The sample means ranged from 5.1–5.8, but day-to-day variation was not significant. Sandard deviations were homogeneous and of ca. the same order as in the past. The pooled standard deviation for the 10 days was 0.79.

This light test may be compared with other stability tests by examining the relative standard deviations or coefficients of variation (Table V). Both organoleptic evaluation and peroxide values vary less than methods now used. When compared with AOM values, the variation of the organoleptic test is only slightly more than the AOM method for testing stability. This degree of precision, plus the shorter time required for the test, appears favorable to most testing programs.

In France, peanut oil is being marketed in disposable, translucent plastic bottles (1). The light test was used to compare the effect of container on the stability of soybean oil. The 1-liter translucent plastic bottle from France, a 1-qt clear glass bottle and a 1-qt brown glass bottle were filled with soybean oil and exposed to light for 2 hr. The results are shown in Table VI. The oil stored in the brown glass bottle was scored only slightly lower than the control sample, which came from a 5-gal tin, whereas the oils stored in both the clear and the plastic bottles were scored significantly lower than either the control or the oil stored in brown glass.

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Mustard Seed Processing: Improved Methods for Isolating the Pungent Factor and Controlling Protein Quality[1]

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Abstract

A modified cooking and extraction process for mustard seed is reported in which the pungent factor, allyl isothiocyanate, is separated from the seed to yield triglyceride oil and protein meal. Although removal of the pungent factor from the oil and meal products was previously reported, investigations were continued to develop critical improvements in the process. A reduction in conversion time, combined with steam stripping and shorter heating preiods, resulted in quantitative recovery of the essential oil and in improved protein quality, as measured by the basic amino acids. Biological testing with rats showed the processed meals to be free of toxic and goitrogenic factors and to be well utilized nutritionally. Preliminary estimates indicate that process costs are nearly the same as for a comparable soybean plant.

Introduction

MODIFIED COMMERCIAL OILSEED techniques, such as those used in processing soybeans and cottonseed, have been applied successfully to mustard seed at this Laboratory. In previous studies (7,8) the basic method was developed. The integrated enzymatic and lipid extraction process leads to three products—triglyceride oil, a palatable protein meal and the pungent factor, allyl isothiocyanate. This paper presents new studies which obtained significantly improved separation of the pungent factor to give near theoretical recovery, along with process modifications which improved oil meal quality.

[1] Presented at AOCS Meeting in Toronto, 1962.
[2] No. Utiliz. Res. & Dev. Div., ARS, USDA.

Materials, Methods, and Equipment

In these studies, oriental mustard seed, *Brassica juncea,* was obtained from two lots of seed grown in Montana and received during 1960 and 1961, respectively. The seed lots averaged 7% moisture, 38.3% oil, 22.5% protein and approx 10% hull content. Glucoside content, expressed as converted allyl isothiocyanate, averaged 0.7% moisture-free basis. Commercial grade *n*-hexane was used as solvent in the filtration-extraction.

Allyl isothiocyanate was determined by Wetter's procedure (10). Purity of the essential oil was determined by a modification of procedure in which an aliquot of the oil in ethanol was added directly to the ammoniacal silver nitrate solution. Purity of the allyl isothiocyanate was also analyzed by GLC on a Beckman GC-2A (6,10) packed with Apiezon-L on Celite (40–60 mesh) with a nitrogen flow of 60 ml/min at a temp of 115C. Crude fat was determined by extracting with pentane-hexane in a Bütt extractor for six hr and drying overnight in a vacuum oven at 80C. Amino acid analyses were obtained by hydrolyzing

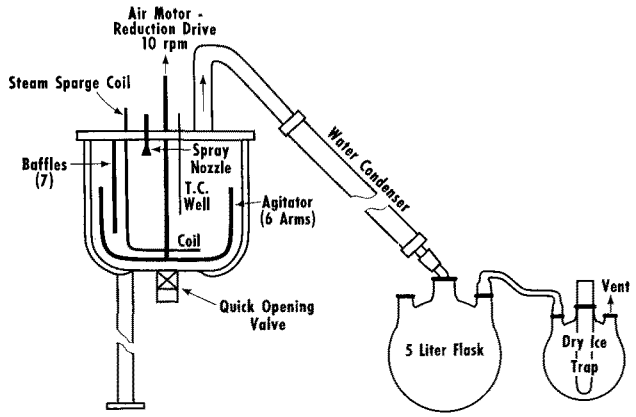

FIG. 1. Conversion-cooking equipment.

the meal protein with hydrochloric acid followed by analysis on a Spinco MS amino acid analyzer (9).

Mustard seed was cracked on 6-in. diam corrugated rolls (10 corrugations/inch) and flaked in 12-in. diameter smooth rolls.

Moistening, converting and cooking steps were conducted in a 5-gal steam-jacketed stainless-steel vessel (Fig. 1). This unit was designed to give thorough mixing of the mustard seed flakes and water with control of temp. The assembly includes an insulated electrically heated cover, an intermeshing rod agitator with rod baffles, a spray nozzle, a steam sparge coil and a thermocouple well. For steam distillation of the essential oil, the top vapor opening was connected to a condenser, receiver and solid carbon dioxide trap.

Apparatus similar to that previously described (5) was used for evaluating the characteristics of mustard seed for filtration-extraction. The cooker also served as a slurrying unit. The design of the extraction-filter unit is shown in Figure 2. This unit consisted of a 16-in. diam funnel mounted over a 10-gal stainless-steel receiver tank with a connecting 3-way valve to permit both blowback and vacuum conditions. A vacuum pump with a control valve provided 4–6 in. Hg on the receiver tank.

Procedure

A flowsheet of the process is shown in Figure 3. The clean seed was tempered to approx 8% moisture and milled to flakes of ca. 0.005 in. thickness. Milling consisted of either cracking through corrugated rolls followed by flaking through smooth rolls, or by flaking the whole seed through smooth rolls directly. The full-fat flakes were charged to the converter-cooker apparatus, adjusted to 30% moisture by use of the spray nozzle, and agitated for approx 5 min. While

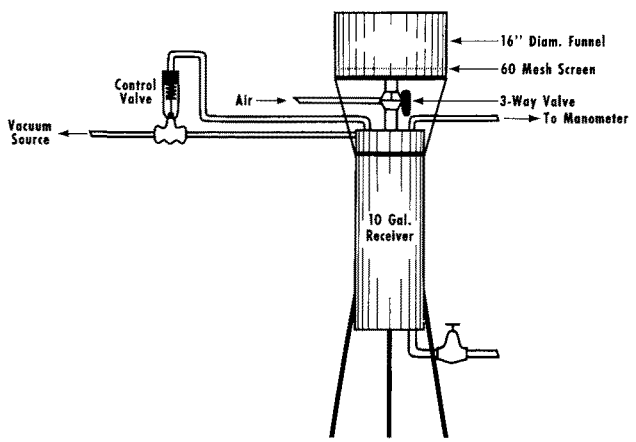

FIG. 2. Extraction filter.

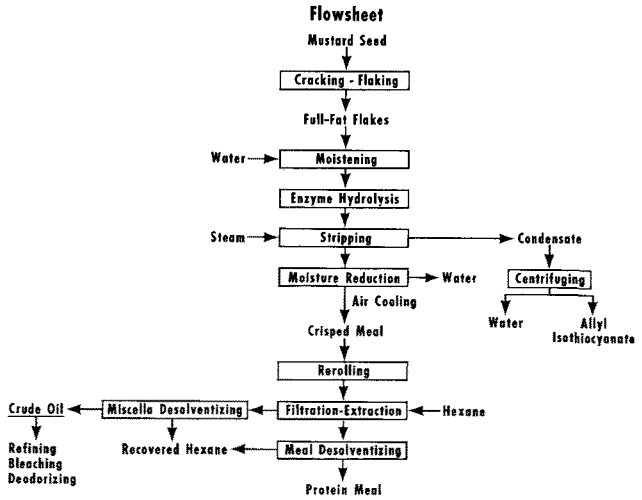

FIG. 3. Flowsheet of mustard seed process as followed in pilot plant.

mixing was continued, the flakes were heated to 130F and held 15 min to carry out the enzymatic conversion of sinigrin. This step was followed by direct steam stripping of the meal through the bottom sparge coil for 30 min. Simultaneously, indirect steam at 25 psig was added to the cooker jacket. The direct steam raised the meal temp rapidly to ca. 215F and simultaneously stripped out the essential oil. Indirect steam to the vessel wall helped prevent excessive condensation. After 30 min, sparge steam flow was shut off and the meal was dried to ca. 17% moisture with jacket steam. In some cases, a slight vacuum was used to facilitate the removal of moisture. The hot meal was discharged by rubbing over a 5-mesh screen to break up moisture balls, followed by air cooling to obtain crisping. Crisping and cooling to room temp resulted in coagulating the mass to larger gritty and granular particles, which were then rerolled through smooth rolls before solvent extraction. Filtration-extraction was carried out generally according to the bench procedure of A. V. Graci et al. (5) using a 60-min slurry, solvent ratio of 1.3, three washes, 1.75-in. cake thickness, and extraction temp of 140F. Slurry miscella contained 10% oil in hexane, and washes contained 5, 1 and 0% oil in hexane. Meals prepared for the feeding studies were extracted by the double-soak process described by

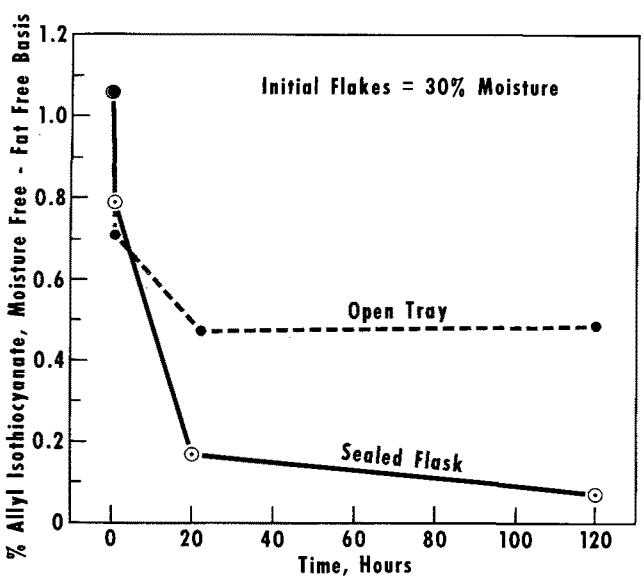

FIG. 4. Effect of storage time on allyl isothiocyanate.

TABLE I
Essential Oil Recovery from Mustard Seed by Different Recovery Procedures

Process	Residual essential oil in crisped meal g/100 g meal (mfb)	Percentage recovery in condensate[a]
Simple distillation	0.062	71.7
Simple distillation plus additional water and redistillation	0.011	85.0
Superheated steam stripping 30 min	0.066	87.4
Atmospheric steam stripping 30 min	0.007	99.2

[a] Based on grams of essential oil recovered/100 g of essential oil in the original seed.

E. L. D'Aquin et al. (3), using pure hexane rather than miscella for the slurry liquids and washes.

Recovery of essential oil from the converted meal was studied by various separation techniques, which were, in order of study: 1) simple distillation of the volatiles from the meal, 2) simple distillation followed by redilution with water and subsequent redistillation, 3) meal indirectly heated and simultaneously sparged with open superheated steam for 30 min, and 4) meal indirectly heated and simultaneously sparged with open atomspheric steam for 30 min.

Results

Separation of the Pungent Factor. Previous observations indicated that when ground mustard seed was moistened and allowed to stand at room temp, the allyl isothiocyanate produced by hydrolysis, could not be detected by chemical analysis. Figure 4 shows the results of an experiment where the allyl isothiocyanate content of both sealed and open samples declined rapidly during the first hour and thereafter more rapidly in a sealed flask than in an open tray. This reduction suggested that the losses in essential oil were not due to vaporization but that a reaction occurred which decomposed or chemically bound the allyl isothiocyanate as it was liberated from the thioglucoside. The disappearance of isothiocyanate was not as great for the open sample which lost moisture by evaporation; thus, the myrosinase enzyme was probably less reactive in the drier state to free isothiocyanate from the thioglucoside and make it available for reaction. Under temp conditions of 55C used in the process, the hydrolysis goes rapidly and directly to the product allyl isothiocyanate, whereas under conditions of room temp hydrolysis, the enzymatic conversion products might be considerably different. This could explain the lower yields of allyl isothiocyanate at room temp. Further experimental work would be desirable on the nature of the reaction or reactions occurring to explain these observations.

On the basis of these findings, the hydrolysis time was reduced to 15 min. This length of time proved sufficient for complete conversion, yet did not allow the essential oil to decompose or react.

Comparison of essential oil recovery by the procedures used is shown in Table I. The degree of essential oil removal is indicated also by the amount remaining in the crisped meal. Since the methods of analysis employs a conversion period the isothiocyanate detected in the crisped meal may be bound as thioglucoside. Simple distillation recovered 71.7% of the essential oil, but atmospheric steam stripping increased recovery to 99.2%. An excellent material balance was obtained in the atmospheric steam-stripping process where the quantity of essential oil in the condensate and crisped meal was essentially equal to that in the original feed meal. This equality suggests that the losses incurred in the other processes may be largely due to reaction of essential oil with the meal. A short

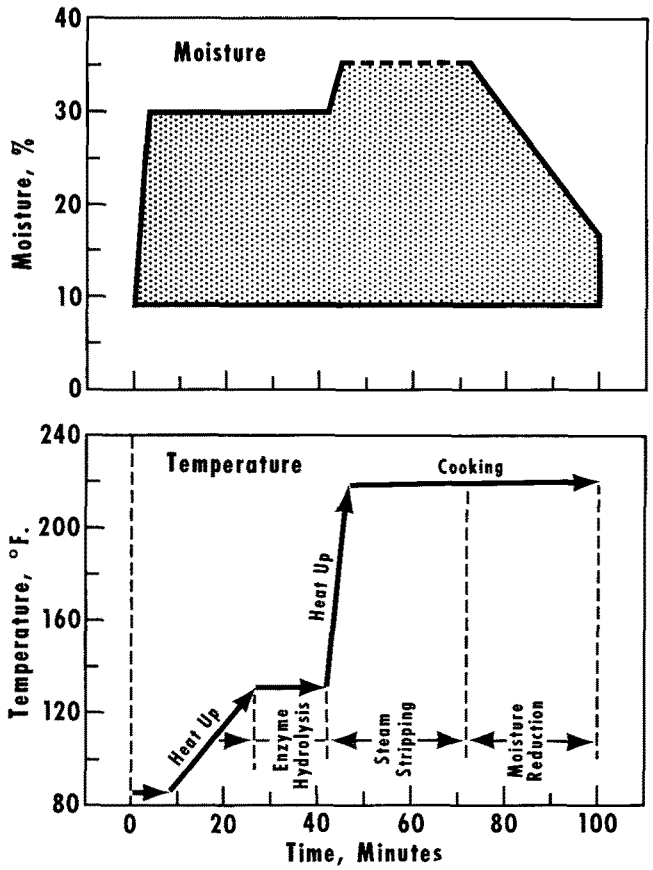

FIG. 5. Temp and moisture profiles during processing.

conversion period followed by steam stripping for approx 30 min, therefore, gives the highest recovery of allyl isothiocyanate.

Temp and moisture profiles of the modified hydrolysis and cooking process are shown in Figure 5.

The simplest method for recovery of essential oil from cook condensates is that of gravity settling. The rate of settling, however, is slow due to surface tension effects and the proximity in densities of the two liquids. Centrifugation was, therefore, examined as a more rapid and efficient means of separation. The efficiency of this operation is limited theoretically by the solubility of allyl isothiocyanate in water (0.124 g/100 g of water at 20C). Approx 99% of the essential oil in the feed flakes was recovered in the steam distillate and the remaining 1% was retained in the crisped meal either as free allyl isothiocyanate or as unconverted thioglucoside. Approx 80% of the essential oil was recovered in the centrifuged oil layer.

Recovered crude, essential oil contained approx 90% allyl isothiocyanate and 10% of an impurity previ-

TABLE II
Effect of Heat Treatment Upon the Basic Amino Acid Content of Mustard Meals

Process conditions	Original seed composition	Test 1	Test 2	Test 3
Steam stripping time, min		20	20	30
Moisture reduction time, min		38	38	63
Temperature of meal leaving cooker, °F		221	221	225
Moisture of meal leaving cooker, %		17.5	17.5	13.4
Spent meal steaming period, min		0	30	0
Basic amino acids, g/16 g N[a]				
Lysine	5.5	5.2	3.1	4.3
Arginine	6.7	7.0	5.6	6.3
Histidine	2.6	2.6	2.4	2.2

[a] Other amino acids in mustard meal protein were not affected by heat treatments and, therefore, are not shown here.

TABLE III

Analysis of Mustard Meal[a]

Crude fat, % mfb	1.8
Protein, % mfb	44.5
Allyl isothiocyanate, % mfb	0.007
Basic amino acids, g/16 g N	
Lysine	5.3
Arginine	6.9
Histidine	2.8

[a] Prepared by 15-min conversion at 130F, 30% moisture; 20-min steam stripping period and 30-min moisture reduction preiod, maintaining 2-in. water vacuum. The crisped meal was extracted with hexane at 140F and air dried.

ously unidentified (6). The unknown impurity had an emergence time equivalent to allyl thiocyanate by gas-liquid chromatography. Since the thiocyanate isomerizes to isothiocyanate during atmospheric distillation, possibly isothiocyanate in the essential oil can be increased.

Lipid Extraction. Mustard seed was readily adapted to the filtration-extraction technique for lipid removal. After the flakes, processed by preparative cooking, were air cooled, they gave a crisp, granular material that extracted readily at good filtration throughput rate (2,000 lb/hr/sq ft). Since extraction conditions were established previously (7), the same conditions were used, and no attempt was made to carry out additional extraction studies.

Protein Quality. Lysine, arginine and histidine contents of mustard meal protein varied appreciably, depending upon the degree of heat treatment received during processing. Destruction of these amino acids during processing was noted when the meals were dried excessively during cooking or when the defatted and desolventized meals were steam-treated. Experimental data that demonstrate these results are compared in Table II, along with lysine, arginine and histidine contents of an unprocessed meal. Typical results obtained when heating conditions in the cook step were minimized are shown in Test 1. The results of additional steaming of the spent or extracted meal for 30 min on a Büchner funnel are shown in Test 2. This technique resulted in a definite "browning" of the meal and a very significant decrease in both lysine and arginine. Finally, in Test 3, cooking the meal for slightly over an hour resulted in a significant amt of amino acid destruction, particularly lysine.

Evidently prolonged heating and drying to low moisture levels, with high temp, should be avoided. Recommended temp are 220F or below; also, time intervals for hydrolysis, heat up, steam stripping and moisture reduction periods should be held to a minimum.

Toxicological and Nutritional Evaluation of Mustard Meal. Rat bioassays on the meals were carried out in cooperation with the Pharmacology Laboratory of the Western Regional Research Laboratory, Albany, Calif., to evaluate the suitability of mustard meal as a feedstuff. A nutritionally adequate basal diet was used, the major constituents of which were corn meal, linseed meal and crude casein (1). A processed meal (analyses in Table III) was fed to rats at 20 and 30% levels substituted at the expense of the entire basal diet (i.e. 20 g mustard meal + 80 g basal diet). Good growth and utilization resulted when the 20% level of mustard meal was fed, which was equal to that obtained when soybean meal was fed at a 30% level. Food intake for the experimental and the basal diets was approx the same. When the mustard meal level was increased to 30%, growth was significantly inhibited. Histopathological examination of thyroid tissues failed to reveal any abnormal effects for any of the mustard meals tested regardless of the level used.

In a second study, the mustard meal was fed as a 50% blend with soybean meal, which was the sole source of protein in the diet. A 20–20% mustard-soybean meal blend was compared to a 40% soybean meal control and the blended protein showed growth respenses essentially equal to the soybean meal diet. Similar results were reported earlier by Goering et al. (4), with mustard meals prepared by a different process. On the basis of these preliminary rat feeding tests of 90 days duration or less, it appears that mustard meal as a supplementary source of protein will be satisfactory.

The effects of allyl isothiocyanate on the growth rate of rats fed at levels of 0.01–0.4% were also investigated. Significant growth inhibition was not shown until the level reached 0.2%. However, when the essential oil was added in combination with 20% mustard meal in the diet, significant growth inhibition was encountered with only 0.05% allyl isothiocyanate. These results suggest the desirability of reducing the allyl isothiocyanate content of the mustard meal to approx 0.01%

Proposed Commercial Process and Cost Estimates. Figure 6 is a flowsheet for a proposed commercial process, wherein mustard seed is processed with techniques similar to those used for soybeans and cottonseed. The process should lead economically to three products—triglyceride oil, a palatable protein meal

TABLE IV

Estimated Fixed Capital Investment for a Plant Processing 100 Tons Mustard Seed Daily (Operating 330 Days/Year, 24 Hr/Day)

Installed cost of processing equipment	$ 539,000
Piping, wiring, instruments, etc.	164,000
Fire protection and miscellaneous service facilities	100,000
Machine shop, storeroom, and locker room facilities	48,000
Office and laboratory facilities	30,000
Grain storage units for incoming seed	227,000
Building for flake preparations and meal processing equipment	40,000
Building for solvent extraction equipment	40,000
Land	12,000
Engineering and contracting fees	240,000
Contingencies	120,000
Estimated fixed capital investment	$1,560,000[a]

[a] Cost of steam generating plant not included.

TABLE V

Processing Costs for 100-Ton/Day Mustard Seed Plant (Operating 330 Days/Year, 24 Hr/Day)

Item	Dollars/ton of seed
Hexane, $0.16/ gal, 10 lb (1.85 gal) loss/ton of seed	0.30
Utilities:	
Steam, 1400 lb/ton, $0.80/M	1.12
Water, 1500 gal/ton, $0.10/M	0.15
Electricity 25 kwh/ton, $0.015/kwh	0.38
	1.65
Maintenance:	
Equipment, 3%/yr on $1,063,000	0.97
Bldg and grain storage, 2%/yr on $497,000	0.30
	1.27
Fixed charges:	
Depreciation, equipment 5%/yr on $1,063,000	1.61[a]
Bldg and grain storage, 3%/yr on $497,000	0.45
	2.06
Taxes and insurance, 3%/yr on $1,560,000	1.42
	3.48
Labor:	
10 Operators, $2.40/hr	1.92
1 Helper, $2.10/hr	0.17
1 Laboratory technician, $20/day	0.20
3 Foremen, $24/day	0.72
1 Plant superintendent, $30/day	0.30
Overhead	0.50
	3.81
Miscellaneous factory supplies and expenses	0.19
Charge on working capital, 5%/yr on $600,000	0.90
General plant overhead	2.03
Processing cost, total	13.63

[a] If estimate is based on 10 yr depreciation, estimated processing cost would be $15.24/ton.

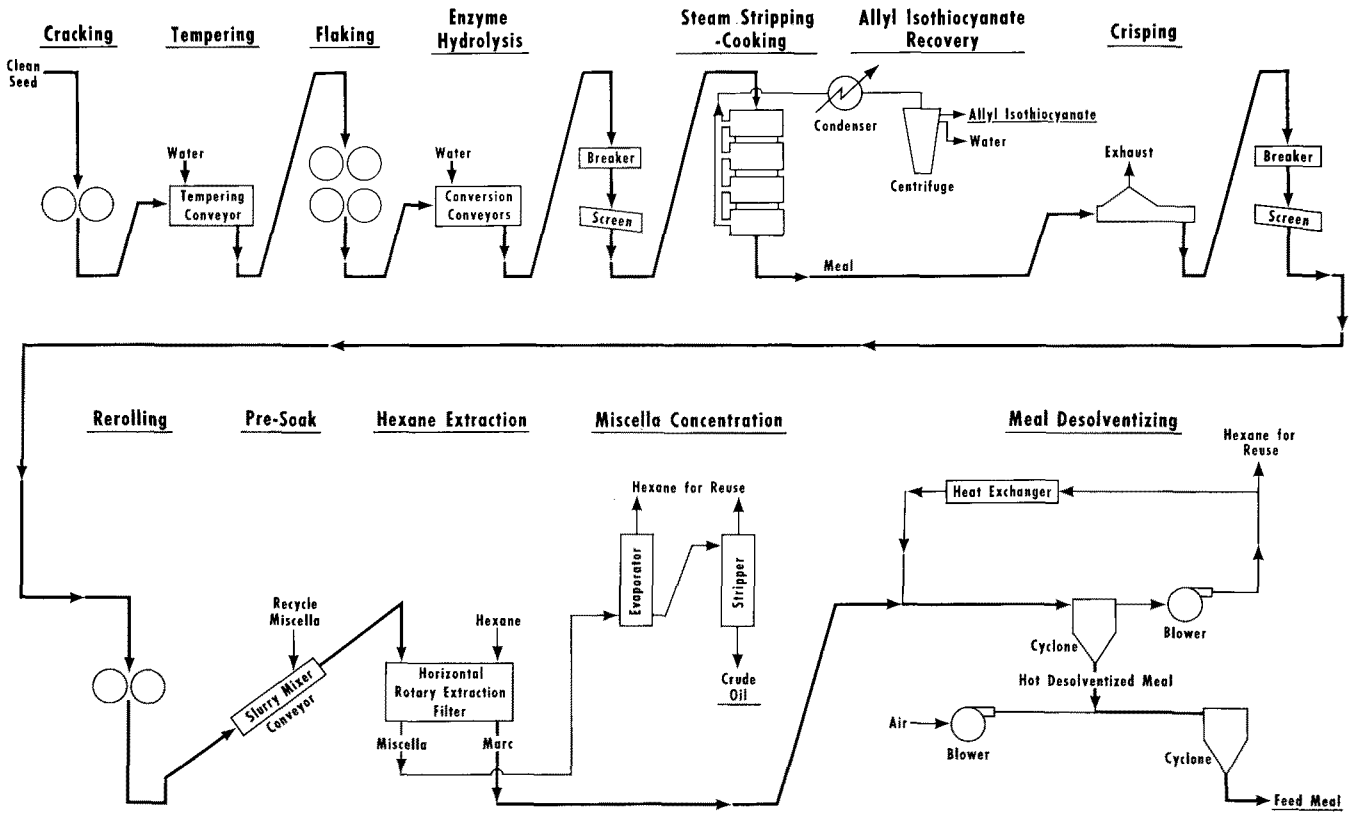

FIG. 6. Proposed commercial process for mustard seed.

and the byproduct essential oil allyl isothiocyanate—all of which have potential edible or industrial uses.

A preliminary cost estimate has been prepared for a hypothetical plant processing 100 tons of mustard seed daily. Operations in such a plant would follow the procedure in the flowsheet (Fig. 6) and are assumed to be conducted 330 day/year, 24 hr/day. Daily production for the plant would be approx 75,200 lb crude oil, 117,200 lb oilseed bulk meal and 1,150 lb allyl isothiocyanate (90% purity). The fixed capital investment (Table IV) for such a plant is estimated at $1,560,000. In addition to the cost of processing equipment and related items, this estimate includes the cost of units sufficient for storing seed for an 80-day operation, as well as the cost of office, laboratory, shop, fire protection, and similar auxiliary facilities. The cost of a steam generating plant is not included in the fixed capital investment however, a charge of 80¢/1,000 lb steam is included in calculating processing costs. Information reported by Brewster (2) on plant investments and processing costs for soybean processing plants, which involve operations similar to those proposed for a mustard seed plant, was adjusted to current values and served as a guide in the preparation of this cost estimate.

Processing costs for a mustard seed plant (Table V) are estimated at ca. $13.63/ton, not including the cost on the mustard seed, sales costs and administrative expenses. Based on pilot-plant tests, the loss of hexane in the process should not exceed 10 lb/ton of seed, and a charge for the loss of hexane is included in the processing costs. Such a loss is experienced in other oilseed extraction processes.

An estimated processing cost of $13.63/ton of mustard seed is considerably higher than the cost generally reported when soybeans are processed. In the estimate for the mustard seed plant, calculations were based on a completely new installation with all necessary auxiliary facilities, except steam. In an all-new installation, the cost to the process for fixed charges naturally is greater than the cost of these items in many soybean plants of similar capacity that have been established for some years and that have been depreciated considerably already, or may be totally depreciated. Depreciation of the mustard seed plant was calculated at 5%/year for equipment and 3%/year for buildings, storage and auxiliary facilities. These percentages are comparable to those reported by Brewster (2). In addition, because most soybean processing plants have an operating capacity well above 100 tons/day, reduction in costs occur in such installations through economies which usually accompany operations in plants of large capacity. By extrapolation of the data, processing cost in a 1,000-ton/day mustard seed plant is roughly estimated to be of the order of $7.00/ton, or nearly as low as the cost of processing soybeans in a plant of comparble size.

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