Plastein Reaction for Food Protein Improvement

Michiko Yamashita,* Soichi Arai, and Masao Fujimaki

It has been elucidated that plastein synthesis is initiated by peptidyl-enzyme formation with liberation of water. The peptidyl-enzyme can be attacked by another substrate peptide as a nucleophile to form a new larger peptide. Water concentration in the reaction system is thus expected to affect plastein formation. In fact, addition of organic solvent to the system was effective in forming a plastein. Amino acid ethyl esters added to the system also acted as nucleophiles and were incorporated during the plastein synthesis. Papain was most effective as an enzyme in this case. The specificity of the incorporation by papain of the amino acid ethyl esters is discussed in relation to structure. The results are applicable to the improvement of amino acid composition of food proteins. In particular, the process was successful in improving the essential amino acid patterns of unconventional proteins from photosynthetic origin.

Interest in plastein reaction probably is as old as the history of protein biosynthesis research. During a few decades prior to 1960, several studies were carried out aimed at elucidating the relationship between plastein reaction and protein synthesis in biological systems (Haurowitz and Horowitz, 1955; Tauber, 1951; Virtanen et al., 1949; Wasteneys and Borsook, 1930). However, since a modern aspect of the peptide chain elongation in vivo was established, the plastein research has been directed toward the subjects of enzymological interest (Determann and Koehler, 1965; Wieland et al., 1960). Our research group began to reinvestigate the plastein reaction and provided information applicable to the protein processing area (Fujimaki et al., 1970; Yamashita et al., 1970a–c, 1971b; Tsai et al., 1974). What may be more useful in this area is the plastein reaction accompanied by amino acid incorporation. Yamashita et al. (1971a) have found that, when a mixture of a soybean protein hydrolysate and L-methionine ethyl ester is incubated in the presence of papain, a plastein can be obtained in which the methionine has been incorporated in a peptide-bonding state. It is also possible to prepare a water-soluble material from soybean protein when glutamic acid is incorporated in a similar manner (Yamashita et al., 1975). Such plastein reactions may thus be useful for the improvement of the nutritional and functional properties of food proteins. On the other hand, it is of enzymological interest to investigate the characteristics of the plastein reaction and the specificity of the amino acid incorporation reaction during the plastein synthesis process. The information obtained from these basic studies is expected to be applicable to improving the amino acid compositions of food proteins. In this regard the current paper primarily describes our recent work on the improvement of the essential amino acid patterns of unconventional proteins, i.e., algal protein from *Spirulina maxima*, bacterial protein from *Rhodopseudomonas capsulatus*, and a leaf protein from white clover (*Trifolium repens L*).

MATERIALS AND METHODS

Proteins. The following conventional proteins were obtained from commercial sources in Japan: purified ovalbumin from Tokyo Kasei Co., soybean protein isolate from Fuji Oil Mills Co., and gluten from Kanto Kagaku Co. Unconventional proteins were prepared from a blue-green alga (*S. maxima*), a nonsulfur purple bacterium (*R. capsulatus*), and a type of white clover (*T. repens L*). In each case the dried material (10 g) was treated with 500 ml of ethanol to remove photosynthetic pigments and other ethanol-soluble substances. The residue was suspended in 0.1 N NaOH and ground with a mechanical grinder. The alkaline extract was dialyzed against running water for 3 days to obtain a crude protein as a nondialysate. The protein contents (N × 6.25) were 64.5% in the algal protein, 55.5% in the bacterial protein, and 72.7% in the leaf protein on a dry-matter basis.

Peptic Hydrolysates of Proteins. Peptic hydrolysates of ovalbumin, soybean protein, and gluten were prepared according to Fujimaki et al. (1970). The unconventional proteins also were hydrolyzed with pepsin (recrystallized preparation from Sigma Chemical Co.) in an almost similar manner; each protein (10 g) and the pepsin (100 mg) were dissolved in an aqueous medium at pH 1.6 and incubated at 37 °C for 24 h. The resulting degree of hydrolysis was determined by the use of trichloroacetic acid (Cl_3CCOOH) as reported by Yamashita et al. (1970b) and expressed as (10% Cl_3CCOOH-soluble nitrogen/total nitrogen) × 100 (%). Each protein hydrolysate was freeze-dried and used as substrate for the following plastein synthesis.

Plastein Synthesis. A papain preparation (Difco NF VIII) was used as the enzyme in most cases. In one case an immobilized chymotrypsin preparation was used which had been obtained by attaching α-chymotrypsin (recrystallized preparation from Sigma Chemical Co.) to porous glass (Pierce CPG/P-Amino Aryl 550) through diazo coupling (Royer and Uy, 1973). Details for the plastein synthesis procedures are described later in the respective cases. The plastein productivity was determined with Cl_3CCOOH as reported by Yamashita et al. (1970b) and expressed as (10% Cl_3CCOOH-insoluble nitrogen/total nitrogen) × 100 (%).

RESULTS AND DISCUSSION

Enzymological Characteristics of Plastein Synthesis. The protein hydrolysis by protease is generally known to proceed effectively at a very low concentration of substrate. This process is usually carried out at a substrate concentration of 1% or less. It is expected that, as the substrate concentration increases, the hydrolysis reaction becomes slow. Our recent study with an ovalbumin hydrolysate (degree of hydrolysis, 67%) disclosed that no apparent reaction occurred when this was incubated at a concentration of 7.5% in the presence of α-chymotrypsin. Apparently, both the hydrolysis and the synthesis reactions proceeded at the same rates. At a concentration of greater than 7.5% it was observed that the synthesis reaction was predominant over the hydrolysis reaction. Reportedly, the most preferable concentration

Department of Agricultural Chemistry, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

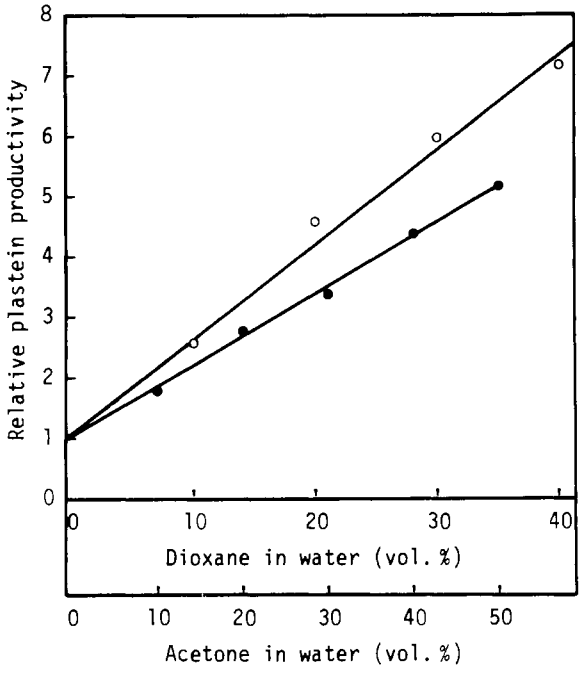

Figure 1. Effects of concentrations of dioxane (open circles) and acetone (filled circles) on the plastein production from an ovalbumin hydrolysate by immobilized α-chymotrypsin.

seems to lie in the range of 30–50% (Tsai et al., 1974). A higher substrate concentration is considered to be more preferable in the sense that this causes a decrease in the water concentration of the reaction system. In order to decrease the water concentration more positively, we tried to add organic solvent to the plastein reaction system. Various concentrations of dioxane and acetone in water were used. An ovalbumin hydrolysate (degree of hydrolysis, 85%) was used as substrate and incubated with immobilized α-chymotrypsin under the following conditions: substrate concentration in reaction medium, 50%; enzyme amount, a large excess; pH 6.5; temperature, 37 °C; and incubation time, 2 h. The plastein productivity was determined as already described. At the same time, the activities of the immobilized enzyme to hydrolyze *N*-benzoyl-L-tyrosine *p*-nitroanilide in the corresponding solvent systems were determined according to the method of Bundy (1962). Each plastein productivity was divided by a hydrolytic activity and the value obtained when the reaction system did not contain any organic solvents was set at unity. Figure 1 shows the relative plastein productivities thus obtained at various concentrations of dioxane and acetone, indicating that a higher solvent concentration, i.e., lower water concentration, is more effective in the plastein formation.

Studies on the main mode of the plastein reaction have been carried out with α-chymotrypsin. Tanimoto et al. (1972) have elucidated the involvement of His-57 and Ser-195 in the catalysis of the plastein synthesis as well as in the usual protein hydrolysis by this enzyme. Yamashita et al. (1974), using a soybean protein hydrolysate whose carboxyl groups had been labeled with ^{18}O, have demonstrated that $H_2{}^{18}O$ can be liberated with an initial burst within the first 1 h during the incubation of this substrate with α-chymotrypsin at pH 5.3. The result suggests that a substrate peptide can react with α-chymotrypsin to form a peptidyl-enzyme intermediate with liberation of water. It has also been demonstrated that the ninhydrin response of the reaction system decreases as the water liberation proceeds. This implies that the

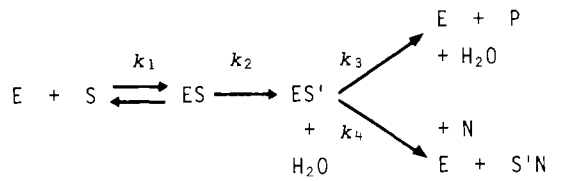

Figure 2. Estimated scheme for the formation of a peptidyl-enzyme intermediate and its degradation by water or by nucleophile peptide: E, enzyme; S, substrate peptide; ES, Michaelis complex; ES', peptidyl-enzyme; P, product (the same as S); N, another peptide as nucleophile; S'N, higher molecular weight product; k_1, k_2, k_3, and k_4, rate constants at the respective reaction steps.

peptidyl-enzyme can be almost simultaneously attacked by another substrate peptide through a nucleophilic reaction. As a result a peptide–peptide condensation reaction can be completed and this is recognized as the initiation for the peptide chain elongation during the plastein reaction. There must be competition between the peptidyl-enzyme degradation by water and that by a nucleophile peptide (Figure 2). In case a substrate concentration is very low, the hydrolytic degradation must be predominant. In such a case it appears that there is no reaction occurring, since exactly the same peptide is reproduced. A higher substrate concentration is more favorable for the degradation of the peptidyl-enzyme by the peptide to give rise to a higher molecular weight peptide. An extremely high substrate concentration is thus necessary for synthesizing a plastein, not only in view of mass action but also in the sense that this results in a decrease in the water concentration of the system as mentioned above. Tanizawa and Bender (1974) have studied the effect of the water concentration on the hydrolysis of *N*-benzoyl-L-tyrosine *p*-nitroanilide by immobilized α-chymotrypsin and disclosed that a lower water concentration is more effective in lowering the deacylation rate constant (k_3) without affecting the acylation process. The data are useful in understanding the effectiveness of the organic solvents for the plastein synthesis.

Amino Acid Incorporation during Plastein Synthesis. As previously mentioned, it is possible to incorporate methionine during the plastein synthesis by papain, provided this amino acid has been esterified prior to being submitted to the reaction (Yamashita et al., 1971a). A detailed analysis has demonstrated that the incorporated methionine molecules are located primarily at the C terminals of the plastein chains (Yamashita et al., 1972). This result indicates that the peptidyl-enzyme can be attacked nucleophilically by the amino acid ester as well as by peptides in the deacylation process (Figure 2). Not only methionine but also other amino acids, when esterified, are expected to act as nucleophiles and to be incorporated in accordance with their respective specificities. In order to characterize the specificity in relation to the amino acid side-chain structure we carried out the following experiment using an ovalbumin hydrolysate (degree of hydrolysis, 85%) as a substrate and a series of L-amino acid ethyl esters as nucleophiles. The ovalbumin hydrolysate (300 mg) was dissolved in water and adjusted to pH 6.0 with NaOH and to 0.9 ml with additional water. To the solution was added each amino acid ethyl ester so as to be 0.02 M. The mixture was preincubated at 37 °C for 15 min. On the other hand, papain (3 mg) was dissolved in 0.1 M L-cysteine (0.1 ml). This enzyme solution was combined with the above-mentioned preincubation mixture. The incubation was carried out at 37 °C. After 6 h, 0.1 N NaOH (10 ml) was added to stop the papain action and this alkalized solution was allowed to stand for

Table I. Percent Incorporation of α-Ethyl Esters of L-Amino Acids during the Plastein Synthesis from an Ovalbumin Hydrolysate by Papain

Amino acid	Incorporation, %
Lysine	33.3
Glutamic acid	25.0
Glycine	13.8
Alanine	22.0
α-Aminobutyric acid	62.0
Methionine	70.0
Norvaline	77.7
Valine	10.4
Leucine	72.5
Isoleucine	12.5
Norleucine	78.0
Glutamic acid γ-ethyl ester	80.1
Tyrosine	72.6
Phenylalanine	80.3
Tryptophan	84.5

3 h at room temperature to hydrolyze the amino acid ethyl ester remaining unreacted. The free amino acid thereby produced was determined with an amino analyzer. A reactivity of each amino acid ethyl ester was obtained by subtracting the determined value from the initial concentration (0.02 M). Table I shows the results obtained from this experiment, indicating that the reactivity of the nucleophiles depends primarily on the hydrophobicity of their amino acid side chains, except for the cases of the β-branched amino acids valine and isoleucine. As described later, the data in Table I can have practical use in incorporating several amino acids during the plastein synthesis from food protein hydrolysates. In particular, the different specificities observed with lysine, methionine, and tryptophan are related to the different amounts of addition of their ethyl esters to the hydrolysates of algal, bacterial, and leaf proteins, when these unconventional proteins are improved with respect to their essential amino acid pattern.

Improvement of Amino Acid Compositions of Conventional and Unconventional Proteins. As mentioned repeatedly, L-methionine ethyl ester can be effectively incorporated during the plastein synthesis from a peptic hydrolysate of soybean protein (Yamashita et al., 1971a); the resulting content of methionine in the plastein has been reported to be almost 8% (Table II). A similar process can produce a glutamic acid enriched plastein when α,γ-diethyl ester of glutamic acid is used according to the results shown in Table I. Yamashita et al. (1975) have reported that the glutamic acid content in this plastein is over 40% (Table II). It is thus possible to obtain a material with great solubility in water even at the isoelectric point of soybean globulin. Recently, we attempted preparing a gluten plastein with a high content of lysine. A gluten hydrolysate (degree of hydrolysis, 75%) was used as substrate. Various amounts of L-lysine ethyl ester dihydrochloride were mixed with this substrate. Each mixture was incubated with papain in an aqueous medium (pH 7.0) containing 20% acetone and 0.01 M L-cysteine. The condition of the incubation was as follows: substrate concentration, 50%; enzyme–substrate ratio, 1:50; temperature, 37 °C; and incubation time, 24 h. After the incubation, each reaction mixture was diluted with a 100-fold volume of 10% ethanol and treated with an Amicon membrane filter (UM-05) to obtain a plastein fraction whose molecular weight was higher than 500. The lysine content in this fraction was determined and expressed as a function of the amount of L-lysine ethyl ester dihydrochloride added (Figure 3). An almost maximal content of lysine was observed when the gluten hydrolysate

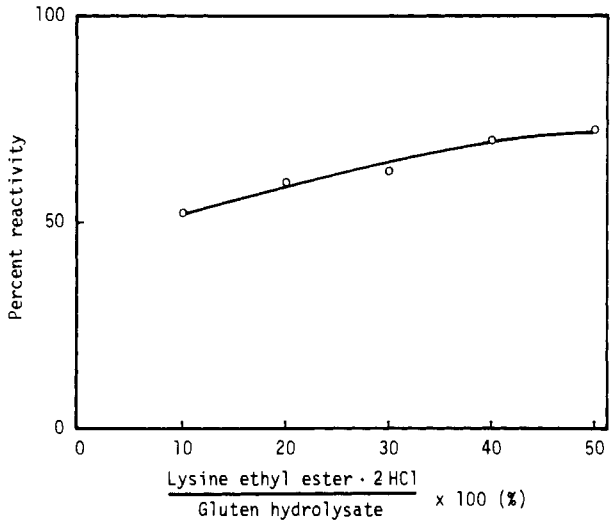

Figure 3. Relationship between the amount of addition of L-lysine ethyl ester dihydrochloride and its reactivity in the plastein synthesis from a gluten hydrolysate by papain.

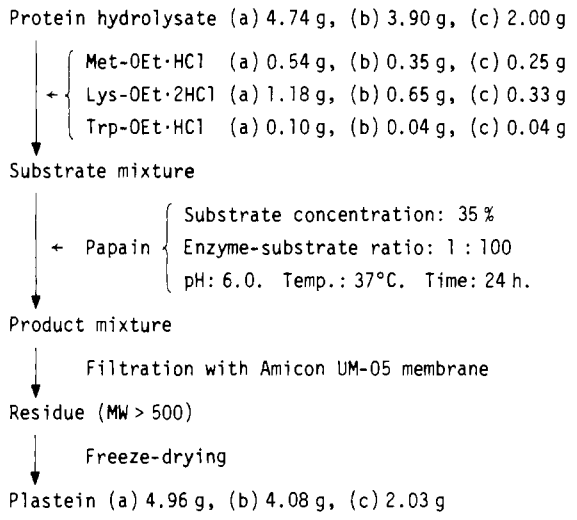

Figure 4. Process for producing plasteins with the incorporation of methionine, lysine, and tryptophan: (a), (b), and (c), processes in the cases of plastein production from *Spirulina maxima*, *Rhodopseudomonas capsulatus*, and *Trifolium repens L.*, respectively.

was formulated with half its amount of L-lysine ethyl ester dihydrochloride. The amino acid composition of the plastein prepared in this case is shown in Table II.

As a next attempt we carried out an experiment of simultaneously incorporating three essential amino acids, methionine, lysine, and tryptophan, during the plastein synthesis from unconventional protein hydrolysates. Peptic hydrolysates of proteins from *Spirulina maxima*, *Rhodopseudomonas capsulatus*, and *Trifolium repens L.* were used as substrates, their degrees of hydrolysis being approximately 80%. Each substrate was formulated with ethyl esters of L-methionine, L-lysine, and L-tryptophan. The amounts of the formulation of these ethyl esters and the procedures for synthesizing and obtaining the respective plasteins are shown in Figure 4. As Table II describes, it can be found that the methionine, lysine, and tryptophan levels have been greatly improved as a result of the process mentioned above.

It may be more interesting to pick out the eight kinds of essential amino acid (EAA) and to compare their patterns of the plasteins with the FAO/WHO suggested

Table II. Amino Acid Compositions of the Material Proteins and the Plasteins Produced with Incorporation of Selected Amino Acids

	Weight %										
	Soybean				Gluten plastein	*S. maxima*		*R. capsulatus*		*T. repens L.*	
Amino acid	Protein	Plastein[a]	Plastein[b]	Gluten		Protein	Plastein	Protein	Plastein	Protein	Plastein
Lysine	5.28	4.73	3.05	1.7 →	15.79	4.59 →	7.75	5.37 →	7.38	6.06 →	8.23
Histidine	2.04	2.20	1.22	1.9	1.01	1.77	1.91	2.35	2.44	1.94	2.01
Arginine	5.94	5.61	3.82	3.3	3.47	6.50	6.70	6.27	6.05	3.66	3.84
Aspartic acid	8.70	7.76	11.76	9.6	4.53	8.60	11.87	8.57	10.21	11.10	10.45
Threonine	2.63	2.11	3.08	2.5	2.35	4.56	5.42	5.07	4.36	5.60	5.93
Serine	3.53	2.75	4.46	4.0	3.78	4.20	4.43	3.16	4.63	4.17	4.00
Glutamic acid	15.00	10.20 →	41.93	27.0	30.99	12.60	14.68	10.03	9.77	16.00	15.54
Proline	4.32	2.18	2.88	8.0	6.17	3.90	3.62	4.26	3.45	3.75	3.57
Glycine	4.38	2.55	3.64	7.0	2.24	4.75	4.76	4.53	5.98	5.01	4.79
Alanine	3.98	2.65	2.51	3.0	2.43	6.80	5.80	8.74	8.32	6.23	6.05
Valine	3.36	4.29	4.34	4.1	4.33	4.69	6.00	6.59	6.56	7.45	7.00
Isoleucine	3.00	5.72	2.37	4.2	3.22	6.03	6.32	4.96	5.30	4.98	4.55
Leucine	5.17	7.26	3.65	7.0	5.12	8.02	8.98	8.45	8.47	8.86	8.24
Aromatic amino acid	7.03	9.46	3.38	5.4	6.02	8.92	8.96	8.09	8.16	9.61	9.00
Tyrosine	2.83	3.52	0.88	1.3	2.07	3.95	3.98	3.21	3.53	4.11	3.88
Phenylalanine	4.20	5.94	2.50	4.1	3.95	4.97	4.98	4.88	4.63	5.50	5.12
S-Containing amino acid	2.94	9.96	2.80			1.77	8.75	3.73	9.06	1.82	8.13
Half-cystine	1.76	1.98	1.60			0.40	0.53	0.76	0.77	0.97	0.99
Methionine	1.18 →	7.98	1.20			1.37 →	8.22	2.97 →	8.29	0.85 →	7.14
Tryptophan	1.34	1.30	0.70			1.40 →	2.72	2.05 →	2.56	1.51 →	2.73

[a] Methionine-incorporated plastein. [b] Glutamic acid incorporated plastein.

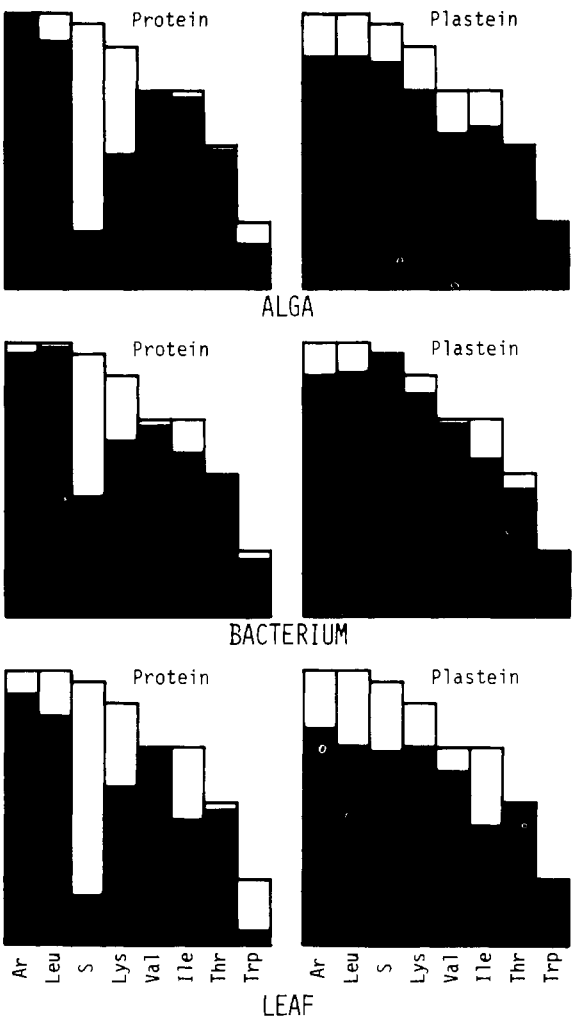

Figure 5. Essential amino acid patterns (filled columns) of the proteins from a blue-green alga (*S. maxima*), a non-sulfur purple bacterium (*R. capsulatus*), and a leaf (*T. repens L*), and of the plasteins produced from these proteins, with the FAO/WHO pattern in each background (open columns).

patterns (cf. Report of a Joint FAO/WHO *Ad Hoc* Expert Committee, 1973). In Figure 5 there are EAA patterns of the three material proteins and the three corresponding plasteins, with the FAO/WHO pattern in each background. It can be seen that the material proteins are more or less different from the FAO/WHO suggestion, especially with respect to methionine, lysine, and tryptophan levels, whereas the EAA patterns of the plasteins have become almost similar to that suggested by FAO/WHO.

CONCLUDING REMARKS

Plastein reaction seems to be greatly applicable to the improvement of amino acid composition of food proteins. Particularly, nutritional improvement of proteins may thus be feasible. Also, the creation of some new functional properties might be possible. However, what is of pressing importance for this reaction to obtain economic feasibility is to find out how to retain a maximum amount of enzyme, because the cost of production of plastein is primarily dependent on the cost of enzyme. In order to solve this problem an approach should be made from the standpoint of enzyme kinetics. Finally, what may be more important must be the confirmation of the wholesomeness of the plastein as human food. We are going to carry out animal feeding tests with the plasteins in which appropriate amounts of essential amino acids have been incorporated.

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LITERATURE CITED

Bundy, H. E., *Anal. Biochem.* **3**, 431 (1962).
Determann, H., Koehler, R., *Justus Liebigs Ann. Chem.* **690**, 197 (1965).
Fujimaki, M., Yamashita, M., Arai, S., Kato, H., *Agric. Biol. Chem. (Tokyo)* **34**, 1325 (1970).
Haurowitz, F., Horowitz, J., *J. Am. Chem. Soc.* **77**, 3138 (1955).

Report of a Joint FAO/WHO *Ad Hoc* Expert Committee: Energy and Protein Requirements, World Health Organization, Geneva, 1973.
Royer, G. P., Uy, R., *J. Biol. Chem.* **248**, 2627 (1973).
Tanimoto, S., Yamashita, M., Arai, S., Fujimaki, M., *Agric. Biol. Chem. (Tokyo)* **36**, 1595 (1972).
Tanizawa, K., Bender, M. L., *J. Biol. Chem.* **249**, 2130 (1974).
Tauber, H., *J. Am. Chem. Soc.* **73**, 1288 (1951).
Tsai, S.-J., Yamashita, M., Arai, S., Fujimaki, M., *Agric. Biol. Chem. (Tokyo)* **38**, 641 (1974).
Virtanen, A. I., Kerkkonen, H. K., Laaksonen, T., Hakala, M., *Acta Chem. Scand.* **3**, 520 (1949).
Wasteneys, H., Borsook, H., *Physiol. Rev.* **10**, 111 (1930).
Wieland, T., Determann, H., Albrecht, E., *Justus Liebigs Ann. Chem.* **633**, 185 (1960).
Yamashita, M., Arai, S., Aso, K., Fujimaki, M., *Agric. Biol. Chem. (Tokyo)* **36**, 1353 (1972).
Yamashita, M., Arai, S., Gonda, M., Kato, H., Fujimaki, M., *Agric. Biol. Chem. (Tokyo)* **34**, 1333 (1970a).
Yamashita, M., Arai, S., Kokubo, S., Aso, K., Fujimaki, M., *J. Agric. Food Chem.* **23**, 27 (1975).
Yamashita, M., Arai, S., Matsuyama, J., Gonda, M., Kato, H., Fujimaki, M., *Agric. Biol. Chem. (Tokyo)* **34**, 1484 (1970b).
Yamashita, M., Arai, S., Matsuyama, J., Kato, H., Fujimaki, M., *Agric. Biol. Chem. (Tokyo)* **34**, 1492 (1970c).
Yamashita, M., Arai, S., Tanimoto, S., Fujimaki, M., *Biochim. Biophys. Acta* **358**, 105 (1974).
Yamashita, M., Arai, S., Tsai, S.-J., Fujimaki, M., *J. Agric. Food Chem.* **19**, 1151 (1971a).
Yamashita, M., Tsai, S.-J., Arai, S., Kato, H., Fujimaki, M., *Agric. Biol. Chem. (Tokyo)* **35**, 86 (1971b).

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Fortification of Foods by Fermentation with Lysine-Excreting Mutants of Lactobacilli

David C. Sands* and Lester Hankin

Most microorganisms regulate amino acid biosynthesis by mechanisms such as feedback inhibition and repression. Thus, they do not usually produce more amino acids than they need and in fact deplete those in the medium. However, some wild-type microorganisms and mutants have been found that do excrete specific amino acids. Some of these microorganisms are used to manufacture amino acids through fermentation procedures but none have been used directly to augment the amino acid content of food produced by fermentation. We used lysine analogues to select for spontaneous mutants of lactobacilli that over-produce and excrete lysine. Wild type *Lactobacillus acidophilus* and *L. bulgaricus* and lysine-excreting mutants were used to ferment soybean milk to yogurt. In all tests, mutants increased the lysine content of yogurt over that obtained with the wild-type. Mutants of *L. plantarum* were used to increase the lysine content of corn silage. The potential use of lysine-excreting mutants in producing fermented foods is suggested.

Many raw products can be fermented to provide different food, and the range of raw materials includes animal products such as milk, beef, and fish and such plant products as cereal grains, soybeans, coconuts, and peanuts (Hesseltine, 1965). Examples of fermented products are shown in Table I. Although fermentation may not improve the nutritive value of the raw material (van Veen and Steinkraus, 1970), it may enhance taste, sometimes making an inedible material palatable. In some parts of the world fermented foods are an important part of the diet and are classed as "traditional". The nutritive quality of some of these fermented foods could be improved by the use of mutant strains of the fermenting organisms that would excrete an essential amino acid into the food.

Lysine, one of the ten essential amino acids in animal nutrition, is often low in plant materials, and food has been fortified by adding lysine, either synthesized or purified from fermentation liquors (Altschul, 1974). Lysine intake may also be increased by eating varieties of plants that are richer in lysine (Brock et al., 1973). Mutant plants that contain up to twice the lysine in their protein than wild types have been described and used, e.g., high lysine maize, barley, and sorghum (Mertz, 1974).

Department of Plant Pathology and Botany and Department of Biochemistry, The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504.

Table I. Examples of Fermented Foods and Starting Materials

Place	Product fermented	Product
United States	Milk	Cheese, yogurt
Orient	Grain	Soy sauce
Japan	Rice	Tamari sauce
Indonesia	Soybeans	Tempeh
Orient	Fish	Sauce
Hawaii	Taro	Poi
China	Eggs	Pidan
Java	Peanuts	Ontjon
Java	Coconuts	Bongkrek
Indonesia	Rice or cassava	Tape'
World-wide	Grain	Beer, bread

We suggest a third method to increase the lysine intake by selecting, from the normal food fermenting organisms, mutant strains that excrete this amino acid into the food and then use these strains to ferment food (Sands and Hankin, 1974). The key is finding organisms now used for fermentation that can be selected to excrete lysine.

To find a lysine excreting mutant for fermentation, several methods could be tried. Although many colonies from natural fermentations could be screened and tested for lysine excretion, our experiments with lactobacilli showed that none of the wild-type strains examined excreted lysine. However, most of the species tested on a lysine-free medium (Lysine Assay Medium, Difco, Detroit, Mich.) did produce sufficient lysine for their own growth.