The PRO-XAN Process: The Design and Evaluation of a Pilot Plant System for the Coagulation and Separation of the Leaf Protein from Alfalfa Juice

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A pilot plant coagulation system capable of handling over 90 gal of plant juice per hour was assembled and evaluated for the preparation of leaf protein concentrate. The effect of pH on the coagulation of the green juice protein and the resulting effect on the pilot plant coagulation system was determined. The best starting material for the system was found to be juice, prepared from ammoniated freshly chopped alfalfa, with pH of 8.0–8.5. The ammonia treatment essentially eliminated carotenoid losses and caused denser and larger curds to form during coagulation, which facilitated their separation in the processing operation.

Fractionation of fresh alfalfa into several valuable animal products, better suited than dehydrated alfalfa meal for monogastric and ruminant animals is the objective of a new processing method (Kohler et al., 1968; Spencer et al., 1970). The new process, named after the protein-xanthophyll concentrate produced, involves four basic steps: (1) expression of the juice; (2) dehydration of the dewatered alfalfa; (3) coagulation and separation of the protein and xanthophyll in the juice; and (4) drying of the coagulum. In the first step of the process the alfalfa is crushed to express some of the liquid from the plant cells to yield a green, low-fiber juice containing 7¹/₂ to 8% solids and a pressed cake. The pressed alfalfa, with a third to half of the water removed, is dehydrated to produce a dehydrated alfalfa which will meet market grades and be saleable without penalty. In related studies by Hartman et al. (1967), it had been suggested that the whole green juice could be spray-dried and used as a protein concentrate. These workers pointed out that toxic factors such as saponins may lower the feed value but that the spray-dried product may be suitable in limited amounts for some species. For example, the amount of alfalfa that can be fed in poultry rations is limited; however, hogs thrive on alfalfa alone.

In the case of alfalfa, water-soluble saponins would be concentrated by the preparation of a whole juice concentrate (Walter et al., 1954). Therefore, in the third step of the process, the green juice is processed by heat-coagulation to form a green protein-xanthophyll coagulum containing 12 to 15% solids and a brown juice fraction (alfalfa solubles) containing 5% solids. After drying, the protein fraction (PRO-XAN) is suitable for use in the poultry industry as a low fiber, high xanthophyll pigmenter for broilers and laying hens. The alfalfa solubles, containing amino acids, sugars, minerals, and vitamins, can be useful at low levels as a liquid feed supplement for ruminant animals such as cattle (Perry et al., 1969) or as a source of other unidentified growth factors (Kohler, 1953).

Although the literature on leaf protein contains numerous reports on the equipment used to mechanically express juice from green plant material (Pirie, 1942, 1956; Tilley and Raymond, 1957), there has been very little information on methods for automating the overall process of coagulation and curd separation. Nozzles which can be used to coagulate the juice protein have been described by Ahmed and Singh (1969) and Pirie (1957). The objective of the work reported here was to design a simple continuous system which would coagulate the protein in the green juice, separate it from the brown juice, and produce the protein-xanthophyll concentrate suitable for final drying. In addition, the system had to be capable of scale-up to juice volumes of at least 2000 gal per hr. It has been reported that commercial equipment such as vacuum filters or centrifuges is available to handle the coagulated material (Chayen et al., 1961; Pirie, 1957, 1966; Raymond and Tilley, 1957). Although this type of equipment could be used, the capital outlay and operating cost were considered excessive for a feed grade product.

EXPERIMENTAL

Analytical Methods. SAMPLE PREPARATION. All wet products were freeze-dried for analytical determination. Results were calculated on a dry weight basis.

PROXIMATE ANALYSIS. Minerals, ether extractives, and crude fiber were determined by the standard AOAC methods (Official Methods of Analysis, 1965). Kjeldahl nitrogen $\times$ 6.25 was used for crude protein determination.

CAROTENE-XANTHOPHYLL DETERMINATIONS. The coagulum was analyzed by the Kohler procedure (Kohler et al., 1967). The green juice was analyzed by the Quackenbush procedure (Quackenbush et al., 1970). The percent transmittance of each of the carotenoid solutions was determined on an Evelyn colorimeter using a No. 440 Evelyn filter for carotene and a No. 470 filter for xanthophyll. The transmittance readings were converted into absorbance using a calibration curve which had been prepared by comparing identical solutions on the colorimeter and on a Cary Model 15 spectrophotometer.

MOISTURE CONTENT. The amount of moisture in each sample was determined by drying samples at 110° for 2 hr in a forced draft oven.

Effect of pH on the Curd Properties. CURD SIZE. Ammonia was added to the alfalfa before rolling to give green juice with pH values of 6.5, 7.5, and 8.5. For comparison, samples of untreated green juice (pH 5.8) were adjusted to comparable pH levels with aqueous ammonia. The green juice samples (200 ml) were heated to 82° C on a steam bath with gentle stirring, removed from the heat, and stirred vigorously for 1 min with an overhead stirrer. After standing for 5 min, the particle size of the coagulum was determined by weighing the wet material retained, respectively, on No. 14
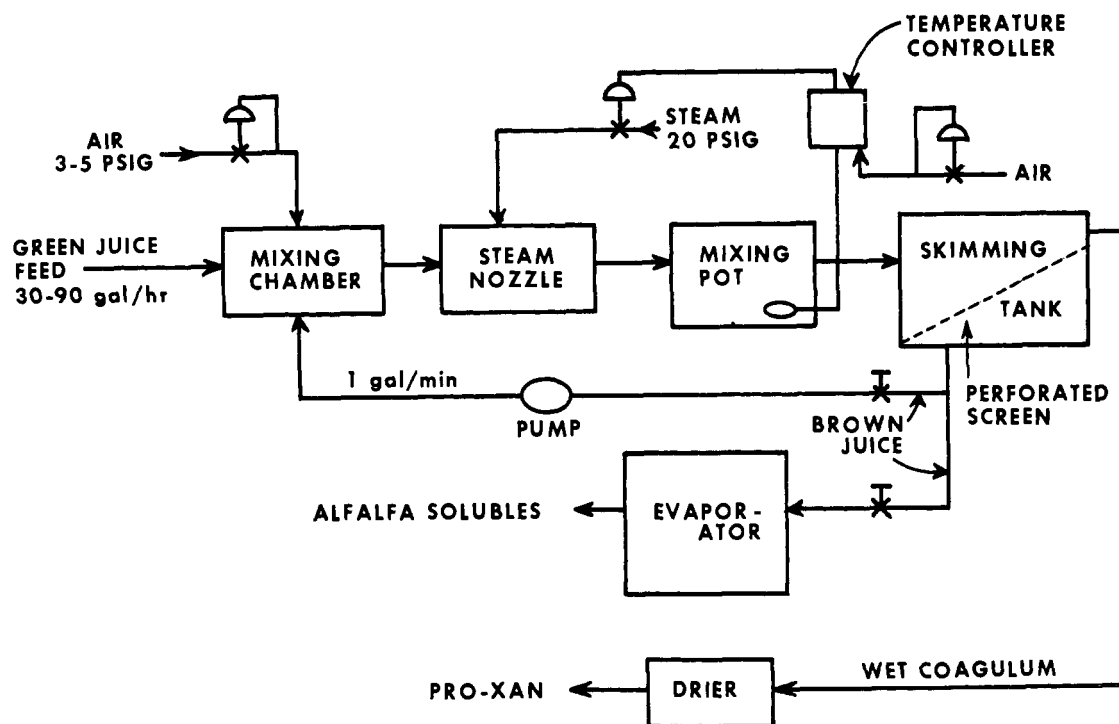
Figure 1. Flow sheet of the pilot plant PRO-XAN process

and 20 U.S. standard screens. The weight of the sediment which passed through both screens into the bottom pan was also determined.

CURD HARDNESS. Curd hardness was measured by means of a penetrometer (Precision Scientific Co.). The pH of the juices used in these tests ranged from 5.8–8.8, depending on the amount of ammonia added before rolling. Each juice sample was coagulated with the steam nozzle and discharged into a 3-gal tank. After standing for 1 hr, the coagulated mixture was uniformly mixed and poured onto a No. 20 U.S. standard screen. The coagulum was allowed to drain for 3 min then transferred to an aluminum weighing dish (9 cm in diameter, 5 cm high). The contents were scraped level with the top of the dish and the penetrometer readings were obtained by employing a cone-shaped plunger weighing 14.8 g which was connected to a shaft weighing 15 g. The distance these weights penetrated (mm) was averaged for plotting in the following pH ranges: 6.0 to 6.5, 6.6 to 7.0, 7.1 to 7.5, 7.6 to 8.0, 8.1 to 8.5, and 8.6 to 9.0.

EFFECT OF AMMONIA ON COAGULATION AND SEPARATION OF THE CURD FROM THE BROWN JUICE. Thirty-gallon batches of green juice were prepared from untreated and ammoniated alfalfa. The latter produced juice pH levels of 6.5, 7.5, 8.0, and 8.5. The untreated alfalfa yielded juice with a pH of 5.8. The coagulation system described below was charged with brown juice, preheated to 80–82° C, and the green juice was pumped into the system at a flow rate of 1 gal per min. From the wet and dry weights of the green juice, green coagulum, and brown juice, material balances through the system could be calculated. Any loss in total solids through the system was assumed to be sediment that had settled to the bottom of the skimming tank. The values were obtained from duplicate runs carried out over 4 separate days.

PILOT PLANT EQUIPMENT. The approximate size of the pilot plant equipment (Figure 1) is as follows. The mixing chamber was a standard pipe cross with a ³/₈ in. inside diameter. The dimensions of the steam nozzle are given in Figure 2. The mixing pot was 10 in. in diameter and 13 in. high,

with a liquid capacity of 4¹/₂ gal. The skimming tank was 20 in. in diameter and 24 in. high, with a liquid capacity of 30 gal. The skimming tank was equipped with an overflow lip and an overhead S-shaped paddle. The metal paddle was a little smaller than the diameter of the tank and was provided with rubber tips to scrape the sides of the skimming tank.

DESIGN AND OPERATION OF THE
COAGULATION SYSTEM

A flow sheet of the coagulation system is shown in Figure 1. The equipment was designed to handle total liquid flow rates up to 150 gal per hr (gph). The green juice was prepared as previously described (Knuckles et al., 1970). A steam injector, used for the rapid heat processing of fluid foods (Brown et al., 1951; Morgan and Carlson, 1960), was tested as a means of rapidly heating the green juice. Due to the back pressure caused by the discharge orifice and low flow rate, the coagulum plugged the injector. The unit was modified to discharge directly to the atmosphere (Spencer et al., 1970). This nozzle functions similarly to units described by Ahmed and Singh (1969) and Pirie (1957), except that in our unit the green juice is introduced into the center of the steam flow, as shown in Figure 2. In this way the protein in the juice is coagulated before it comes into contact with the hot outer walls of the nozzle, which helps to reduce plugging at flow rates less than ¹/₂ gal per min.

At flow rates greater than ¹/₂ gal per min the nozzle performed satisfactorily when used in a batch type operation. The coagulated mixture was discharged into a 30-gal container and processed in a similar manner to that previously described (Morrison and Pirie, 1961). However, when adapting the nozzle to a continuous process, incomplete coagulation occurred at flow rates over 1 gal per min. In the continuous process the coagulum was skimmed off the surface of the fluid before complete coagulation occurred. In order to assure complete coagulation in our system, we added more heat to the green juice by blending hot brown juice with the freshly expressed green juice. This raised the temperature of the
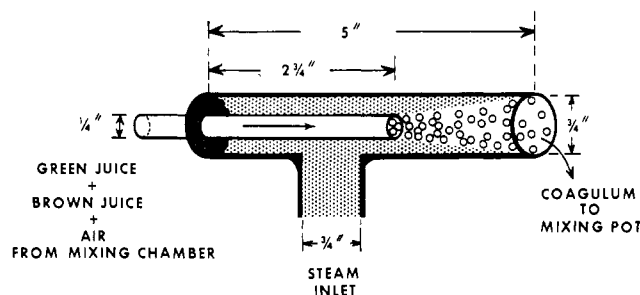
Figure 2. Steam nozzle for coagulation

juice mixture to 45–55° C. The recirculating brown juice both preheated the green juice and increased the flow rate, so that green juice flow rates less than $1/2$ gal per min could be handled successfully. The brown juice also returned some of the remaining fine coagulum from the bottom of the skimming tank to the nozzle, where it was removed from the system by coagulating green juice. The brown juice flow rate was generally set at 60 gal per hr.

The coagulum prepared in this manner was inconsistent in its density. From some batches of alfalfa a higher percentage of curd would float than from others. Since the juice can be foamed quite easily, we took advantage of this property in our pilot plant process to effect a froth-flotation separation of the coagulum from the brown juice by the introduction of air. The air pressure, generally 3–5 psig, was adjusted to give a rich green lather-like foam. The addition of air did not harm the quality of the product.

The resulting air–liquid mixture was discharged directly into the steam nozzle where steam at 20 psig was used to coagulate the green xanthophyll-protein concentrate. The coagulated foamy mixture passed into a mixing pot where it was well mixed by the force of material discharging from the nozzle. The mixing pot also allowed time for the curd to coalesce and float. The temperature of the pot was maintained at 80–82° C by modulating the steam flow to the nozzle. From the mixing pot the mixture emptied into the skimming tank for the separation of the curd from the brown juice.

Since the green foam would not flow off the skimming tank by itself, a screw was designed to remove the coagulum. Although this system worked satisfactorily, the size of the skimming tank was limited to the diameter of the screw. An S-shaped skimming paddle rotating at 8 rpm was found to work as well and permitted the use of larger skimming tanks. A 10–15% increase in total liquid volume was contributed by the steam, which is in agreement with previously published values (Morrison and Pirie, 1961).

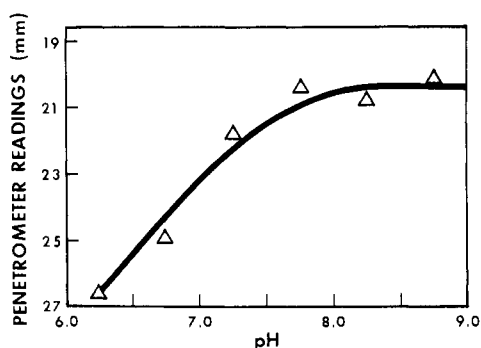A fine perforated screen at the bottom of the skimming

tank filtered the fines and settled material from the brown juice. The accumulation of settled material did not cause any problem in test runs up to 6 hr in length. The brown juice was clarified and concentrated to 60% solids content in a vacuum evaporator. These alfalfa solubles did not spoil when stored at 104° F for 60 days in closed containers which contained a large headspace of air.

RESULTS AND DISCUSSION

The addition of ammonia to the alfalfa before rolling affected the physical properties of the coagulated curd. Larger curds resulted from the coagulation of the green juice protein as the pH increased. This is shown in Table I, where the screen with the larger mesh size (No. 14) retained over three times more coagulum from pH 8.5 juice than was retained from the untreated juice (pH 5.8). The use of ammonia before rolling had little effect on the fines or sediment that passed the No. 20 screen (Table I, sediment A). However, when ammonia was added to the juice after rolling, more fines passed through the screens at each of the pH levels (Table I, sediment B). The remaining wet weight distribution was similar. Thus, for the preparation of large curds, ammonia must be added prior to pressing the juice from the green plant material.

Ammonia added before rolling also beneficially affected the hardness of the curd (Figure 3). Each point on the curve represents an average of three to five different determinations from four different batches of alfalfa. The hardness of the curd increased as the pH increased, leveling off in the 8.0 region. A harder curd is easier to handle in the processing operation.

Weight balances of the coagulation and separation process further emphasized the effect of pH on the coagulation of the green juice protein (Table II). The separation of the untreated juice into coagulum and brown juice fractions showed that 14% of the solids originally present settled to the bottom of the skimming tank. The amount of settling decreased as the pH increased. Only 2% of the total weight from the pH 8.0 or 8.5 juice ended up in the sediment fraction.

Table I. Effect of Ammonia on Curd Size of Wet Coagulum

| | Coagulum Distribution | | | | |
| | Screen Number | | Total | Sediment[c] | |
Sample pH	14[a] %	20[a] %	Screened[a] %	A[a] %	B[b] %
5.8	19.7	72.5	92.2	7.8	...
6.5	23.6	69.2	92.8	7.2	16.8
7.2	34.4	60.3	94.7	5.3	16.1
8.5	63.8	30.0	93.8	6.2	13.8

[a] From juice, ammonia treated before rolling. [b] From juice, ammonia added to juice after rolling. [c] Fine coagulum that passed both screens.

Table II. Effect of Ammonia on Weight Balances through Coagulator[a]

Sample pH	Separated Coagulum Solids[a] %	Brown Juice Solids %	Sediment %
5.8	37	49	14
6.5	41	48	11
7.5	40	52	8
8.0	48	50	2
8.5	46	52	2

[a] Includes insoluble solids plus a fraction of the soluble solids proportional to the moisture content. Calculated on a dry weight basis.

Figure 3. The effect of pH on curd hardness

Table III. Carotene-Xanthophyll[a] Loss During Coagulation

Sample pH	Carotene Loss %	Xanthophyll Loss %
5.8	10.6	36.5
6.5	17.5	42.8
7.5	5.8	29.7
8.0	5.4	26.6
8.5	0.0	5.8

[a] Average initial carotenoid content of whole juice; carotene 120 mg/lb, xanthophyll 292 mg/lb calculated on a dry weight basis.

Table IV. Proximate Composition of the Two Products[a] of the Coagulation Process

Sample pH	Protein %	Crude Fat %	Fiber %	Ash %
		PRO-XAN		
5.8	49.0	9.49	1.26	11.1
7.5	49.2	7.62	1.43	12.8
8.5	53.2	7.10	1.67	12.1
		Alfalfa Solubles		
5.8	16.3	0.66	0.74	21.4
7.5	17.8	0.53	0.68	21.2
8.5	20.8	0.53	0.70	20.5

[a] Dry weight basis.

The carotenoid losses during coagulation are summarized in Table III. The greatest carotene-xanthophyll loss occurred at pH 6.5. This would be expected from previous studies on the carotene destruction in alfalfa (Bondi et al., 1968; Grossman et al., 1969). Maximum loss was found to occur in the pH range of 6.6 to 6.8. The addition of ammonia completely eliminated the carotene loss at pH 8.5 and substantially reduced the xanthophyll loss. Ammonia had some effect on the composition of the xanthophyll-protein concentrate (Table IV). There was a small increase in the percentage of crude protein (N × 6.25) in both the concentrate and the brown juice fractions, as would be expected in part from the added ammonia and from the greater amounts of protein extracted by base (Crook, 1945).

Morrison and Pirie (1961) reported that the pH of the green juice from other leaf species should not be greater than 6 or the texture of the curd would not be satisfactory. However, the changes in alfalfa curd properties caused by adjustments to

higher pH values are beneficial to our coagulation and separation process. The ammonia stabilized the carotenoids and the resulting coagulum was easier to handle in our pilot plant. Larger and denser curds were formed which separated more readily from the brown juice. The optimum pH range for these effects has been found to be 8.0 to 8.5.

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