Processes for Making Animal Feed and Protein Isolates from Jojoba Meal

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Hexane-extracted jojoba (*Simmondsia chinensis*, Link.) seed yields meal that contains 25–30% crude protein (N × 6.25) and the antinutritional factors (ANF) simmondsin, simmondsin 2′-ferulate, 5-demethylsimmondsin, and 4,5-didemethylsimmondsin. The ANF were water-extracted or degraded enzymatically or microbiologically and resulting meals fed to mice. At levels up to 20% of the diet, meals treated with ammonia or sodium hydroxide showed no toxicity and supported growth equal to that of the control diet, whereas microbiologically treated or ammonia plus steam treated meals supported a lower growth rate at 10 and 20% substitution. ANF-degrading enzymes inherent in the meal were inactivated in the steam-sparging recovery of hexane after 10 min of steaming when the meal reached 93 °C, but they were still active after 5 min when the meal was processed at 66 °C. The water extraction and inherent enzyme processes were scaled up to 4- and 15-kg batches. Proteins were extracted from the meal with 0.1% ethylenediaminetetraacetic acid disodium salt or 1% ascorbic acid in water and separated by membranes to give white water-soluble protein isolates.

Jojoba is a new oilseed crop being grown in the desert southwestern United States and in other countries. It produces a highly marketable oil which is a unique mixture of unsaturated liquid wax esters (Spencer and Plattner, 1984). While the oil, 50–60% of the seed weight, is valuable, the hexane-extracted meal is underutilized because it contains antinutritional factors (ANF) for cattle. The same compounds are toxic to monogastric animals. Much of the pressed cake meal produced in the United States is hexane extracted in a plant in Mexico. The meal contains 25–30% crude protein, is high in dietary fiber, and could serve as an animal feed supplement. Practically, the meal cannot be fed directly because it contains 11% ANF—5-demethylsimmondsin (DMS), 4,5-didemethylsimmondsin (DDMS), simmondsin (S), and simmondsin 2′-ferulate (SF)—that have adverse effects on animals (Elliger et al., 1973, 1974a,b; Verbiscar and Banigan, 1978). In monogastric animals the ANF decompose on ingestion and apparently cause death by cyanide poisoning on the basis of reports of CN^- and its metabolites in mice fed simmondsin (Weber et al., 1983). Ruminants are somewhat more tolerant of the ANF but do not use the protein efficiently or gain weight well on unmodified meal substituted at a 10% level in a normal diet (Manos et al., 1986). Some wild animals can live on a total diet of jojoba seeds (Sherbrooke, 1976), and range cattle will browse the leaves and seed (Roundy et al., 1989).

Chemical and biological means of decomposing ANF have been proposed, and ANF can be extracted with various solvents to yield an edible meal. Concentrated (10 N) aqueous ammonia was shown to reduce ANF to acceptable levels for rat feeding (Elliger et al., 1975). The 30-day reaction period and high chemical costs make this method impractical. Later, ammonical hydrogen peroxide was shown to reduce ANF content and result in a nontoxic product (Banigan and Verbiscar, 1980). Reaction times were substantially reduced from the concentrated ammonia method to only 8 days, but chemical costs remained substantial, hydrogen peroxide was not easily mixed and retained, and heat is generated in the process. All of these chemical treatments are likely to decrease the nutritional quality of jojoba proteins (Slump and Schreuder, 1973; Maga, 1984).

Cotageorge et al. (1979) examined several biological methods for decreasing ANF in jojoba meal. They noted a significant reduction in simmondsin when seeds were germinated for 5 days. Allowing the ground meal or ground germinated seeds to incubate for 48 h with added water resulted in meals that appeared to have reduced simmondsin levels but still caused high mortality in mice-feeding trials. Incubating with β-glucosidase also did not yield an acceptable meal for mice. Initial deglucosation and possible rearrangement to a more toxic metabolite in the first 3 days have been proposed (Verbiscar and Banigan, 1982). Rumen fluid has been shown to degrade S in only 6 h. The microorganisms or enzymes responsible for the degradation were not isolated (Temple, 1979).

Verbiscar et al. (1981) tested *Lactobacillus* spp. for 10 days on autoclaved meal. Nine of the 15 *Lactobacilli* species reduced S and SF contents of the meal. These *Lactobacilli* did not attack the pure ANF in culture and the need for a cofactor has been suggested by Verbiscar and coworkers. Three of the active *Lactobacilli* were tested on unsterilized meal with added ammonia for 21 days, and S and SF were reduced even further. Large-scale (100 kg) batches of unsterilized meal with either of two added *Lactobacillus* strains yielded feeds that were palatable to animals but showed poorer protein efficiency than other protein sources, or reduced weight gain compared to the control diets, when substituted at the 10% level. Only lambs fed *Lactobacilli*-treated meals had comparable weight gains and feed efficiencies when the meals were added to a basal diet at the 10% level. We have been unable to duplicate detoxification by *Lactobacillus* of autoclaved jojoba meal using the procedure of Verbiscar et al. (1981). However, we concluded that inherent enzymes can utilize the ANF on ensilage and that some of the enzymes involved must be membrane bound (Abbott et al., 1988). Optimum conditions for ANF degradation by

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endogenous enzymes were determined to be 20 days at 28–46 °C in 85% water content slurries to reduce the sum of S, DMS, and DDMS to less than 1%. SF is not attacked by endogenous enzymes, but addition of 0.25% NH_3 on the basis of added water temporarily raises the pH, aiding in the hydrolysis and degradation of SF.

Solvent extraction of ANF by methanol (Cotageorge et al., 1979; Verbiscar et al., 1980), ethanol (Cotageorge et al., 1979), 2-propanol–H_2O (7:3) (Medina et al., 1988), or butanol–water–acetic acid (Sodini et al., 1979) removes about one-third of the meal weight, including the ANF and sugars. Solvent extractions are generally too expensive for a feed-type of product with an expected market value of $100–200/ton. Alcohols also extract water from the meal and must therefore be rectified in the solvent recovery step; this would be another negative economic factor for alcoholic extraction. Some solvents, such as methylene chloride–methanol, will simultaneously extract oil and ANF, but residual SF contaminates the high-value product, the oil. Although extraction of oil, then ANF, with 91% 2-propanol yielded acceptable oil and an apparently edible meal, this process is very sensitive to moisture levels in the flaked meal seed (Johnson and Sadek, 1987). There may be residual SF in the extracted oil as well.

Water extraction removes ANF, sugars, and some protein from jojoba meal. If the protein is first denatured with phosphoric acid, less is extracted in the water (Verbiscar et al., 1980). Even with ANF extracted or degraded, tannins and enzyme inhibitors remain in the meal. Tannins can be removed by methanol (Sanchez, 1988), and enzyme inhibitors can be inactivated by heating (Storey et al., 1983). Tannins probably originate in the seed coat, which loosens during germination so that it is easily removed. Thus, the potential for a much more nutritious protein from germinated seeds exists. Seed coats also have been removed by soaking in alkali (Sanchez, 1988). Germination likewise reduces toxin levels, but concomitant oil degradation, microbial growth, and water contamination in the pressed oil would be serious economic impediments to the removal of seed coats before pressing unless a high-value protein product results.

Several protein characterizations have shown that principal components of jojoba proteins are 25- and 47-kDa water-soluble proteins (Wolf et al., 1988). Extracting these proteins from meal that contains seed coats is not difficult, but concentrating the aqueous extract by membrane filtration to remove salts and other nonprotein constituents yields a dark brown solution and an intractable proteinaceous precipitate. Informal discussions with workers in the field indicate that jojoba proteins isolated from deoiled whole-seed meal always appear as a brown intractable material. In addition to the tannins that likely bind to the proteins and cause precipitation, anthocyanins or other phenolic seed components may contribute to the color of protein isolates.

Part of the problem with meals that are reportedly free of simmondsin, but have high mortality in mice-feeding trials, has been the methods of analysis for ANF. Acetone extraction does not completely remove ANF for analysis and, in most studies, only SF and S were measured. A more rapid method, using 90:10 dioxane–water and ultrasonic vibration, extracts ANF more completely, and all four major ANF can be measured on HPLC (Abbott et al., 1988).

This study was performed to develop practical processes for converting jojoba meal to cattle feed and protein isolates. Initial laboratory-scale trials led to three processes for detoxification: an enzymatic process, a fermentative process, and a water extraction process. Each of these was scaled up to produce enough meal for mice-feeding trials, and pilot-plant processes were tested on up to 18.2 kg of meal.

Table I. Experimental Design of Mice Feeding Trials

	no. of mice				
	jojoba meal substitution level, %				
treatment	0	5	10	20	total
none (rodent chow control)	9	0	0	0	9
none (untreated JM[a])	0	3	3	3	9
NH_3	0	3	3	3	9
NaOH	0	3	3	3	9
Lactobacillus	0	3	3	3	9
NH_3 + steam	0	3	3	3	9

[a] JM, jojoba meal.

MATERIALS AND METHODS

Jojoba, *Simmondsia chinensis* (Link) Schneider, seed was obtained from Desert King, Inc., Chula Vista, CA, in 1988.

Analytical Methods. All four ANF were measured by HPLC (Abbott et al., 1988), and their percentages were combined for total ANF. Crude protein by the Kjeldahl method (AOAC, 1984) was calculated as % N × 6.25. Dietary fiber was measured by the Novo BioLabs (Danbury, CT) procedure using their Fiberzym kit.

Viability of the ANF-degrading enzymes endogenous in the meal was assayed by combining 2 g of meal with 1 g of sterile water in a sealed vial for 10 days and measuring ANF remaining. Fully active enzymes typically degrade 50% of the ANF under these conditions.

Moisture levels in samples removed from the toaster–desolventizer were determined by Karl–Fisher titration.

Laboratory-Scale Enzymatic Ensilage and *Lactobacillus* Fermentation. Meal (500 g) was mixed with distilled water (1600 mL) whose pH had been adjusted to 8.0 with either NaOH (10%) or NH_4OH (12 N). The resulting alkali content was low (0.2% NaOH or 0.25% NH_3). The mix was sealed in double plastic bags and held at 29 °C for 20 days. Samples were taken at 0, 3, 6, 10, and 20 days and analyzed for Kjeldahl nitrogen, pH, and ANF. Fermentation was done in static culture. Minimal movement for sampling took place, and samples were remixed at 7 days. In a separate experiment, *L. acidophilus* NRRL B-629 was added as described by Verbiscar et al. (1981) to finely ground defatted jojoba meal at the same water level they used. After 20 days, the meals were freeze-dried. Aged, freeze-dried meals from the NH_4OH treatment (JM + NH_3), from the NaOH treatment (JM + NaOH), and from the *Lactobacillus* treatment (JM + LB) and untreated jojoba meal were tested in mice-feeding trials. A portion of the meal incubated with NH_3 and water was autoclaved to denature antinutritional proteins and then freeze-dried (JM + NH_3 + steam) for mice-feeding trials.

Mice-Feeding Trials. Freeze-dried meals were rehydrated to 10% moisture, and 4.5% corn oil was added to make them isocaloric with the control feed, Purina Rodent Laboratory Chow 5001, meal form. The treated jojoba meals were ground and mixed with control feed in the ratios of 95% control feed to 5% treated jojoba meal, 90% control feed to 10% jojoba meal, and 80% control feed to 20% jojoba meal. After thorough mixing, 2% powdered sucrose was added to eliminate feed avoidance by the mice because of bitter components. The jojoba meals were fed as powders to test for toxicity and to determine whether the treated meals supported growth equivalent to that with the control chow.

The design of the feeding tests was a balanced, full factorial experiment (Table I). Juvenile male and female Institute for Cancer Research mice averaging 23.5 ± 2.9 g were fed either a rodent chow control diet (nine mice of mixed sexes) or a chow diet in which 5–20% of the chow had been replaced with jojoba meals (three mice of mixed sexes per diet). A total of 54 mice was used. The mice were housed individually in metal cages with bedding. They were weighed weekly for 8 weeks and then euthanized by halothane overdose. Average daily gain (ADG)

Table II. Percent Antinutritional Factors in Jojoba Meal (JM) Ensilaged with H_2O or Fermented

	day															
	0 (3–4 h)				3				6				10[a]			
treatment[b]	SF	S	DMS	DDMS	SF	S	DMS	DDMS	SF	S	DMS	DDMS	SF[c]	S	DMS	DDMS
JM + NaOH	1.0	0.68	0.45	2.0	1.2	0.05	0.03	0	1.3	0.05	0.02	0	1.6	0.04	0	0
JM + NH_3	1.33	2.7	1.0	3.0	0.56	0	0.015	0	0.77	0.03	0	0	1.0	0.03	0	0
JM + LB[d]	0.94	1.9	0.68	1.9	0.61	0.06	0.04	0.06	0.85	0.04	0.04	0	0.58	0.05	0.020	0

[a] Toxin levels at 20 days were not significantly changed from those at 10 days. [b] See text for experimental conditions. SF, simmondsin ferulate; S, simmondsin; DMS, demethylsimmondsin; DDMS, didemethylsimmondsin. [c] Measuring SF in fermented meals is not always accurate by HPLC because some fermentation products overlap the SF peak and IR analysis detects no R–CN at 0.1% (5 basis) or lower levels, whereas HPLC methods detect 0.2% or more SF (Abbott et al., 1988). [d] LB, *Lactobacillus* fermented.

was estimated for each mouse by final weight minus initial weight divided by the 49 days on test. The first week on the feed was used as an adjustment period because of initial problems with feed avoidance. The ADG values were analyzed by an analysis of variance procedure with a model which had gender of mouse and diet type as main effects and initial weight as a covariate. Linear contrasts within the diet type were used for specific comparisons among treatments. Inclusion of gender and initial weight in the analysis of variance procedure allowed comparisons to be made independent of the influences of gender and initial weight. At necropsy, the livers, spleens, and kidneys were examined for macroscopically visible lesions. Histopathological examination of liver specimens were carried out by a veterinary pathologist.

Pilot-Plant Deoiling. Seeds (43.2 kg) were cracked and flaked in a Wolf mill and then extracted at ambient temperature in a stirred tank with solvent-grade commercial hexane and drained. Five washes resulted in 203 g of oil in the last wash. Meal (19.4 kg) was air-dried for 3 days and then stored at 4 °C for enzyme and fermentation studies. Flaked seeds for toaster–desolventizer studies were similarly extracted, but the drained, hexane-laden meal was transferred to the Baeuerle and Morris toaster–desolventizer (Mustakas et al., 1981).

The toaster–desolventizer is steam jacketed, and steam is introduced through the rotating blade stirrer in the bottom of the tank. An 18.2-kg batch had a bed depth of 23 cm.

Pilot-Plant Water Extractions. For extraction of ANF or proteins, meal slurried in distilled water was drained on a Prater vibrating screen; the water passing through the screen was centrifuged in a Sharples type N supercentrifuge. The residual meal was dried in a forced-air oven on perforated trays with an 8–10 cm bed depth at 70 °C for 3 days.

Pilot-Plant Membrane Separation of Jojoba Proteins. Water extract (114 L) from jojoba meal (6 kg) was centrifuged in a Sharples supercentrifuge at 15 000 rpm and then concentrated by pumping through a Pellicon 0.5 m^2 area membrane apparatus at various pressures. A Millipore PLGC cassette, 10 000 nominal molecular weight cutoff and low protein binding, was used.

For one 11.4-L trial concentration to 750 mL of concentrate, pressure differential on the membrane was 82 kPa, filtrate rate was 140 mL/min decreasing to 80 mL/min, retentate flow rate was 110 mL/min, and bypass was 1.44 L/min.

RESULTS AND DISCUSSION

Laboratory-Scale Enzymatic Ensilage and *Lactobacilli* Fermentation. ANF levels in jojoba meals (JM) aged at 20% solids in water containing 0.25% NH_3 and no added microorganisms (JM + NH_3) were similar to ANF levels in *Lactobacillus*-treated meals (JM + LB, Table II). The initial pH of 8 in all three treatments decreased to 5 or less in 3 days. Some hydrolysis of SF may have taken place; however, this could not account for the total drop in pH to 5. Recovery for the NaOH-amended, aged jojoba meal (JM + NaOH) was 87.4%, for JM + NH_3 was 87.7%, and for JM + LB was 91.6%. Nitrogen content of JM + NaOH was 4.95%, of JM + NH_3 was 5.38%, and of JM + LB was 5.34%. The percent solids in the JM + LB fermentation was 70%, while that in the JM + NaOH and JM + NH_3 was 20%. This resulted in a dark brown, crusty JM + LB product on freeze-drying.

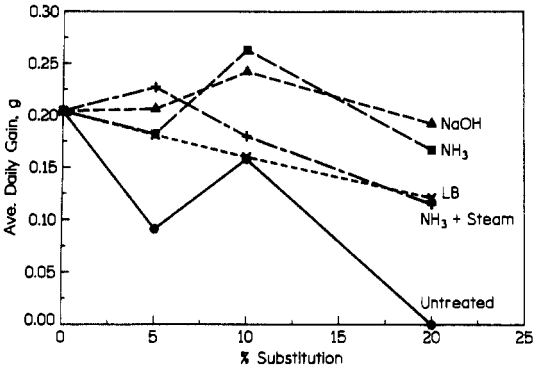

Figure 1. Average daily gains for mice on diets substituted at 0, 5, 10, or 20% with treated and untreated jojoba meal. NH_3 and NaOH represent results on diets containing enzymatically detoxified meals whose pH was initially adjusted to 8 with NH_4-OH or NaOH solution, respectively. NH_3 + steam is the same as NH_3 except autoclaved after 20 days of ensilage. LB represents results on diets containing *Lactobacillus*-treated jojoba meal.

Other 20-day ensilages of meal from sodium hypochlorite sterilized seed at 20% solids gave a tan product (JM20) and at 70% solids gave a dark brown product (JM70), demonstrating the influence of water level on product. ANF were reduced from 11% to less than 1% in both JM20 and JM70. The reduced water content of the JM70 would lower drying costs, but the meal may not be as nutritious after this treatment because the meal is apparently more degraded.

Mice-Feeding Trials. *Comparing Enzymatically Detoxified Meal to Lactobacillus-Treated Meal.* Figure 1 shows the average daily gain of mice in grams per day on the various diets for 7 weeks after 1 week of adjustment on the new diet. Contrasts tested (and the significance found) are shown in Table III. A probability near 1.0 in the last column of Table III indicates that there are essentially no differences between growth rates of mice fed as group 1 and those fed as group 2. Linear tests 1–4 test for an overall linear trend caused by substitution at 0, 5, 10, and 20% rates. Table III can be used for comparison of levels shown in Figure 1. When all treated diets were considered as a group, no differences in ADG of the mice were found when diets were substituted at 5 or 10% (contrasts 1 and 2). A decline in ADG occurred when diets were substituted at 20% (contrast 3). All treated diets considered together or individually had higher ADGs than the untreated diets (contrasts 4–7). All mice on diets containing 20% untreated jojoba meal died within 2 weeks of having been placed on the diet. One mouse on the 10% untreated jojoba meal diet died in the second week. One mouse on the 20% JM + NaOH diet died during the adjustment period for unknown causes. The other 49 mice survived the 8-week test period. One mouse fed 5% untreated JM had an ADG of −0.01 g. This weight loss accounts for the low ADG for the 5% substitution level of untreated meal shown in Figure 1.

Table III. Probabilities That Mice on Two Groups of Diets Have Similar Growth Rates[a]

	group 1	group 2	probability
contrast			
1	all treatments—5% substitution	control	0.85
2	all treatments—5% substitution	all treatments—10% substitution	0.53
3	all treatments—10% substitution	all treatments—20% substitution	<0.01
4	all treated	untreated	<0.01
5	NH_3	untreated	<0.01
6	NaOH	untreated	<0.01
7	LB	untreated	<0.01
8	NH_3	NaOH	0.67
9	NH_3	LB	0.02
10	NaOH	LB	0.02
11	NH_3	NH_3 + steam	0.14
linear test			
1	linear effect of substitution rate of NH_3		0.70
2	linear effect of substitution rate of NaOH		0.80
3	linear effect of substitution rate of LB		0.03
4	linear effect of substitution rate of NH_3 + steam		<0.01

[a] Abbreviations: NH_3, jojoba meal aged with initial pH at 8 by NH_3; NaOH, jojoba meal aged with initial pH at 8 by NaOH; LB, *Lactobacilli*-treated jojoba meal; NH_3 + steam, same meal as NH_3 but autoclaved for 20 min at 120 °C.

Table IV. Analyses of Desolventizer–Toaster-Processed Meal Showing Inactivation of Inherent Enzymes

		indirect heat	steam sparged for			
analysis	meal + residual hexane	for 5 min, meal at 150 °F	5 min, meal at 66 °C	10 min, meal at 93 °C	25 min, meal at 99 °C	air-dried
hexane, % by weight	54	54	5.2	3.0	<1.0	
meal, % by weight	46	46	73	71	73	92
water, % by weight	0	0	22	26	26	7.8
toxins, % of dry meal	9	9	8	10	8	9
toxins, % of dry meal after incubation[a]	4.3	4.0	3.1	8.8	8.2	8.8

[a] Two grams of meal + 1 g of H_2O incubated for 10 days.

The NH_3- and NaOH-treated diets had similar effects on the gains of mice (contrast 8), and both had higher responses than the LB-treated diet (contrasts 9 and 10).

Adding steam treatment to the NH_3 treatment did not consistently change the gain of the mice (contrast 11). The percent substitution in this trial had little effect on gain for the NH_3 (linear test 1) or the NaOH treatments (linear test 2). Higher levels of substitution were associated with lower ADG for both the LB (linear test 3) and NH_3 plus steam (linear test 4) treatments. On the basis of this analysis, we conclude that all mice gained weight on the treated meal diets and at low levels of substitution they gained similar amounts. Autoclaving the NH_3-treated meals after the 20th day denatured proteins, rendering them insoluble. Soluble proteins such as casein have optimal availability for monogastric animals. Similarly, in *Lactobacilli*-treated meals, soluble protein has been converted to bacterial protein, which may reduce quality as well as accessibility to monogastric animals. These differences in proteins may not be significant in ruminants. Liver histopathological examination revealed no pattern of abnormalities—only one liver abnormality in a mouse on a control diet and one indicating hepatitis on a mouse consuming a diet substituted at 10% with JM + NH_3 + steam.

Comparing Enzymatically Detoxified Meal to Water-Extracted, Steam-Denatured Meal and Fungally Detoxified Meal. A second feeding trial comparing the above three meals was reported earlier for fungally detoxified and enzymatically detoxified meal (Abbott et al., 1990). Included in this second trial, but not previously reported, were the results for jojoba meal that was steam desolventized in the Baeuerle and Morris toaster–desolventizer and water extracted as described under Materials and Methods. Statistical analysis of mice growth rates showed no significant differences ($P < 0.01$) in average daily gain of mice on control diets compared to that of mice on diets substituted with 5, 10, or 20% of the above three treated meals.

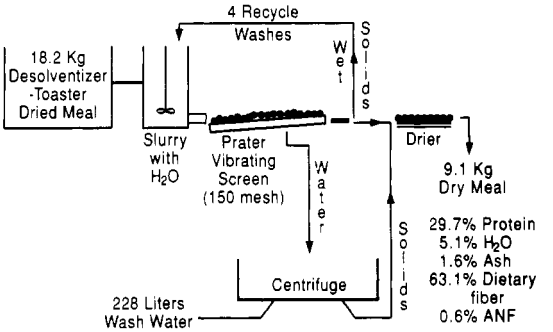

Figure 2. Pilot-scale water extraction of steamed jojoba meal.

Pilot-Scale Processes. Deoiled jojoba meal can be converted to animal feed by extraction with water, by ensilage with endogenous enzymes, or by fermentation with microorganisms that degrade ANF. The method of choice depends on the economics of each process, the stability of the endogenous enzymes, and the solubility of proteins after the deoiling process.

Water Extraction of Steam-Denatured Meal. If the meal temperature reaches 93 °C during steam recovery of hexane, proteins including enzymes will be denatured. Table IV shows the results of pilot-plant desolventization of 18.2 kg of hexane-deoiled meal in a toaster–desolventizer. An assay of toxin-degrading activity shows that after 10 min of sparging (meal reached 93 °C), the enzymes are no longer active. Soluble proteins were denatured and became insoluble. This meal was processed by water extraction retaining 60% of the protein, as shown in Figure 2. Material loss was significant, and in more carefully controlled laboratory tests, up to 75% of the protein was retained in the washed meal. The resulting meal should be an adequate animal feed, but material loss and waste-

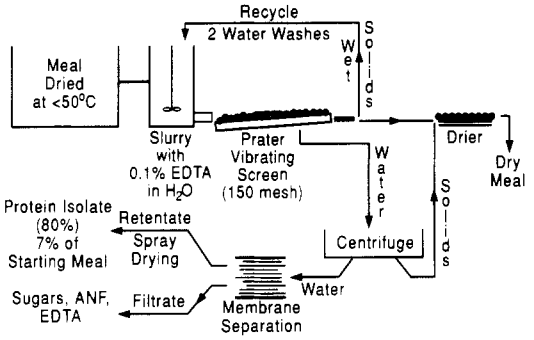

Figure 3. Process for isolating jojoba protein and byproduct feed.

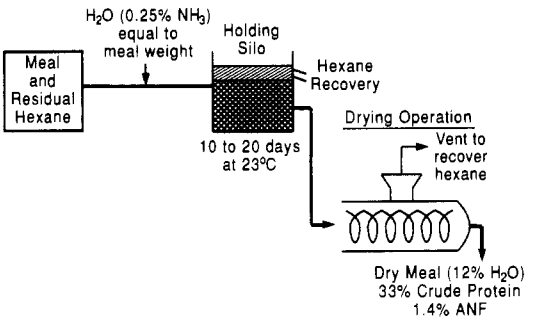

Figure 4. Enzymatic process to biodegrade antinutritional components in jojoba meal and produce animal feed.

water treatment would add to the cost of this process. The effluent water contains sugars, ANF, salts, and other components which would be degraded in the soil if the effluent water were used for irrigation of jojoba fields near the processing plant. Although salts are low in jojoba meal, they could build up in the soil.

Extraction of Protein Isolates. If the hexane-extracted meal is desolventized by flash desolventization or other processes which do not denature proteins, then soluble protein isolates may be extracted as outlined in Figure 3. In the initial pilot-scale trials using room temperature dried, hexane-extracted meal, water alone was used to extract proteins, but the protein precipitated from the water solution as it was being concentrated and the fine precipitate clogged the membrane filtration unit. The extract was also tea brown in the initial extract and became darker and more cloudy as it was concentrated. This is probably caused by tannin–protein complexes. Several procedures to overcome these problems failed, including passing the extract through ion-exchange resins or powdered nylon, raising the pH to 8, adding antimicrobial preservatives, or increased centrifuging and filtering of the dilute extract. The addition of 1% or more ascorbic acid or 0.1% ethylenediaminetetraacetic acid disodium salt (EDTA) to the water before extraction gave a clear extract which, when dialyzed and freeze-dried, yielded a white protein isolate (13.3% N). This isolate was completely water soluble and added no visible color to a generic "tearless" baby shampoo when added at the 5% level. Gel electrophoresis showed it to be composed of proteins similar to those isolated from dehulled jojoba seed (Wolf et al., 1988). The protein extract using EDTA has not been scaled up to a pilot-plant operation, and in that sense Figure 3 is a proposed process because pilot-scale tests were done with water alone as the solvent. Meals recovered from EDTA and ascorbic acid extractions are typically 50% of pre-extraction weight and contain 3.9% N.

Enzymatic Process. Hexane-laden meal, before it is steam sparged, is nearly sterile and contains active ANF-degrading enzymes (Abbott et al., 1988). Inherent enzymes degrade ANF under anaerobic conditions, and hexane inhibits microbial growth. As shown in Figure 4, hexane wet meal (4 kg of dry weight) with 4 L of H_2O containing 40 mL of 12 N NH_4OH was aged for 20 days. This experiment tested the activity of the enzyme system under reduced water conditions and in the presence of hexane, which prevents uncontrolled fermentation by spurious microorganisms.

Some advantages of the enzymatic process are that the meal is nearly completely recovered as an animal feed (87–91%), there is no conversion of soluble proteins to bacterial protein, and there is no loss of material as CO_2 produced by microorganisms and there is no waste water to treat if the meal is dried. Hexane was partially displaced by the water and could be drawn off the top. We suggest that a process for hexane recovery could be tested on the basis of water addition to the drained hexane wet meal, compressing the wet meal and drawing off the hexane from the top layer that would form over the water. This would avoid distillation or heating of the hexane and is potentially safer. ANF were reduced to 1.4% in 20 days from an initial 14% on the basis of HPLC analysis. In 80% water to 20% meal slurries, endogenous enzymes reduced ANF to 0.36% in some tests. The disadvantages of the process are the drying cost of the meal and the 20-day storage time.

Fermentation Process. A fermentation process to degrade ANF is possible with *Pseudalescheria boydii* NRRL 13766 and *Fusarium moniliforme* (Sheldon) NRRL 13767. These two fungi are among four microorganisms found to degrade specific ANF. Details of their isolation and testing in combination on jojoba meal were described elsewhere (Abbott et al., 1990). The advantage of fermentation for reducing ANF is that there is no byproduct waste stream. However, the process requires aeration and results in weight loss due to catabolic CO_2 evolution. Finished fermentations should be heated to stop further microbial activity, adding to the cost of any fermentation approach. No mycotoxins, which are normally associated with other strains of *F. moniliforme*, were found on jojoba meal or cracked corn fermented with these strains. *P. boydii* is a human pathogen and should be handled appropriately. The next step in developing this process would be to isolate the enzyme system that specifically attacks ANF and use it alone or try to introduce the same enzyme-producing genes into a less harmful organism.

CONCLUSION

All processes investigated result in jojoba meal that does not adversely effect growth rates and appears nontoxic. Because the jojoba industry uses 45-min steam desolventizing after the oil is extracted, the enzymatic process cannot be used on that type of meal. Also, water extraction of the meal yields an undesirable byproduct-contaminated water stream. Thus, the choice of fermentation for currently produced defatted jojoba meal seems inevitable. Only a nontoxigenic *F. moniliforme* or other organism that degrades ANF in the meal should be used, and the resulting meal should be tested for the absence of toxins and retention of nutritional value.

Adapting the desolventizing procedure to flash desolventizing or reduced temperatures would make the more desirable enzymatic process possible and this should be considered for future jojoba extraction facilities.

Presscake contains residual oil and active enzymes. The enzymatic process for detoxification should work on presscake meal, but antinutritional jojoba oil should still be removed. The EDTA–water extraction process for protein

isolates would provide the most valuable byproducts from presscake if the oil can be separated from the protein solution.

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