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Effect of γ-Irradiation on the Lipid Profile of Nutmeg (*Myristica fragrans* Houtt.)

ZAREENA NIYAS, PRASAD S. VARIYAR,* ACHYUT S. GHOLAP, AND ARUN SHARMA

Food Technology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

The effect of γ-irradiation on the lipid constituents of nutmeg (*Myristica fragrans*) was examined at radiation doses between 2.5 and 10 kGy. The fatty acid composition of the triacylglycerol, the major lipid component, was found to be made up of myristic (90%), palmitic (6%), lauric (3%), petroselinic (0.13%), and stearic acids (0.5%) as determined by gas chromatography−mass spectrometry. A dose-dependent decrease in the triacylglycerol content and a concomitant increase in free fatty acids characterized the lipid profile of the irradiated spice. This suggested a breakdown of acylglycerols during radiation processing, resulting in the release of free fatty acids. These changes were found to be significant at doses above 5 kGy. The impact of the above changes on the flavor of the spice is discussed. These studies suggest that radiation processing of nutmeg should be limited to a dose of 5 kGy.

KEYWORDS: Flavor; γ-irradiation; lipids; nutmeg; gas chromatography−mass spectrometry (GC-MS)

INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) is a spice widely used for its aromatic and flavoring properties. The characteristic flavor of the spice is attributable to its steam volatile essential oils. The volatile oil content of nutmeg is reported to be 10−15% (*1*).

Nutmeg also contains 25−50% lipids as fixed oil comprising mainly myristic, petroselinic, and palmitic acids (*1*). Although steam volatile essential oils are mainly responsible for the aroma and flavor properties of nutmeg, lipids have a modifying effect on its flavor (*1*). Salzer (*2*) attributed the strong and coarser note of nutmeg to its fixed oil that, due to its higher saturated fatty acid content and melting point, modifies the impression of the essential oil on the tongue.

Like other spices, nutmeg is also prone to microbial contamination and infestation by insect pests during processing and postharvest storage. Although fumigation is a widely used method for the decontamination of spices, it has several drawbacks. It leaves a harmful residue and is unsafe to workers and the environment. The process is being phased out in several countries. Exposure to ionizing radiation such as γ-rays provides an effective alternative. It is a nonthermal process and leaves no toxic residue (*3*). Doses ranging from 5 to 10 kGy are normally recommended for the sterilization of spices without adversely affecting their flavor quality (*4*). Increasing importance is being accorded to food irradiation the world over in order to counteract the prevalence of foodborne illness. One major drawback in the irradiation of high-fat-containing foods is the production of off-odor due to radiation-induced breakdown of lipids (*3*). Because nutmeg is a high-fat-containing spice, it was of interest to ascertain whether at doses recommended for the decontamination of spice changes in any of the flavor characteristics of the spice occur. The effect of γ-radiation on the volatile oil constituents of nutmeg obtained by steam distillation has been reported earlier. No detectable changes in these components up to a dose of 10 kGy were reported (*5*). However, there are no reports so far on the effect of radiation processing on nutmeg lipids. The present work therefore attempts to determine the changes, if any, in the lipid composition of irradiated nutmeg.

EXPERIMENTAL PROCEDURES

Commercial samples of dry nutmeg (1000 g) were obtained from three different local markets of Mumbai, India. Each of the three samples was divided into two lots. One lot (200 g) was used as non-irradiated control. The other lot was further subdivided into four equal lots of 200 g each and then exposed to γ-radiation at 25 °C to overall average doses of 2.5, 5, 7.5, and 10 kGy at a dose rate of 14 Gy/min using a ^{60}Co package irradiator (AECL, Ottawa, Canada). Samples were analyzed within 1 week of storage. For lipid analysis 1 g each of the above samples was analyzed in triplicate. Thus, the data collected for each assay are the averages of three independent determinations, each carried out in triplicate, and standard errors were then calculated. Thus, a total of nine estimations have been carried out for each sample. Reagents and solvents used were obtained from E. Merck India Ltd. (Mumbai, India) and were of analytical grade. All solvents were distilled before use.

Sensory Analysis. Sensory evaluation of the above samples was carried out for both non-irradiated and irradiated samples of nutmeg. Different samples of finely powdered nutmeg placed separately in airtight stoppered 50-mL conical flasks were sniffed to carry out aroma evaluation of the spice. The flask was sniffed after 15 min for any difference in aroma. A panel of five judges who were familiar with nutmeg aroma carried out the sensory evaluation. Evaluation was made on a 5 point hedonic scale: 1 = very poor (rancid/soapy odor); 2 =

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poor (soapy odor); 3 = less acceptable (phenolic odor); 4 = acceptable (spicy and phenolic odor, nutmeg-like); and 5 = highly acceptable (characteristic nutmeg odor). Data used for evaluation are the average of three independent determinations in all cases.

Isolation and Analysis of Lipids. *Isolation of Total Lipids.* Powdered nutmeg (1 g), controls (non-irradiated) as well as irradiated samples (2.5, 5, 7.5, and 10 kGy), were subjected to solvent extraction. Samples in each case were soaked in chloroform/methanol (2:1) for 90 min and then individually extracted in an Omnimixer for 1 min (3 × 10 mL) at room temperature. The resulting slurry in all cases was separately filtered under suction and the residue re-extracted with the same solvent mixture until the filtrate was colorless. The filtrate from each sample was then individually concentrated to dryness under vacuum using a Buchi flash evaporator to obtain a dried residue. A 20% stock solution in chloroform of total lipids was prepared in all cases.

Analysis of Total Lipids. Analytical thin-layer chromatography (TLC) of both control and irradiated samples was carried out on ammonium sulfate (5%) impregnated silica gel G plates (0.25 mm thickness). The plates were developed using petroleum ether (60−80 °C)/diethyl ether/acetic acid (90:10:1) as developing solvent system. Spots were visualized either by exposing the plate to iodine vapors or by heating the plate for 15 min at 180 °C. The spots of interest were identified by comparing the R_f values with those of standard compounds.

Isolation of Fatty Acids. Preparative TLC (0.5-mm thickness) of total lipid of both control and irradiated samples was performed on silica gel G plates using the same solvent system as above. The bands at R_f values corresponding to free fatty acids (FFA) were scraped and eluted with chloroform.

Analysis of Fatty Acids. Free fatty acids (FFAs) obtained by preparative TLC of unhydrolyzed control and irradiated samples (20 mg) as described above were converted to their fatty acid methyl esters (FAMEs) using diazomethane gas in diethyl ether (50 mL) as methylating agent (room temperature, overnight). Complete conversion of fatty acids to their methylated derivative was confirmed on analytical TLC. FAMEs thus obtained were isolated by preparative TLC and then analyzed using a Shimadzu QP-5050A gas chromatography−mass spectrometry (GC-MS) instrument (Shimadzu Corp., Kyoto, Japan). The instrument was equipped with a GC-17A gas−liquid chromatograph (GLC) and provided with a DB-1 (dimethyl polysiloxane, J&W Scientific, Folsom, CA) capillary column (length = 30 m, i.d. = 0.25 mm, and film = 0.25 μm). The operating conditions were as follows: column temperature, programmed from 140 to 200 °C at the rate of 4 °C/min, held at initial temperature and at 200 °C for 5 min, and further programmed to 280 °C at the rate of 10 °C/min, and held at the final temperature for 20 min. Injector and interface temperatures were maintained at 210 and 230 °C, respectively. Helium was used as carrier gas at a linear flow rate of 0.9 mL/min. Ionization voltage was 70 eV. Electron multiplier voltage was 1 kV. Compounds of interest were identified by comparing their mass fragmentation pattern with that of standard spectra available in the spectral library (Flavor and Fragrance and Wiley/NIST libraries) of the instrument as well as by comparing the retention time of each standard fatty acids (Sigma, St. Louis, MO) injected separately under the same conditions.

Colorimetric Estimation. Colorimetric estimation of FFAs was carried out according to the Duncombe method (*6*) using palmitic acid as standard fatty acid.

For preparation of the standard curve, aliquots of standard fatty acid in chloroform (5 mL) were prepared ranging in concentration from 10 to 100 μM. To each aliquot was added 2.5 mL of copper reagent [10 volumes of a 6.45% solution containing 1 M aqueous triethanol amine (9 volumes), 1 N acetic acid (1 volume), and 6.45% copper nitrate (10 volumes)]. The tubes were shaken vigorously for 2 min and then centrifuged (10 min, 40 rpm) to separate into two clear layers. After removal of the aqueous phase using a hypotonic needle, 0.5 mL of diethyl dithiocarbamate (0.1% w/v) was added to 3 mL of organic phase containing fatty acids of interest. The absorption of the resultant yellow complex was then measured spectrophotometrically at 440 nm. A graph of concentration versus optical density was then drawn to obtain a standard curve. The plot was found to be linear up to a concentration of 50 μM.

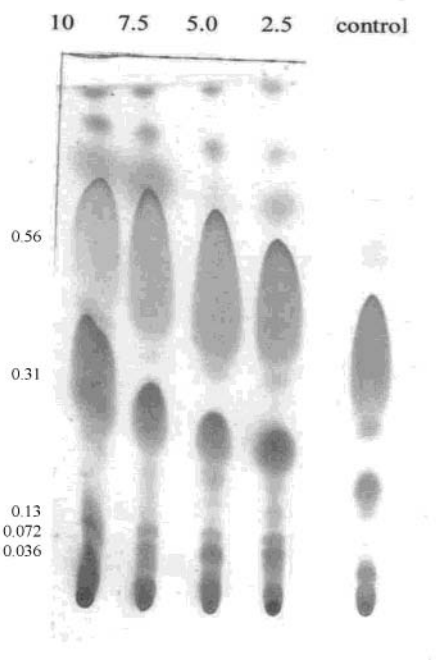

Figure 1. TLC chromatogram of lipid extract isolated from control and irradiated (kGy) samples of nutmeg.

For assay, the samples under investigation (20% stock solutions) were appropriately diluted, and 5 mL each of these solutions was then treated with the above reagents in the same manner as described for the preparation of standard curve. The content of FFAs was calculated from the standard curve and expressed as milligrams of palmitic acid equivalent per gram of nutmeg. All samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Nutmeg fat has been reported to have a significant modifying effect on the aroma of the spice (*1*). It was therefore of interest to determine changes, if any, in these constituents at doses used for radiation processing of nutmeg. Chloroform/methanol extract of nutmeg yielded a pale yellow solid with an average yield of 30% in both the control and irradiated samples. The fixed oil content of sound nutmeg has been reported to range from 25 to 40% (*1*). γ-Irradiation does not bring about any alteration in the yield of lipid up to a dose of 10 kGy.

Figure 1 shows the TLC chromatogram of lipid extract isolated from control and irradiated samples. The extract resolved into several spots of which triacylglycerol (R_f 0.56) was identified as the major lipid class by comparison of its R_f value with that of the standard compounds. Other lipid classes identified include FFAs (R_f 0.31), sterols (R_f 0.13), diacyl glycerols (R_f 0.072), and monoacyl glycerols (R_f 0.036). Phospholipids were found to be in low amounts in this spice. As shown in the figure an increase in the content of FFAs and a decrease in triacylglycerol content with increasing dose could be noted. In fact, a rapid degradation of triglycerides could be clearly distinguished at doses above 7.5 kGy. Concentration of most of the other spots was more or less similar between different samples analyzed. Increased breakdown of triacylglycerols and release of FFA with increasing radiation dose could thus be inferred.

To determine the nature of the above fatty acids, the FFAs (R_f 0.31) present in both control and irradiated samples were isolated by preparative TLC. Myristic acid was the major compound identified, accounting for 90% of the total FFAs in both the control and irradiated samples, respectively. The relative

Table 1. Content of Free Fatty Acids in Different Nutmeg Samples (Mean ± SE)[a]

sample	free fatty acids (mg/g of nutmeg)
control	168.96 ± 0.10
2.5 kGy	169.21 ± 0.04
5.0 kGy	178.54 ± 0.07*
7.5 kGy	245.54 ± 0.07*
10 kGy	265.16 ± 0.11*

[a] Data are the average of three independent determinations each analyzed in triplicate ($n = 9$). * indicates significant difference in values from control sample at the 0.05 level ($p < 0.05$).

Table 2. Sensory Evaluation of Various Powdered Nutmeg Samples[a]

sample	non-irradiated	2.5 kGy	5.0 kGy	7.5 kGy	10.0 kGy
score	5.0	4.8	4.6	1.8	1.4
SE	0.0	0.20	0.24	0.37	0.24

[a] Overall aroma was evaluated using a 5 point scale where 1 = very poor, 2 = poor, 3 = less acceptable, 4 = acceptable, and 5 = highly acceptable. $n = 45$.

distribution of other fatty acids identified in control was lauric acid (3%), palmitic acid (6%), petroselinic acid (0.13%), and stearic acid (0.5%). Results were in conformity with that reported earlier (*1*). The corresponding values for irradiated samples were 0.50, 7.3, 1.7, and 0.45%, respectively.

A quantitative approach was made to determine the increase in FFAs with increase in radiation dose. The content of FFAs (millgrams per gram of dry weight of nutmeg) present in different nutmeg samples is presented in **Table 1**. An increase in FFA content could be clearly noted in the nutmeg samples treated at doses above 5 kGy. This is in accordance with TLC data given above.

Sensory evaluation of whole nutmeg subjected to various doses of γ-radiation indicated clear and perceivable differences between the control and irradiated powdered spice (**Table 2**). In fact, at doses beyond 5 kGy a discernible rancid/soapy odor could be noted by the majority of the trained panelists. As it has been already reported (*5*) that radiation treatment does not have a noticeable effect on the aroma constituents of the spice, it can therefore be presumed that the unacceptable odor perceived beyond 5 kGy may be attributed to the effect of ionizing radiation on the lipid constituents of the spice.

The contribution of short-chain saturated fatty acids formed by the hydrolytic/lipolytic rancidity to the off-flavor development in food products has been reported in the literature (*7*). Ohren and Tucky (*8*) reported that the contribution of specific FFAs had an important influence on the flavor of cheese. Samples that had abnormally high amounts of capric (C_{10}), lauric (C_{12}), and myristic (C_{14}) acids had unclean, rancid, and soapy flavors. The olfactory perception threshold values of lauric and myristic acids in oil were shown by Kinsella (*9*) to be 700 and 5000 ppm, respectively. Thus, when the concentrations of these compounds attain high levels, these particular off-flavors ensue. The breakdown of triaclyglycerols to liberate FFAs when exposed to ionizing radiation has been reported earlier in seafood with subsequent development of off-flavors at higher dose (*10*). The contribution of myristic acid to soapy rancid odors at high concentrations can therefore be proposed.

The present study has thus shown that the dose range of 5−10 kGy for the purpose of microbial decontamination of spices cannot be applied to nutmeg due to the presence of a high lipid content. No odor difference persists between the control and irradiated nutmeg samples up to a dose of 5 kGy. Beyond this dose, olfactory perception of FFAs released during radiolysis increases beyond their threshold values, resulting in a discernible off-odor. Because nutmeg is mainly prone to insect infestation, doses up to 5 kGy are normally sufficient to disinfest the spice. Thus, 5 kGy can be considered as the threshold dose for nutmeg.

ABBREVIATIONS USED

FAME, fatty acid methyl ester; FFA, free fatty acids; GC-MS, gas chromatography−mass spectrometry; GLC, gas−liquid chromatography; TLC, thin-layer chromatography.

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