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Evaluation of antioxidant potential of African Nutmeg (*Monodora myristica*) on the peroxide value of soya bean oil

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Target audience*:* Researchers, farmers, feed millers and policy makers.

Abstract

The antioxidative activity of African Nutmeg (Monodora myristica) was evaluated using ethanol extract of the spice in soya bean oil over a period of 28 days. The peroxide value was used to monitor the development of rancidity in the oil. The peroxide values of soya bean oil treated with 2.5ml, 5.0ml, 7.5ml and 10ml concentrations of the Monodora myristica were measured. The peroxide value of the 10ml Monodora myristica- treated soya bean oil was not raised (P<0.05) up to 20mg/g oil until about 21 days of storage. The peroxide value of the varying concentrations of Monodora myristica crude extract revealed that the quantity of peroxide generated increased with time. Between day 0 and day 7, the peroxide value of all the samples treated with the varying concentrations of the Monodora extracts and the control were low and were different (P<0.05) from one another. In the Monodora extracted oil, the peroxide value between this period and the 7th day were below 10mg/g oil while the control recorded 10.52mg/g oil. The peroxide values increased in all the samples above 10mg/g oil between the 14th day and 28th day. From the results, Monodora myristica appears to be effective in the improvement of the shelf life of soya bean oil and so promises to improve the shelf life of broiler feeds under storage.

Description of the problem

Spices are known for their antioxidative properties (1). They prevent rancidity and extend the shelf life by slowing down the oxidation of fats and enzymes. Fats are broken down into peroxides (free radicals) which damage the cells and limit their ability to fight off cancer, aging and memory loss on exposure to air or oxygen and finally into aldehydes and alcohols that give rancid taste (1). Spices have components that act as anti-oxidants that protect cells from free radicals (1,2). The compounds responsible for the anti-oxidative properties of spices are mainly phenolic compounds such as phenolic diterpenes, diphenolic diterpenes (16). They are effective against oxidative rancidity of fats and colour deteriorations of carotenoid pigments. The anti-oxidative activity of rosemary (*Rosmarinus officinalis*) was reported (3). The anti-oxidative compounds identified in rosemary spice are carnosol, carnosonic acid, epirosmanol, isorosmanol and rosmanol (3; 4). The essential oil components of *Afromomum danielli* spice were reported to be more potent than synthetic anti-oxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). With an antioxidant effectiveness of 70.7% compared to that of 46.1% for α-tocopherol, stabilization of

soybean oil was more effective using a crude antioxidant extract of *A. danielli* (5). The ability of *A. danielli* antioxidant extract (200ppm) to stabilize soybean oil and palm oil (9) against lipid oxidation was observed to be similar to that reported by (4) who identified the active component of the extract of *A. danielli.*

African nutmeg (*Monodora myristica*) or Ehuru in Igbo Language is an attractive small tree found in the rain forest (6). It is a tree of 15m high and 1.2m in girth and belongs to the family – *Myristicaceae* (1). Nutmeg seed is a light brown or greyish wrinkled seed with a smooth hard blackish brown nut. This research work was carried out to investigate the anti-oxidative activity of methanol extract of African nutmeg (*Monodora myristica*) on soybean oil.

Materials and methods

The research work was carried out at the Nutrition Laboratory of the College of Animal Science and Animal Production of the Michael Okpara University of Agriculture, Umudike of Abia State, Nigeria. It is situated at latitude $5^0$29"N and longitude $7^0$23'E in the rainforest zone of Nigeria. It is characterized by a mean annual rainfall of 2238mm, maximum and minimum temperature of 32^0C and 23^0C respectively and relative humidity of 63%-80% (21).

Procurement of Test Material

Seeds of *Monodora myristica* were bought from Ndoro market in Ikwuano local Government Area of Abia State. They were cleaned, milled and stored in a container for chemical analysis.

Proximate Composition Analysis

Determination of the proximate composition of *Monodora myristica* was done according to the procedure of the (7), employing the microkjeldahl method for crude protein and soxhlet extraction procedure for ether extract. Gross energy of the sample was assayed using the adiabatic bomb calorimetric technique.

Phytochemical components

The alkaloids and saponins were determined according to the procedure of (10). Total phenols and tannin were determined by the spectrophotometric method according to (11). The flavonoids were determined according to (12)

Antioxidant activity of *Monodora myristica*

20g sample of the fresh milled spice was mixed with 70% methanol (1000ml) and kept in the shaking incubator at 250^0C for 3 days and then filtered in vacuum using Whatman, No. 1 filter paper. After solvent fractionation, both aqueous and organic fractions were evaluated for antioxidant activities. The antioxidant activities of *Monodora myristica* were determined by assessing the peroxide value of the spice. The effect of using 2.5ml, 5.0ml, 7.5ml, 10.0ml and 12.5ml of antioxidant crude extract obtained from *Monodora myristica* on lipid oxidation was determined by measuring the peroxide value of treated soybean oil using the method of (13). The antioxidant activities of the spice were compared with the conventional antioxidant butylated hydroxytoluene (BHT). 10ml of soybean oil was used for each level of the crude extract. The peroxide values were measured for four weeks. For the peroxide value determination, 1g of *Monodora* was weighed out into a clean boiling tube and while still liquid, 1g of powered potassium iodide and 20ml of solvent mixture (2 vol glacial acetic acid and 1 vol chloroform) was added. The tube was then placed in boiling water so that the liquid boils within 30 seconds and was allowed to boil vigorously for 30 seconds. The content of the tube was poured into a flask containing 20ml of potassium

iodide solution (5%) and the tube washing out twice with 25ml water and titrated with 0.002 m sodium thiosulphate solution using starch. A blank was prepared at the same time. The antioxidant effectiveness (AE) was calculated as shown below;

$$AE=\frac{\text{Peroxide value of control - peroxide value of test sample}}{\text{Peroxide value of control}} \times 100$$

Where, AE represents antioxidant effectiveness.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using Completely Randomised Design. Where significant differences were obtained, means were further subjected to Duncan's multiple Range test (14) as packaged in (15).

Results and Discussion

Chemical Composition of Raw *Monodora myristica*

The proximate and phytochemical composition of African nutmeg (*Monodora myristica*) is shown in Table 1. Raw *Monodora myristica* was observed to contain 89.90% dry matter. The ash, crude fibre, ether extract, crude protein and Nitrogen-free extract were 8.84%, 9.64%, 28.60%, 15.75% and 37.17% respectively. The proximate composition suggests that *Monodora myristica* is a good source of protein and carbohydrates and ether extract.

The alkaloid and flavonoid contents were higher than other phytochemicals in the *Monodora myristica.* The following were observed; 3.84% alkaloid, 12.88% flavonoid, 0.32% saponins, 0.15% phenol, 0.32% tannin and 0.03% phytate. The high level of flavonoids observed may imply higher inflammatory activity. Flavonoids from spices were reported to be effectively used in the treatment of arthritis in herbal medicine(16). Flavonoids from *Magnifera indica* have been reported to aid protection against allergies, inflammation and free radical scavengers (17). Saponins were reported to lower plasma cholesterol (18). Alkaloids have been reported to be soluble in water but highly poisonous (19). Phytate can affect the digestibility of minerals by chelating with calcium or by binding with substrate or proteolytic enzymes (20).

Table 1. The Proximate and antinutritional contents of raw *Monodora myristica*

Chemical constituents		**SEM**
Dry Matter (%)	89.90	0.007
Ash (%)	8.84	0.003
Crude Fibre (%)	9.64	0.000
Ether Extract (%)	28.60	0.003
Crude Protein (%)	15.75	0.003
Nitrogen-free Extract (%)	37.17	0.00
Alkaloids (%)	3.84	0.003
Flavonoids (%)	12.88	0.003
Saponins (%)	0.32	0.003
Phenol (%)	0.15	0.003
Tannin (%)	0.32	0.003
Phytate (%)	0.03	0.003

Values in the table are means of triplicate determinations

Antioxidative Activity of methanol Crude Extract of *Monodora myristica* Spice

The result of the antioxidant activity of *Monodora myristica* Spice is shown in Table 2. The peroxide value was used to monitor the development of the rancidity of the oil. The peroxide value of the soya bean oil treated with the varying concentrations of *Monodora myristica* crude extract revealed that the quantity of the peroxide generated increased with time. Within day 0 and day 7, the peroxide value of the samples treated with the varying concentration of the *M. myristica* extract and the control were low and were significantly ($P<0.05$) different from one another. In the *Monodora* extract- treated oil, it was observed that the peroxide value between this period and 7th day were below 10mg/g oil while the control recorded 10.52mg/g oil. The peroxide value increased in all the samples above 10mg/g oil between the 14th day and 28th day. As the concentration of the extract increased, the peroxide value decreased with a resultant increase in the antioxidant effectiveness while, peroxide value of each concentration of the Monodora myristica extract increased over time. The sample treated with 12.5ml of *Monodora* extract recorded an increase in the peroxide value from 8.67mg/g oil on the 7th day to 19.11mg/g oil at the end of the experiment while the soya bean oil treated with 10ml extract yielded a peroxide value of 8.86mg/g oil on the 7th day which increased to 21.41mg/g oil on the 21st day of study. Low concentration of *Monodora* extract *of* 2.5ml in soya bean oil resulted in peroxide value of 9.73mg/g oil implying an antioxidative effectiveness at only 7.51% compared to 15.78% and 17.58% observed in the 10ml and 12.50ml concentration respectively within the 1st week of study.

The low peroxide values observed in all the treatments during the first week of the experiment was an indication that the oils were fresh and had not undergone much oxidation probably as a result of the presence of terpenes which were still active and had not undergone much transformation reaction due to oxidation. Terpene is a major component of the phenols-a phytochemical constituent of *monodora myristica* (2). From the result, there was a slight oxidation after the first week in all the samples indicating slight deterioration as the phenols present in the spice extract must have been able to scavenge the aldehydes resulting from the breakdown of the fats. The high rate of increase in the peroxide values observed in the oil treated with the various *Monodora* extract between the 7th day and 21st day suggests that the free radicals may have been liberated from the breakdown of the oils with the result that the speed of oxidation increased. However, the oxidation in most of the treatments did not reach actual rancidity level until 21st day of experiment. This implies that the *Monodora* was able to slow down oxidation and the soya bean oil treated with the varying concentration of *M.myristica* extract did not develop any rancid taste until after the 3rd week of the experiment. It was reported that a rancid taste that was a sign of spoilage in oil occurs at a peroxide value of between 20-40mg/g oil (18). Rancidity observed at the 3rd week must have come from the aldehydes resulting from the breakdown of the soya bean oil into peroxide. In the oil treated with 12.5ml *M.myristica* extract, the peroxide value did not rise up to 20mg/g oil even after the 28th day indicating that *M.myristica* at such high concentrations could be used to stabilize soya bean oil effectively and improve the shelf life of soya bean oil. The ability of the crude extract of *M.myristica* to stabilize soya bean oil against lipid oxidation is attributed to the phenolic diterpenes (1).

Table 2. Peroxide values of Soya bean oil treated with crude methanol extract of *Monodora myristica*

Sample	Day 0 Mg/g oil	Day 7 Mg/g oil	Day 14 Mg/g oil	Day 21 Mg/g oil	Day 28 Mg/g oil	SEM
Control	8.30	10.52	16.83	22.90	22.94	1.60
MEC 1	8.30	9.73^{ab} (7.51)	16.22^{b} (3.62)	22.70^{a} (0.87)	22.87^{a} (0.31)	1.93
MEC 2	8.30	9.70^{ab}-(97.79)	15.62^{b} (7.19)	21.52^{b} (6.03)	21.82^{b} (4.88_	1.52
MEC 3	8.30	9.59^{b}(8.84)	15.14^{d} (10.04)	21.45^{b} (6.33)	21.03^{c} (8.33)	1.47
MEC 4	8.30	8.86^{c}(15.78)	12.84^{e}(23.71)	20.41^{c} 10.87)	20.70^{c} (9.76)	1.48
MEC 5	8.30	8.67^{d} (17.58)	12.10^{f} (28.10)	18.05^{d}21.18()	19.11^{d} (16.70)	1.28

Means a-f in the same column with the same superscripts are not significantly (P<0.05) different from one another. MEC 1, 2, 3, 4 and 5 represent Monodora Extract Concentrations 2.5ml, 5.0ml, 7.5ml, 10ml and 12ml respectively. Figures in parenthesis represent the antioxidant effectiveness of the treatments over the control

Conclusion and Application

1. The antioxidative activity of African Nutmeg (*Monodora myristica)* was evaluated using ethanol extract of the spice in soya bean oil over a period of 28 days.
2. The peroxide value of the *Monodora myristica*-treated soya bean oil increased with time while as the level of *Monodora myristica* increased in the soya bean oil, the peroxide value decreased.
3. The shelf life of oil rich ingredients such as ground nut meal and soya bean could be enhanced by *Monodora myristica* through a delay in the oxidation of the oil. This is evident in the reduced peroxide value of soya bean treated with 12ml of *Monodora myristica* at various days especially at days 21 and 28.
4. Feed spoilage due to rancidity and resultant aflatoxicosis could be reduced using *Monodora myristica* extract. This is good news for feed millers and animal farmers who lose lots of money through feed spoilage.

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