Note on an Enlarged-Scale Method for Preparing a Methionine-Enriched Plastein from Soybean Protein

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In a laboratory-scale experiment we have found that a mixture of a soybean protein hydrolysate and L-methionine ethyl ester, when incubated with papain under specific conditions, reacts to form a plastein which contains a large amount of methionine (1). The present note deals with a revised method for preparing such a plastein on a practical scale and also with its amino acid composition compared with that of a soybean protein preparation used as starting material.

METHODS AND RESULTS

A soybean acid-precipitated protein isolate (Fuji Oil Mills, Ltd.) was used as starting material. This material (1 kg.) was dissolved in 1N NaOH (5 liters) and the solution (pH 12.0) allowed to stand for 4 hr. at room temperature to hydrate and denature the protein. Subsequently, the solution was acidified with HCl to pH 5 to recover an acid-precipitated protein fraction. This was dissolved in 0.1N HCl (50 liters) and the solution adjusted with HCl to pH 1.6. To the solution was added 20 g. of pepsin (a recrystallized preparation, Sigma Chemical Co.) and the mixture incubated at 37°C. for 24 hr. After peptic hydrolysis the reaction mixture was concentrated to less than 3 liters. In this concentrate were dissolved L-methionine ethyl ester (100 g.) and L-cysteine (3.6 g.) as an enzyme activator, and the resulting solution was adjusted with aqueous NaOH to pH 5.3 and to 3 liters in final volume. Twenty grams of papain (a purified preparation; Sigma Chemical Co.) was added and the mixture incubated at 37°C. for 48 hr. to synthesize a plastein. The entire plastein-reaction mixture was suspended in 1N NaOH (6 liters) and the suspension (9 liters total; pH 11.2) stirred for 4 hr. at 30°C. to hydrolyze the ethyl ester linkage. Ethanol (21 liters) was added and the resulting 70%-ethanol suspension of the plastein-reaction product was stirred gently overnight at room temperature. By centrifuging this suspension a plastein was obtained with a yield of 650 g. on a dry-matter basis. This plastein was free from taste, odor, and color.

Direct analysis with an amino acid analyzer confirmed that the plastein contained no free methionine (Fig. 1A). When the plastein was hydrolyzed with 6N HCl *in vacuo* at 110°C. for 24 hr., the amino acid analysis of the resulting hydrolysate gave a result as in Fig. 1B. Similar hydrolysis of the soybean protein used as the starting material showed another result (Fig. 1C).

It is further noted that there is a 70%-ethanol-soluble plastein which is obtainable as a water-insoluble precipitate with a yield of 75 g. on a dry-matter basis. The amino acid pattern of this plastein was similar to Fig. 1B.

Thus, the total plastein yield was 725 g. from 1 kg. of the starting material.

It should also be noted that, in spite of the alkali treatments at the two steps mentioned above, little if any lysinoalanine formed (4) since no clear lysinoalanine peak is observable just before the lysine elution (Fig. 1B).

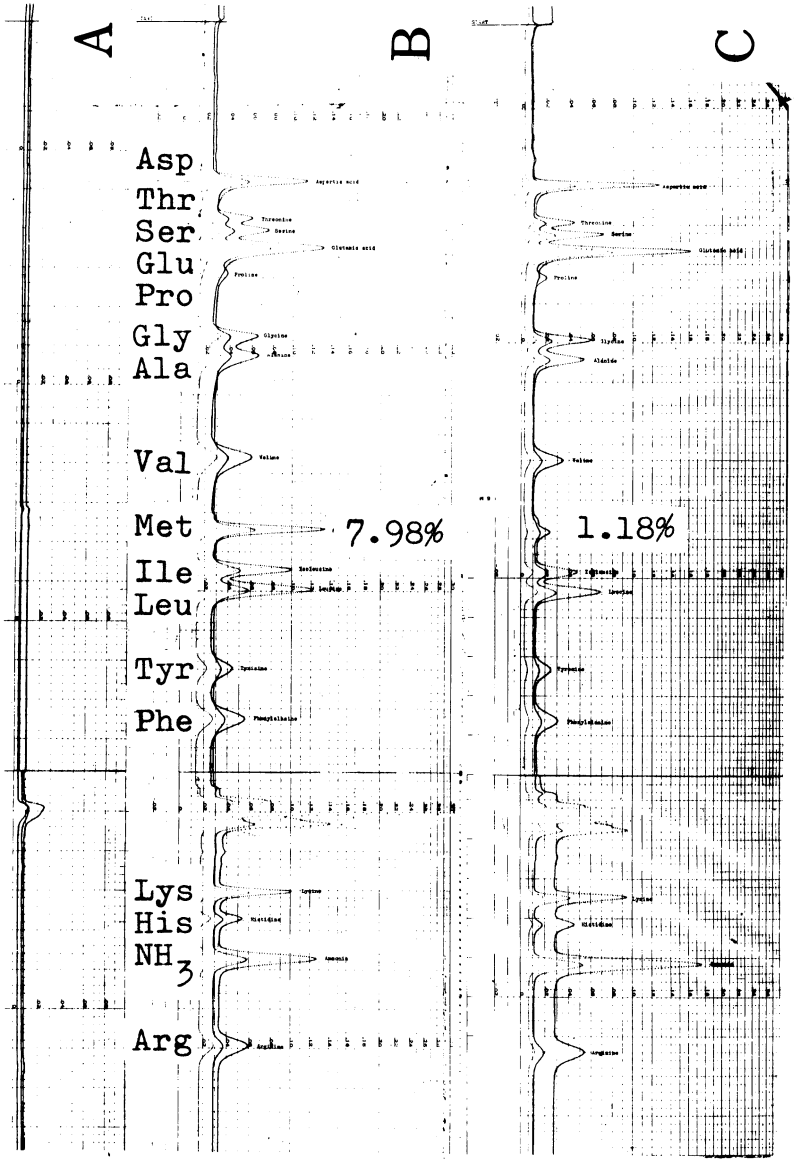

Fig. 1. Amino acid patterns. A = methionine-enriched plastein before hydrolysis. B = the same plastein after hydrolysis. Asp, 7.76; Thr, 2.11; Ser, 2.75; Glu, 10.20; Pro, 2.18; Gly, 2.55; Ala, 2.65; Val, 4.29; Met, 7.98; Ile, 5.72; Leu, 7.26; Tyr, 3.52; Phe, 5.94; Lys, 4.73; His, 2.20; Arg, 5.61; Trp, 1.30; and Half Cys, 1.98% on a weight basis. C = soybean protein after hydrolysis. Asp, 8.70; Thr, 2.63; Ser, 3.53; Glu, 15.00; Pro, 4.32; Gly, 4.38; Ala, 3.98; Val, 3.36; Met, 1.18; Ile, 3.00; Leu, 5.17; Tyr, 2.83; Phe, 4.20; Lys, 5.28; His, 2.04; Arg, 5.94; Trp, 1.34; and Half Cys, 1.76% on a weight basis. (Tryptophan and half-cystine were determined by the methods specific to their analyses (2,3).)

DISCUSSION

From the present results (Fig. 1, A and B) as well as our previous report (5) it appears certain that the plastein produced through the above-mentioned procedures contains methionine in peptide linkage. The content is as high as 7.98% (Fig. 1B); this value is nearly seven times the methionine level, 1.18%, of the unmodified protein (Fig. 1C). Although such a content (7.98%) is considered to be in excess for optimum amino acid nutrition, it is possible to obtain a plastein with a satisfactory methionine content if the amount of L -methionine ethyl ester is controlled on use. Another convenient means of decreasing the methionine content to a nutritionally suitable level is to dilute the methionine-enriched plastein with soybean proten. A 1:5 mixture, for example, of plastein and protein contains approximately 2.3% methionine and 1.8% half-cystine. Such a mixture resembles the ideal protein proposed (6), in respect to the levels of the sulfur-containing amino acids.

It is expected that a similar technique employing the plastein reaction may be applicable even to alter the general amino acid patterns and functionalities of cereal proteins.

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