to emulsify oil. Enzymatic digestion of proteins completely destroyed the emulsifying capacity of the flour. Apparently hydrolysis substantially altered protein surface activity strengths and the ability of peanut protein to stabilize oil-in-water emulsions. This agrees with an earlier report showing decreased emulsion capacities of peanut flour fermented with fungi (Quinn and Beuchat, 1975).

Liquid Retention. Water- and oil-retaining data for control and test flours are shown in Table I. Results showed that heating the flour slurries at acidic or alkaline pH greatly improved the water-imbibing capacity of flour. Hydrolysis by pepsin, bromelain, and trypsin during heating progressively lowered water absorption capacity. Capacities remained higher than the nontreated control, however, even after 50 min of pepsin and bromelain treatment. Trypsin hydrolysis resulted in a product having less water-retaining capacity than the nontreated flour.

Test samples were consistently more lipophilic than hydrophilic. Although heating at pH 2.0 did not affect oil-retaining characteristics of the flour, heating at pH 4.5 and 7.6 tended to enhance these characteristics. Oil retention by peanut flour was not significantly changed from respective controls as a result of hydrolysis with pepsin, bromelain, or trypsin.

Liquid retention properties of peanut protein may affect food processing conditions where water or oil is incorporated as ingredients along with the peanut flour. Overall qualities of food products, such as shrinkage during processing, mouthfeel, and storage stability, are affected by liquid retention properties of their constituent ingredients.

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Pilot Plant Production of an Edible White Fraction Leaf Protein Concentrate from Alfalfa

Richard H. Edwards,* Raymond E. Miller, Donald de Fremery, Benny E. Knuckles, E. M. Bickoff, and George O. Kohler

This paper describes the development of a pilot plant scale wet fractionation process to obtain an edible white protein fraction from fresh alfalfa (*Medicago sativa L.*). Expressed alfalfa juice is given a flash heat treatment to agglomerate preferentially the green pigmented proteins which can then be separated by continuous high-speed centrifugation. The chlorophyll-free soluble protein remaining in the supernatant is precipitated by heating to 80° and separated by centrifugation. An off-white to light-tan, bland, protein concentrate containing approximately 90% protein is obtained. The product and its processing behavior can be improved by the addition of sodium metabisulfite to the fresh alfalfa prior to processing. The major product from the process, called the Pro-Xan II process, is a dehydrated alfalfa meal. The remaining products include the alfalfa solubles fraction and a feed-grade protein–xanthophyll concentrate. The latter is prepared by heat coagulating, pressing, and drying the agglomerated green protein fraction. Yields, compositions, and other processing data from the pilot plant operation are discussed.

Leaf protein concentrates (LPC's) were first described over 200 years ago (Rouelle, 1773) and have been studied extensively during the past 30 years (Bickoff et al., 1947; Kohler et al., 1968; Pirie, 1971a). However, the use of LPC in the human diet has remained almost nonexistent while other newly developed protein concentrates and isolates from soy, cottonseed, peanut, whey, and other sources are already being incorporated into human foods at a rapidly expanding rate (Hammonds and Call, 1972; Holsinger et al., 1973; Rooney et al., 1972). In spite of its high protein content and good nutritive value (Singh, 1967; Woodham, 1971; de Fremery, 1972; Olatunbosun et al., 1972), LPC has been rejected by most human consumers and food product

Western Regional Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Berkeley, California 94710.

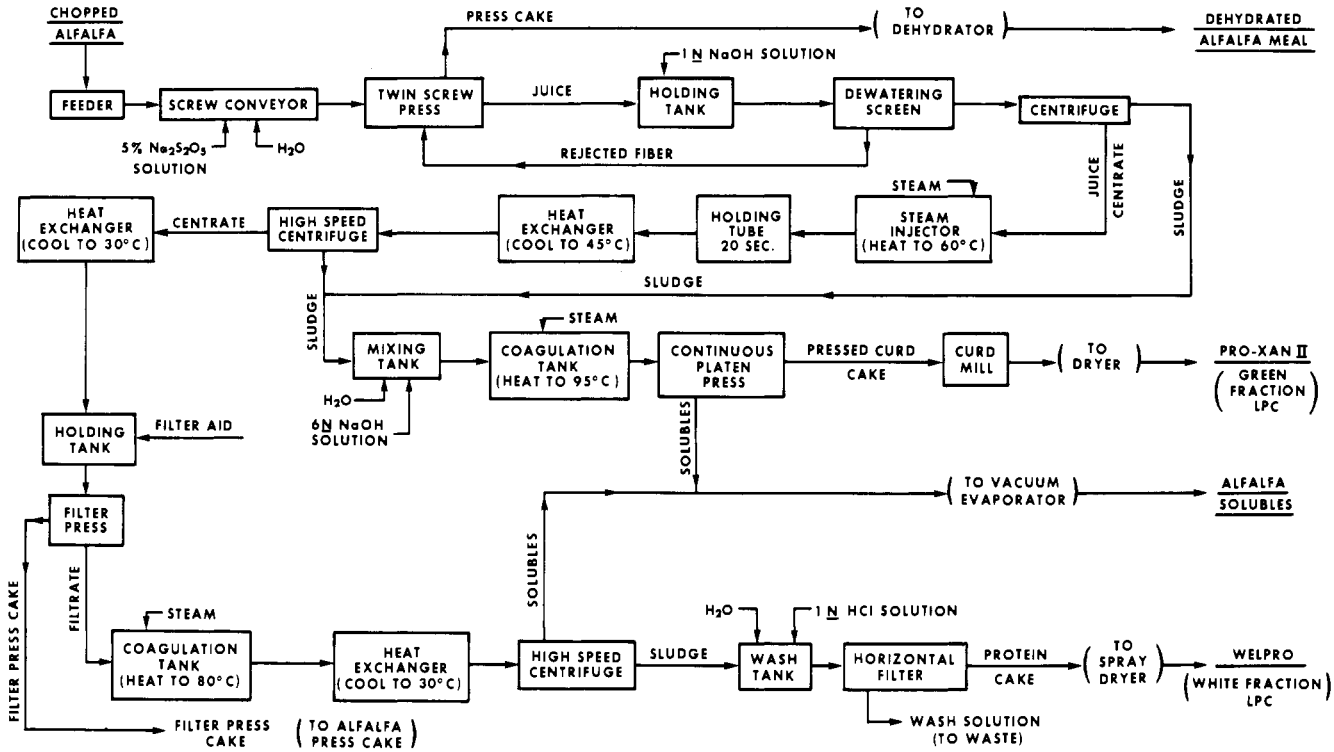

Figure 1. A flow diagram for the Pro-Xan II pilot plant process.

formulators because of its bitter, grassy flavor and dark green color.

Previous workers (Chayen et al., 1961; Hartman et al., 1967; Huang et al., 1971; Wilson and Tilley, 1965) have produced bland, light colored, chlorophyll-free LPC's by extraction of the plant juice or the precipitated LPC with polar solvents. Others have separated the soluble white protein present in the juice from that insoluble protein associated with the chloroplasts and cell debris by employing a differential heat treatment (Byers, 1967; Cowlishaw et al., 1956; de Fremery et al., 1973; Henry and Ford, 1965; Lexander et al., 1970; Subba Rau et al., 1969).

Since the solvent extraction process would involve high capital and operating costs, we turned our attention to developing a practical differential heat process. The colloidally dispersed chlorophyll-containing material coagulates more rapidly and at lower temperatures than most of the soluble protein fraction. The agglomerated green protein fraction may then be sedimented by normal centrifugal techniques. If properly carried out, the chlorophyll-free soluble white protein remains in the supernatant and may be precipitated by a second heating step at a higher temperature or by other means. The chlorophyll-containing green protein fraction will be referred to as green LPC or Pro-Xan II and the white protein precipitate as white LPC or Welpro (white edible leaf protein). Previous work at this laboratory (de Fremery et al., 1973) has established that direct steam injection heating of juice to a temperature of 56–62° for times of 10–60 sec appears adaptable for continuous, large scale separation of the green and white protein fractions from alfalfa.

Since previous work has been done only on a small laboratory scale little has been reported regarding process parameters for making white LPC by this method. The objective of the present work was to develop and test a pilot plant process for the production of a white, bland, leaf protein concentrate from alfalfa (*Medicago sativa L.*) which would have potential applications as a food grade protein product. Elements of the Pro-Xan process (Kohler et al., 1968; Knuckles et al., 1970, 1972; Lazar et al., 1971; Miller et al., 1972; Spencer et al., 1970, 1971), previously developed by this laboratory for producing an animal grade whole leaf protein concentrate called Pro-Xan, have been incorporated into the present pilot plant operation wherever practical.

In addition to the white LPC (Welpro), the process products are the green LPC (Pro-Xan II), a dehydrated alfalfa meal, and an alfalfa solubles fraction. This process will be referred to as the Pro-Xan II process.

EXPERIMENTAL SECTION

Equipment. The following pieces of equipment were employed in the pilot plant operation as shown in Figure 1: alfalfa feeder (Arnold Dryer Co., Model A632-44); twin screw press (see Knuckles et al., 1972) with 9-in. diameter main screws and cross screw discharge (Bauer Bros. Co., Helipress No. 585 with 0.035-in. screen openings); dewatering screen with 0.020-in. screen openings (Bauer Bros. Co., No. 552 Hydrasieve); imperforate bowl centrifuge, 17-in. bowl diameter, 1500 rpm, RCF_{max} (relative centrifugal force) = 540*g* (Fletcher Works, Fletcher Standard, size 17); high-speed, disk-type, solids discharging centrifuge, 31-cm bowl diameter, 6000 rpm, RCF_{max} = 14,500*g* (De Laval Separator Co., Model BRPX-207S); plate and frame filter press, 25.0 ft^2 filtration area (Sperry and Co., size 12, type 37); horizontal filter, 14.5 ft^2 filtration area (Sparkler Mfg. Co., Model 18-D-8); continuous platen belt press (see Miller et al., 1972) (Josef Willmes Co., Bensheim, W. Germany, Contipak press, demonstration model); plate-type heat exchanger (Creamery Package Co., Model SC-3196); tube-type heat exchanger, constructed at this laboratory; steam injector, 0.5 in. IPS pipe with 0.136 in. diameter hole for tangential steam entry, constructed at this laboratory; steam injector (Hydro-thermal Corp., Hydroheater Model B-300); insulated 1.5 in. diameter sanitary tubing or 1-in. creamery hose; coagulation tanks constructed at this laboratory, capacities of 4 and 6 gal with overflow spouts and rotating skimming paddles; Conical-type spray dryer (Bowen Engineering Co., Laboratory model); curd mill with 0.25 in. screen (F. J. Stokes Corp., Stokes Granulator, Model 43-B).

Analytical Methods. *Sample Preparation.* All wet

products were freeze dried and ground prior to analytical determination. Results were calculated on a dry weight basis.

Proximate Composition. Ether extractives, crude fiber, and ash content were determined by standard AOAC methods (AOAC, Official Methods of Analysis, 1970).

Protein. Crude protein was calculated as Kjeldahl nitrogen × 6.25. That nitrogen soluble in 10% trichloroacetic acid solution was assumed to be nonprotein nitrogen. True protein nitrogen was calculated as the difference between the total nitrogen and the nonprotein nitrogen in a sample.

Carotenoids. Carotene and total xanthophyll contents were determined by the method of Knuckles et al. (1971) and Livingston et al. (1971).

Moisture. The moisture content was determined by drying samples for 2 hr at 110° in a forced draft oven.

Pilot Plant Calculations. Mass balances for each unit operation were calculated on the basis of wet solids, dry solids, and crude protein. In those cases where the mass of all inflow and outflow streams could not be measured directly, calculations have assumed that no solids are lost in the process. Where measurements showed actual losses, the composition of the lost material was estimated by calculation and apportioned among the stream flows in amounts to maintain the correct composition of each stream. The mass balance of each operation was then brought to the common starting basis of 10,000 lb of fresh alfalfa.

General Pilot Plant Procedure. The following procedure applied to all pilot plant runs. Approximately 6000 to 8000 lb of field chopped alfalfa was fed through the screw press at a rate of 7000 lb/hr 1–2 hr after cutting. Juice was produced at a rate of approximately 10 gal/min and pumped into holding tanks. The juice was processed through the remaining unit operations at approximately 1.5 gal/min, with the exception of the filtration steps and the centrifugation of the precipitated white protein coagulate. These operations were completed at rates up to 10 gal/min. As many unit operations as feasible were run simultaneously.

Inflow–outflow masses of the feed and product streams were measured for each unit operation wherever possible on either a rate basis or a total operation yield. All unit operation streams were sampled for moisture and proximate analyses.

The same high-speed centrifuge was used for both the separation of the agglomerated green LPC solids from the soluble white LPC as well as for the separation of the precipitated white LPC from the noncoagulable alfalfa solubles.

All processing with the exception of the washing of the white protein was accomplished in 1 work day. The white LPC sludge was diluted with tap water (1:5, w/w) and adjusted to pH 4.5 for storage overnight at 2°. Washing and filtration of the white LPC were completed the following day.

Centrifugation Studies. The separation of the agglomerated green LPC solids from the soluble white LPC fraction by the high-speed centrifuge was studied with respect to feed solids level, feed rate, feed temperatures, juice pH, juice heat treatment, and juice age. The solids in the feed and centrate were measured on a volume percent (vol %) basis by spinning 10-cm^3 samples in calibrated tubes for 3 min in a test centrifuge (RCF_{max} = 9200*g*, De Laval Separator Co., Gyro-Tester). Centrate is defined as the predominantly liquid phase from a centrifuge following a solid–liquid separation.

Filtration Studies. Filtration rates of the two filtration unit operations were measured as a function of several process variables. Measurements of filtrate volume vs. time were made for each experimental run.

The filtration runs varied in length from 25 to 84 min. The filter press and horizontal filter with filtration areas of 25.0 and 14.5 ft^2, respectively, were used for large volume runs. Units with filtration areas of 1.2 ft^2 (Sparkler Mfg. Co., Model C) and 3.60 $in.^2$ (Dicalite Division, Grefco, Inc., Dicalite test bomb filter) were used for small volume runs. The measured values of filtrate volume per square foot of filter area vs. time were plotted on log–log graph paper. The filtration rate in gal/(ft^2 hr) after 60-min filtration time was determined from the graph.

PILOT PLANT SYSTEM: DESIGN AND OPERATION

The Pro-Xan II process shown in Figure 1 is best examined by separating it into its major parts: (1) the expression of juice from the alfalfa and the treatment of that juice to yield a green fraction containing the green LPC solids and a clear liquor fraction containing the soluble white LPC; (2) precipitation of the proteins in the clear liquor to give a white protein suspension which is subsequently separated from the solubles, washed, and dried; (3) high-temperature coagulation of the green fraction, separation of the precipitated green LPC curd from the solubles, and subsequent drying of that curd; and (4) further processing of the alfalfa press cake and solubles fractions.

Juice Extraction and Separation of Protein Fractions. Basically, juice was extracted from the fresh alfalfa in the twin screw press and flash heat treated to agglomerate the green LPC solids in the juice. The agglomerated green LPC solids were separated from the mother liquor containing the soluble white LPC by high-speed centrifugation. A heat treatment of 60° for 20 sec was severe enough to cause sufficient green LPC agglomeration to allow centrifugal separation. Higher temperatures resulted in lower white LPC recoveries and an increased amount of fouling in the holding tube and the plate heat exchanger. Lower temperatures did not cause sufficient agglomeration of the green LPC solids with some batches of juice. Increasing the holding time to 60 sec had little effect on the centrifugal separation.

Additional unit operations prior to the flash heat treatment are also required to obtain the desired separation in the high-speed centrifuge. Depending on the batch of alfalfa processed, juice heated to 60° for 20 sec and quickly cooled contained 17 to 30 vol % sediment when spun down in the test centrifuge. Additionally, as the pH of the treated juice was increased from 5.5 to 8.5, the sediment level also increased. This type of high-speed centrifuge became ineffective when the suspended solids level exceeded 10 to 12 vol %. Therefore, water was added to the fresh alfalfa in such amounts as needed to lower the suspended solids level in the treated juice to that range.

Approximately 200 ppm of SO_2 was added to the fresh alfalfa using a 5% sodium metabisulfite solution adjusted to pH 6.1 with sodium hydroxide. This resulted in various improvements in processing behavior and product quality. These effects will be discussed in a later section.

The expressed juice was adjusted to pH 6.0–6.1 with 1 *N* NaOH solution. This juice pH was low enough to permit a reasonable centrifugal separation after heat treatment with a minimum water addition to the fresh alfalfa but high enough to probably reduce the problem of juice foaming (McArthur, 1972). The juice was pumped over a dewatering screen to remove residual suspended fiber and into the imperforate bowl centrifuge to remove suspended sand, dirt, and large fragments of cell debris. These materials could not be successfully discharged from the high-speed centrifuge and led to clogging of the centrifuge bowl.

Studies showed that the centrate clarity following heat treatment (60°, 20 sec) was highly dependent on both feed temperature and feed rate. Centrate clarity of heat treated juice at the natural juice pH (5.8–6.0) improved dramatically as the feed temperature was increased from room temperature to 45°. At feed temperatures of 50° and higher, the white LPC in the centrate partially precipitated in

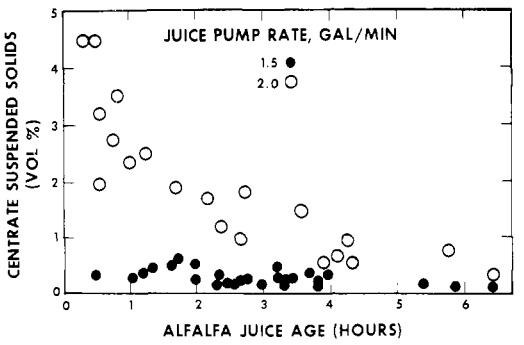

Figure 2. The effect of some process variables on centrate clarity.

the centrifuge and formed a film of white protein on the interior surfaces. Hence, 45° was adopted as the centrifuge feed temperature. At this temperature, centrate clarity was acceptable at a feed rate of 1.5 gal/min, marginally acceptable at 2 gal/min, and unacceptable at higher feed rates.

Centrate clarity improved as the age of the juice increased from the time of juice expression. This effect, shown in Figure 2, was clearly visible at a feed rate of 2 gal/min. At 1.5 gal/min the effect was largely overcome by the increased liquid residence time in the centrifuge bowl.

Following high-speed centrifugation, the residual suspended solids (0.2–0.4 vol %) were removed from the centrate by filtration with filter aid in the plate and frame filter press. Experimentation showed that a fine grade filter aid such as Celite 505 or Dicalite UF was required to prevent penetration of the precoat layer by the green suspended solids. The filter aid loading was 0.25 to 0.35 wt %. Although the filtration rate was found to increase with filtration temperature, filtration was accomplished at 30° in these runs to reduce protein losses due to proteolysis, which increases rapidly with increasing temperature (de Fremery et al., 1972).

Precipitation and Recovery of the White Protein Fraction. Following filtration, the solution containing the soluble white LPC was heated to 80° by direct steam injection through a sparger nozzle in a 6-gal stirred coagulation pot. The precipitate obtained was off-white in color, curdlike in appearance, and soft in consistency. At first, the majority of the curd floated, but settled readily if deaerated by mechanical agitation. The hot slurry was cooled to 30° in the parallel tube heat exchanger to avoid the unknown effects of standing in a hot condition.

The precipitate was separated from the alfalfa solubles by continuous high-speed centrifugation. The sludge produced was a smooth, creamy, off-white paste containing 13 to 16% total solids. Feed rates to 10 gal/min left only trace amounts (0.01 vol %) of precipitate in the centrate.

The sludge was easily redispersed in water by a three-bladed propeller mixer. A redispersed sludge–water mixture in the ratio of 1:5 by weight was adjusted to pH 4.5 with 1 *N* HCl solution and held overnight in refrigerated storage. Washing was resumed the following day.

Washing and subsequent filtration of the Welpro wash suspension in the horizontal filter were done at a final sludge:water ratio of 1:50 by weight. This dilution was judged more than adequate to reduce water-soluble compounds in the precipitate to a satisfactory level. A single washing was chosen over multiple washings at lower dilutions to simplify the operation and reduce the processing time required. Our high-speed centrifuge could not be used for this operation because the sludge could not be properly discharged from the bowl.

Washing was accomplished at a pH of 4.5 and tap water temperature (20°). The Welpro suspension was found to filter faster under acidic conditions and low temperatures as seen in Table I. Higher filtration rates at acidic pH

Table I. Effect of Process Variables on the Filtration Behavior of Welpro Wash Suspensions

Feed solids, vol %	pH	Temp, °C	Filtration rate,[a] gal/(ft^2 hr)
1.5	4.5	20	12.4
2.0	4.5	20	9.2
1.5	4.5	20	12.4
1.5	6.8	20	8.2
1.5	4.5	5	13.2
1.5	4.5	20	12.4
1.5	4.5	35	11.4

[a] For filtration time of 60 min at 40 psig.

values during the washing of whole leaf protein concentrates have been noted by Cowlishaw et al. (1956) and Morrison and Pirie (1961). Removal of flavors and possible harmful alkaloids from leaf protein was reported by Pirie (1969, 1971b) to be more efficient at acidic pH values.

Samples of the washed protein cake were freeze dried. When ground, the freeze-dried product was a fine, nongritty, bland powder, off-white to light tan in color. In some cases, the remainder was spray dried at an inlet temperature of 400°F and outlet temperature of 225–240°F. The spray dried product was a fine, light tan, bland tasting powder. Drum drying and hot air drying were unacceptable, producing hard, gritty material that was dark brown in color.

Coagulation and Recovery of the Green Protein Fraction. The green LPC solids from the imperforate and high-speed centrifuge were combined to form a thick, soft, mudlike mass containing 22–23% total solids. Heating to temperatures above 80° produced protein curds from which additional solubles could be expressed (Byers, 1967). Water was added to the feed solids to simplify pumping and steam injection heating with the Hydroheater injection unit. The feed was adjusted to pH 8.5 prior to heating to preserve the green color and carotenoid content during heating and drying.

The feed was heated to 95° and passed into a stirred, 4-gal coagulation pot. The continuous platen belt press was used to express additional solubles from the hot coagulate. The pressed Pro-Xan II curd averaged 41% total solids, a level that is noticeably higher than that produced by coagulation at lower temperatures. The pressed Pro-Xan II curd was easily crumbled when passed through the granulator. The granulated Pro-Xan II feed produced in this way on a commercial basis would be dried in a rotary drier, an air flotation drier, or a tunnel drier (Miller et al., 1972).

Further Processing of the Press Cake and Soluble Fractions. Neither the press cake nor alfalfa soluble fractions were processed to final product form in the present series of experiments since earlier work has covered these aspects (Spencer et al., 1970, 1971). For the most energy-efficient operation, the alfalfa solubles would be concentrated in multieffect vacuum evaporators to a serum containing 50 to 60% total solids.

PILOT PLANT SYSTEM: RESULTS AND DISCUSSION

The composition of the process fractions is shown in Table II and the process yields are presented in Table III. The Welpro contains almost 90% protein and negligible amounts of fat, fiber, and ash. This protein level is substantially higher than that obtained by Cowlishaw et al. (1956) and Subba Rau et al. (1969) for white LPC preparations from alfalfa. The light color and bland taste should help solve the acceptability problems encountered by earlier leaf protein products. The Welpro should be useful in upgrading the protein content and nutritional value of human

Table II. Composition of the Process Fractions

Fraction	Component[a]					
	Protein,[b] %	Fat, %	Fiber, %	Ash, %	Carotene, mg/lb	Xantho-phyll, mg/lb
Chopped alfalfa	21.5	4.0	24.6	9.6	132	273
Press cake	17.8	3.4	33.1	7.0	86	194
Welpro (white LPC)	88.7	<0.5	<0.5	<0.5		
Pro-Xan II (green LPC)	47.2	13.7	4.1	16.0	409[c]	647[c]
Alfalfa solubles	18.2	0.8	0.6	19.6		
Filter press cake	7.6	3.0	0.7	86.1	42	84

[a] Dry basis; average of four replicate runs. [b] N × 6.25. [c] Average of three replicate runs.

Table III. Yields of the Pilot Plant Process Fractions[a]

Fraction	Yield,[b] wt %	
	Av	Range
Welpro (white LPC)	2.0	1.9–2.2
Pro-Xan II (green LPC)	6.8	5.7–7.9
Press cake	72.9	70.9–74.2
Alfalfa solubles	15.3	14.8–16.1
Filter press cake	2.1	1.8–2.2
Wash solution	0.9	0.7–1.0
	100.0	

[a] Based on four replicate runs. [b] Basis: total dry input solids. To obtain yield on whole alfalfa dry solids basis, multiply values by 1.023.

Table IV. Distribution of Protein in the Pro-Xan II Process

Item[a]	Dry wt, lb	Crude protein,[b] % of original	Soluble protein, % of original
Total solids	100		
Total crude protein	21.4	100	
Total soluble protein	7.1[c]	33	100
Welpro	2.1	8.7	26.1
Pro-Xan II	6.9	15.1	
Alfalfa solubles	15.7	13.3	1.9
Press cake	74.6	61.7	54.4
Filter press cake	2.1	0.7	
Wash solution	0.9	0.5	

[a] Basis: 100 lb dry weight whole alfalfa; average of two replicate runs. [b] N × 6.25. [c] Assumed value.

foods. Some preliminary work on functional properties and the incorporation of Welpro into cookies and soups have been reported (Betschart and Kohler, 1975).

The green Pro-Xan II product should be a good protein concentrate for monogastric animals. It contains almost 50% protein, is high in lipids, low in fiber, and very high in total xanthophyll (more than 600 mg/lb). Like Pro-Xan, it should make an excellent chick (Kuzmicky et al., 1972) or swine (Cheeke and Myer, 1973) feed ingredient.

The press cake constitutes almost 75% of the process dry product output. It retains enough protein to produce a high quality dehydrated alfalfa meal. Alternately, enough moisture has been removed from the fresh alfalfa to allow ensiling the cake without prior field wilting (Oelshlegel et al., 1969). The cake may also be fed directly to ruminants as is, or sun dried into hay. In each case, the juice removal or dewatering step improves the basic operation for the preservation or utilization of the forage crop. In dehydration, one has less water to evaporate, saving on energy costs and conserving energy supplies. In direct feeding, one is transporting less water from plant to feedlot. In silage making, one can start with a less mature, higher quality fresh alfalfa, and field wilting is not required, thus reducing the farmer's dependence on weather conditions. In hay making, drying is more rapid due to the crushing of the stems and the lower moisture content.

The solubles fraction contains valuable vitamins, sugars, amino acids, and minerals. The solubles can be recycled back onto the field as fertilizer, used as a separate feed ingredient, used as a growth medium for single cell protein, or put back onto the press cake to become part of the alfalfa meal.

The yields of Pro-Xan II and Welpro are 6.8 and 2.0%, respectively. These results were obtained using field chopped alfalfa and a single pass through the twin-screw press. The yield of the LPC products could have been increased by grinding the alfalfa prior to pressing or by multiple extractions of the alfalfa. These procedures are expensive on a large scale basis, however, and their merits must be confirmed by future economic analysis of the process.

Of the 21.5% crude protein (dry basis) in the fresh young alfalfa, approximately ⅓ is soluble true protein, ⅓ is insoluble true protein, and ⅓ is nonprotein nitrogenous compounds. Using these assumptions, the juice yields actually obtained, and the determination of the true soluble protein content in the expressed juice, it was calculated that 45.6% of the total soluble true protein was extracted during the juicing operation, leaving 54.4% remaining in the press cake (see Table IV). The Welpro represents 8.7% of the original crude protein and 26.1% of the total soluble true protein present in the fresh alfalfa. The Welpro also represents a recovery of approximately 57% of the total true soluble protein in the expressed juice. Twenty percent of the extracted soluble protein becomes denatured during the flash heat treatment. Another 7% is present in the liquid phase in the chloroplastic solids from the imperforate bowl and the high-speed centrifuges. Most of this soluble protein becomes part of the Pro-Xan II upon final heat coagulation. About 4% of the extracted soluble protein is not precipitated by the final heat coagulation steps and remains in the alfalfa solubles. Finally, the alfalfa solubles fraction also contains a portion of the soluble protein which has been hydrolyzed to peptides and amino acids by the active proteases in the early steps of the process.

A typical material balance for the process is shown in Table V. The largest inflow–outflow component on an as-is basis is the wash water. The wash water volume must be reduced substantially for the washing step to be considered practical.

Table V. Typical Material Balance for the Pilot Plant Process[a]

Component	Weight, lb		
	As-is basis	Dry basis	Crude protein[b]
Inflow			
Chopped alfalfa	10,000	2114	472.7
Water, dilution	2,993		
Bisulfite solution	83	4.1	
NaOH solution	27	3.7	
HCl solution	60	2.2	
Steam	1,064		
Filter aid	34	33.7	
Water, wash	17,010		
Total	31,271	2157.7	472.7
Outflow			
Alfalfa press cake	5,244	1599	299.4
Welpro cake	242	42.6	38.3
Pro-Xan II cake	330	142.6	68.9
Alfalfa solubles	8,194	319.4	61.5
Filter press cake	93	39.9	2.7
Wash solution	17,168	14.2	1.9
Total	31,271	2157.7	472.7

[a] Calculated for a process feed weight of 5 tons fresh alfalfa. [b] N × 6.25.

Table VI. Effects of the Addition of Sodium Metabisulfite to Fresh Alfalfa during Processing

Effect of bisulfite addition	Net value to process
Increases whiteness of final Welpro product	Positive
Decreases off-flavors in final Welpro product	Positive
Increases softness of bulk Welpro precipitate	Positive
Increases uniformity of bulk Welpro precipitate	Positive
Increases pH stability of fluids during processing	Positive
Increases the nutritional value of final Welpro product	Positive
Does not affect the centrifugability of the final heat treated green juice	Neutral
Decreases curd size of bulk Pro-Xan II precipitate	Negative
Decreases curd firmness of bulk Pro-Xan II precipitate	Negative
Increases cost of process	Negative

Effects of Bisulfite Addition. A 5% sodium metabisulfite solution was sprayed on the fresh alfalfa to add approximately 200 ppm of SO_2 to the fresh alfalfa. The bisulfite addition produced a freeze dried Welpro product with a slightly lighter color, and also appeared to improve the blandness of the product.

Color development may be due to one or more causes. Phenolic compounds such as chlorogenic acid, which are present in the juice, may be oxidized by polyphenol oxidases to *o*-quinones which may polymerize to brown colored compounds, or may react with peptides and proteins to produce brown or other colored products (Smith and Johnsen, 1948; Pierpoint, 1969a,b). Browning may also occur by the Maillard reaction (Ellis, 1959). Bisulfite additions are known to reduce both enzymatic and nonenzymatic browning reactions (Schroeter, 1966).

Higher levels of bisulfite were not used in the current series of runs because they had some undesirable effects on processing (see Table VI). While the addition increased the whiteness and palatability of the Welpro product, it also decreased the curd size and firmness of the Pro-Xan II coagulum. At bisulfite addition levels above 200 ppm of SO_2, the curd was more difficult to dewater. This difficulty might be eliminated by the use of a centrifuge to dewater the Pro-Xan II coagulum. The increased uniformity of the Welpro precipitate enabled use of the high-speed centrifuge to separate the precipitate from the solubles. Without bisulfite, the bulk Welpro precipitate contained small amounts of fibrous protein curds. These small fibrous curds were not discharged from the centrifuge bowl and eventually led to bowl clogging and shutdown. The bisulfite addition also increases the nutritional value of the Welpro to a level equivalent to that of casein. An evaluation of the protein nutritional quality of both Welpro and Pro-Xan II may be found in other publications (Bickoff et al., 1975a,b).

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Soluble Proteins of Alfalfa (*Medicago sativa*) Herbage. Fractionation by Ammonium Sulfate and Gel Chromatography

Subodh K. Sarkar,[1] Ronald E. Howarth,* Mitsuru Hikichi,[2] and John M. McArthur[2]

Procedures were devised for the isolation of three soluble protein fractions: (1) fraction I protein, (2) high molecular weight fraction II proteins, and (3) low molecular weight fraction II proteins. Fraction I protein was isolated by sodium sulfate fractionation and Sepharose 6B chromatography. It was completely dissociated into two subunits at pH 11.7. The sedimentation coefficient of alfalfa fraction I protein was similar to those reported for other species. The two groups of fraction II proteins were isolated using ammonium sulfate fractionation and chromatography on Sephadex G-25 and G-150. Average sedimentation coefficients of the high and low molecular weight fraction II protein groups were 6.8 and 3.8, respectively. The high molecular weight fraction II group contained two predominant proteins while the low molecular weight fraction contained many proteins. *o*-Diphenol oxidase activity was most effectively inhibited by storing the forage under a nitrogen atmosphere and by extraction in the presence of 5.0 m*M* metabisulfite.

The soluble leaf proteins are the predominant foaming agents in legume forage crops and are responsible for pasture bloat in ruminant animals grazing alfalfa or clover pastures. In the course of our studies on pasture bloat we wished to know whether or not there are differences among soluble alfalfa leaf proteins in their ability to stabilize persistent foams in the rumen. If differences do occur, they should be indicated by certain physical–chemical properties related to surface activity, i.e., isoelectric point (Cumper, 1953), solubility, hydrophobicity, and stability of tertiary configuration (Evans et al., 1970). Isolation of the protein fractions under consideration was required for the study of these parameters.

The soluble leaf proteins are classified into two major

Agriculture Canada Research Station, Saskatoon, Saskatchewan, Canada, S7N OX2.
[1] Present address: Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2E1.
[2] Present address: Agriculture Canada Research Station, Summerland, British Columbia, Canada, VOH 1Z0.