric acid might have broken down to less or non-detectable substances in the products during the storage period [29].

Conclusion

The spice, (African nutmeg) possess antioxidant properties as exhibited in scavenging DPPH radical and in suppressing malonaldehyde formation (TBA values) in stored African breadfruit seed dhal and flour. The antioxidant activity in both DPPH and stored African breadfruit increased as the concentration of its extract or oil increased in both samples. Its antioxidant activity in African breadfruit decreased with increasing storage time. The effective period for protection by the antioxidants is about one month. The spice could therefore be likely used to quench radical formation in both food and live tissues, on a short term basis.

Author Contributions

Fabian Ugwona carried out the research and participated in the write -up.

Jane Onweluzo designed the work.

Philippa Ojimelukwe analysed the data and did most of the writing.

Conflict of Interest

The authors do not have any conflict of interest in publishing this work.

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