Effects of γ-Irradiation on the Enzyme Relating to the Development of Characteristic Odor of Onions

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Effects of γ-irradiation on the activity of cysteine sulfoxide lyase in onions and on the development of di-*n*-propyl disulfide in macerates of onions were investigated to elucidate the effects of irradiation on the quality of onions. The lyase activity was decreased apparently by irradiation with relatively lower doses utilized for sprout inhibition, 7 to 15 krad, but they were restored up to the level of the unirradiated control during storage for 3 months at room temperature. The formation of the disulfide was also decreased by irradiation with the same doses, which corresponded to that observed in the lyase activity. These relations between the lyase activity and the amount of sulfide were not so conspicuous at higher doses, and some other factors such as radiolysis of the sulfoxide may be involved.

The sprout inhibition of vegetables by γ-irradiation is considered to be one of the most promising uses of radiation preservation of food. Sprouting of onions can be effectively prevented by γ-irradiation with doses of 7 to 15 krad when it is performed immediately after harvesting. However, some problems remain to be investigated, especially concerning the biochemical aspects of radiation effects and the practical conditions for storage after irradiation. As to the quality changes in the irradiated onions, it has been noted that irradiation treatment makes their characteristic odor milder (Hetherington and Lillie, 1961), though this has not yet been demonstrated from chemical and biochemical aspects. The odor of onions has been studied extensively by many workers, and it has been shown that this characteristic odor consists principally of various sulfur-containing compounds which are developed through an enzymatic degradation process, with several alkyl-L-cysteine sulfoxides as precursors. Generally, onions contain appreciable amounts of the sulfoxide, and main parts of the sulfoxide are known to be *S*-(1-propenyl) (Spåre and Virtanen, 1963), *S*-*n*-propyl-, and *S*-methyl-L-cysteine sulfoxide (Virtanen and Matikkala, 1959). These sulfoxides are decomposed by the action of their lyase [termed cysteine sulfoxide lyase (Mazelis, 1963)] to give propenyl sulfenic acid, *n*-propyl-, and methylthiosulfinate, and also to give pyruvic acid and ammonia as their common decomposition products (Kupiecki and Virtanen, 1960; Schwimmer *et al.*, 1960; Mazelis, 1963). Among these decomposition products, propenyl sulfenic acid is considered to be a lachrymatory factor in crushed onions (Spåre and Virtanen, 1963), and dialkyl disulfides are presumed to be the principal part of a characteristic odor of *Allium* species, in which di-*n*-propyl disulfide gives the common odor of fresh onion and its contents are higher than that of dimethyl disulfide (Bernhard, 1968; Saghir *et al.*, 1964).

In a previous paper, the γ-radiolysis of *S*-*n*-propyl-L-cysteine sulfoxide, a precursor of onion odor, was reported in aqueous solution (Nishimura *et al.*, 1970), and it was shown that di-*n*-propyl disulfide can also be formed from *n*-propyl-L-cysteine sulfoxide by radiation chemical process.

This paper is concerned with the investigations on the effects of γ-irradiation at low doses on cysteine sulfoxide lyase activity in onions and changes in its activity during storage after irradiation. The correlation between the lyase activity and the formation of di-*n*-propyl disulfide in the irradiated onions is also examined.

EXPERIMENTAL

Material. Onions (*Allium cepa* L. strain: Sensyu Yellow, bulb's weight: 250 to 300 g) were obtained from local orchards at the time of harvest (July, 1968). One lot of the onions was irradiated immediately after harvest, and the other was irradiated after about 3 months storage at 0° C and room temperature (25° to 30° C). The irradiated onions were stored at room temperature (20° to 30° C) until used—6 and 3 months, respectively.

Irradiation. Irradiation was carried out with γ-rays from a ^{60}Co source at 20° C, and the dosages employed were 7, 15, 50, and 320 krad at a dose rate of 20 krad per hr.

Preparation of the Crude Extracts of Cysteine Sulfoxide Lyase from Onion. To avoid individual variation in the lyase activity in onion bulbs, the samples used for these experiments were prepared by mixing cut pieces of onion obtained from each of five bulbs which were freed of skin, top, and disk. The onion pieces, 50 g, were blended with 50 ml of 0.2 *M* phosphate buffer, pH 6.8, for 1 min at 0° C. For irradiation of the crude lyase solution, the enzyme solutions were prepared by using 50 ml of water, phosphate buffer solution (pH 6.8), phosphate buffer solution + sucrose (0.1 *M*), and phosphate buffer solution + sucrose + EDTA (0.1 mM), respectively. The thick suspension was filtered through cotton gauze to make 100 ml with phosphate buffer and this extract was used as an enzyme solution.

Determination of Activity of Cysteine Sulfoxide Lyase. The activity of cysteine sulfoxide lyase was determined from the amounts of the liberated pyruvic acid measured by the colorimetric method using 2,4-dinitrophenylhydrazine (Schwimmer and Weston, 1961). The enzyme reaction was carried out at pH 8.0 for optimal activity (Namiki and Kawakishi, 1968) and a synthetic (±)-*S*-*n*-propyl-L-cysteine sulfoxide was used as a substrate for the enzyme reaction. The composition of the reaction mixture was as follows:

0.1 *M* Phosphate buffer solution (pH 8.0)	2 ml
Substrate solution (*S*-*n*-propyl cysteine sulfoxide 20 μmole per ml)	1 ml
Enzyme solution	1 ml
Total volume	4 ml

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This reaction mixture was incubated at 37° C for 20 min. Four milliliters of 10% TCA were added after incubation, the material was filtered and made to 100 ml with water, and 2-ml aliquots were used in the determination of pyruvic acid. A control solution without substrate was treated in the same manner. The lyase activities are expressed as μmoles of pyruvate produced by the lyase from 100 g of onion for 20 min.

Determination of Di-*n*-Propyl Disulfide by Gas Chromatography. Di-*n*-propyl disulfide in headspace gas of crushed onions was determined by use of gas chromatography. Cut pieces of onion, 180 g, from three skin and disk-free bulbs were blended for 1 min without additional water, and the macerated tissues were transferred to a 500-ml flask, and the flask sealed with silicone rubber. After standing for 2 hr at 20° C, 2 ml of the headspace gas were withdrawn by a syringe and injected to the gas chromatograph under the following conditions: Hitachi Model K53 gas chromatograph; Column, 20% Reoplex 400 on acid washed 40- to 60-mesh C-22, 0.4 × 100 cm stainless steel column; Temperature, 120° C (injection port 210° C); Carrier gas N_2 20 ml per min; Detector, Flame ionization.

Identification of di-*n*-propyl disulfide was followed by the comparison with its retention time on the chromatogram to that of an authentic sample. The main peak (3) (Figure 1) was collected in a cold trap by repeated preparative gas chromatography using Hitachi Model KGL-2A, having the same conditions as for the analytical procedure. This component was also identified by means of mass spectrometry using a Hitachi Model RMU-6. Identification of the other components in the headspace gas will be reported elsewhere.

RESULTS AND DISCUSSION

The enzyme solution used in this study was a crude extract from onions. It was preliminarily examined to determine whether there is some linear relation between the enzyme concentration (dilution of enzyme solution) and its activity. From determination of the activity on the diluted enzyme solution, it was found that a linear relation exists between both matters. Therefore, a 1-ml aliquot of double folded dilutions of the original enzyme solution was used for assays of the lyase activity. There were considerable differences in the lyase activity among individual bulbs of onions, but these were eliminated by using mixed macerates obtained from several bulbs.

Changes in the Activities of Cysteine Sulfoxide Lyase in γ-Irradiated Onions. With γ-irradiation immediately after harvest, there was a marked decrease in the lyase activity at doses below 15 krad, and an inverse relation between the lyase activity and the dose (Table I). At higher doses, however, there was some increase in the activity, and the reason for this effect is not yet clear. Also, with irradiation after storage for 3 months at 0° C and room temperature, the lyase activity was lowered with increasing doses, especially in the former case. Thus, apparently some damage in the lyase system caused by γ-irradiation resulted in a lowering of its activity.

The onions irradiated immediately after harvest were stored for 6 months at room temperature, and changes in the lyase activity were examined at intervals throughout storage (Table II). The activity in unirradiated onions increased during storage and reached a maximum after 1 month and then decreased gradually with storage time. In the onions irradiated with 7 and 15 krad, lyase activities increased with storage time and reached a maximum level after 3 months, which was approximately the same level as that of the unirradiated sample. Also, the increase in activity during 1 month could be observed even with the samples irradiated with 50 krad. In the onions irradiated with higher doses (320 krad), however, activity rapidly decreased during the 2 weeks after irradiation and only a little remained after 1 month, when putrefaction of the onions occurred. From these results, it is clear that lyase in onions is partially inactivated by irradiation with doses of 7 and 15 krad, which are usually used for sprout inhibition, but its activity is restored to the level in unirradiated onions with storage for 3 months at room temperature. On the other hand, its activity could not be completely recovered when the onions are irradiated with 50 krad. When the onions were stored at 0° C for 3 months after harvest, the lyase activity was kept at the intact level, and was also lowered considerably with the irradiation (Table III). With storage after irradiation, the activity was gradually decreased in every case except that of the 7 krad dose, in which partial recovery of the lyase activity was also observed. With the onions stored for 3 months at room temperature prior to irradiation, the changes in lyase activity by irradiation exhibited a similar tendency to that observed in storage at 0° C

Table I. Relative Residual Activities of Cysteine Sulfoxide Lyase in Onions Immediately after Irradiation

Irradiation Doses, krad	Relative Activities: Irradiated Immediately After Harvest	Irradiated After Storage, 0° C 3 Months	Irradiated After Storage, Room Temp[a] 3 Months
0	100	100	100
7	82.3	53.4	92.3
15	58.0	46.7	75.6
50	52.4	27.5	37.8
320	81.0	...	...

[a] Room temperature 20° to 30° C.

Table II. Changes in Time Course of Cysteine Sulfoxide Lyase Activities in Onions Irradiated by Several Doses Immediately after Harvest

Irradiation Doses, krad	Activities[a]: Time after Irradiation in Month 0	0.5	1	3	6
Unirradiated	535	545	620	450	210
7	440	520	525	585	220
15	310	390	420	570	275
50	280	375	450	240	...
320	440	50	25	...	...

[a] Activities were expressed as pyruvate μmole/20 min/100 g of onion.

Table III. Changes in Time Course of Cysteine Sulfoxide Lyase Activities in Onions Irradiated by Several Doses after Storage at 0° C for 3 Months Since Harvest

Irradiation Doses, krad	Activities[a]: Immediately after Harvest	Time after Irradiation in Month 0	1	3
Unirradiated	535	600	165	100
7		320	470	70
15		280	115	160
50		165	80	...

[a] Activities were expressed as pyruvate μmole/20 min/100 g of onion.

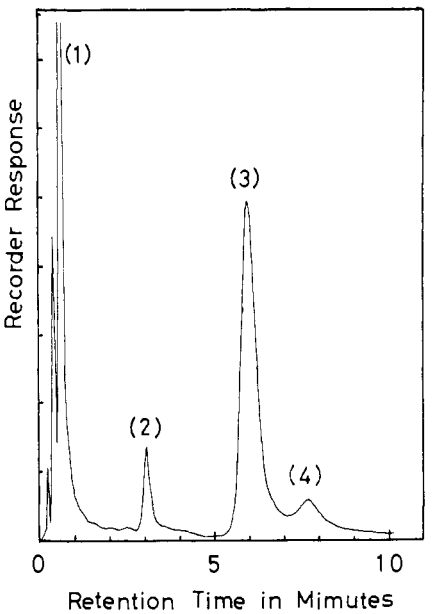

Figure 1. Gas chromatogram of headspace gas of the macerated onion. (1) *n*-propyl mercaptan and propionaldehyde. (2) 2-methyl-2-pentenal. (3) di-*n*-propyl disulfide. (4) unidentified

Table IV. Changes in Time Course of Cysteine Sulfoxide Lyase Activities in Onions Irradiated by Several Doses after Storage at Room Temperature[a] for 3 Months Since Harvest

Irradiation Doses, krad	Activities[b] Immediately after Harvest	Time after Irradiation in Month 0	1	3
Unirradiated	535	450	305	...
7		415	440	200
15		340	190	...
50		170	125	...

[a] Room temperature 20° to 30° C. [b] Activities were expressed as pyruvate μmole/20 min/100 g of onion.

Table V. Relative Residual Activities of Cysteine Sulfoxide Lyase in Irradiated Onion Extracts

Irradiation Doses, krad	Relative Activities Extractants[a] (i)	(ii)	(iii)	(iv)
Unirradiated	100	100	100	100
50	85	89	95	97
100	85	87	77	94
200	75	90	80	86
320	50	77	38	78

[a] (i) Water, (ii) phosphate buffer solution (pH 6.8), (iii) phosphate buffer solution + sucrose (0.1 *M*), (iv) phosphate buffer solution + sucrose + EDTA (0.1 mM).

(Table IV). These results indicate that lowered activity of lyase in the onion irradiated with a lower dose (7 krad) could be partially restored, although the preservation conditions prior to γ-irradiation were different.

From these experimental results it seems reasonable to say that the γ-irradiation causes some damages in the cysteine sulfoxide lyase system of the onion and, consequently, the activity is reduced, while a reason for the damage in the lyase system is not yet known. In lower doses of 7 to 15 krad, applied to inhibit sprouting of the onion, it is likely that this damage was perfectly recovered by the storage and the lyase was reactivated to the unirradiated level.

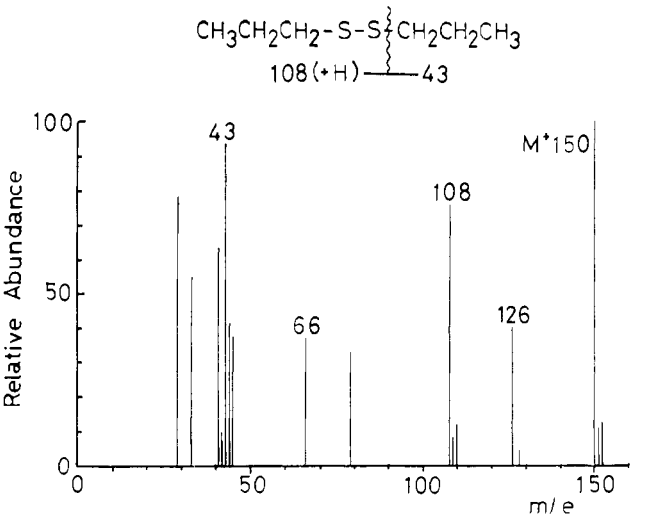

Figure 2. Mass spectrum of volatile component (3) in headspace gas of the macerated onions

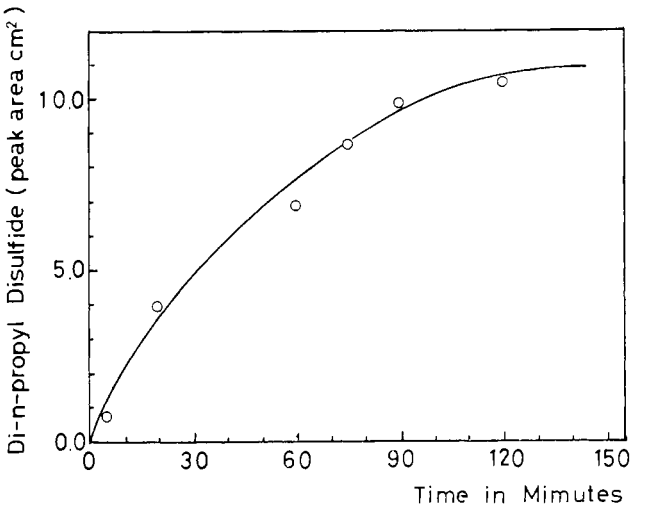

Figure 3. Changes in time course of peak areas depends upon di-*n*-propyl disulfide on gas chromatograms of headspace gas of the macerated onions

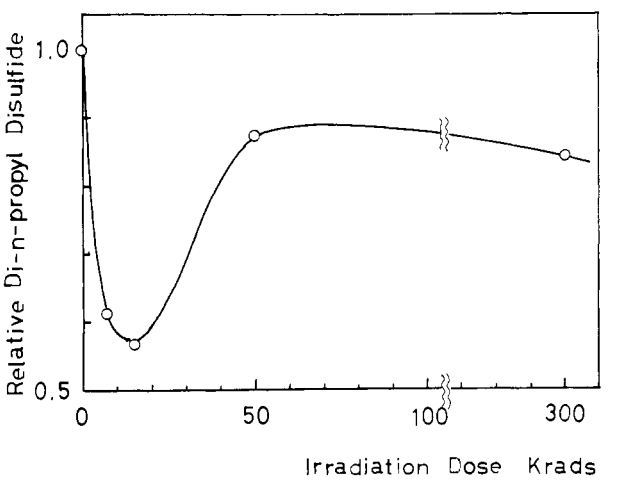

Figure 4. Effect of irradiation dose on the formation of di-*n*-propyl disulfide in headspace gas of the macerated onions irradiated on several doses

Effects of γ-Irradiation on the Crude Extracts of Cysteine Sulfoxide Lyase Prepared from Unirradiated Onions. As shown in Table I, irradiation of the onions appeared to effect the activity of the cysteine sulfoxide lyase. In order to ascertain the radiation sensitivity of the lyase, the effect of irradiation on the crude lyase freed from the onion tissues was also examined. The crude lyase solution prepared from the unirradiated onions, as in the preceding experiment, was irradiated with several doses, and lyase activities were assayed. In this case (Table V), lyase activities, as compared with those of the irradiated onions, were not decreased as much by irradiation less than 100 krad, though there were slight differences in the lyase activities of each extractant. From these facts, it does not always follow that lyase is highly sensitive to γ-irradiation. These differences in radiation sensitivity of the cysteine sulfoxide lyase were presented between intact onion and cell free extract, but this reason was not yet clarified.

Formation of Di-*n*-Propyl Disulfide from γ-Irradiated Onion Macerates. It is known that di-*n*-propyl disulfide is a main component of onion odor (Saghir *et al.*, 1964; Brodnitz and Pollock, 1970) and that it is formed through the enzymatic degradation of *n*-propyl-L-cysteine sulfoxide. This disulfide could be produced not only by the enzymatic process, but also by γ-radiolysis of its precursor (*n*-propyl-L-cysteine sulfoxide) in aqueous solution (Nishimura *et al.*, 1970). A gas chromatogram of the headspace gas developed from a crushed onion, shown in Figure 1, in which the retention time of three peaks (1), (2), and (3) agreed with *n*-propyl mercaptan and propionaldehyde, 2-methyl-2-pentenal, and di-*n*-propyl disulfide, respectively. Peaks (2), (3), and (4) were collected fractionally by preparative gas chromatography, and peak (3) was identified as di-*n*-propyl disulfide by its mass spectrometry (Figure 2). Identification of the other peaks will be reported elsewhere. Di-*n*-propyl disulfide is a main component in the headspace gas from this chromatogram (Figure 1). Various disulfides, except this disulfide, such as methyl *n*-propyl, methyl allyl, dimethyl, and diallyl disulfide, have been demonstrated in the volatiles from fresh onion (Bernhard, 1968). The disulfide formation proceeds from the enzymatic reaction by the crushing of onion tissue, so the correlation between the amount of di-*n*-propyl disulfide in the headspace gas and the time after the blending of onion tissue was examined by gas chromatography. As shown in Figure 3, this disulfide in the headspace gas reached a maximum level approximately 2 hr after blending. Therefore, the determination of di-*n*-propyl disulfide in the volatiles of crushed onion was performed after the crushed onion had stood for 2 hr at room temperature, and amounts of the disulfide were expressed as the ratio of its peak area to the peak area of the unirradiated sample on the gas chromatogram.

Changes in the amount of di-*n*-propyl disulfide which developed from the onions irradiated at the earlier time after harvest, are shown in Figure 4. The disulfide was considerably decreased compared with the unirradiated sample by irradiation with 7 and 15 krad, but the decrease was not so remarkable at higher doses than at 7 and 15 krad levels. Decreases in disulfide formation with the irradiation by 7 and 15 krad corresponded well with the decreases of cysteine sulfoxide lyase activity in the onion irradiated with these doses. On the radiation chemical study in our preceding paper (Nishimura *et al.*, 1970), it was confirmed that di-*n*-propyl disulfide is also formed from *n*-propyl-L-cysteine sulfoxide in aqueous solution by γ-radiolysis. At higher doses, 50 or 300 krad, accordingly, it may be considered that there exists some contribution from the radiolysis of the sulfoxide, and for this reason the disulfide level at higher doses increases compared with the case of low doses.

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LITERATURE CITED

Bernhard, R. A., *J. Food Sci.* **33,** 298 (1968).
Brodnitz, M. H., Pollock, C. L., *Food Technol.* **24,** 78 (1970).
Hetherington, C. H., Lillie, A. B., Proc. 4th Japan. Conf. Radioisotopes, p. 1054 (1961).
Kupiecki, F. P., Virtanen, A. I., *Acta Chem. Scand.* **14,** 1913 (1960).
Mazelis, M., *Phytochemistry* **2,** 15 (1963).
Namiki, M., Kawakishi, S., *Food Irrad. Japan* **3,** 178 (1968).
Nishimura, H., Kawakishi, S., Namiki, M., *Agr. Biol. Chem.* **34,** 609 (1970).
Saghir, A. R., Mann, L. K., Bernhard, R. A., Jacobsen, J. V., *Proc. Amer. Soc. Hort. Sci.* **84,** 386 (1964).
Schwimmer, S., Carson, J. F., Makower, R. U., Mazelis, M., Wong, F. F., *Experientia* **14,** 449 (1960).
Schwimmer, S., Weston, W. J., J. AGR. FOOD CHEM. **9,** 301 (1961).
Spåre, G. C., Virtanen, A. I., *Acta Chem. Scand.* **17,** 641 (1963).
Virtanen, A. I., Matikkala, E. J., *Acta Chem. Scand.* **13,** 1898 (1959)

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