Precursors and Enzymatic Development of Caucas Flavor Components

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Sulfoxide amino acid, such as S-alkyl-L-cysteine sulfoxides (alkyl: methyl, n-propyl, allyl, 1-propenyl), are abundantly found in Allium,2) Brassica3) and Phaseolus4) plants. Yurugi et al.5) have investigated the biological activity of allithiamine from the reaction between thiamine and diallyl thiosulfinate which is produced by the reaction of S-allyl-L-cysteine sulfoxide with C-S lyase. Recently, Fujiwara et al.6) have reported anti-hypercholesterolemic effect of S-methyl-L-cysteine sulfoxide isolated from cabbage. Since S-alkyl-L-cysteine sulfoxides have a variety of biological activities, the studies on sulfoxide amino acids are important for chemists and biologists.

We have studied on γ-radiolysis7)-10) and UV-photolysis11) of sulfur-containing amino acids, such as S-alkyl-L-cysteines and those sulfoxides (alkyl: n-propyl, allyl, 1-propenyl), in connection with food-flavor deterioration caused by irradiation. It is very interesting to compare enzymatic reaction of S-alkyl-L-cysteine sulfoxides with radiolytic reactions of those.

We have reported the volatile flavor components of caucases in terms of the enzymatic development of flavor and elucidated that the main volatile components attributed to the caucas flavor were methyl allyl disulfide, diallyl disulfide and methyl allyl trisulfide.11) Leaves and stems of the caucas, which is widely distributed in Hokkaido under the name Ainu-negi or Gyoja-ninniku, are nutritious vegetables.

The enzymatic-formation mechanism of unsymmetric disulfides, such as methyl allyl disulfide attributed to the caucas flavor,11) has not yet been elucidated.

This paper deals with the identification of precursors of the caucas flavor components and enzymatic developments of unsymmetric disulfides.

The synthesis of S-alkyl-L-cysteines and those sulfoxides was reported previously.7) These compounds were confirmed by IR spectra and elementary analysis. Authentic volatile disulfides were prepared by the procedure of Kirner and Richter.12)

About 8 kg of fresh caucases were treated with boiling water to deactivate the C-S lyase and extracted with distilled water (30 liters). The extracts were passed through a column of Dowex 50 W × 2 (H+) and amino acid fractions were eluted with 2 N ammonium hydroxide from the column. Two components A and B, which are positive to both of ninhydrin and potassium iodoplatinate reagents, were isolated by cellulose column chromatography (100–200 mesh) and preparative paper chromatography (Toyoda No. 50) in solvent systems of n-butanol-acetic acid-water (4:1:1, v/v) and phenol-water (4:1,v/v). The Rf values of A and B were 0.25 and 0.13 in the former solvent, respectively. Each component was crystallized from ethanol-water, and was obtained in yields of 20 mg and 25 mg, respectively.

By comparing Rf values, infrared spectra and mass spectrometric fragmentations of component A and B with those of authentic compounds, A and B were identified as S-allyl-L-cysteine sulfoxide (ACSO) and S-methyl-L-cysteine sulfoxide (MCSO), respectively.

In order to elucidate enzymatic developments of unsymmetric disulfides, we examined reactions of S-alkyl-L-cysteine sulfoxides with alliinase which prepared from garlic by the method of Stoll and Seebeck.13) The time course of enzymatic reaction with mixture of ACSO and MCSO is shown in Fig. 1. The specific activity which was measured at a linearly increasing stage was 17.8. A gas chromatogram of head space vapor in 10 min after the enzymatic reaction is shown.

FIG. 1. Time Course of Enzymatic Splitting of Sulfur-containing Amino Acids.

Assay solutions (1 ml), which contain 0.2 M borate buffer (pH 7.8), 10 μmole of ACSO, 10 μmole of MCSO, 0.82 mg of alliinase and 0.1 μmole of pyridoxal-5-phosphate, were incubated at 37°C. The enzymatic reaction was stopped by adding 1 ml of 2 N sulfuric acid. One unit of enzyme activity was defined as the amount of enzyme to form 1 μmole of pyruvate per min and the specific activity of the enzyme was expressed as units per mg protein.
in Fig. 2. The sum of twice amounts of dimethyl disulfide, methyl allyl disulfide and diallyl disulfide was approximately equal to the amount of produced pyruvate. Furthermore, methyl allyl disulfide was not formed by alkyl exchange reactions of each mixture of dimethyl and diallyl disulfides or thiosulfinates in crude enzyme solutions at 37°C. These facts indicate that methyl allyl disulfide is not formed thermochemically but enzymatically from mixture of ACSO and MCSO.

We also measured an electron spin resonance spectroscopy at 77°K after the enzymatic reaction and observed an anisotropic signal (g=2.00~2.05). The signal might indicate that such volatile disulfides as methyl allyl disulfide are produced via free radicals formed in the first stage of the enzymatic reaction.

REFERENCES

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