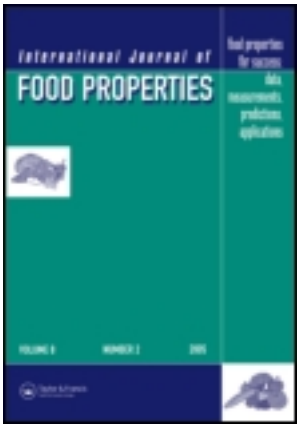

International Journal of Food Properties
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ljfp20

Chemical Composition and Antioxidant Activity of Essential Oil and Oleoresins of Nutmeg (Myristica fragrans Houtt.) Fruits

I.P.S. Kapoor [a], Bandana Singh [a], Gurdip Singh [a], Carola S. De Heluani [b], M. P. De Lampasona [b] & Cesar A.N. Catalan [b]

[a] Chemistry Department, D.D.U. Gorakhpur University, Gorakhpur, India

[b] Instituto de Quimica, Organica Universidad Nacinal de Tucuman, S.M. de Tucuman, Argentina

Accepted author version posted online: 27 Sep 2012.Published online: 02 Apr 2013.

To cite this article: I.P.S. Kapoor , Bandana Singh , Gurdip Singh , Carola S. De Heluani , M. P. De Lampasona & Cesar A.N. Catalan (2013): Chemical Composition and Antioxidant Activity of Essential Oil and Oleoresins of Nutmeg (Myristica fragrans Houtt.) Fruits, International Journal of Food Properties, 16:5, 1059-1070

To link to this article: http://dx.doi.org/10.1080/10942912.2011.576357

International Journal of Food Properties, 16:1059–1070, 2013

ISSN: 1094-2912 print / 1532-2386 online
DOI: 10.1080/10942912.2011.576357

Taylor & Francis
Taylor & Francis Group

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL AND OLEORESINS OF NUTMEG (*MYRISTICA FRAGRANS* HOUTT.) FRUITS

I.P.S. Kapoor[1], Bandana Singh[1], Gurdip Singh[1], Carola S. De Heluani[2], M.P. De Lampasona[2], and Cesar A.N. Catalan[2]

[1]*Chemistry Department, D.D.U. Gorakhpur University, Gorakhpur, India*
[2]*Instituto de Quimica, Organica Universidad Nacinal de Tucuman, S.M. de Tucuman, Argentina*

Essential oil and oleoresins (ethanol, ethyl acetate, and iso-propyl alcohol) of* Myristica fragrans *were extracted by using Clevenger and Soxhlet apparatus, respectively. Gas chromatography-mass spectrometry analysis of essential oil showed the presence of 38 components representing about 99.6% of the total weight. Sabinene (29.4%) was found to be a major component along with beta pinene (10.6%), alpha pinene (10.1%), terpene-4-ol (9.6%), and several other minor components. The major component of all oleoresins contained elemicin. It has been observed that the essential oil and ethanol oleoresin showed better activity compared to other tested oleoresins and synthetic antioxidants, butylated hydroxyl anisole and butylated hydroxyl toluene. Furthermore, the activity of essential oil and oleoresins was measured for the inhibition of primary and secondary oxidation products in mustard oil by using peroxide, thiobarbituric acid, and p-anisidine values. In addition, these experiments were further supported by other complementary antioxidant assays, such as ferric thiocyanate method in a linoleic acid system, reducing power, chelating effect, and scavenging effects on 1,1′-diphenyl-2-picrylhydrazyl radical. Hence, the essential oil and ethanol oleoresin of* M. fragrans *could be considered as a natural food preservative.

Keywords: Myristica fragrans*, Essential oil, Oleoresins, GC–MS, Antioxidant activity.*

INTRODUCTION

Free radical and reactive oxygen species are responsible for lipid oxidation, which is the major chemical change involved in the deterioration of food during processing and storage.[1] Antioxidants have been widely used as food additives to provide protection from oxidative degradation of foods and oils. The most extensively used synthetic antioxidants are propylgallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ),[2] but there are some arguments about the safety and adverse effects of these substances when used as food additives.[3,4] In fact, in recent years, researchers have focused on spicy and medicinal plants for extracting natural antioxidants,[5–7] which play an important role in the food industry to combat food deterioration.

Received 30 December 2010; accepted 26 March 2011.
Address correspondence to Gurdip Singh, Chemistry Department, D.D.U. Gorakhpur University, Gorakhpur 273009, India. E-mail: gsingh4us@yahoo.com

The nutmeg tree (*Myristica fragrans* Houtt.) is a large, leafy evergreen plant originating from Moluccas (the spice island) and now cultivated in the West Indies.[8] It belongs to the family Myristicaceae and produces two spices: mace and nutmeg. Nutmeg is the seed kernel inside the fruit and mace is the lacy covering (aril) of the kernel. Nutmeg has aromatic, stimulant, narcotic carminative, antifungal, antidysentric, and anti-inflammatory activities.[9]

As far as a literature survey is concerned, the chemical constituents of the nutmeg volatile oil are well studied,[10,11] but potential antioxidative properties of its various oleoresins have not yet been reported. In continuation of our research programmed[12–15] on chemistry, antioxidant, and antimicrobial activity of various spicy essential oils and oleoresins of spices, the present work deals with the chemistry and antioxidant activities of volatile oil and various oleoresins (ethanol, ethyl acetate, and iso propyl alcohol) of *M. fragrans* for their possible utilization as food preservatives.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA), diphenylpicrylhydrazyl radical (DPPH), and linoleic acid of Across (NJ, USA); and BHT, BHA, PG, and 2,4-dinitrophenylhydrazine (s.d. Fine-Chemicals, Ltd., Mumbai, India) were used as received. Crude mustard oil was purchased from a local oil mill, Gorakhpur, India.

Plant Material

Fruits of *M. fragrans* were purchased from the local market of Gorakhpur, India during November 2008 and a voucher specimen has been deposited in the Herbarium of the Science Faculty of DDU Gorakhpur University, Gorakhpur, India.

Isolation of the Volatile Oil

The powdered fruits of nutmeg were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h in accordance with European pharmacopoeia procedure,[16] to get colorless volatile oil (yield 3.4%) with characteristic odor and sharp taste. The oil was dried over anhydrous sodium sulphate to remove traces of moisture, filled in a bottle, and stored in a refrigerator in the dark at 4°C until use.

Isolation of the Oleoresins

The oleoresins were obtained by extracting 25 g of powdered fruits of nutmeg with 250 mL of each solvent (ethanol, ethyl acetate, and iso-propyl alcohol) for 3 h in a Soxhlet extractor, and solvent was distilled out. The obtained oleoresins (yield 3.8, 3.2, and 2.5%, respectively) were filled in bottles and stored in a refrigerator in the dark at 4°C until use.

Chemical Investigation

The chemical analysis of volatile oil and oleoresins of *M. fragrans* were undertaken by gas chromatography-mass spectroscopy (GC-MS). The gas chromatograph, (Hewlett-Packard 6890 Hewlett-Packard, Agilent Technologies, Buenos Aires,

Argentina) coupled to a quadruple mass spectrometer (Hewlett-Packard 5973, Agilent Technologies) equipped with a Perkin Elmer Elite-5MS capillary column (Argentina, 5% phenyl methyl siloxane, length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm) and a selective mass detector was used for GC/MS detection, an electron ionization system with ionization energy 70 eV was used. Helium was taken as a carrier gas at a flow rate of 1.0 mL/min. Injector and ion source temperatures were set at 230 and 280°C, respectively, and split ratio is 80:1. The oven temperature for the volatile oil was programmed as follows: 60°C (1 min), 60–185°C (1.5°C min^{-1}), 185°C (1 min), 185–275°C (9°C min^{-1}), 275°C (2 min), and for oleoresins: 60°C (0 min), 60–300°C (3°C/min), 300°C (10 min).

The components were identified on the basis of comparison of their retention indices and mass spectra with published data,[17–22] and computer matching was done with the Wiley 275 and National Institute of Standards Technology (NIST) libraries provided with the computer controlling GC-MS systems. The retention indices were calculated using a homologous series of *n*-alkanes C_8–C_{22} and the results of volatile oil and oleoresins are reported in Tables 1 and 2.

Antioxidant Activity in Mustard Oil

Antioxidant potential of nutmeg oil and oleoresins for mustard oil have been evaluated by different methods. Crude mustard oil having an initial peroxide value of 6.3 meq/kg was taken for present investigation. The nutmeg oil and oleoresins were added individually to unrefined mustard oil at the concentration of 200 ppm (v/v). Synthetic antioxidants, such as BHA, BHT, and PG, were also added to mustard oil at the same concentration, i.e., 200 ppm (w/v). A control sample was prepared under similar condition without any additive. They were subjected to the Schaal oven test[23] in 100-mL open beakers at 60°C.

The antioxidant activity of oil and oleoresins for mustard oil was examined by comparing the activity of known antioxidants, such as BHA, BHT, and PG, by peroxide,[24] TBA,[25] and p-anisidine values[26] according to the methods reported earlier. Complementary antioxidant activity of nutmeg oil and oleoresins in linoleic acid system,[27] scavenging effect on DPPH,[28] reducing power,[29] and chelating effect[30] was determined by the methods reported earlier.

Statistical Analysis

Duplicate samples of mustard oil for each treatment/period were taken. Furthermore, each sample was analyzed individually in triplicate. The data were presented as mean ± standard deviation of three determinations (data were not shown).The quantitative data of essential oil and oleoresin were statistically examined by Student's *t*-test by using a Microsoft Excel statistical analysis program (Microsoft Office, 2003, India). A probability value of $P \leq 0.05$ was considered to denote the statistically significant differences.

RESULTS AND DISCUSSION

GC-MS analysis of *Myristica fragrans* volatile oil showed the presence of 38 components representing about 99.6% of the total weight (Table 1). Sabinene (29.4%) was found to be a major component along with beta pinene (10.6%), alpha pinene (10.1%), and terpenen-4-ol (9.6%) along with several other minor components. The oleoresins in ethanol, ethyl acetate, and iso-propyl alcohol showed, respectively, the presence of 40 components (84.0%), 51 components (95.3%), and 37 components (95.7%) of the total amounts (Table 2). The major components of each oleoresin are:

Table 1 Chemical composition of nutmeg essential oil.

Compounds	%	RI[#]	Identification[Φ]
α-Thujene	0.9	921	MS, RI, co-GC
α-Pinene	10.1	929	MS, RI, co-GC
Camphene	0.2	945	MS, RI, co-GC
Sabinene	29.4	967	MS, RI, co-GC
β-Pinene	10.6	973	MS, RI, co-GC
Myrcene	1.8	985	MS, RI, co-GC
α-Phellandrene	0.6	1004	MS, RI, co-GC
3-Carene	1.7	1006	MS, RI, co-GC
α-Terpinene	1.3	1012	MS, RI, co-GC
p-Cymene	1.2	1021	MS, RI, co-GC
Limonene	3.6	1025	MS, RI, co-GC
β-Phellandrene	2.7	1026	MS, RI, co-GC
γ-Terpinene	2.5	1053	MS, RI, co-GC
cis-Sabinene hydrate	2.3	1062	MS, RI
Terpinolene	1.6	1081	MS, RI, co-GC
trans-Sabinene hydrate	3.1	1104	MS, RI
cis-p-Menth-2-en-1-ol	0.7	1124	MS, RI
trans-p-Menth-2-en-1-ol	0.4	1142	MS, RI
Terpinen-4-ol	9.6	1175	MS, RI, co-GC
p-Cymen-8-ol	Trace	1181	MS, RI
α-Terpineol	1.2	1190	MS, RI, co-GC
cis-Piperitol	Trace	1201	MS, RI
trans-Piperitol	Trace	1207	MS, RI
Citronellol	Trace	1228	MS, RI, co-GC
Bornylacetate	0.4	1292	MS, RI
Safrole	1.1	1294	MS, RI
α-Cubebene	Trace	1347	MS, RI
Terpenyl acetate	0.3	1348	MS, RI
Citronellyl acetate	0.5	1352	MS, RI
α-Copaene	0.6	1373	MS, RI
Geranyl acetate	Trace	1378	MS, RI, co-GC
Methyl eugenol	1.1	1401	MS, RI, co-GC
β-Caryophyllene	0.9	1415	MS, RI, co-GC
trans-α-Bergamotene	Trace	1427	MS, RI
α-Humulene	Trace	1450	MS, RI, co-GC
Myristicin	3.8	1515	MS, RI
Elemicin	5.6	1551	MS, RI
Docosane	Trace	2200	MS, RI, co-GC
Total	99.8%		

Trace: <0.05; [#]the retention index was calculated using a homologous series of *n*-alkanes C8-C22; [Φ]Co-GC: co-injection with an authentic sample.

Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.

- Ethanol oleoresin: elemicin (9.3%), myristic acid (7.3%), 2-(4-allyl-2,6-dimethoxy phenoxy)-1-(3,4,5-trimethoxyphenyl)-1-propanol (7.0%), dehydrodiisoeugenol + licarin E (6.4%), and myristicin (4.2%), respectively.
- Ethyl acetate oleoresin: glycerine-1,3-dimyristate (29.6%), elemicin (11.5%), myristic acid (5.0%), dehydrodiisoeugenol + licarin E (5.9%), and myristicin (5.0%), respectively.
- Iso-propyl alcohol oleoresin: elemicin (17.8%), 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)-1-propanol (7.0%), dehydrodiisoeugenol + licarin E (9.4%), myristicin (8.1%), and myristic acid (5.3%), respectively.

Table 2 Chemical composition of various oleoresins of nutmeg.

Compounds	B2%	B3%	B4%	RI*	Identification$^{\Phi}$
Sabinene	—	—	Trace	967	MS, AI, co-GC
α-Terpinene	—	—	Trace	1012	MS, AI, co-GC
γ-Terpinene	—	—	Trace	1053	MS, AI, co-GC
cis-Sabinene hydrate	0.5	—	Trace	1062	MS, AI
Terpinolene	—	—	Trace	1081	MS, AI, co-GC
Linalool	—	—	Trace	1098	MS, AI, co-GC
trans-Sabinene hydrate	0.7	—	Trace	1104	MS, AI
Terpinen-4-ol	2.6	3.0	1.7	1175	MS, AI, co-GC
α-Terpineol	0.6	—	0.3	1190	MS, AI, co-GC
Safrole	Trace	—	1.1	1294	MS, AI
δ-Elemene	Trace	—	Trace	1335	MS, AI
Citronellyl acetate	Trace	—	Trace	1352	MS, AI
α-Copaene	Trace	—	0.4	1373	MS, AI
Geranyl acetate	Trace	—	Trace	1378	MS, AI, co-GC
Methyl eugenol	1.4	1.1	1.4	1401	MS, AI
β-Caryophyllene	1.1	0.3	0.7	1415	MS, AI, co-GC
trans-α-Bergamotene	Trace	—	0.2	1427	MS, AI
trans-Isoeugenol	Trace	1.1	0.3	1453	MS, AI
trans-Methylisoeugenol	Trace	0.4	0.4	1496	MS, AI
δ-Amorphene	Trace	—	0.3	1511	MS, AI
Myristicin	8.1	4.2	5.0	1515	MS, AI
Elemicin	17.8	9.6	11.5	1551	MS, AI
Methoxyeugenol	1.2	3.2	0.8	1558	MS[a]
Isoelemicin	1.2	0.9	0.6	1570	MS, AI
Myristic acid	5.3	7.3	2.9	1739	MS[φ]
Myristic acid ethyl ester	—	0.6	—	—	MS[φ]
Palmitic acid	—	1.5	—	—	MS
Palmitic acid ethyl ester	—	Trace	—	—	MS
Oleic acid	—	2.4	—	—	MS
Oleic acid ethyl ester	—	Trace	—	—	MS
Otobaphenol stereoisomer I	—	1.5	0.4	—	MS
(4-Hydroxy-3-methoxy-1'-allyl-3',5'-dimethoxy)-8-O-4'-neolignan	—	0.3	0.3	—	MS
2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(3,4-methylenedioxyphenyl)-1-propanol	—	Trace	0.3	—	MS
Otobaphenol stereoisomer II	—	0.3	0.3	—	MS
Guaiacin	—	2.6	0.3	—	MS
Dehydrodiisoeugenol + licarin E	9.4	*6.4*	*5.9*	—	*MS*
Virolongin B	1.2	*0.9*	*0.7*	—	*MS*
Acuminatin	1.1	*0.6*	*1.0*	—	*MS*
Austrobailignan-7	—	—	*0.4*	—	*MS*
2-(4-Allyl-2-methoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-1-propanol	*3.5*	*0.5*	*0.3*	—	*MS*
4,4'-Dihydroxy-3,3'-dimethoxy-7,7'-epoxylignan stereoisomer (probably malabaricanol)	—	*1.1*	*3.7*	—	*MS*
Myrislignan [erythro-(1R,2S)-2-(4-allyl-2,6-dimetoxyphenoxyl)-1-(4-hydroxy-3-methoxyphenyl)-propan-1-ol]	*2.5*	*2.1*	*1.8*	—	*MS*
$\Delta^{8'}$-7-Hydroxy-3,4,3',5'-tetramethoxy-8-O-4'-neolignan stereoisomer I	*9.8*	*6.1*	*6.2*	—	*MS*
4,4'-Dihydroxy-3,3'-dimethoxy-7,7'-epoxylignan stereoisomer (probably fragransin A_2)	*2.1*	*4.1*	*1.6*	—	*MS*
$\Delta^{8'}$-7-Hydroxy-3,4,3',5'-tetramethoxy-8-O-4'-neolignan stereoisomer II	*1.9*	—	*1.0*	—	*MS*

(*Continued*)

Table 2 (Continued).

Compounds	B2%	B3%	B4%	RI*	Identification$^{\Phi}$
Tricosane	—	*0.6*	—	—	*MS*
$\Delta^{8'}$-7-Hydroxy-3,4,3',5'-tetramethoxy-8-O-4'-neolignan stereoisomer III	*4.6*	*3.4*	*3.2*	—	*MS*
Licarin C	—	*0.8*	*0.3*	—	*MS*$^{\varphi}$
Surinamensin	—	*1.1*	*0.6*	—	*MS*
2,3-Didydro-7-methoxy-2-(3-methoxy-4,5-methylenedioxyphenyl)-3-methyl-5-(1-propenyl)-benzofuran	*4.7*	*2.7*	*2.7*	—	*MS*
5'-methoxy dehydrodiisoeugenol	*3.1*	*3.2*	*1.6*	—	*MS*
2-(4-Allyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)-1-propanol	*7.7*	*7.0*	*3.8*	—	*MS*
Fragransin D_3	*1.0*	*1.2*	*0.5*	—	*MS*
O-Methyl derivative of fragransin D_3	*0.7*	*0.8*	*0.4*	—	*MS*
Grandisin stereoisomer	*0.7*	*0.7*	*0.5*	—	*MS*
Sitosterol	*Trace*	*0.4*	*0.3*	—	*MS*$^{\varphi}$
Glycerine-1, 3-dimyristate	*1.2*	*Trace*	*29.6*	—	*MS*$^{\varphi}$
Total	*95.7*	*84.0*	*95.3*		

B2: Isopropyl alcohol oleoresin; B3: ethanol oleoresin; B4: ethyl acetate oleoresin.

Trace: <0.05; *RI: retention index. RI was calculated using a homologous series of *n*-alkanes C8-C18; $^{\Phi}$Co-GC: co-injection with an authentic sample.

Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.

$^{\varphi}$Available in the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library, Version 2.0.

Antioxidant Activity for Mustard Oil

The curves in Fig. 1 demonstrate peroxide value changes in mustard oil of all investigated samples at 60°C. It must be noted that peroxide value is widely used to measure the primary lipid oxidation indicating the amount of peroxides formed in the fats and oils during oxidation. Mustard oil oxidation was measured at time intervals of 7 days for 28 days of storage. During this time, the peroxide value of the blank sample was higher than oleoresins, essential oil, BHA, BHT, and PG. The effectiveness of the additives to inhibit the formation of primary oxidation products at a concentration of 200 ppm increases in the order:

$$\text{Control} < \text{BHT} < \text{BHA} < \text{Et. acetate ole.} < \text{Iso pro. alcohol ole.}$$

$$< \text{Ethanol ole.} < \text{Nutmeg oil} < \text{PG.}$$

Primary oxidation products are unstable compounds that produce a number of secondary products, such as alkenes, alcohols, aldehydes, and acids, some of which smell badly at low threshold values. Many of these secondary oxidation products are highly reactive themselves[31] and may initiate the oxidation chain processes. Hence, with the peroxide value, the changes of the secondary oxidation products, such as malonaldehyde and 2-alkenals, measured by thiobarbituric (Fig. 2) and p-anisidine (Fig. 3) after every 7 days. The effects of oil and oleoresins on malonaldehyde formation for mustard oil in terms of incubation time versus TBA value at 60°C are shown in Fig. 2. Malonaldehyde, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative.[32] The malondehyde formation of all the additives increases with storage time. The oil showed a moderate inhibition at 200 ppm concentration, and was comparable to

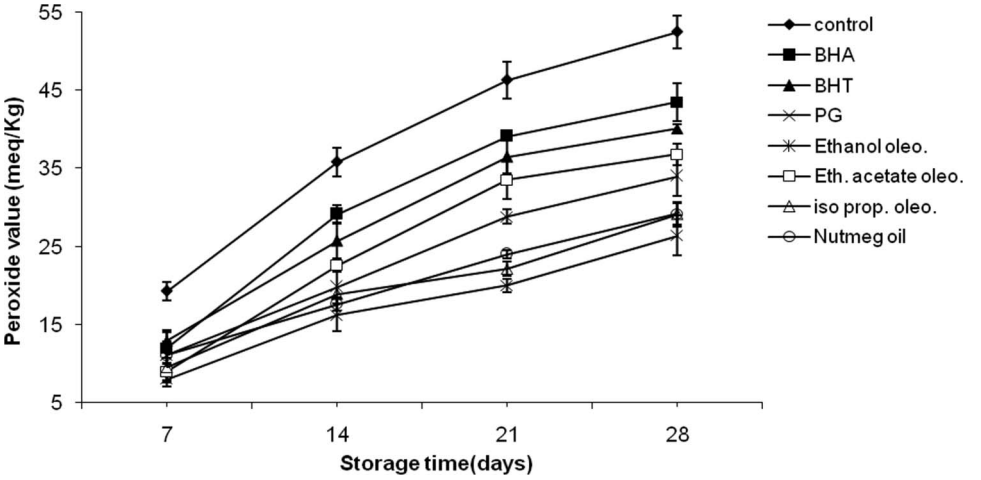

Figure 1 Antioxidant activity of nutmeg oil and oleoresins in terms of peroxide values.

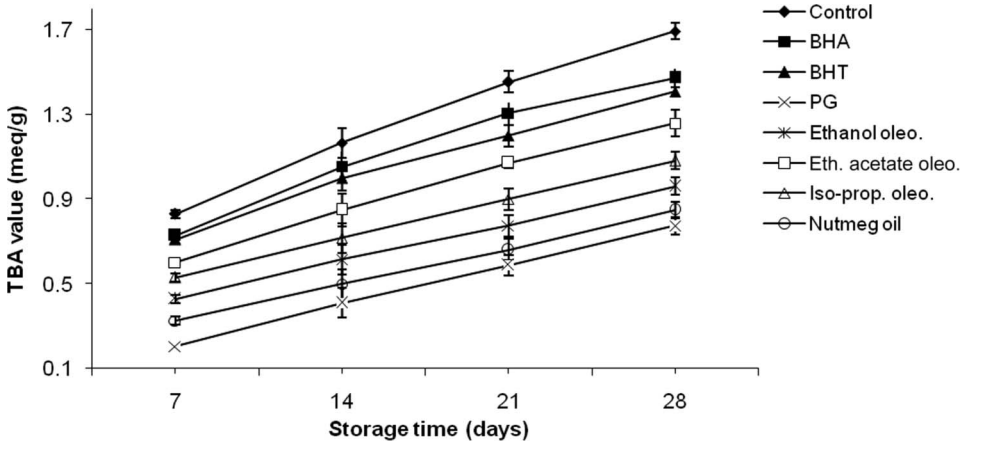

Figure 2 Antioxidative effect of nutmeg oil and oleoresins in terms of thiobarbituric acid values.

BHA and BHT but much lower than PG. These results were well correlated with p-anisidine values (Fig. 3).

From the above results, it is inferred that the formation of the primary oxidation species, peroxides, were also quite similar with the secondary oxidation products, and the changes of both oxidation characteristics are in a good correlation. Hence, the inhibition activity of volatile oil was excellent among all the additives and there was a significant difference between the blank and antioxidants at the $P < 0.05$ level.

Antioxidant Activity in Linoleic Acid System

High absorbance is an indication of a high concentration of formed peroxides. The absorbance values of volatile oils and oleoresins of nutmeg along with synthetic antioxidants are shown in Fig. 4. The absorbance of linoleic acid emulsion without additive

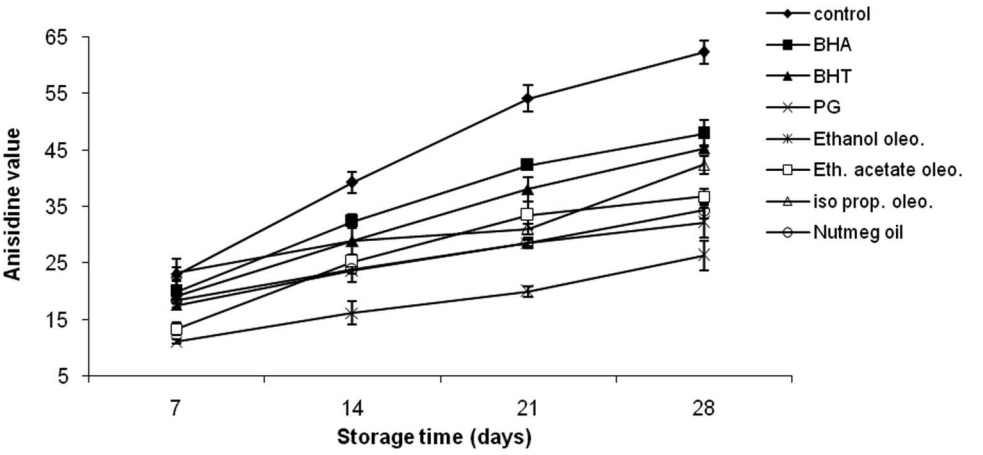

Figure 3 Antioxidative effect of nutmeg oil and oleoresins in terms of p-anisidine values.

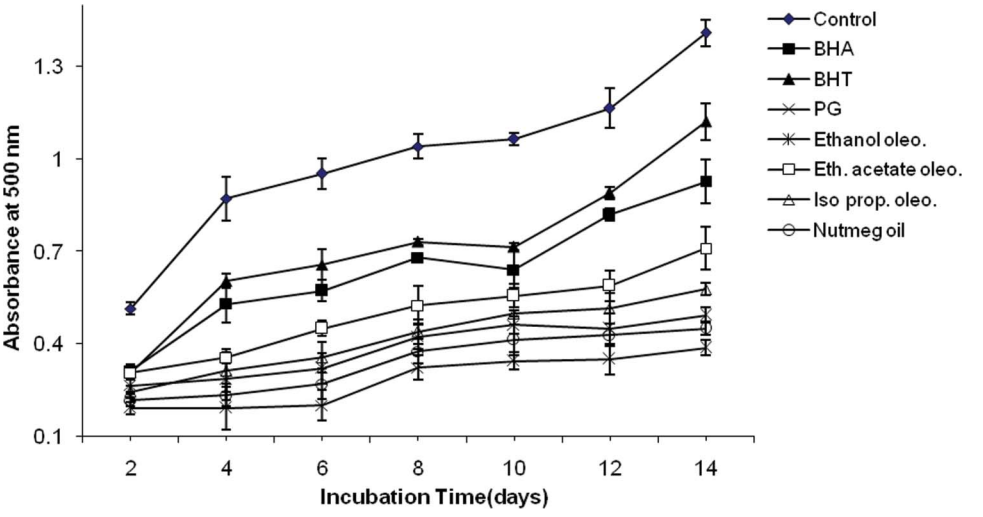

Figure 4 Antioxidant activity of nutmeg oil and oleoresins in linoleic acid.

increased rapidly, and there was a significant difference between blank and antioxidants at the $P < 0.05$ level. It is clear from Fig. 4 that nutmeg volatile oil was most effective among all the additives followed by ethanol oleoresin. However, there are no significant ($P < 0.05\%$) differences between antioxidative activities of oleoresins, oils, BHA, and BHT.

Scavenging Effect on 1,1-Diphenyl-2-Picrylhydrazyl Radical (DPPH)

DPPH radical is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction either by the process of hydrogen- or electron-donation. Substances that are able to perform this reaction can be considered as antioxidants

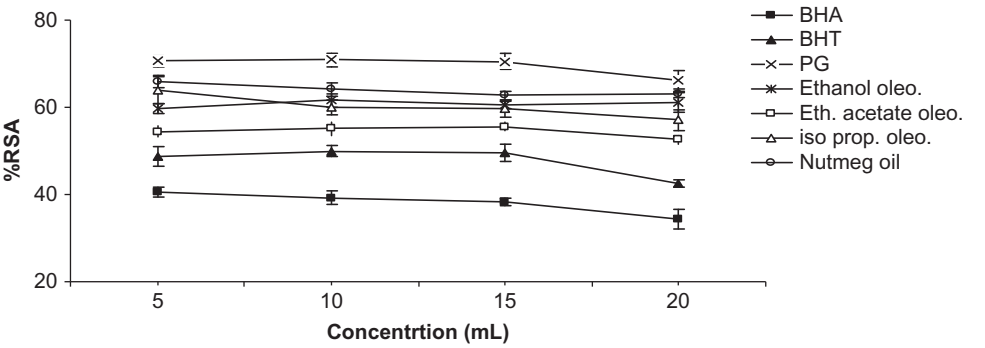

Figure 5 Radical scavenging effect of nutmeg oil and oleoresins on DPPH radical.

and, therefore, radical scavengers.[33] The radical scavenging activity of nutmeg oil and oleoresins is shown in Fig. 5 and expressed as percentage reduction of the initial DPPH absorption by the test compound. The result shows that volatile oil is an excellent radical scavenger of DPPH scavenged under the experimental conditions in comparison with BHA and BHT.

Ferrous Ion Chelating Ability

Ferrozine can quantitatively form complexes with Fe^{+2} but in the presence of ion chelating agents, the complex formation is disrupted, resulting in a decrease in the red color of the complexes. Among essential oil, oleoresins of *M. fragrans* and synthetic antioxidants essential oil showed the highest ferrous ion chelating ability. These abilities were significantly higher ($P < 0.05$) than that of BHA and BHT but lower than PG (Fig. 6). The second highest abilities showed in ethanol oleoresin, which was also significantly ($P < 0.05$) higher than the values of synthetic antioxidants. The iron (II) chelating property of nutmeg essential oil may be attributed to their endogenous chelating agent, mainly phenolics,[34] such as methyl eugenol, trans-isoeugenol, etc.

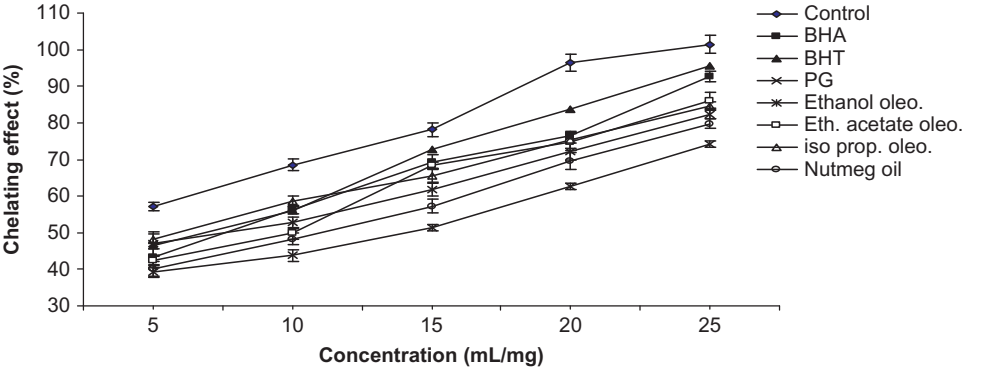

Figure 6 Antioxidative effect of nutmeg oil and oleoresins in chelating activity.

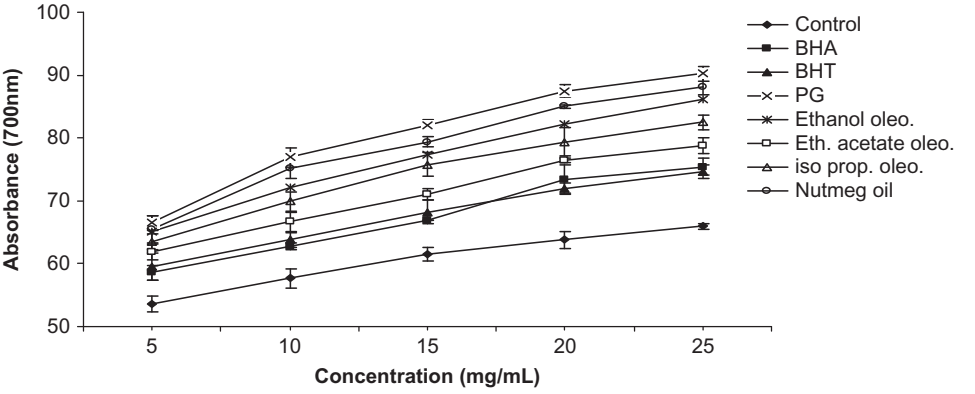

Figure 7 Antioxidative effect of nutmeg oil and oleoresins in reducing power system.

Reducing Power

The reducing ability of a compound generally depends on the presence of reductones[35] that have exhibited antioxidant potential by breaking the free radical chain donating a hydrogen atom.[36] The reducing power of the nutmeg oil and oleoresins along with BHA, BHT, and PG increased with concentration (Fig. 7). The presence of reductants (i.e., antioxidants) in the volatile oil and ethanol oleoresin showed that lower reducing power was found to be better than BHA, BHT, and others, which would lead to stabilization and termination of free radical chain reactions.

Phenolic groups play an important role in antioxidant activity.[37] Hence, the presence of phenolic compounds, such as terpene-4-ol, methyl eugenol, trans-isoeugenol, and trans-methyl eugenol in nutmeg essential oil and ethanol oleoresin, are responsible for the antioxidant activity in all antioxidant assays studied. Strong antioxidant activity of nutmeg volatile oil might be due to the presence of more phenolic components in comparison with oleoresins. It is reported[38] that an aromatic ring having an electron donating substituent (such as terpene-4-ol, elemicin, eugenol, etc.) could increase the antioxidant activity. In addition, antioxidant activity in essential oil and oleoresins could be the synergistic effect of more than two compounds that may present in the system. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidant activity that create an effective defense system against free radical.[39] Based on the result obtained, it is highly possible that several compounds of different polarity may contribute to the antioxidant activity of nutmeg essential oil and oleoresins.

CONCLUSIONS

In conclusion, our study can be considered as the first report on the *in vitro* antioxidant properties of the essential oil and various oleoresins extracted from *M. fragrans*, which introduce a unique natural source that possesses strong antioxidant substances. The results indicate that these are as potent as those of known antioxidants (BHA and BHT). Antioxidant properties of essential oil and oleoresins from nutmeg are of great interest in

the food industry, since their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones.

ACKNOWLEDGMENTS

The authors are thankful to the Head, Chemistry Department, D.D.U. Gorakhpur University, Gorakhpur for providing laboratory facilities. Financial assistance by CSIR for Emeritus scientist to Dr. Gurdip Singh and to CST, U.P. for research assistant to Bandana Singh is highly appreciated.

REFERENCES

1. Haraguchi, H.; Ishikawa, H.; Kubo, I. Antioxidative action of diterpenoids from Podocarpus nagi. Planta Medica **1997**, *63*, 213–215.
2. Sherwin, E.R. Antioxidants. In: *Food Additives*; Branen, R.; Ed.; Marcel Dekker: New York, 1990; 139–193.
3. Hudson, B.J.F. *Food Antioxidants*; Elsevier: Amsterdam, The Netherlands, 1991.
4. Shahidi, F.; Wanasundara, P.K.J.P.D. Phenolic antioxidants. Critical Review Food Science Nutrition **1992**, *32*, 67–103.
5. Lindenschmiidt, R.C.; Trika, A.F.; Guard, M.E.; Witchi, H.P. The effect of dietary hydroxy toluene on liver and colour tumour development in mice. Toxicology **1986**, *38*, 151–160.
6. Okmer, B.; Sigva, H.O.; Mutlu, S.; Doganlar, S; Yemenicioglu, A.; Frary, A. Total antioxidant activity and total phenolic contents in different Turkish eggplant (*Solanum melongena* L.) cultivars. International Journal of Food Properties **2009**, *12* (3), 616–624.
7. Szabo, M.R.; Radu, D.; Gavrilas, S.; Chambre, D.; Iditoiu, C. Antioxidant and antimicrobial activities of selected spice extracts. International Journal of Food Properties **2010**, *13* (3), 535–545.
8. Jukic, M.; Potiteo, O.; Milos, M. Chemical composition and antioxidant effect of free volatile aglycones from nutmeg (*Myristica fragrans* Houtt.) compared to its essential oil. Crotica Chemical Acta **2006**, *79* (2), 209–214.
9. Nadkarni, K.M. Myristica fragrans. In: *Indian Material Medica*, 3rd Ed.; Bombay Popular Parkashan: Bombay, 1988; 830–834.
10. Ogunwande, I.A.; Olaware, N.O.; Adeleke, K.A.; Ekundayo, O. Chemical composition of essential oil of *Myristica fragrans* Houtt. (nutmeg) from Nigeria. Journal of Essential Oil Bearing Plants **2003**, *6*, 21–26.
11. Singh, G.; Marimuthu, P.; Heluani, C.S.; Catalan, C. Antimicrobial and antioxidant potentials of essential oil and acetone extract of *Myristica fragrans* Houtt. (Aril part). Journal of Food Science **2005**, *70*, 2.
12. Singh, G.; Maurya, S.; De Lampasona, M.P.; Catalan, C. A composition of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents (Part 57). Food and Chemical Toxicology **2007**, *45*, 1650.
13. Singh, G.; Marimuthu, P.; Catalan, C.; De Lampasona, M.P. Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract (Part 45). Journal of the Science of Food and Agriculture **2004**, *84*, 1878–1874.
14. Singh, G.; Kapoor, I.P.S.; Singh, P..; Heluani, C.S. de; and Catalan, C.A.N. Chemical composition and antioxidant potential of essential oil and oleoresins from Anise seeds (*Pimpinella anisum* L.). International Journal of Essential Oil Therapeutics **2008**, *2*, 122–130.
15. Singh, G.; Kapoor, I.P.S.; Singh, P.; Heluani, C.S. de; De Lampasona, M.P.; Catalan, C.A.N. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. Food and Chemical Toxicology **2008**, *46*, 3295–3302.
16. Maisonneuve, S.A.; Sainte. Ruffine Europen Pharmacopoeia, 1983, Part 1, P.V.4.5.8.

17. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Quadruple Mass Spectrometry*; Allured Publishing: Carol Stream, Illinois, 2001.
18. Massda, Y. *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*; Halsted/Wiley: New York, 1976.
19. Hattori, M.; Hada, S.; Shu, Y.Z.; Kakiuchi, N.; Namba, T. New acyclic bis-phenylpropanoid from the aril of *Myristica fragrans*. Chemical and Pharmaceutical Bulletin **1987**, *35*, 668–674.
20. Hada, S.; Hattori, M.; Tezuka, Y.; Kikuchi, T.; Namba, T. New neolignans and lignans from the aril of *Myristica fragrans*. Phytochemistry **1988**, *27*, 563–568.
21. Kasahara, H.; Miyazawa, M.; Kameoka, H. Absolute configuration of 8-O-4′-neolignans from *Myristica fragrans*. Phytochemistry **1995**, *40*, 1515–1517.
22. Isogai, A.; Murakoshi, S.; Suzuki, A.; Tamura, S. Isolation from nutmeg of growth inhibitory substances to silkworm larvae. Agricultural and Biological Chemistry **1973**, *37*, 889–895.
23. Bandoniene, D.; Gruzdiene, D.; Venskutonis, P.R. Antioxidant activity of sage extracts in rape seed oil irradiated with UV rays. Nahrung/Food **2001**, *45*, 105–108.
24. Hortwitz, W. (Ed.). 41.1.16. AOAC official method 965.33; Peroxide value of oils and fats. In: *Official Methods of Analysis of AOAC International*, 17th Ed.; AOAC International: Gaithersberg, MD, 2002.
25. Marcuse, R.; Johansson, L. Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. Journal of American Oil Chemist Society **1973**, *50*, 387–391.
26. AOCS. Official methods: p-Anisidine value. In: *Official Methods and Recommended Practices of the American Oil Chemist's Society*, 5th Ed.; AOCS Press: Illinois, 1998; 18–90.
27. Mitsuda, H.; Yuasumoto, K.; Iwami, K. Antioxidation action of indole compounds during the autooxidation of linoleic acid. Eiyo to Shokuryo **1996**, *19*, 210–214.
28. Cunndet, M.; Hostettmann, K.; Potterat, O.; Dyatmilko, W. Iridoid glucosides with free radical scavenging properties from Fagraea blumei. Helvetica Chimica Acta **1997**, *80*, 1144–1152.
29. Senevirathne, M.; Kin, S.H.; Siriwardhana. N.; Ha, J.H.; Lel, K.W.; Jeon, Y.J. Antioxidant potential of Ecklonia cava on reactive oxygen species, scavenging, metal chelating, reducing power and lipid peroxidation inhibition. Food Science Technology International **2006**, *12* (1), 27–38.
30. Decker, E.A.; Welch, B. Role of ferritin as a lipid oxidation catalyst in muscle food. Journal of Agricultural and Food Chemistry **1990**, *38*, 674–677.
31. Shahidi, F. Natural antioxidants: An overview. In: *Application*; Shahidi, F.; Ed.; AOCS Press: Champaign, Illinois, 1997; 1–11.
32. Botsoglou, N.A.; Fletouris, D.J.; Papageorgion, G.E.; Vassilopoulos, V.N.; Mantis, A.J.; Trakatellis, A.G. A rapid sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissues, food and feedstuffs samples. Journal of Agricultural and Food Chemistry **1994**, *42*, 1931–1937.
33. Brand-Williams, W.; Cuvelier, M.; Berset, C. Use of a free radical method to evaluate antioxidant activity. LWT–Food Science Technology **1995**, *28*, 25–30.
34. Thompson, M.; Williams, C.R. Stabilty of flavanoid complexes of Copper (II) and flavanoid antioxidant activity. Analytica Chemica Acta **1976**, *85*, 375–381.
35. Duh, P.-D. Antioxidant activity of Budrock (*Arctium laooa* Linn): Its scavenging effect on free radical and active oxygen. Journal of the American Oil Chemists Society **1998**, *75*, 455–461.
36. Gordon, M.H. The mechanism of the antioxidant action in vitro. In: *Food Antioxidants*; Hudson, B.J.F.; Ed.; Elsevier: London, 1990; 1–18.
37. Baratta, M.T.; Dorman, H.J.D.; Deans, S.G.; Figueiredo, A.C.; Baroso, J.G.; Ruberto, G. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and Fragrance Journal **1998**, *104*, 286–292.
38. Zhang, H.Y. Theoretical methods used in elucidating activity differences of phenolic antioxidants. Journal of the American Oil Chemists Society **1999**, *76*, 745–748.
39. Lu, F.; Foo, L.Y. Phenolic antioxidant component of evening primrose. In: *Nutrition, Lipids, Health and Disease*; Ong, A.S.H.; Niki, E.; Packer, L.; Eds.; American Oil Chemists Society Press: Champaign, Illinois, 1995.