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Monitoring of Volatile Flavor Components and Amino Acids in Fresh Mushrooms (*Agaricus bisporus*) Associated with Shelf-Life Extension

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Abstract

Some chemical constituents were monitored to evaluate the biochemical and nutritional aspects of γ-irradiated mushrooms associated with shelf-life extension. Volatile components identified by GC and GC-MS were composed primarily of 1-octen-3-ol(68%), benzaldehyde(13%), 3-octanone(8%), benzyl alcohol(5%), 3-octanol(2%), 1-octen-3-one(1%), etc. Treatment with 2 kGy-irradiation and subsequent storage for 17 days at 9±1°C and 80±7% RH resulted in appreciable changes in their contents, even though negligible changes were observed in GC patterns between the nonirradiated and 2 kGy-irradiated samples. Most of the amino acids were resistant to ionizing energy of 2 kGy, while sulfur-containing free amino acids were affected significantly by γ-irradiation.

Key words: fresh mushrooms (*Agaricus bisporus*), γ-irradiation, volatile components, amino acids

Introduction

Mushrooms are usually consumed for their unique flavor properties. They have become an indispensable raw material of modern diet.

The chemical constitution of various mushroom species has been extensively studied. The early few studies appeared to be in direct conflict relative to which fraction is primarily responsible for the flavor properties of the mushrooms. Craske and Reuter[1] placed major emphasis on the nonvolatile nitrogenous constituents. Specifically they reported that with dried *Boletus edulis*, the high basic amino acids contributed most to its characteristic flavor. Likewise, Altamura *et al.*[2] in working with *Agaricus campestris* reported on the isolation and identification of a series of novel free amino acids which they assumed could relate to the characteristic flavor of this mushroom, especially upon heating.

On the contrary, most recent reports have put primary emphasis on the volatile fractions as being the main contributor to characteristic mushroom flavor. The actual number of different volatile compounds identified in various mushroom species approaches 150 and represents a wide variety of compound classes.[3] Currently, it is generally agreed that a series of compounds containing eight carbons are the primary volatiles contributing to mushroom flavor.

Curtured mushrooms are highly perishable vegetables, and can be kept in prime conditions for only 3 to 5 days at commercial preservation conditions.[4,5] There has been considerable progress in the marketing of fresh mushrooms during recent years with an increasing use of cool storage and prepacking to faciliate handling and to obtain optimal shelf-life. Some limitation in these techniques, however, still remain. Since the first finding of Staden[6] that ionizing radiations have a beneficial effect on the shelf-life extension of fresh mushrooms, similar results have been reported by many workers.[7-10] Most research, however, has emphasized the physical and physiological aspects of stored mushrooms.

Since fresh mushrooms are high in moisture, any form of processing usually result in apparent changes in their overall composition including their flavor composition. Drying or dehydration which has been the time-honored way of preserving fresh mushrooms, was generally known to bring about the changes in flavor properties of mushrooms. Cooking can also result in major volatile changes. Picardi and Issenberg[11] compared the volatiles present in the raw product of *Agaricus bisporus* with the volatiles produced after cooking for up to 3 hrs.

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The major difference with heating was the appearance of 1-octen-3-one.

The shelf-life extension of mushrooms is one of the most promising fields in food irradiation and recently irradiation technology has been legally approved as a new technique for mushroom preservation in different countries including Korea.[12] However, the chemical or biochemical aspects of irradiation effects on mushroom flavor have not yet been investigated.

The present work was undertaken to determine the influence of γ-irradiation on volatile flavor components and amino acids of fresh mushrooms associated with quality changes during post-irradiation storage.

Materials and Methods

Materials

Locally cultured mushrooms (*Agaricus bisporus*, white strain) were harvested at the stage of 45 days after cultivation, having a pileus diameter of 3.5 to 4.0 cm. The fresh mushrooms were aerobically packed into a corrugated paper box (18 × 11 × 17 cm) and the boxes were wrapped up in polyethylene film (0.06 mm thickness).

The packaged mushrooms were irradiated by a 7.4 nBq Co-60 gamma irradiator (dose rate: 20 Gy/hr) with doses of 0,1,2 and 3 kGy, respectively at room temperature. Treated mushrooms were used for analyses immediately after irradiation or during the course of storage at 9 ± 1 °C and 80 ± 7% relative humidities for three weeks.

Analysis of volatile components

Five hundred grams of fresh mushrooms were washed and blended with 1000 m*l* of distilled water in a Waring Blender at low speed for 3 min. The slurry was transferred to a 3000 m*l* round-bottomed flask connected in a Likens-Nickerson continuous distillation-extraction apparatus modified by Schultz *et al.*[13] for four hours' extraction with a solvent mixture (n-pentane/diethyl ether, 1:1, v/v). The resulting solvent extract was concentrated to 2 m*l* by nitrogen blowing and injected into a Hewlet-Packard 5890 A flame ionization gas chromatograph equipped with 30m × 0.32mm i.d. fused silica capillary column packed with carbowax 20M at flow rate of one m*l* of nitrogen per min. The column temperature was programmed linearly from 60 °C (5 min) to 200 °C (30 min) at an increasing rate of 3 °C per min. The peak area reported by the detector was integrated through a built-in integrator (Hewlett-Packard 5890 A GC terminal). GC-MS was carried out by a Hewlett-Packard 5985 B system, and operation parameters were as follows: carrier gas, helium; ionization voltage, 70 eV; acceleration voltage, 3 kV; ion source temperature, 200 °C.

Analysis of amino acids

Analyses of total amino acids were carried out after HCl hydrolysis. One m*l* of 6 N HCl was added to 1 mg of sample protein in a pyrex tube that was then sealed *in vacuo*. It was heated at 110 °C for 24 hrs to allow for a complete hydrolysis. After cooling, the solution was filtered and evaporated to dryness under reduced pressure. Subsequently, 0.5 m*l* of 0.01N NaOH solution was added to the sample and allowed to stand at room temperature for 4 hours. Oxidation of cysteine into cystine took place during that period. The volume was adjusted to 2 m*l* by the addition of 0.02 N HCl. The final solution was then injected into an amino acid analyzer (Hitachi model 835-50).

Free amino acids were extracted three times with 75% ethanol for 20 minutes. The combined extracts were evaporated by heat to obtain an aqueous solution to which an equal volume of diethyl ether was added and shaken to remove the residual lipids from the sample. After evaporating the aqueous layer, a 0.01 N NaOH solution was added to allow for the oxidation of cysteine into cystine. A solution of 0.02 N HCl was added to obtain an appropriate volume for injection. Tryptophan was not measured in this study.

The significance of each factor was determined according to the T-test and ANOVA test. All figures reported here represent the mean of triplicate determinations.

Results and Discussion

Volatile flavor components

Since fresh mushrooms contain numerous reactive classes of compounds, any form of processing usually resulted in significant changes in their overall composition including their volatile com-

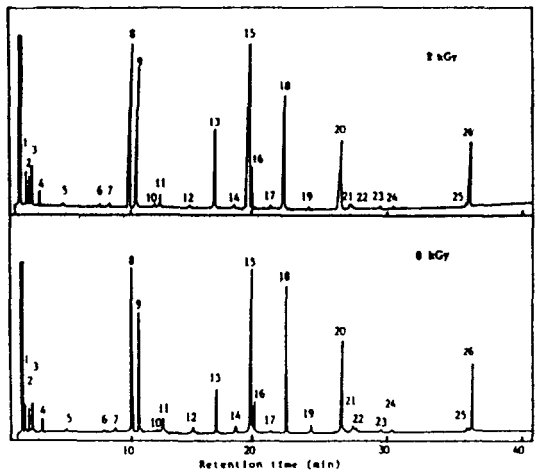

Fig. 1. Gas chromatograms of volatile components of gamma-irradiated mushrooms (*Agaricus bisporus*)
Solvent: n-pentane/Et_2O(1:1)

ponents. It has been known that undesirable quality changes can occur when foods are exposed to high levels of radiation. The most noticeable and prevalent of these changes are the development of objectionable flavors and odors. The levels needed to first observe these off-flavor and odors were found to vary widely with the commodities.[14] However, little has been reported on adverse changes to the flavor of irradiated fruits and vegetables.

The present study was focused on the determination of major volatile constituents in fresh mushrooms used and of changes which occur in their pattern and composition as a result of irradiation process and subsequent storage. Fig. 1 shows the gas chromatograms of the extracted volatiles from the 2 kGy-irradiated and nonirradiated control mushrooms. A total of 26 volatiles were extracted by the simultaneous distillation-extraction method and no significant changes in the chromatographic profiles were observed between the control and irradiated samples.

Table 1 summarizes the composition of identified volatile components and their changes induced by irradiation and subsequent storage of mushrooms. Identification was accomplished by comparing the mass spectra of the components with the published mass spectral data and their GC retention time with authentic samples.[15-17]

About 13 volatile components were identified. Fig. 2 shows mass spectra of major volatile components identified in the sample, which were undergone an apparent change due to irradiation and subsequent storage. The major volatile components were composed of 1-octen-3-ol(68%), 3-octanone (8%) and 3-octanol(2%) and of aromatic components like benzaldehyde(13%) and benzyl alcohol(5%). These results confirmed previous reports[3,11,18] that a series of compounds containing eight carbon atoms are the primary volatiles contributing to

Table 1. Volatile flavor components of gamma-irradiated mushrooms (*Agaricus bisporus*) during storage[a]

Peak number[b]	Compounds identified	Relative peak area (%)			
		0 days[c]		17 days[c]	
		0 kGy	2 kGy	0 kGy	2 kGy
3	Ethyl alcohol (g.c)	0.03	0.06	trace	0.08
5	n-Hexanol (g.c)	0.15	0.18	0.09	0.15
7	Iso amyl alcohol (g.c)	0.07	0.27	trace	trace
9	3-Octanone (g.c,m.s)	7.72	8.08	7.57	6.00
11	1-Octen-3-one (g.c,m.s)	1.07	1.61	0.83	3.67
12	n-Hexanol (g.c,m.s)	0.39	0.34	0.38	0.26
13	3-Octanol (g.c,m.s)	2.36	7.41	4.60	5.56
14	2-Octenal (g.c)	0.68	0.36	0.30	0.55
15	1-Octen-3-ol (g.c,m.s)	67.54	62.89	63.66	58.76
16	Furfural (g.c,m.s)	1.77	2.06	0.28	1.08
18	Benzaldehyde (g.c,m.s)	12.62	11.74	15.55	20.16
19	n-Octanol (g.c,m.s)	0.63	0.29	0.31	0.24
26	Benzyl alcohol (g.c,m.s)	4.89	4.71	6.42	3.47

[a] Sample was stored at 9 ± 1 °C and 80 ± 7% RH.
[b] Peak numbers are refered to Fig. 1.
[c] Storage period

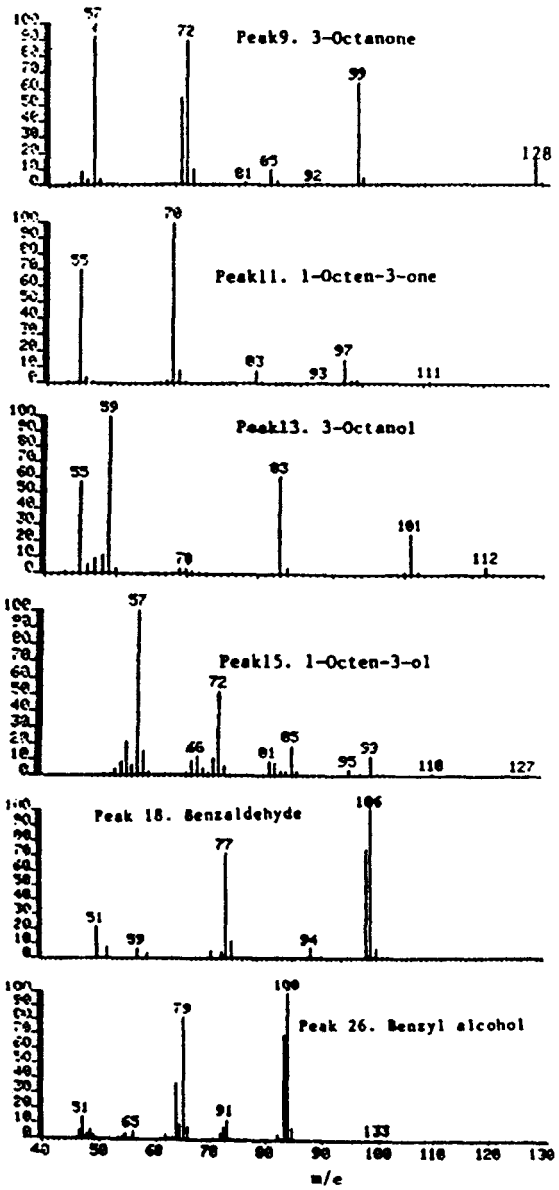

Fig. 2. Mass spectra of major volatile components identified from fresh mushrooms (*Agaricus bisporus*)

mushroom flavors and that the high concentration of benzaldehyde and benzyl alcohol in the composition of aroma compounds of *Agaricus bisporus* is a specific difference from that in wild mushrooms.

Relative amounts of identified volatile components indicated that both the irradiation processing for extending the shelf-life of mushrooms and storage time brought about detectable changes in 3-octanol, 1-octen-3-one, 1-octen-3-ol, benzaldehyde and benzyl alcohol.

Table 2. Amino acids of mushrooms (*Agaricus bisporus*) immediately after gamma irradiation[a]

Amino acids	Total amino acid		Free amino acid	
	0 kGy	2 kGy	0 kGy	2 kGy
Aspartic acid	1.65	1.83	0.98	0.97
Threonine	0.65	0.71	5.79	5.81
Serine	1.08	1.10	6.36	6.35
Glutamic acid	11.79	11.38	15.64	15.42
Proline	4.31	4.34	23.49	24.00
Glycine	7.91	7.89	1.78	1.80
Alanine	10.52	10.68	16.42	16.62
Cystine	0.75	0.60	0.17	0.12[b]
Valine	7.24	7.20	0.33	0.34
Methionine	0.94	0.84	0.26	0.18[c]
Isoleucine	8.39	8.50	2.32	2.30
Leucine	5.21	5.23	4.58	4.30
Tyrosine	1.63	1.64	0.14	0.15
Phenylalanine	2.54	2.58	3.17	3.16
Histidine	11.97	11.62	6.15	6.10
Lysine	4.38	4.40	3.66	3.90
NH_3	15.94	16.46	7.73	7.38
Arginine	3.10	3.00	1.12	1.10

[a] Amino acid contents are expressed as relative percentage.
[b] Significantly different from control ($p<0.05$)
[c] Significantly different from control ($p<0.01$)

The fluctuations in the amount of major volatile components may be explained by the reports,[19,20] suggesting that the harvested mushrooms are subjected to significant enzymatic changes relating to carbohydrate catabolism and that the enzymic reactions are involved in the formation or reduction of mushroom alcohol and aromatic compounds of mushrooms. It has been reported that maturity and storage of fresh mushrooms had an influence on their flavor properties[3,21] and particularly, considerable changes were found in aroma composition of mushrooms upon heating.[22,23] The literature contains very few reports only dealing with sensory evaluations on the changes in flavor and aroma of mushrooms under irradiation processes.[24,25]

Therefore, it is desired that more quantitative analyses should be followed for definite evaluations about the irradiation.effects on mushroom flavor.

Amino acids

Seventeen different amino acids were analysed for mushrooms which were divided into two groups, nonirradiated control and 2 kGy-irradiated samples. Table 2 shows the immediate effect of γ-irradiation

at 2 kGy on the contents of total and free amino acids of fresh mushrooms. Total amino acids were composed mainly of histidine(12%), glutamic acid (12%), alanine (11%), isoleucine (8%), glycine (8%) and valine (7%). Negligible changes were observed in their contents.

Chemical changes induced by irradiation of food proteins have been extensively studied and the changes were known to be principally related to high doses of irradiation. In this respect, the influences of irradiation are well documented by Elias and Cohen,[26] who indicated that the primary effects involve deamination, decarboxylation and denaturation of the irradiated proteins and that secondary effects include the decomposition or recombination of free radicals that originate from the primary reactions.

Although sulfur-containing amino acids like cystine ($p<0.05$) and methionine ($p<0.01$) decreased significantly due to γ-irradiation, insignificant changes were observed in the content of most of free amino acids which were mainly composed of proline(23%), alanine(16%) and glutamic acid (16%). These findings were similarly observed in a study on the amino acids of γ-irradiated ginseng powders.[27]

Thiol or disulfide moieties found in amino acids are known to be particularly sensitive to ionizing radiations. This is despite the fact that no consistent pattern has been presented to account for the resistance of each amino acid that is subjected to irradiation in relation to the nature of the food and to the physical state of amino acid and the proteins.

Based upon the preliminary observations of irradiation influence on the chemical contituents of fresh mushrooms, it was indicated that 2 kGy irradiation for extending the shelf-life of mushrooms and subsequent storage seemed to cause detectable changes in volatile flavor components and sulfur-containing amino acids.

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양송이의 숙도지연에 따른 휘발성 향기성분과 아미노산의 변화

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양송이 버섯의 신선도 연장을 위한 전리에너지 처리가 버섯의 품질에 관련된 휘발성 향기성분과 아미노산에 미치는 영향을 검토하였다. 시료의 주요 휘발성 성분으로 확인된 1-octen-3-ol(68%), benzaldehyde(13%), 3-octanone(8%), benzyl alcohol(5%), 3-octanol(2%), 1-octen-3-one(1%) 등의 eight-carbon 화합물과 방향족 화합물들은 2 kGy의 감마선 조사와 저장조건(17일간, 9± 1℃, 80± 7% RH)에 의해 함량의 증감현상이 나타났으나 대조시료와 감마선 조사시료에서 각각 추출된 휘발성 성분의 GC pattern에는 큰 차이를 보이지 않았다. 함유황 유리아미노산은 2 kGy의 감마선 조사에 의해 유의적으로 감소되었고, 그밖의 대부분의 아미노산은 안정한 것으로 나타냈다.