Mustard Seed Processing: Simple Methods for Following Heat Damage to Protein Meals[1]

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Abstract

Processed mustard seed contains a considerably higher content of reducing sugar than other oilseeds. During processing, the natural reducing sugar is supplemented with glucose released by enzyme hydrolysis of the thioglucoside, and this total content reaches a value of more than 3% of the defatted mustard meal. This quantity of reducing sugar in mustard seed is three times more than that in soybean meal. Consequently, the browning reaction, which degrades protein, presents a greater problem in processing oilseeds containing thioglucosides than oilseeds free of thioglucosides. In developing the processing of mustard seed, the degree of heat treatment given the protein meals had to be determined. Several indirect methods were developed for following the effects of heat treatment on protein quality. Nitrogen solubility index, optical density of aqueous extracts, and reducing sugar content correlated well with degradation of heat-labile amino acids, such as lysine, arginine, and histidine, and gave an index of protein quality.

Introduction

IN THE DEVELOPMENT of a commercial process for mustard seed (10), engineering studies were initiated to remove the pungent essential oil allyl isothiocyanate and to produce a palatable high-quality protein meal. To accomplish the first objective, the isothiocyanate precursor was converted by the natural enzyme, myrosinase, to yield 1 mole each potassium hydrogen sulfate, allyl isothiocyanate, and glucose per mole of thioglucoside. Unprocessed mustard seed contains considerably more reducing sugar than do other oilseeds, which contain no thioglucosides, such as soybeans. Unprocessed soybeans contain approximately 1% reducing sugars, whereas unprocessed mustard seed contains twice this amount before enzyme hydrolysis of the thioglucoside. After complete hydrolysis of the thioglucoside in mustard seed, the total reducing sugar content increases to more than three times the amount in mature soybeans.

When mustard meals were heated to remove the essential oil, a loss in the basic amino acids, lysine, arginine, and histidine, was observed (9,10). The loss of amino acid is attributed primarily to a reaction with reducing sugars in the protein meal. Conceivably, the browning reaction presents a greater problem in processing oilseeds containing thioglucosides, which release additional reducing sugar upon enzyme hydrolysis, than the processing of thioglucoside-free oilseeds.

Because the engineering studies were directed toward the production of a protein meal of high quality with a minimum of heat treatment, simple methods were needed to follow the degree or extent of heat treatment given the meals during processing. The process consists of the following steps: a) moistening the flaked seeds, b) heating to a 130F temp for enzymatic release of the essential oil allyl isothiocyanate from its glucoside precursor, c) steam stripping to remove the essential oil, d) extracting to remove the triglyceride oil, and e) desolventizing to remove hexane from the oil extract and the marc.

The damaging effect of heat upon amino acids of vegetable protein has been noted in the processing of several oilseeds including soybeans, cottonseed, peanut, and sunflower (1,2,12). Many workers have studied the problem and, although some results are conflicting, the knowledge already obtained has helped to improve control of meal quality.

The basic amino acids, lysine, arginine, and histidine, are the most affected. Damage to these is believed to result from a) condensation with other amino acids in the protein chain, and b) reaction with nonprotein constituents, generally carbohydrates. The first of these reactions is reversible by acid hydrolysis, whereas the second is usually believed to be nonreversible by acid hydrolysis at least in the latter stages. Because lysine is the most heat sensitive of all of the amino acids in mustard seed, it was used as an index of destruction of the heat-labile amino acids.

Preparations of Protein Meals

The meals were prepared under conditions differing in severity of heat treatment. For simplicity, the letters A,B,C, and D are used to distinguish between preparation methods. Meals A,B, and C were prepared in previous bench-scale studies (10,11); whereas meal D was prepared previously in the pilot plant (9). For meal A, ca. 9 lb mustard flakes were charged to a steam-jacketed vessel equipped with an agitator. The flakes were moistened to 30%, heated to 130F, and held at this temp for 15 min for enzyme conversion. At the end of this period, live steam was admitted through a sparger, together with jacket steam at 30 psig, to strip off the pungent oil. After 30 min steaming, the sample was dried for 20 min, using jacket steam alone, and discharged from the cooker at 21% moisture and 215F. After air cooling to room temp, the meal was extracted to 140F by filtration-extraction technique in which the meal was slurried for 30 min in hot hexane and then filtered, washed, and air-dried.

Meal B was prepared similarly to A except that the steam-drying period was extended to 77 min to evaluate the effect of heat on meal protein at this stage of the process. The meal was discharged at 12.8% moisture and 225F.

For meal C approximately 1 lb mustard flakes was charged to an electrically heated vessel equipped with an agitator. The flakes were moistened to 15.5%, heated to 130F, and held at this temp for 45 min for enzyme conversion. At the end of this period the meal was heated to 185F and moisture was sprayed in to 25%. The additional moisture served as a stripping agent to remove the pungent oil during the subsequent drying step. The meal was dried for 15 min to a 215F discharge temp, and then air-cooled. The extraction step was the same as for preparations

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TABLE I

Effect of Heat Treatment on Basic Amino Acid Content of Mustard Oils Meals [a]

Preparative method	Condition of preparation: Moisture reduction period in cook (min)	Condition of preparation: Maximum temp (F) in cook	Spent meal resteaming period (min)	Lysine content, g/16 g N	Arginine content, g/16 g N	Histidine content, g/16 g N
A	20	221	0	5.0	7.0	2.7
B	77	225	0	4.3	6.3	2.6
C	15	221	20	3.3	5.9	2.6
D	15	212	60	1.8	4.9	2.3

[a] Basic amino acid analysis of mustard meal before processing in g/16 g N: lysine 5.5, arginine 6.7, histidine 2.6.

A and B. The air-dried meal was steamed for 20 min in a heat-jacketed Büchner funnel holding the meal at ca. 7% moisture.

In preparing meal D, a 470-lb lot of flakes was moistened to 30%, converted at 130F for 60 min, steamed, and dried for 60 min at 212F before air-drying the hot meal. The meal was extracted at 140F in the pilot-plant filtration-extraction facilities of the Southern Regional Research Laboratory, by using a 30-min slurry time. The meal was desolventized for 60 min, reaching a final temp of 212F (jacket steam 25 psig), and was steamed and dried for 60 additional min at 212F. The pilot-plant meal was heat-treated severely to insure removal of the essential oil and was prepared prior to a knowledge of the protein damage occurring under these conditions.

Analytical Methods

Amino acid analyses were obtained by hydrolyzing the meal protein with HCl (14), followed by analysis on a Spinco MS amino acid analyzer.

The nitrogen solubility index (NSI) was determined on the seed before and after processing by a modification of the method of Smith and Circle (13). By this method, a 2.5-g meal sample in 100 cc water was agitated for 2 hr at 25C with a flat-blade paddle revolving at 125 rpm. NSI was calculated as water-soluble protein ÷ total protein × 100.

Browning of the meal was visual during heating. A portion of the color was extracted to distilled water, and the optical density of an aliquot of the filtrate was measured as follows: a 0.6-g solid meal sample was agitated in 25 ml distilled water at room temp for 30 min. The mixture was filtered through a No. 42 Whatman filter paper. The residue was washed with a few ml distilled water; the total vol was made up to 30 ml. An aliquot of the filtrate was read on a Beckman Model DB spectrophotometer at a wavelength of 502 mμ.

Reducing sugar content of the various meals produced was determined as follows: a 10-g powdered meal sample was added to 100 ml boiling, distilled water; and the mixture was agitated with a glass stirring rod to obtain a homogeneous medium. The solution was cooled to 25C on a constant temp bath before filtering through No. 1 Whatman filter paper. The residue was washed twice with 20 ml hot water. The total volume was made up to 100 ml. An aliquot (5 ml) of the filtrate was analyzed for reducing sugars, according to Munson and Walker (8). Duplicate preparations were run on all meal samples, and the results are highly reproducible. All results are reported on a moisture-free and fat-free basis.

Results and Discussion

Any heat damage to basic amino acid content of the mustard seed meals was recorded in Table I. Lysine destruction increased as the severity of heat treatment increased for preparation A to D. Because meal A was exposed to very mild processing conditions, very little lysine was destroyed. Although the

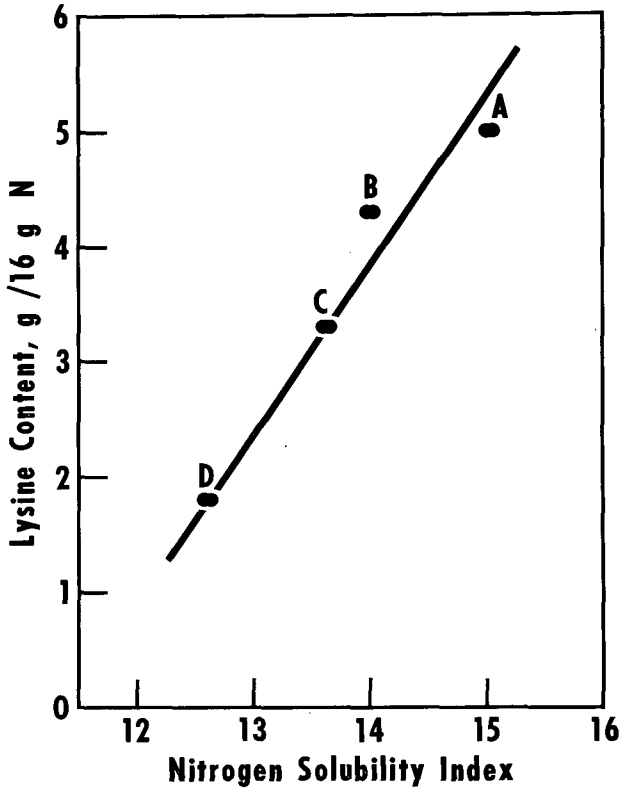


FIG. 1. Lysine vs. protein solubility of processed mustard meals.

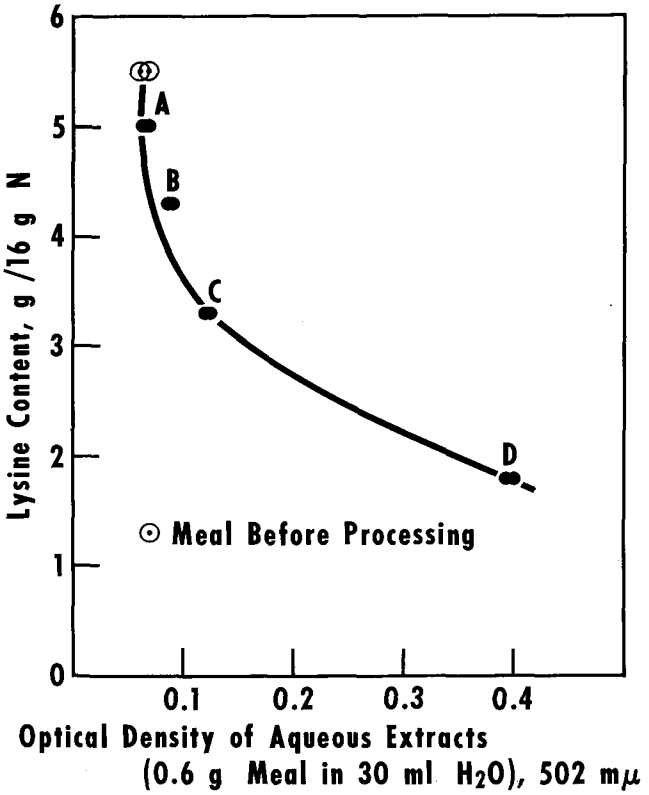


FIG. 2. Lysine destruction as reflected by optical density measurements of aqueous meal extracts.

max temp and total heating time for meal B were greater than for meal C, the lysine content of meal C was lower. This difference is attributed to re-steaming of the spent meal 20 min. Meal D, heated to a lower temp in the cook, showed an even greater loss of lysine. Losses of the basic amino acids occurred during periods of exposure to high temp at low-moisture levels; namely, during drying of the meals at the time of, or following, removal of the volatile oil before solvent extraction and also during steaming of the spent meals. The nonbasic remaining amino acids were relatively unaffected by heat treatment during processing.

Protein solubility was the first evaluation as an indicator for heat treatment. The correlation between lysine content and NSI show in Figure 1. Unprocessed mustard meal had a protein solubility of 32 which is approximately twice the percentage solubility of meal protein from preparation A. Unprocessed mustard meal had the following basic amino acid contents: lysine 5.5 g/16 g N, arginine 6.7 g/16 g N, and histidine 2.6 g/16 g N.

Water extracts of the meals showed increasing optical density with heat treatment. Variation of color intensity with lysine content shows in Figure 2. Extracts from preparation A that were high in lysine were pale yellow-green. Extracts of meals B and C were dark green, whereas meal D had brown extracts. The unprocessed meal and preparation A had aqueous extracts that showed no dark color, possibly because they contained no water-soluble melanoidines, which are products of the browning reaction.

When the aqueous extracts were analyzed for reducing sugars, it was observed that the concn of these reducing sugars in the meal decreased as lysine decreased (Fig. 3). As shown by copper reduction values, substantial amounts of reducing sugar [other reducing substances are formed during the latter stages of the browning reaction (5)] were present in mustard meals. An additional quantity was released in the form of glucose in the enzymatic conversion of the thioglucoside.

The total reducing sugar content of unprocessed soybeans, analyzed by the same copper reduction method, was approximately 1% of the defatted meal (12.4 mg/g). This quantity of reducing sugar compares favorably with the amount reported in mature soybeans by Markley (7). Completely hydrolyzed mustard seed contains more than three times (40.0 mg/g) the quantity of reducing sugar found in mature soybeans. The mustard thioglucoside reaction, according to Ettlinger and Lundeen (3), is:

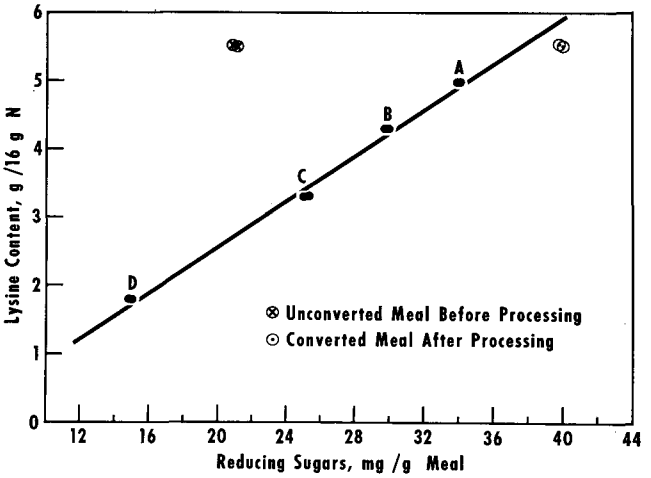
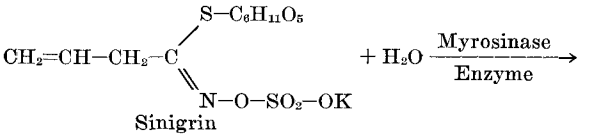


FIG. 3. Correlation of reducing sugars with lysine content in mustard seed meal.



$CH_2=CH-CH_2-N=C=S$
Allyl isothiocyanate
$+ C_6H_{12}O_6 + KHSO_4$
Glucose

The decrease of reducing sugars and basic amino acids observed during processing mustard meals, together with the corresponding increase in meal color, indicates the occurrence of a Maillard-type browning reaction (6). Maillard's representation of the amino acid reaction with glucose is:

$$RNH_2 + \text{Glucose} \longrightarrow \underset{-CO_2 \downarrow\ -H_2O}{\text{Condensation products}} + H_2O$$

Brown products
(Melanoidines)

Molar ratios of reducing sugars condensing with basic amino acids during processing of the mustard meal preparations were calculated from the reducing sugar and amino acid analyses of these meals before and after processing. These M ratios (Table II) are, except for preparation A, near a 1:1 ratio. Previously, Hannan and Lea (4) found that primary amino compound and glucose condensed in a 1:1 ratio. The 2:1 ratio calculated for preparation A may not be significant since at this level of reaction a slight error in any of the amino acid determinations will affect the ratio greatly. The browning reaction has been referred to by Altschul (1) as "destructive" because

TABLE II
Molar Ratios of Reducing Sugar to Basic Amino Acids After Heating of Mustard Meals

Preparative method	Basic amino acid loss during processing							Reducing sugar loss during processing		M ratio of reactants [e]
	G/16 g N [a]			Moles/g meal × 10^{-5} [b]				Mg glucose/g meal [c]	Moles glucose/g of meal × 10^{-5} [d]	Moles glucose/moles amino acid
	Lysine	Arginine	Histidine	Lysine	Arginine	Histidine	Total			
A	0.5	....	....	1.47	.....	.....	1.47	5.5	3.05	2.08
B	1.2	0.4	0.0	3.53	0.99	.....	4.52	10.5	5.83	1.29
C	2.2	0.8	0.0	6.52	1.97	.....	8.49	14.5	8.05	0.95
D	3.7	1.8	0.3	10.90	4.44	0.80	16.14	24.5	13.60	0.84

Sample Calculations—Meal C

[a] Lysine = lysine content of meal before processing—lysine content of meal C = 5.5 − 3.3 = 2.2 g/16 g N.

[b] Moles of lysine loss $= \frac{(\text{lysine loss})\,C}{100} \times \frac{\%\ \text{protein (sample wt)}}{100} \times \frac{1}{\text{mol wt lysine}} = \frac{2.2}{100} \times \frac{43(1)}{100} \times \frac{1}{146} = 6.52 \times 10^{-5}$.

[c] Reducing sugar loss = mg reducing sugar in hydrolyzed meal before processing—mg reducing sugar in processed meal = 39.5 − 25.0 = 14.5.

[d] Moles of reducing sugar loss $= \frac{(\text{mg reducing sugar loss})\ (\text{sample wt})}{(1000)\ \text{mol wt glucose}} = \frac{14.5\ (1)}{(1000)\ (180)} = 8.05 \times 10^{-5}$.

[e] M ratio $= \frac{\text{moles reducing sugar loss}}{\text{total moles basic amino acid loss}} = \frac{8.05 \times 10^{-5}}{8.49 \times 10^{-5}} = 0.95$.

it cannot be reversed by acid hydrolysis. Because the amino acid analyses reported here were run on acid hydrolyzates, and also because of good correlation found between lysine destruction and severity of heat treatment, apparently the reaction is irreversible. The preceding data indicate that although some processed meals show extensive amino acid losses, little destruction of basic amino acid occurs if the meals are processed with controlled heat, as illustrated by preparation A. In that method, the moisture reduction step during cooking is not allowed to continue longer than 30 min, and there is no steaming after solvent extraction. The basic amino acid destruction appears to be greatest when meals are dried near the end of the cooking step (13% moisture) and during dry steaming of the spent meal (moisture 7%).

Conclusions

The basic amino acids—lysine, arginine, and histidine—are the most heat-labile in mustard meals. The analytical methods proposed—nitrogen solubility index (NSI), optical density of aqueous extracts, and reducing sugars—provide rapid and simple ways of following amino acid destruction by heat processing.

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Preparation and Analysis of Some Food Fats and Oils for Fatty Acid Content by Gas-Liquid Chromatography

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Abstract

The fatty acid compositions of some food fats and oils were determined by gas-liquid chromatography (GLC) before and after application of reagents and conditions of some extraction procedures. The extraction procedures studied had a nonsignificant effect on the fatty acid compositions. Procedures leading to methyl ester formation through a series of room temp reactions were selected over procedures requiring higher temp reactions, on the basis of yield of products, fatty acid compositions of food lipids of simple composition, or both. These procedures were then used to prepare some food fats and oils for analysis by GLC and the fatty acid compositions determined in this manner are presented.

Introduction

THE PRESENT STUDY was undertaken to determine the effects of the environment created by several different extraction procedures on the apparent fatty acid composition of some food fats and oils. Several procedures employing mild conditions have been combined for the quantitative fatty acid analysis of food lipids of relatively simple composition. GLC analysis aided in the selection of these preparative procedures. Following the validation of these procedures, they were applied to some separated food fats and oils and the resulting fatty acid methyl esters were analyzed with GLC.

Experimental

Materials

Fats and Oils. A butter-margarine blend, the covering fat from cured, smoked ham, and a cottonseed oil, No. 2, and a soybean oil, No. 4 (salad or cooking oils from government stocks) were available from other laboratory work. Soybean oil No. 3, a "pure soya salad oil," was purchased from a wholesale dealer in June, 1961. All other samples analyzed were purchased at local retail markets between June and August, 1962.

The fat or oil sample from each container was blended thoroughly under nitrogen before subsamples were taken. The ham fat had been ground, sealed in a coated, tinned container, and stored at −40C for approximately 5 months before analyses were carried out. Replicate analyses were made on material taken from a single container in all cases except the processed soybean oil, values for which are means of 4 analyses on the contents of each of four containers.

Organic Solvents. Ethanol, 95%, refluxed and distilled over KOH.

Methanol, absolute, acetone-free, certified reagent grade.

Benzene, certified reagent grade.

Normal-hexane (purified, bp 65–67C) and petroleum ether (certified reagent grade, bp 30–60C), dried, distilled over KOH.

Mixed ethers: equal volumes of ethyl ether (anhydrous, reagent grade) and petroleum ether.

Apparatus and Procedures

Analysis of Fatty Acids by GLC. The gas chromatographs used were the argon ionization detection systems of the Barber-Colman Co. Some instrumental and operational details appear in Table I. The polar liquid phase was ethylene glycol succinic acid polyester (15%, w/w) on 100–140 mesh Gas-Chrom P. This commercially-prepared packing was used for all quantitative analyses. Apiezon L at a level of 14%

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