THE DEVELOPMENT OF ALFALFA LEAF PROTEIN CONCENTRATES FOR HUMAN AND ANIMAL USES

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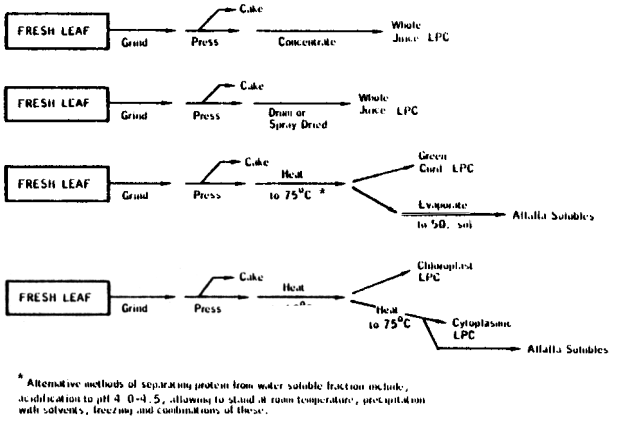
Protein, the basic constituent of all living things, and a nutritional essential for animals, occurs in varying amounts in almost all of our foods. It is of interest to note that most of the world's protein is found in grasses, alfalfa, and other forage plants. They in turn provide the basis for animal agriculture. In the United States alone, more than 130 million tons of grass and legume hays are produced annually, over half of which is provided by alfalfa (U.S. Stat. Rept. Ser., Crop Prod., 1969).

Because of the great amount of research effort that has been expended on forages, their production and utilization have become most efficient. This efficiency has resulted in the continuing evolution of a highly sophisticated dehydration industry, both here and abroad. The value of the protein that is contained in dehydrated alfalfa can be calculated as 10 to 15¢ per pound, based on the fact that dehy at 17 to 20 percent protein sells for 40 to $60 per ton. Fresh forage in the field sells for 10 to $20 per ton (dry), or 2.5 to 5¢ per pound of protein. Since soybean meal costs about $70 per ton (10¢ per pound of protein), forages provide a comparatively low cost raw material for protein recovery. Additionally, the amino acids of alfalfa protein are nutritionally well balanced and equivalent to those of soy protein.

The relative abundance of protein in leaf material and the basic need for protein in the human diet are the reasons why scientists have tried to prepare edible proteins from green plant tissue for more than 50 years. The stumbling block has proved to be the economical separation of protein from the indigestible components of plant cells. As Osborne et al. stated in 1920, "If we can learn to separate the contents of the cell from these (i.e., cell walls and water), we shall obtain a food product of very great value." Our laboratory initiated experiments along these lines in the early 1940's, about the time that Dr. N. Pirie began his work in England. Keys to the more recent literature are found in the bibliographies of the papers of Pirie (1969a,b), Akeson (1966), Akeson et al. (1966), and Oelshlegel et al. (1969). The earlier literature has been reviewed by Tilley et al. (1957). In the present paper we shall examine the technological and economic factors which have, until recently, prevented commercialization of leaf protein concentrate (LPC). We shall also describe our experimental solution to many of these problems.

The basic steps for the production of LPC, shown in Figure 1, have not changed during the past 30 years. The first step is the grinding and pressing of the plant material to rupture the cells and express the juice from the insoluble fiber. As shown in Processes 1 and 2, the juice, containing soluble protein and suspended chloroplasts (Kohler et al., 1951), may be concentrated or dried to provide whole juice LPC (Hartman et al., 1967). Alternatively, the juice may be heated to 75°C (Process 3) to provide an insoluble protein coagulum and a clear brown juice containing the non-protein components. If heating to 60°C takes place as the first step (Process 4), the chloroplastic protein precipitate contains the chlorophyll and carotenoid pigments, leaving about half of the protein in solution. The cytoplasmic proteins can be precipitated from this solution by raising the temperature to 75°C. Although heat treatment is a simple way to precipitate the leaf proteins, they may also be precipitated by treatment with acid, solvents, salts, etc. In both processes 3 and 4, the LPC can be preserved by drying and the protein-free brown juice can be stabilized by concentrating to 50% solids or higher (Bickoff et al., 1947; Chayen et al., 1961; Crook, 1946; Duckworth et al., 1961; Henry et al., 1965 Kohler et al., 1951; Lugg, 1939; Morrison et al., 1961)

Although this brief description of the four processes makes LPC production sound very simple indeed, a number of factors combine to make the recovery of plant protein relatively low. In the first place, only about 75% of "crude" protein (Kjeldahl nitrogen times 6.25) is "true" protein; the rest is non-protein nitrogen (NPN). Although more than half of the NPN consists of free amino acids, they are not coagulated with LPC but remain in the alfalfa solubles. Drying or concentrating of the whole juice (Processes 1 and 2, Figure 1) will retain these amino acids in the product, but Pirie (1969b) has shown that the soluble fraction of leaves may contain undesirable flavors and even nutritionally undesirable alkaloids and glycosides. Thus, any commercial process for the production of LPC should utilize a method in which the soluble materials have been removed from the coagulated proteins.



* Alternative methods of separating protein from water soluble fraction include, acidification to pH 4.0-4.5, allowing to stand at room temperature, precipitation with solvents, freezing and combinations of these.

Figure 1.--Several processes for preparing LPC and byproducts

A second factor which limits the yield of LPC is the fact that it is virtually impossible to rupture all of the cells in the plant tissue during the grinding and pressing operations. From the yields obtainable by various workers, it seems likely that 75 percent breakage is the maximum achievable under practical conditions.

The third factor which severely limits yields during pressing is the fact that the fiber mat acts as a filter which prevents passage of many of the chloroplasts (Davies et al. 1965). Since about half of the leaf protein is associated with the chloroplasts, these losses can be serious. Ways in which the filtration loss can be reduced include press design to minimize the thickness of the plant tissue layer (Davies et al., 1965; Morrison et al., 1961), pulping with an excess of water (Chayen et al., 1961; Morrison et al., 1961), and rewashing the pressed residue (Bickoff et al., 1947; Morrison et al., 1961).

Although the yields of LPC may be lower than one might hope for, the amino acid content of the heat-coagulated soluble leaf proteins is very encouraging. The amino acid composition, as published by Byers (1971), is shown in table 1. The composition of LPC

Table 1.--Amino acid composition of heat-coagulated leaf protein (g. amino acid per 100 g. recovered amino acids)

Amino acid	Concentrations
Aspartic acid	9.9
Threonine	5.1
Serine	4.5
Glutamic acid	11.7
Proline	4.7
Glycine	5.6
Alanine	6.3
Cystine	1.6
Valine	6.2
Methionine	2.0
Isoleucine	4.8
Leucine	9.5
Tyrosine	4.6
Phenylalanine	6.2
Lysine	6.8
Histidine	2.4
Arginine	6.6

is relatively constant from species to species; in all cases, the first limiting amino acid is methionine. Although the methionine contents of the chloroplastic and cytoplasmic proteins are the same, the cystine content is higher in the cytoplasmic proteins than in the chloroplastic fraction. This is encouraging since cystine exerts a sparing action on the nutritional need for methionine. Temperatures should be controlled carefully during processing, since the protein digestibility of LPC is quite sensitive to heat damage (Davies et al., 1965; Duckworth et al., 1961; Henry et al., 1965). Lipid oxidation and the Maillard reaction (carbonyl-amino interaction) have both been implicated in this heat-sensitivity.

In general, the biological value of the protein of carefully prepared LPC is superior to that of soybean and other seed proteins and approaches that of milk protein. Within LPC itself, the chloroplastic proteins have lower biological value and digestibility than do the cytoplasmic proteins (Davies et al., 1952; Henry et al., 1965).

Perhaps the most important drawback to the acceptance of LPC as a food is its objectionable color and flavor (Pirie, 1969b). Although Pirie (1969a) has made heroic efforts to introduce LPC successfully into the developing countries, it is our belief that a food product should be essentially free of lipid to prevent autoxidative flavors during storage. Such a product can be made by solvent extraction of LPC (Akeson, 1966; Henry et al., 1965) or by separation and precipitation of the cytoplasmic proteins. Although the yield in the latter case would be lowered drastically, the chloroplastic proteins could still serve as an excellent source of proteins for animal feeds.

The successful commercial production of LPC has been hindered by the problems that have been discussed, as well as by competition from oilseeds and peanuts. In our reentry to this area of research, we developed a program whose prime objective was the development of superior animal feeds. Once this objective had been achieved, it could serve as a sound economic base for the development of a low-cost LPC that was colorless, bland, odorless, and suitable for human nutrition.

The primary research goals were: (a) reduction of processing cost of dehydrated alfalfa by mechanical dewatering, (b) production of a high-protein-high-xanthophyll product (PRO-XAN) eminently suitable as a pigmentation supplement for poultry, and (c) recovery of the water-soluble components from the coagulation step in concentrated form as a source of unidentified growth factors. Once these goals had been attained, the second phase of the program would be the production of two leaf protein fractions: (a) a modified PRO-XAN containing all the carotenoid pigments but little or no cytoplasmic protein, and (b) an edible protein product that was bland, colorless, and odorless. As a result of our Phase I objectives, the PRO-XAN process has been developed (Figure 2) and the results have been

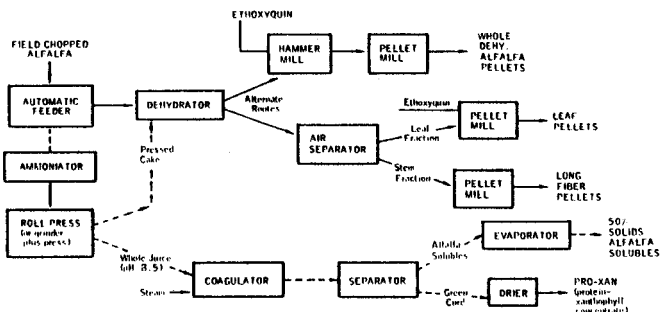


Figure 2.--The PRO-XAN Process

described in several publications (Knuckles et al., 1970; Lazar et al., 1971; Spencer et al., 1971; Witt et al., 1971). Basically, the PRO-XAN process follows process 3 of Figure 1, although some modifications had to be made.

As the first step in the process, a sugar cane roll press was used to perform both the grinding and pressing operations. Although roll presses are considered to be relatively inefficient (Morrison et al., 1961), they are used in our process because of their large throughput. They also are available for large scale operations, thus requiring no additional engineering research. In our pilot plant rolls, we can obtain 35 to 50 percent of the alfalfa fresh weight as press juice at a feed rate of 1000 pounds per hour. Table 2 shows the wet and dry yields of various fractions of the process, based on fresh

Table 2.--Yield of various fractions of the PRO-XAN process

Fraction	35 Percent juice expression		50 Percent juice expression	
	Fresh weight	Dry weight	Fresh weight	Dry weight
	Pounds	Pounds	Pounds	Pounds
ıh alfalfa	100	20	100	20
Pressed residue	65	17.6	50	16.5
Green juice	35	2.4	50	3.5
Coagulum	6.6	1.1	9.5	1.6
Brown serum	32.9	1.3	47.0	1.9

alfalfa having a moisture content of 80 percent. The composition of the fractions is shown in table 3. Even at a 50 percent juice expression rate, the level of protein in the

Table 3.--Composition of various fractions of the PRO-XAN process

Component	Fresh alfalfa	Fractions			
		Pressed residue		Coagulum	Serum
		35 Percent juice	50 Percent juice		
Solids, percent*	20	27	33	17	4
Protein, percent	23	21	20	55	22
Fiber, percent	23	26	27.5	3	0

*Solids expressed as percent fresh weight; others based on dry weight.

pressed residue is still 20 percent, starting from fresh alfalfa with 23 percent protein. Because so much of the water has been removed during pressing, the dehydrator throughput has been increased by about 40 percent, a fact which greatly reduces the cost of dehydration.

When alfalfa is crushed and pressed without pH adjustment, xanthophyll is lost at a rapid rate. When the pH is adjusted to 8 or over, this loss is reduced greatly, presumably due to inhibition of lipoxidase in alkaline solutions. The pH adjustment is accomplished by the addition of gaseous ammonia to the alfalfa just prior to rolling. Because of the value of xanthophyll in poultry rations (10 to 20¢ per gram), any modification which improves the yield of xanthophyll should be incorporated. Computer evaluations show that PRO-XAN, with 40 percent protein and 700 mg/lb xanthophyll would have been worth over $300 per ton in a layer ration based on the Kansas City market, December 1966 (Taylor et al., 1968).

The addition of ammonia has a number of beneficial effects. These are summarized in table 4.

Difficulties with steam injection were overcome by use of a redesigned steam injection unit which permitted coagulation without plugging. The coagulated curd may either be floated off the top of a separation tank or allowed to settle prior to separation from the brown juice. A newly designed drag separator (Figure 3) permits continuous separation of all types of coagulum from residual brown juice. This consisted of a trough-like tank with sloping bottom, perforated metal flights. The separated curd is then pressed to yield a cake containing about 40 percent solids which is then crumbled in a granulator and dried in a hot air drier holding the product temperature below 60°C. Under these conditions xanthophyll losses are held to a minimum.

Table 4.--Benefits of addition of ammonia at the grinding-pressing step of PRO-XAN process

1.	Increased recoveries of xanthophyll due to reduced lipoxidase activity.
2.	Maintenance of green color (i.e., chlorophyll).
3.	Improved curd hardness and handling properties.
4.	Increased yield of protein.
5.	Reduced autolyses of protein due to reduced protease activity.
6.	Ammonium salts in the alfalfa solubles byproduct are utilized as N.P.N. for ruminants.

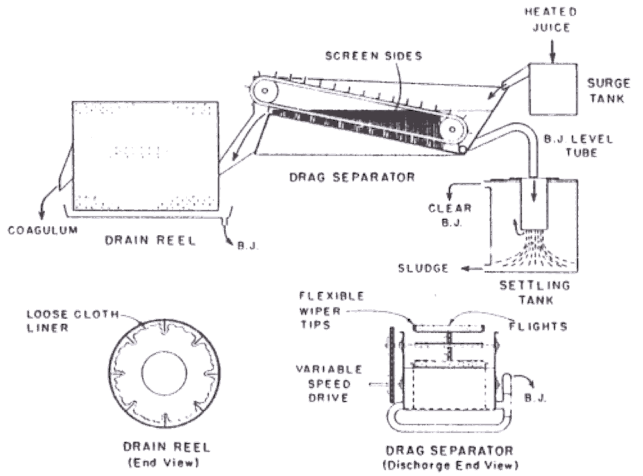


Figure 3.--Pilot plant for separation of coagulum from brown juice in the PRO-XAN process

As our laboratory and pilot plant work progressed, our industry collaborators moved ahead installing full-scale equipment approximately 50 times the size of our pilot plant. This plant has now been in successful operation for two years. While there is still work to be done to increase throughput and product quality and to develop markets, at least several of our main objectives have been accomplished. While continuing to work out details of the Phase I research (PRO-XAN Process), most of our efforts are now directed to the next phase, that of converting the PRO-XAN to human grade LPC plus a stabilized xanthophyll product for poultry.

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