Effect of γ-Irradiation on Flavor Compounds of Fresh Mushrooms

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Fresh mushrooms (*Agaricus bisporus*) were γ-irradiated with doses of 1, 2, and 5 kGy. The volatile compounds were isolated using a Lickens−Nickerson apparatus and analyzed using gas chromatography and gas chromatography−mass spectrometry. The amount of total volatiles was greatly affected by the doses applied. The amounts of benzaldehyde and benzyl alcohol were not affected by γ-irradiation and ranged from 8.94 to 11.79 and from 0.696 to 1.503 μg/g, respectively. The amounts of eight-carbon compounds decreased as the doses of γ-irradiation increased, from 41.73 for the control (0 kGy) to 20.06 (1 kGy), 8.77 (2 kGy), and 4.04 μg/g (5 kGy irradiated mushrooms). The major eight-carbon compound was 1-octen-3-ol, and its amount decreased from 30.34 (the control) to 14.18 (1 kGy), 6.22 (2 kGy), and 2.92 μg/g (5 kGy).

Keywords: *Mushrooms; γ-irradiation; flavor compounds; 1-octen-3-ol*

INTRODUCTION

Because of the extremely perishable nature of cultivated mushrooms (*Agaricus bisporus*) and their high commercial value, the possibility of extending mushrooms' shelf life using ionizing radiation has been studied. Staden (1967) found that cap opening and stipe elongation in harvested mushrooms could be inhibited by γ-irradiation (2 kGy). Furthermore, Casalina (1971), Langerak (1972), Yamaguchi and Campbell (1973), and Wahid and Kovacs (1980) also confirmed γ-irradiation to be a very effective method of controlling deterioration and improving the quality and shelf life of fresh mushrooms.

In addition, Kramer (1986) found that a dose of 0.25 kGy was ineffective in controlling senescence, while a dose of 2 kGy showed no significant improvement over 1 kGy in terms of keeping quality. Zhang *et al.* (1981) indicated that a dose of 1 kGy could extend the shelf life of fresh mushrooms to 20−40 days in terms of cap opening at 4−10 °C. Ajlouni (1991) found no significant changes in the rate of water loss, soluble sugar contents, or absolute fresh weight of the various tissues of irradiated and unirradiated mushrooms during postharvest storage. Also, sensory evaluation showed that the examined attributes, including appearance, odor, flavor and texture, were not affected by irradiation (Lescano, 1994). Narvaiz (1994) evaluated the texture of irradiated mushrooms and found that only the force required to compress the mushroom cap between its edges decreased more readily in the control than in irradiated samples. However, doses applied ranged from 0.065 to 4 kGy, and the different results could be attributed to the physiological age of the mushrooms at harvest, time elapsed between harvest and irradiation, different strains, storage conditions, and type of radiation sources (Thomas, 1988).

Flavor represents one of the most important quality attributes contributing to the widespread consumption of edible mushrooms. The characteristic volatile compounds present in mushrooms are eight-carbon compounds, including 1-octanol, 3-octanol, 3-octanone, 1-octen-3-ol, 2-octen-1-ol, and 1-octen-3-one (Cronin and Ward, 1971; Pyysalo and Suihko, 1976; Fisher and Grosch, 1987). Among them, 1-octen-3-ol is the most important compound associated with fresh mushroom flavor, and its formation in mushrooms has been extensively studied (Mau *et al.*, 1992, 1993).

When postharvest treatments such as refrigeration (4 °C) are combined with low-dose γ-irradiation, the shelf life of fresh mushrooms would be extended longer, but the change of volatile flavor in irradiated mushrooms is unknown. Therefore, our objective was to study the flavor components in irradiated mushrooms in order to provide information for commercial application.

MATERIALS AND METHODS

Mushrooms. Cultivated mushrooms, *Agaricus bisporus* (Lange) Imbach (Tainung 3), were obtained from Taichung County, Taiwan. On the peak day of each flush, mushrooms were harvested before the veil was broken. Freshly harvested mushrooms were transported in a cooler with ice within approximately an hour after harvest to the Food Science Building, the Main Campus of National Chung-Hsing University, Taichung City, Taiwan, and placed in cold storage (4 °C) for approximately 2 h before packaging. Mushrooms were sorted on the basis of size and appearance. Diseased, damaged, misshapen, open-veiled, and extremely large and small mushrooms (>40 or <25 mm in cap diameter) were discarded. Mushrooms of uniform size (25−40 mm) and maturity in the button stage (veil intact and tight) were used.

Packaging and Irradiation. The first four successive flushes of mushrooms were randomly selected and packaged by placing 100 g (about five to seven mushrooms) in 600-mL PVC (polyvinyl chloride) trays and overwrapping with MK-PVC film (0.016−0.018 mm, Kabaido Co., Japan) using a B-105 Diawrapper (ARC Co., Japan). After packaging, mushrooms were transported in a cooler with ice to China Biotech Corp., Taichung City, Taiwan, and placed in a refrigerator at 4 °C before γ-irradiation. Mushrooms were either unirradiated or γ-irradiated with doses of 1, 2, and 5 kGy (^{60}Co, 600 000 Ci, 6.78×10^2 kGy/h) at ambient temperature. After γ-irradiation, mushrooms were returned to the refrigerator at 4 °C and transported in a cooler with ice to the Food Science Building on the next day.

Volatile Compound Extraction. A tray of mushrooms (100 g) was cut into small cubes and blended with 300 mL of 0.1 M sodium phosphate buffer (pH 6.5) containing 0.15% Tween 80 (Wako Pure Chemical Co., Osaka, Japan) and 1 mL of methanol containing 1000 μg of 1-nonanol (Sigma Chemical

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Co., St. Louis, MO) as the internal standard. After 1 min of blending, the homogenate was placed into a modified Lickens−Nickerson apparatus and extracted with 25 mL of diethyl ether (Merck, Darmstadt, Germany, glass distilled) and 25 mL of *n*-pentane (Merck, glass distilled). The steam distillation−solvent extraction (SDE) was allowed to proceed for 2 h, and the extract thus obtained was dried over anhydrous sodium sulfate (Merck) and filtered. The filtrate was preconcentrated with a distillation apparatus at 40 °C and carefully reconcentrated to approximately 50 μL using a 10 cm × 0.2 mm i.d. Vigreux column at 40 °C. Three samples from each irradiation dose were examined, and the experiment was conducted four times for the four successive flushes.

Gas Chromatography. The concentrated isolate was analyzed using a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard 3396A integrator. A fused silica column (30 m × 0.53 mm, J&W, Folsom, CA) coated with DB-Wax (0.25 μm thickness) was used. The oven temperature was programmed from 50 to 200 °C at 2 °C/min. The injector and detector temperatures were 250 °C. The carrier gas was nitrogen at a flow rate of 1.5 mL/min. The linear retention indices of the volatile components were calculated with *n*-paraffin (C_5−C_{25}) as references (Schomberg and Dielmann, 1973). The amount of each component was determined using an internal standard method and calculated by each peak area of gas chromatograms.

Gas Chromatography−Mass Spectrometry (GC−MS). The identification of volatile components was carried out using a Hewlett-Packard 5890A II gas chromatograph coupled to a Hewlett-Packard 5971A MSD mass spectrometer. A fused silica column (50 m × 0.32 mm, Chrompack, Middelburg, The Netherlands) coated with CP-Wax 52 CB (0.25 μm thickness) was used. The operating conditions were as follows: injector temperature, 250 °C; GC−MS interface temperature, 265 °C; helium carrier flow rate, 1.0 mL/min. The oven temperature was held at 50 °C for 5 min, then programmed from 50 to 200 °C at 1.5 °C/min, and held at 200 °C for 10 min. A split ratio of 60:1 was used. Mass spectra were obtained from electron multiplier voltage and electron ionization energy at 1500 V and 70 eV, respectively. Volatile compounds were identified by comparing the mass spectral data with those spectra available from the Wiley computer and the EPA/NIH computer library and by comparing GC retention times of the components with those of authentic compounds.

Statistical Analysis. The experimental data were subjected to an analysis of variance for a randomized complete block design to determine the least significant difference among means at the level of 0.05.

RESULTS AND DISCUSSION

The volatile components in mushrooms were found to be 3-octanone, 1-octen-3-one, 3-octanol, 1-octen-3-ol, benzaldehyde, 1-octanol, 2-octen-1-ol, and benzyl alcohol (Figure 1 and Table 1). These results from Tainung 3 strain of *Agaricus bisporus* were consistent with the findings of Wasowicz (1974), Pyysalo and Suihko (1976), Tressl *et al.* (1982), and Fisher and Grosch (1987). The major compound found in unirradiated mushrooms was 1-octen-3-ol, accounting for 57.2% of the total volatiles and 72.7% of the eight-carbon compounds. In irradiated mushrooms, 1-octen-3-ol comprised 43.6% of the total volatiles and 70.7% of the eight-carbon compounds for 1 kGy, 31.4% and 70.9% for 2 kGy, and 21.3% and 72.3% for 5 kGy, respectively. Generally, 1-octen-3-ol was the major compound of eight-carbon compounds in unirradiated and irradiated mushrooms.

The amounts of total volatiles were greatly affected by the doses applied and decreased as the doses increased (Table 2). However, the amounts of aromatic compounds, including benzaldehyde and benzyl alcohol, were not affected by γ-irradiation and ranged from 8.94 to 11.79 and from 0.696 to 1.503 μg/g mushroom,

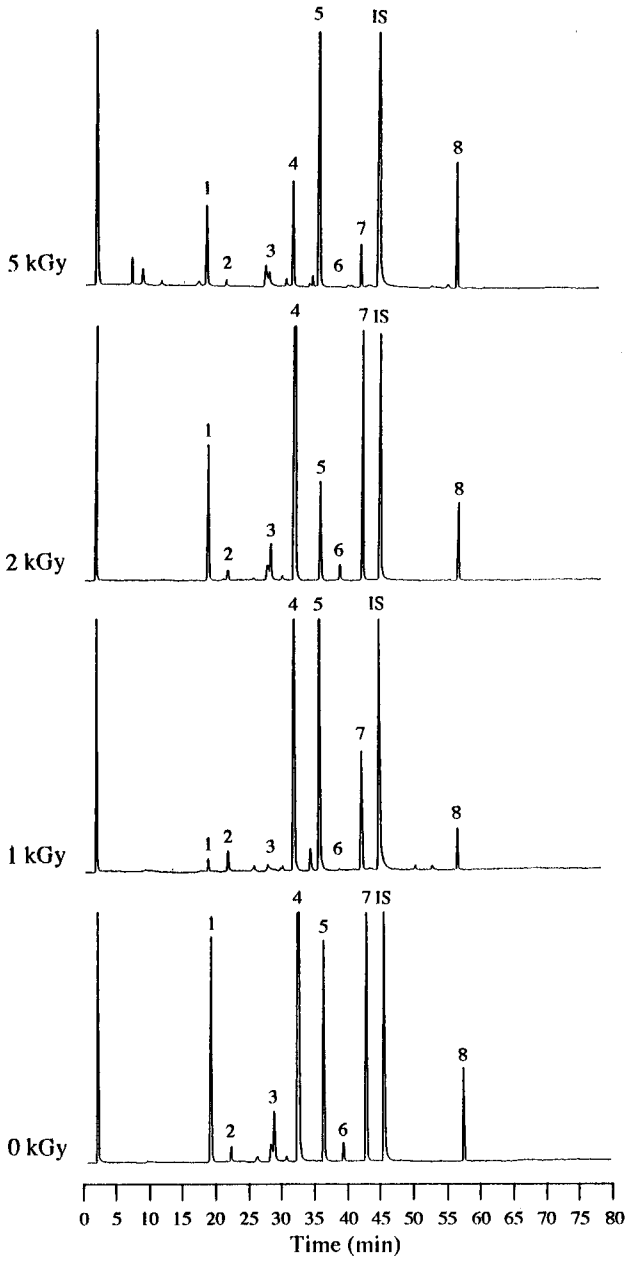

Figure 1. Qualitative gas chromatograms of volatile compounds of fresh mushrooms (*Agaricus bisporus*) irradiated with 0, 1, 2, and 5 kGy. The peak numbers correspond to compounds in Table 1; IS, internal standard.

respectively (Table 1). The amounts of eight-carbon compounds were significantly affected by the doses applied and decreased as the doses of γ-irradiation increased, from 41.73 (100%) for the control (0 kGy) to 20.06 (48%) for 1 kGy, 8.77 (21%) for 2 kGy, and 4.04 μg/g (10%) for 5 kGy irradiated mushrooms (Tables 1 and 2). In addition, the amount of the major component, 1-octen-3-ol, was decreased from 30.34 (100%) for the control (0 kGy) to 14.18 (47%) for 1 kGy, 6.22 (21%) for 2 kGy, and 2.92 μg/g (9.6%) for 5 kGy (Table 2). However, the amounts of aromatic compounds were not affected and markedly increased its percentage in total volatiles as the doses increased and became the major compounds in irradiated mushrooms (Table 2).

Grosch and Wurzenberger (1984) found that 1-octen-3-ol was the major mushroom flavor compound, which was formed from linoleic acid by the action of enzymes lipoxygenase and hydroperoxide lyase, and other eight-carbon compounds were formed from 1-octen-3-ol by

Table 1. Effect of γ-Irradiation on Volatile Compounds of Fresh Mushrooms (*Agaricus bisporus*)

peak no.[a]	compound	RI[b]	content[a] (μg/g mushroom) 0 kGy	1 kGy	2 kGy	5 kGy
1	3-octanone	1270	2.360a[d]	1.378b	0.662c	0.325c
2	1-octen-3-one	1316	0.247a	0.266a	0.203a	0.048b
3	3-octanol	1406	2.582a	1.839b	0.353c	0.237c
4	1-octen-3-ol	1472	30.341a	14.184b	6.218c	2.917c
5	benzaldehyde	1534	9.793a	11.792a	10.282a	8.942a
6	1-octanol	1579	0.198a	0.084b	0.052b	0.021b
7	2-octen-1-ol	1637	6.005a	2.312b	1.287b	0.490c
8	benzyl alcohol	1893	1.503a	0.696a	0.739a	0.763a

[a] The peak numbers correspond to those given in Figure 1. [b] Linear retention index determined on a DB-Wax column using *n*-paraffins (C_8−C_{25}) as reference standards. [c] Content is the average of data collected from three replicate samples for each of four flushes. [d] Means with the same letter within a row are not significantly different ($p = 0.05$).

Table 2. Effect of γ-Irradiation on the Content and Percentage Reduction of Volatile Compounds of Fresh Mushrooms (*Agaricus bisporus*)

compound	content (μg/g mushroom) [% reduction][a] 0 kGy	1 kGy	2 kGy	5 kGy
total volatiles	53.03 [100]	32.55 [61]	19.79 [37]	13.74 [26]
C8 compounds	41.73 [100]	20.06 [48]	8.77 [21]	4.04 [10]
1-octen-3-ol	30.34 [100]	14.18 [47]	6.22 [21]	2.92 [9.6]
aromatic compounds[b]	11.30 [100]	12.49 [110]	11.02 [98]	9.71 [86]

[a] Based on the control (0 kGy). [b] Including benzaldehyde and benzyl alcohol.

other enzyme systems in the mushrooms. Additionally, Chen and Wu (1984) showed the presence of a reductase system for the conversion of 1-octen-3-one to 1-octen-3-ol in mushrooms. These findings support the observation in this study that the effect of γ-irradiation with increased doses on 1-octen-3-ol was consistent with that on eight-carbon compounds (Table 2).

Nishimura *et al.* (1971) found the volatile components of onion were not affected by γ-irradiation up to 0.7 kGy. In cooked potato, γ-irradiation with 1 kGy increased the amount of volatile compounds (Tajima *et al.*, 1967). Narvaiz *et al.* (1989) mentioned that the amounts of total volatiles obtained by steam distillation of spices, including white pepper, coriander, nutmeg, and cinnamon, were reduced by 10 kGy irradiation, but amounts for irradiated clove were almost 3-fold higher than those of the control. These previous results suggested that an optimal dose of irradiation increased the amount of total volatiles but that decreases resulted from high doses. However, in this research, irradiation of as low as 1 kGy seemed sufficient to decrease the amount of total volatiles, primarily the amount of eight-carbon compounds.

In dry shiitake (*Lentinula edodes*), Lai *et al.* (1994) reported that the major flavor compounds, including eight-carbon compounds and sulfur-containing compounds, were significantly reduced by the γ-irradiation with 5 and 10 kGy. However, Yang (1995) irradiated fresh shiitake with 2 kGy and found that the amount of eight-carbon compounds was 1.8-fold higher than that of the control. 1-Octen-3-ol was increased from 30.7 for the control to 53.7 μg/g for 2 kGy irradiated shiitake (Yang, 1995). The discrepancy in the increase of 1-octen-3-ol amount in shiitake and decrease in *Agaricus* mushrooms after irradiation was not clearly understood, and therefore, further study is needed.

At relatively low doses (2−3 kGy), irradiated fruits and vegetables showed loss of texture as a result of changes in cell walls (Urbain, 1985). Similarly, the irradiation applied in this study might damage the cell wall and/or cell membrane of mushrooms. In addition, the two enzymes responsible for 1-octen-3-ol formation are found to be membrane-bound (Gardner, 1989). Therefore, the findings of flavor loss might be due to the reduced activities of lipoxygenase and hydroperoxide lyase, which were adversely affected directly or indirectly by irradiation. Another possibility of flavor loss might be due to less indigenous precursors available for enzymes as a result of postirradiation biochemical changes. To understand the chemical or biochemical basis for the large loss in eight-carbon compounds due to irradiation of mushrooms and the kinetics of this loss, further investigation is needed.

Kramer (1986) found that the irradiation of 2 kGy did not show significant improvement over 1 kGy in keeping mushroom quality. Ajlouni (1991) recommended that the irradiation of 1 kGy in combination with commercial storage condition (12 °C) is an alternative means to retain mushroom quality and extend its shelf life after harvest. Mau and Hwang (1996) studied the effect of γ-irradiation of 0, 1, 2, and 5 kGy on mushroom quality and found that all mushrooms, including various dose-treated groups and the unirradiated control group, showed similar results in weight loss, maturation, cap expansion, and stipe elongation. However, the dose of 5 kGy could cause the microbial counts reduced by 3.4−3.7 logs. In addition, the doses of 1 and 2 kGy could reduced the microbial counts by 1−1.5 logs, while no significant differences were shown between these two treatments. Therefore, Mau and Hwang (1996) summarized that the reduction in microbial counts is the only advantage to extend shelf life of mushrooms by use of γ-irradiation.

For the purpose of extending the shelf life of mushrooms, low-dose γ-irradiation was approved by several countries, including Argentina, China, Hungary, Israel, South Korea, Mexico, and the United States, with doses ranging from 1 to 3 kGy (IAEA, 1995). With the γ-irradiation of 1 kGy, mushroom could retain 48% of eight-carbon compounds and 47% of 1-octen-3-ol contents. After irradiation of mushrooms, this flavor loss is not easily perceived by consumers due to the extremely low threshold of 1-octen-3-ol (0.01−0.43 ppm) (Mau *et al.*, 1994). In addition, the 1-octen-3-ol content of mushrooms decreased during postharvest storage (Mau *et al.*, 1991, 1992). However, mushrooms have long been used as a food or food-flavoring material just because of their unique and subtle flavor. The important findings in this study were the large loss of eight-carbon compounds due to irradiation of mushrooms. Therefore, to minimize the flavor loss by irradiation is an important area of investigation.

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