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Degradation of Methionine in Heated Soybean Protein and the Formation of β-Methylmercaptopropionaldehyde

Michael Shemer and Edward G. Perkins*

Methionine, added to soy protein isolate, when heated in boiling water is destroyed under aerobic conditions. A portion of the added methionine was degraded to the corresponding β-methylmercaptopropionate. This was determined by gas chromatographic separation of the butyl ester of heated soybean protein hydrolysate. A characteristic peak was eluted after 15.30 min from the heated protein hydrolysate. The mass spectrum of this peak indicated that it was *n*-butyl β-methylmercaptopropionate.

The production of flavors in foods upon processing as a result of reactions between sulfur-containing amino acids and sugars has been suggested in several reports. Many studies reporting losses or binding of methionine during thermal processing have been done on meat proteins. Miller *et al.* (1965) reported loss of methionine in cod muscle processed for 27 hr at 85°. Loss of methionine was also reported in defatted herring cake processed for 27 hr at 130°. Similar observations of loss of methionine were observed by Horn (*Agr. Res.*, 1969) who suggested that this was the result of a condensation between the sulfur in the methionine and the carbonyl of the carbohydrate present. Arroyo and Lillard (1970) attributed the overcooked egg odor of a heated cystine–glucose mixture to hydrogen sulfide and mercaptans. Gruenwedel and Patnaik (1971) stated that the black deposits in the headspace area of canned protein rich foods resulted from heat-induced decomposition of the sulfur containing amino acids which produced hydrogen sulfide and, in turn, iron sulfide during the thermal processing.

Wainwright *et al.* (1972) proposed a variety of intermediate compounds of methionine resulting from degradation. The authors suggested that one of these compounds was methional. A general review concerning the Strecker degradation of α-amino acids has been published by Schonberg and Moubacher (1952). According to these authors, the formation of the intermediate Schiff compound between the carbonyl and the amino group is an essential step in the reactions followed by decarboxylation and deamination.

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In the present study, the thermal degradation of methionine, when supplemented to soybean protein and the subsequent formation of methional (β-methylmercaptopropional), was investigated.

EXPERIMENTAL SECTION

Materials. The protein source that was used in the experiments reported herein was Promine D, sodium soy proteinate, a commercially available product, courtesy of the Central Soya Co., Chemurgy Division, Chicago, Ill.

Heat Treatment Procedures. Four methods of heating the protein samples were investigated: (1) dry air heating with a chamber air dryer at 100° for 60 min; (2) steam heating with a steam blancher at atmospheric pressure for 60 min; (3) heating of the protein dispersion (10%) in water at 100° for 60 min; (4) heating under vacuum at 100° for 60 min.

Amino Acid Analysis. Acid hydrolysates were obtained by dissolving 20 mg of protein in 10% 6 *N* HCl in a Pyrex tube. The tube was flushed with nitrogen and closed securely with a Teflon lined cap. Hydrolysis was carried out at 100° for 22 hr. The hydrolysate was filtered through glass wool and the 6 *N* HCl was evaporated by heating in an oil bath at 100° under a stream of nitrogen. The hydrolyzed amino acid residue was dissolved in 10 ml of 0.1 *N* HCl. Ornithine (5 mg) was added as an internal standard and the 0.1 *N* HCl was evaporated again. The sample was redissolved in 10 ml of 3 *N* HCl in *n*-butyl alcohol with the aid of an ultrasonic mixer. Esterification of the amino acids was carried out in open vials, heated at 100° for 15 min. The excess butanol was removed by evaporation and the residue dissolved in 4 ml of methylene chloride–trifluoroacetic anhydride (3:1) solution and acylated for 5 min at 150° in a sealed acylation tube.

Gas Chromatography Conditions. In general, the

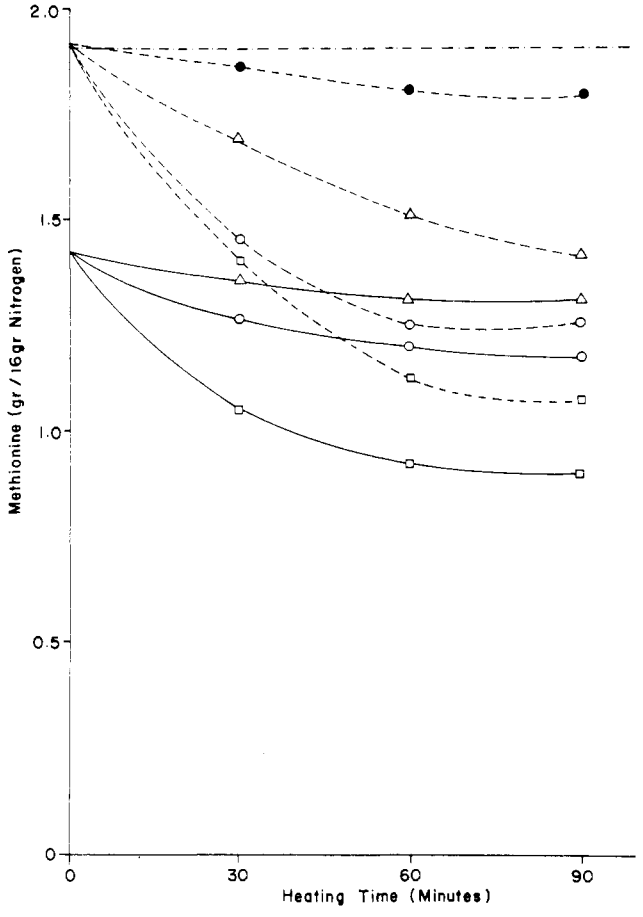


Figure 1. The effect of heating time on the methionine content of a soybean protein isolate: (- - -) isolated soy protein + 0.5% methionine; (—) isolated soy protein; (△) heated with dry air, 100°; (○) heated with steam, 100°, atmospheric pressure; (□) ISP 10% in boiling water; (···) required amount of methionine (FAO pattern); (●) heated under vacuum, 100°.

method developed by Gehrke and coworkers (Gehrke and Stalling, 1967; Gehrke *et al.*, 1971; Roach and Gehrke, 1969; Zumwalt *et al.*, 1970) for amino acid analysis was employed. A Beckman GC-5 flame ionization gas chromatograph was used. The separation of the *n*-butyl trifluoroacetyl derivatives of amino acids was accomplished with a 6 ft × 4 mm i.d. glass column packed with 0.325% (w/w) ethylene glycol adipate on 80–100 mesh acid-washed heat-treated Chromosorb B. After 8 min at 100°, the column was temperature programmed from 100 to 210° in 32 min. The detector signal was transferred to a four-channel digital integrator (Model 3370A, Hewlett-Packard, Inc., Skokie, Ill.). The output data were punched on a paper tape and transferred through an interface to a programmable electronic calculator (Wang 700) with the appropriate program for the printout of the tabulated results.

Synthesis of β-Methylmercaptopropional (Methional). The procedure of Pierson *et al.* (1948) was used in the synthesis of the β-methylmercaptopropionaldehyde intermediate. The reaction was carried out by the addition of methyl mercaptan to acrolein at atmospheric pressure, and was catalyzed by the addition of a small amount of cupric acetate to the reaction mixture. One mole of gaseous methyl mercaptan (48 g) was bubbled for 60 min under the surface of a cooled stirred mixture of 1 mol of acrolein (56 g) and 0.5 g of cupric acetate. The internal temperature of the exothermic reaction was maintained at 30–40° by cooling in an ice bath and adjusting the rate of addition of methyl mercaptan. After addition of reactant gas was completed, the reaction mixture was agitated for 1 hr.

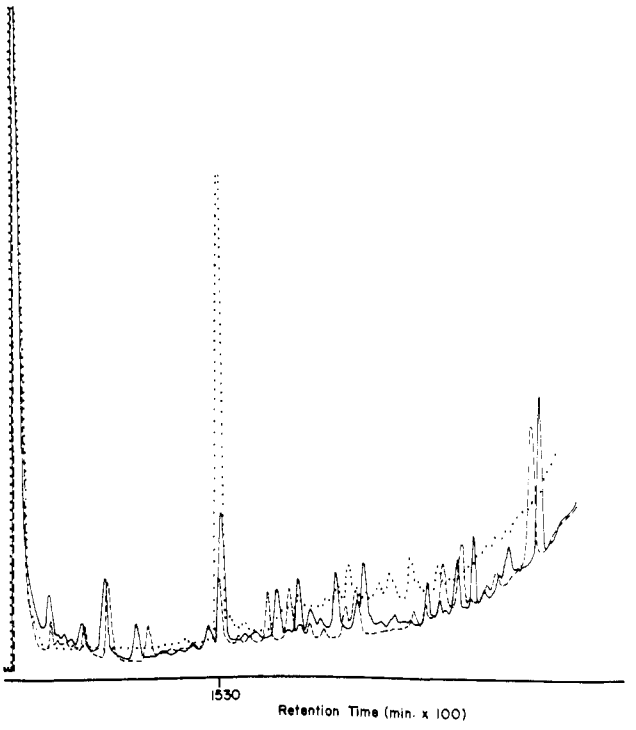


Figure 2. Glc of the butyl esters of a hydrolyzed heated soybean protein isolate: (···) standard; (- - -) steam heated soybean protein; (—) steam-heated soybean protein + 0.5% methionine.

A sample of the reaction mixture (2 ml) was mixed with 0.1 *N* HCl and heated for 15 min at 100° as the first part of the derivatization process. This converted the methional *via* oxidation to β-methylmercaptopropionic acid.

Gas Chromatography–Mass Spectrometry. A Perkin-Elmer Hitachi RMU-6E single focusing mass spectrometer coupled with a gas chromatographic inlet system was used. The helium separator, ion source, and chamber heater were all maintained at 260°. The ionizing potential was 22 eV. Spectra were recorded whenever a peak was eluted from the glc.

A Varian Aerograph Series 1200 gas chromatograph equipped with a 6 ft × ⅛ in. i.d. stainless steel column packed with 0.325% (w/w) ethylene glycol adipate on 80–100 mesh acid-washed heat-treated Chromosorb B was used. Sample sizes of 1–5 μl containing about 5 μg of material were injected. Helium (40 ml/min) was used as carrier gas. The temperature of the column was programmed to 210° at a rate of 4°/min after holding at 100° for 8 min. Most of the column effluent was diverted into the mass spectrometer *via* a fritted glass helium separator. A Varian spectrosystem 100 data processing system was employed for on-line mass spectral data processing.

Samples for glc-mass spectrometric analysis were prepared as butyl esters only. The esterification procedure was the same as previously described in the amino acid analysis section. Following esterification and evaporation of excess *n*-butyl alcohol, the butyl esters were dissolved in methylene chloride prior to injection into the gas chromatograph.

RESULTS AND DISCUSSION

The effect of different heat treatments on isolated soybean protein samples with and without methionine supplementation was determined as a portion of this study. The data shown in Figure 1 indicate that the only treatment that caused loss of methionine in the native protein is heating the solution of 10% protein in water at 100°. About 30% of the methionine was lost in this procedure (from 1.4 to 0.9 g of methionine/16 g of nitrogen). After supplementation of the isolated protein with 0.5% free

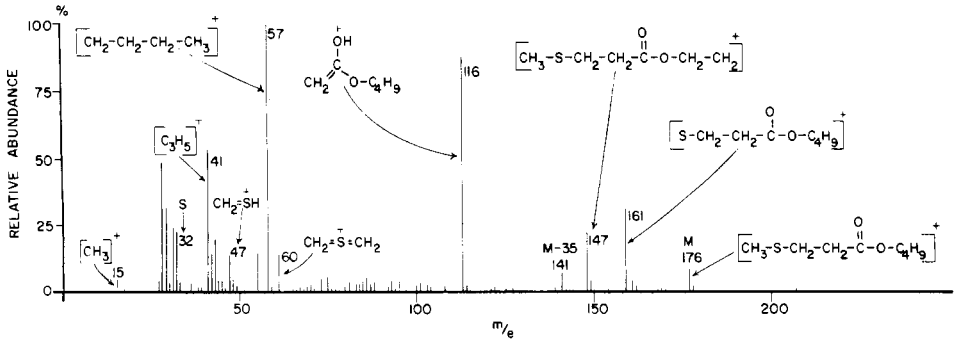


Figure 3. Mass spectrum of butyl β-methylmercaptopropionate.

methionine, the mixture was subjected to the same thermal treatments. Most of the free methionine that was added to simulate supplementation was destroyed during the various treatments (Figure 1). The most severe destruction occurred when the supplemented protein was heated in boiling water for 60 min and essentially all the 0.5% of added methionine was destroyed. Most of the added methionine was destroyed by atmospheric steam heating (from 1.9 to 1.3 g of methionine/16 g of nitrogen) and heating with dry air (100°) caused partial destruction (from 1.9 to 1.5 g of methionine/16 g of nitrogen). Heating under vacuum conditions (0.5 in.) at 100° did not lower significantly the level of methionine in the sample. These data indicated (Figure 1) that most of the methionine destroyed was in the free form in an oxidative type of reaction. The loss of methionine in the native protein in the boiling water may have been due to partial degradation of the protein to small peptides (Shemer *et al.*, 1973) and perhaps free amino acids.

The gas chromatogram of the *n*-butyltrifluoroacetyl esters of the hydrolysate from a steam heated sample of soybean protein separated on an ethylene glycol adipate (EGA) column is shown in Figure 2. A characteristic peak was always eluted at the retention time of 15.30 min. The size of this peak was more than doubled when 0.5% methionine was added and heated with the original protein (Figure 2). Since the acylation step was not performed in the derivatization procedure, amino acids could not possibly appear on the chromatogram obtained from the very polar column. No compounds with proper retention times were found by a similar treatment of unheated soybean protein.

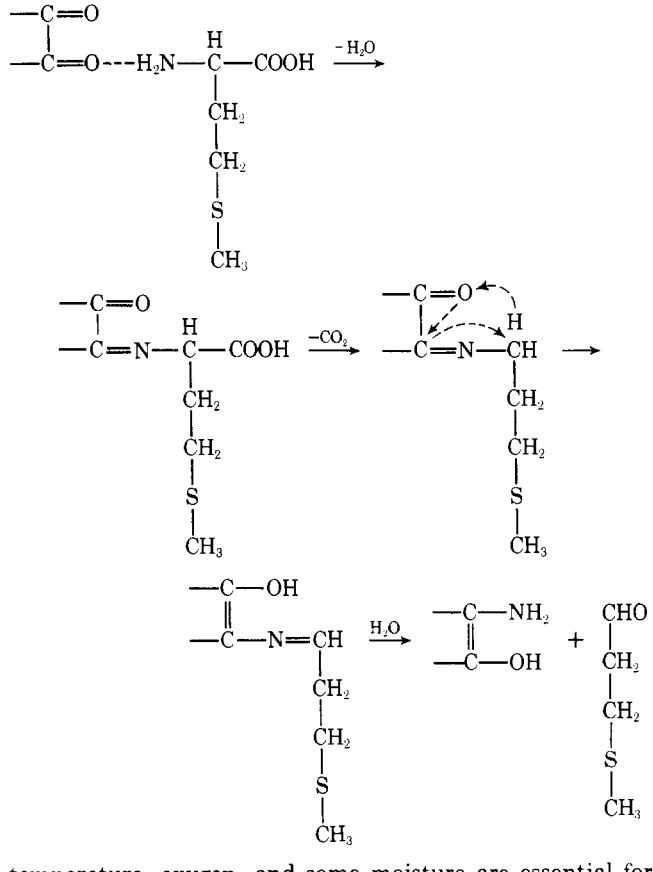
It was logical to expect that the peak at 15.30 min was a butyl ester of a newly formed non-amino acid. However, our attempts to isolate methional, as such or as a derivative, were unsuccessful.

In an attempt to confirm the possibility that this was a product resulting from the Strecker degradation of methionine, β-methylmercaptopropional (methional) was synthesized from methylmercaptan and acrolein, simultaneously oxidized, and converted to the corresponding butyl β-methylmercaptopropionate. Injection of the *n*-butyl β-methylmercaptopropionate into the gas chromatograph column resulted in a peak at the same retention time of 15.30 min.

Gas Chromatography–Mass Spectrometry Study. The mass spectrum of the peak eluted at 15.30 min is shown in Figure 3. The molecular ion at *m/e* 176 agrees with the molecular weight of butyl β-methylmercaptopropionate. The ion at *m/e* 161 indicates cleavage of CH_3S and elimination of the methyl group from the molecule. The ion at *m/e* 141 (M − 35) is prominent in the spectra of short-chain thioethers as a result of loss of SH_3^+ from the molecule. The intense ion at *m/e* 116 is a product of the McLafferty rearrangement of the molecular cleavage of the 2-3 carbon bond and attachment of a hydrogen atom to the ester carbonyl oxygen. The second ion formed is at *m/e* 60. The base peak at *m/e* 57 is a butyl residue from the cleavage of the ester. At *m/e* 47, a characteristic ion of alkyl thioethers and cyclic thioether spectra is formed (Budzikiewicz *et al.*, 1967).

Proposed Mechanism. In order to explain the formation of methional in the present conditions, Schonberg and Moubacher (1952), in their comprehensive review of the Strecker degradation reaction, claimed that an active group, CO—(C=C)$_n$—CO, was involved in catalytic oxidation. Glucose (or another monosaccharide) does not degrade α-amino acids when the reaction is carried out in the absence of oxygen. However, the authors suggested that in the presence of oxygen and at 100°, glucose would be oxidized to give an active substance containing an α-aldehyde group. The active (-C=O)$_2$ group may be involved in the degradation of methionine as shown in Scheme I. This mechanism suggests that high

Scheme I



temperature, oxygen, and some moisture are essential for the sequence of the reactions. Furthermore, the presence of carbohydrates may only have a catalytic role in the observed oxidative degradation of methionine, rather than the active reactant function they play in the Maillard

reaction with lysine. This is supported by the fact that even the minute amounts of residual carbohydrates in the isolated protein are sufficient to exert a catalytic effect.

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Effect of Dietary Carbohydrates and Fats on Inorganic Iron Absorption

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The effects of modifying the dietary carbohydrate and fat upon the availability of iron to rats were studied. In general, iron utilization was greatest with diets containing lactose, less in diets containing sucrose, and least with diets in which the carbohydrate was supplied as starch. However, the effect of the carbohydrate was not uniform when iron sources of differing availability were tested. Diets high in fat favored iron utilization and iron absorption was greater in diets in which the fat was supplied as coconut oil than in those in which the fat was supplied as corn oil.

It is now well known that the availability of dietary iron is dependent not only upon the nature of the iron source in the diet but upon the nature of the diet with which the iron is consumed (Moore and Dubach, 1951; Layrisse *et al.*, 1968; Pla and Fritz, 1970; Amine *et al.*, 1972; Amine and Hegsted, 1974). In previous work (Amine and Hegsted, 1971) we found that the absorption of radioiron was markedly influenced by the kind of dietary carbohydrate. The studies reported here were undertaken to explore this effect further and to evaluate the effects of modifying the kind and amount of dietary fat upon the absorption of dietary iron.

MATERIALS AND METHODS

The composition of the diets used in these studies is indicated in Table I. The kind and amount of carbohydrate and fat in the diet were modified in the various experiments as indicated below. Such diets (Amine and Hegsted, 1971) contain less than 10 ppm of iron.

In the first experiment the diet contained glucose as the source of carbohydrate and 5, 15, or 30% of fat supplied as either coconut oil or corn oil. The absorption of radioiron was studied by the technique previously described (Amine and Hegsted, 1971). Briefly, groups of six female rats (Charles River Breeding Laboratories, Wilmington, Mass.) that were moderately iron deficient were provided with the indicated diets low in iron for 1 week. They were then fasted overnight and given 2 g of the appropriate diet containing 20 μg of iron as ferric ammonium citrate and 0.2 μCi of ^{59}Fe as $FeCl_3$. Two hours later when all or nearly all of the food had been eaten the animals were placed in a whole body counter to determine the amount of ^{59}Fe consumed. The animals were continued on the diets for 9 days after which they were counted again. The difference in counts corrected for physical decay of the isotope was taken as a measure of the iron retained.

In the second experiment the availability of several forms of iron in diets containing different carbohydrate was determined using the "prophylactic assay" previously described (Amine and Hegsted, 1974). Thirty-nine groups of five female weanling rats were used. Three groups received the diets without added iron which were made with either starch, sucrose, or lactose as the carbohydrate source. Three additional groups were used to investigate each iron source with each type of diet. These groups received three arbitrarily selected levels of iron supplied as either ferrous sulfate, reduced iron, sodium iron pyrophosphate, or ferric orthophosphate. All the diets contained 15% of a partially hydrogenated vegetable oil. The animals were fed the diets for a 3-week period. They were weighed at intervals and bled after 3 weeks to determine hemoglobin (Crosby *et al.*, 1954) and hematocrit.

The third experiment also utilized five female animals per group and investigated the effects of varying the kind of carbohydrate and fat on the availability of ferrous sulfate. The diets were made with either starch, sucrose, or a lactose–starch mixture (2:1). The diets contained either 5

Table I. Composition of the Diets

Ingredients	%
Carbohydrate (starch, sucrose, or lactose)	44.2–64.2
Casein (vitamin free)[a]	20.0
Salt mix (iron free)[b]	5.0
Vitamin mix[c]	0.5
Choline chloride	0.3
Dietary fat[d]	5.0–25.0
Cellulose	5.0

[a] General Biochemicals, Chagrin Falls, Ohio. [b] Jones and Foster (1945). Modified by the addition of 0.05 g of sodium selenite and 0.05 g of chromium acetate/2043 g of iron-free salt mix. [c] Hegsted *et al.* (1967). [d] Spry, Lever Brothers Co., New York, N.Y.

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