# PRO-XAN Process: Methods for Increasing Protein Recovery from Alfalfa

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Various pilot plant extractors were compared for their efficiency in separating solids, carotene, xanthophyll, and protein from fresh alfalfa. The equipment studied included a twin-screw press, a single-screw press, a V-press, and sugar cane rolls. The twin-screw was superior to the other expressers, removing about 30% of the solids, 25% of the carotenoids, and 40% of the protein in one pass. A second pressing without added water removed an additional 10% of the carotenoids and solids and 13% of the protein. After removing 40–50% of the initial proteins with the twin-screw press or three rollings through sugar cane rolls, the residual expressed cake prepared from good quality alfalfa still retained about 20% protein and about 100 mg/lb of carotene and 250 mg/lb of xanthophyll.

The PRO-XAN process is oriented toward the removal of protein in excess of the guaranteed minimum for dehydrated alfalfa (Dehy) (Spencer *et al.*, 1970; Knuckles *et al.*, 1970). Because of the establishment of standard grades of Dehy with fixed levels of protein and with set prices, excess protein does not yield its full value for the producer. However, the full value of the protein in alfalfa can be realized by extracting and selling this excess protein in a higher priced concentrate. The success of this process is evidenced by the commercial production of a stable protein–xanthophyll concentrate (Spencer *et al.*, 1971; Witt *et al.*, 1971).

As the market for the low fiber protein–xanthophyll concentrate increases, more efficient extraction of protein and xanthophyll from large quantities of alfalfa will be desirable. Pirie stated that sugar cane rolls have a low efficiency because of the short duration of pressure (Pirie, 1956). However, the extraction efficiency of rolls can be increased by multiple passes through them (Knuckles *et al.*, 1970). Pirie and associates have recommended that a combination of pulper and a belt press be used (Pirie, 1969; Davys and Pirie, 1960, 1965). This combination has good extraction efficiency because 50–70% of leaf protein can be extracted (Davys *et al.*, 1969; Pirie, 1966). Chayen *et al.* (1961) reported a 75% extraction of protein when plant material had been pulped with an excess of water present. In high efficiency extractions, however, the operator runs the risk of lowering the protein concentration of the fiber residue below the minimum accepted for standard grades of Dehy. The twin-screw press, which is capable of dewatering 40 tons of paper pulp per hour, appears to be acceptable for use in the PRO-XAN process. It is an efficient extractor of unpulped material, as are other screw presses (McDonald *et al.*, 1954) and it is relatively inexpensive. The fibrous residue from this press, upon cubing, would supply the "roughage factor" which contributes to the performance of cattle under feed lot conditions (Aughtry, 1971). This paper is concerned with the evaluation of the twin-screw press in relation to other extraction equipment.

## EQUIPMENT

**Twin-Screw Press.** A horizontal screw press (Bauer Brothers Co., Helipress No. 585) consisting of two counter-rotating intermeshing screws was used to press field-chopped alfalfa. This press had a capacity of 9000 lb of alfalfa per hr when the intermeshing screws driven by a 25 hp motor and cross discharge screw driven by a 5 hp motor were operated at near minimum speeds (48 and 19 rpm, respectively). The maximum speeds of the intermeshing and discharge screws are 180 and 45 rpm, respectively.

**Single-Screw Press.** A vertical press (Stearns Roger, experimental model) consisting of a single screw fitted with stops for its full length was also used for extraction. This press, driven by a 10 hp motor, had a capacity of 16,000 lb/hr.

**Sugar Cane Rolls.** Laboratory-scale sugar cane rolls and related equipment (Knuckles *et al.*, 1970) were used to press the field-chopped alfalfa. The rolls, driven by a 5 hp motor, had a capacity of 1000 lb/hr.

**V-Press.** A continuously fed revolving wheel press (Reitz Manufacturing Co., model RVP 36) was used for extraction of leaf components. This press, driven by a 15 hp motor, had a capacity of 4000 lb/hr.

**Disintegrator.** An angle feed disintegrator (Reitz Manufacturing Co., model RP 6K 115) with a screen having $^3/_4$-in.$^2$ openings spaced $^1/_4$ in. apart was used to pulp alfalfa prior to pressing. The disintegrator is driven by a 3 hp motor.

## PROCEDURE

Commercially harvested alfalfa, with or without disintegration, was fed into the sugar cane rolls. Disintegration, when used, was carried out in the presence of a weight of water equal to that of the alfalfa. In the case of multiple rolling, the pressed cake was mixed with an amount of water equal to the amount extracted in the prior pressing.

Alfalfa pressed by V and screw presses was not disintegrated. The resulting pressed cakes and juices were weighed and sampled immediately. The samples were freeze-dried prior to analysis.

## ANALYTICAL METHODS

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

**Proximate Composition.** Ash, ether extractives, crude protein, and crude fiber were determined by the standard AOAC methods (Official Methods of Analysis, 1970). Crude protein refers to Kjeldahl $N \times 6.25$.

**Carotenoids.** Carotenoid determinations on whole and pressed alfalfa were carried out by the method of Knuckles

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**Table I. Percent of Alfalfa Components Extracted by Various Presses**

| Press | Fresh weight, % | Solids, % | Carotene, % | Xanthophyll, % | Crude Protein, % | Ash, % |
|---|---|---|---|---|---|---|
| | Component extracted | | | | | |
| Sugar cane rolls | 30 | 13 | 10 | 9 | 17 | 18 |
| V-Press | 35 | 13 | 8 | 7 | 23 | 25 |
| Single-screw | 54 | 19 | 18 | 15 | 28 | 35 |
| Twin-screw | 63 | 30 | 26 | 25 | 40 | 48 |

**Table II. Percent of Alfalfa Components Extracted by Multiple Rolling[a]**

| Passes | Solids, % | Carotene, % | Xanthophyll, % | Crude Protein, % | Ash, % |
|---|---|---|---|---|---|
| | Component extracted | | | | |
| Field-chopped[b] | | | | | |
| First | 13 | 8 | 8 | 15 | 22 |
| Second | 11 | 7 | 7 | 13 | 12 |
| Third | 5 | 5 | 5 | 12 | 12 |
| Total | 29 | 20 | 20 | 40 | 46 |
| Disintegrated alfalfa[b] | | | | | |
| First | 19 | 17 | 26 | 30 | 34 |
| Second | 6 | 4 | 7 | 9 | 11 |
| Third | 5 | 3 | 4 | 7 | 10 |
| Total | 30 | 24 | 37 | 46 | 55 |

[a] Data obtained from single determinations. [b] An equal weight of water was added prior to first rolling and initial disintegration. Water, equal to weight of juice removed, was added after the first and second pressing.

*et al.* (1971). The carotenoids in extracted juice were determined by the AOAC method (Official Methods of Analysis, 1970).

## RESULTS AND DISCUSSION

The twin-screw press, which occupies a relatively small area (7 × 8 × 12 ft), was capable of processing much larger quantities of alfalfa than previously possible in the pilot plant. The full capacity of the twin-screw press was not achieved because the feed system was capable of supplying material for minimum operation only (9000 lb/hr). At this capacity, about 65% of the fresh weight was removed as juice. This is about twice the extraction obtained by a single pressing in sugar cane rolls or V-press (Table I). The twin-screw press also extracted more juice than the single-screw press. The difference in extraction between these two machines was at least partly attributed to differences in moisture content of the starting material, since extraction and moisture content are directly related (Casselman *et al.*, 1965; Knuckles *et al.*, 1970). The alfalfa pressed in the twin-screw press had a moisture content of 80% while the material pressed in the single-screw press had a moisture content of 74%. The juice extracted by the various machines contained 4–7 g of nitrogen per liter. Of this, about 75% was insoluble in 10% trichloroacetic acid. Thus, the juice contained 20–35 g of true protein per liter.

When extraction of carotenoids and protein was considered, the twin-screw press proved very satisfactory. This press extracted twice the amount of carotenoids and protein extracted by a single pressing in sugar cane rolls (Table I). This extraction was similar to that obtained by triple rolling of field-chopped alfalfa (Table II). Extraction of carotenoids, solids, and protein by the twin-screw press was also greater than their extraction by the single-screw press. The difference in solids and protein extraction may reflect the higher prevailing temperature during extraction with the single-screw press (25 *vs.* 10°C), since solids and nitrogen extractability decreases with higher temperature (Halverson, 1962).

**Table III. Proximate Composition of Juice Solids Extracted by Various Presses[a]**

| Press | Nitrogen, % | Protein, % | Fat, % | Fiber, % | Ash, % |
|---|---|---|---|---|---|
| Twin-screw | 5.9 | 36.6 | 2.2 | 1.0 | 16.9 |
| Sugar cane rolls | 6.2 | 39.0 | 1.4 | 0.7 | 16.7 |
| V-Press | 6.5 | 40.6 | 0.4 | 0.4 | 17.6 |
| Single-screw | 4.4 | 27.0 | 1.0 | 0.9 | 16.4 |

[a] Different alfalfa was used in each press. The alfalfa used in the single-screw press contained 2.9% N; the other batches of alfalfa contained 3.7% N.

**Table IV. Proximate Composition of Pressed Cake Residues[a]**

| Fraction | Nitrogen, % | Protein, % | Fat, % | Fiber, % | Ash, % |
|---|---|---|---|---|---|
| Whole alfalfa[b] | 3.9 | 24.4 | 4.5 | 20.3 | 10.7 |
| Pressed cake | | | | | |
| Sugar cane rolls | | | | | |
| One rolling | 3.5 | 21.7 | 4.0 | 27.5 | 8.7 |
| Three rollings[c] | 3.1 | 19.5 | 3.6 | 33.4 | 6.4 |
| Twin-screw press | 3.1 | 19.4 | 3.7 | 28.5 | 7.4 |
| Single-screw press[d] | 2.7 | 16.9 | 3.2 | 28.0 | 5.5 |
| V-Press | 3.8 | 23.8 | 4.3 | 27.4 | 9.7 |

[a] Data is expressed on a dry weight basis. [b] Representative of alfalfa used in presses other than the single-screw press. [c] Water was added after first and second rolling. [d] Alfalfa pressed in the single-screw press contained 18.1% protein, 3.2% fat, and 7.2% ash.

Double pressing of field-chopped alfalfa in the twin-screw press increased the extraction of protein from 40 to 53%, an increase of 13%. The carotenoid and solids extraction was increased 10%. The extraction in either single or double pressing would have been improved if the starting material had been pulped prior to pressing. Evidence to show that pulping or disintegration increases extraction is given in Table II. In this case, multiple rolling was used to evaluate the extraction of components from field-chopped and disintegrated alfalfa. Disintegration more than doubled the amount of carotenoids removed in the first pressing; the amount of protein removed was doubled. The higher yield reported by Pirie (1966), Chayen *et al.* (1961), Davys *et al.* (1969), and Huang *et al.* (1971) also resulted from pressing of pulped material.

Since screw presses are more efficient extractors (McDonald *et al.*, 1954), disintegration of material would be less important for good extraction. Without prior disintegration of material, a screw press would yield a residue with a greater ratio of long fiber (1 1/8 in.) to short fiber (<1 in.). Such a long fiber product would supply the "roughage factor" for cattle (Aughtry, 1971).

The proximate compositions of juice solids, other than those extracted in the single-screw press, were similar to each other (Table III) as well as to those previously reported (Hartman *et al.*, 1967; Knuckles *et al.*, 1970). The lower nitrogen value for juice solids from the single-screw press was a result of a lower nitrogen content in the alfalfa. In all cases, juice solids show a nitrogen enrichment (1.6 times) over that of whole alfalfa. The juice preparations, on a dry weight basis, contained 150–160 mg/lb of carotene and 295–320 mg/lb of xanthophyll. These values are equal to or greater than those for juice used to prepare a protein–xanthophyll concentrate for poultry feed (Spencer *et al.*, 1970).

The proximate compositions of the residues from the twin-screw press and from triple rolling were similar (Table IV).

These residues contained about 100 mg/lb of carotene and 250 mg/lb of xanthophyll and are high in nitrogen. The residues from the other presses had a protein, fat, and ash content similar to that of the original alfalfa. All residues contain more fiber than is accepted for standard grades of Dehy. The maximum crude fiber in 17 and 20% protein grade Dehys is 27 and 22%, respectively. These residues could meet the standards for good quality Dehy if the nitrogen-containing brown juice from the PRO-XAN process were added back. The addition of brown juice solids would dilute the fiber without significantly lowering the protein. Even without the brown juice added back, the pressed cakes could be used as a ruminant feed. Hollo and Koch (1970) also felt that such residues would be satisfactory for ruminants.

There was almost no loss of crude protein in any of the presses. However, there were losses of carotenoids. In sugar cane rolls, there was a loss of about 10% in a single pass and a loss of about 30% during three rollings. The overall loss of carotenoids in single and double passes through the twin-screw press was 20 and 40%, respectively. A possible explanation for the larger loss of carotenoids in the twin-screw press is that increased rupture of cells would result in the release of increased amounts of enzymes, such as lipoxygenase, which induce carotenoid oxidation (Ben Aziz *et al.*, 1968).

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# Composition of Commercial Wheys

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Commercial whey samples of single cheese types and of blended wheys were analyzed for their protein, carbohydrate, lipid, and ash contents which averaged 9.7, 71.7, 1.28, and 8.2%, respectively. The amino acid compositions of the dialyzable and nondialyzable fractions of selected whey samples were compared to those of a control whey.

The 22–23 billion pounds of whey produced annually by the cheese industry pose a substantial challenge to feed and food technologists, and are a major problem in pollution control. Although approximately one-third of the whey produced is utilized in feed and food formulations, new product development is required for a greater utilization of this by-product. To facilitate this development, we have investigated the nitrogen distribution, protein, carbohydrate, and lipid contents and the amino acid compositions of the dialyzable and nondialyzable nitrogen fractions of several commercial and laboratory wheys.

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## EXPERIMENTAL

**Whey Samples and Fractions.** Whey samples were obtained as dried solids or as pasteurized liquids which were subsequently freeze-dried. The whey samples were obtained from the following sources: sweet whey blends A and B, Kraft Foods, Chicago, Ill.; blend C, Foremost Foods Co., San Francisco, Calif.; blend D, Meinerz Creamery, Fredericksburg, Iowa; and blend E, Pollio Dairy, Campbell, N.Y.; Swiss whey, Star Valley Swiss Cheese Co., Thayne, Wyo.; 25% Cheddar–75% Swiss whey, Cache Valley Dairy Association, Smithfield, Utah; cottage whey A, Kraft Foods, Chicago, Ill.; cottage whey B, Cheddar and skim Cheddar