Note

Lysinoalanine Formation in Gluten:
A Conformational EffectTokuko HARAGUCHI, Keiko ABE,
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Lysinolalanine (LAL) formation in foods has called special attention in recent years. A number of papers on this topic are available.¹⁻⁵ Our laboratory also has contributed to the knowledge of LAL formation in food proteins.⁶ In particular, the condition for maximizing the LAL formation in wheat gluten was well established; the average yield of LAL in that case was found to be about 4 μmol per 100 mg gluten.

However, a conformational effect may possibly exist that favors the LAL formation *via* intramolecular as well as intermolecular reaction between dehydroalanine and lysine residues of gluten. To define the type of this reaction we first investigated the effect of gluten concentration in alkaline medium on LAL formation. Gluten (Wako Junyaku Kogyo Co.) with a protein content of 79.5% was dissolved in dilute NaOH to concentrations of 0.25%, 2.5%, 5.0% and 10%. The solutions were adjusted to pH 13 by controlling the NaOH concentration, prior to heat treatment at 70°C for 1 hr. Each sample after the treatment was analyzed for LAL as described in the preceding paper.⁶ It was observed that the gluten concentration had no appreciable effect on the amount of LAL formed (Fig.

FIG. 1. Formation of Lysinoalanine in Wheat Gluten as a Function of Its Concentration in Reaction Medium.

1). This result indicates that LAL is formed primarily through the intramolecular reaction between dehydroalanine and lysine residues of gluten. Extrapolation gave a value of 3.5 μmol LAL per 100 mg gluten (Fig. 1). The value may reflect that the corresponding amounts of dehydroalanine and lysine residues are conformationally in the vicinity to each other in the molecule.

To remove a conformational effect on LAL formation we attempted to hydrolyze gluten with protease. Pronase (Kaken Kagaku Co.) was used as the protease. The hydrolysis was carried out under the following conditions: concentration of gluten in medium, 1%; pH of the medium, 9.0; Pronase-gluten ratio, 1/200; and temperature, 37°C. The process of the hydrolysis was followed by measuring trichloroacetic acid solubility-insolubility as an index for protein degradation. The hydrolysis for 30 min, 1 hr and 4 hr gave gluten hydrolysates with degrees of hydrolysis of 21.7%, 45.0% and 73.3%, respectively. Each hydrolysate (50 mg on a dry-matter basis) was dissolved in dilute NaOH (2 ml) so as to reach pH 13 and then heated at 70°C for 1 hr. The alkali-treated sample was analyzed for LAL. The result showed that the amount of LAL formed decreased in accordance with the degree of hydrolysis of gluten (Fig. 2).

The above-mentioned two experiments suggest that a conformational effect does exist and the LAL formation in gluten is primarily due to an intramolecular reaction between dehydroalanine and lysine residues of this protein. Hayashi and Kameda,⁷ in investigations with RNase, have observed that its native conformation contributes to effective LAL formation. They have also shown that the possibility of intermolecular cross-linking of this protein is little if any.

An additional experiment was conducted to investigate the effect of added free lysine on LAL formation in gluten. Lysine monohydrochloride was added to gluten (50 mg) in proportions of 0.5%, 1.5%, 2.5%, 3.5% and 5.0% of its weight on a dry-matter basis. Each mixture was alkali-treated as described above, prior to analysis for LAL formed and for free lysine remaining intact. It was found that at the largest proportion, *i.e.*, 2.5 mg lysine monohydrochloride/50 mg gluten, the amount of LAL

FIG. 2. Relationship between the Degree of Hydrolysis of Wheat Gluten and the Amount of Lysinoalanine Formed.

FIG. 3. Effect of Added Free Lysine on the Formation of Lysinoalanine in Wheat Gluten.

formed was approximately $5 \mu\text{mol}$ per 100 mg gluten, whereas the gluten itself, *per se* containing about $9 \mu\text{mol}$ lysine per 100 mg of the protein, produced $4.0 \mu\text{mol}$ LAL (Fig. 3). The increase in total lysine content thus had no great effect; its 4-fold increase from $9 \mu\text{mol}$ to $36 \mu\text{mol}$ caused a 1.2-fold increase in the yield of LAL. A major part of free lysine added was found to remain intact in every case (Fig. 3).

Free lysine is often added to wheat products for

nutritional fortification. They may sometimes be subjected to heat-treatment under alkaline condition. According to the above data, however, it is probable that the added free lysine itself makes no significant contribution to LAL formation in gluten. It is postulated again that the gluten molecule *per se* bears some conformationally favorable lysine residues for effective formation of LAL.

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