Appendix

Technical Papers

AN ALTERNATE USE FOR TOBACCO AGRICULTURE: PROTEINS FOR FOOD PLUS A SAFER SMOKING MATERIAL

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Abstract

Tobacco could be developed as an important food crop in combination with its traditional use for smoking and chewing. As a food crop, tobacco grown in dense spacing could produce about four times more protein per acre than soybeans and about five times more dry tobacco for smoking than conventional tobacco crops. A simple method has been developed for extracting the proteins from the aerial portions of fresh tobacco and other plants. The water soluble proteins have unique nutritional and functional properties that could make them valuable for the package food industry and for medical use. The insoluble proteins have properties similar to soy protein. A byproduct of growing tobacco for food proteins is a deproteinized smoking tobacco that has lower concentrations of chemical compounds known or suspected to have adverse effects on cigarette smokers,

Plant, Extraction Products, and Extraction Techniques

Plant

Ninety-nine percent of the tobacco used for smoking and chewing is obtained from *Nicotiana tabacum*. This plant was a weed believed to have been domesticated in Latin America long before Columbus' discovery of the New World. Today tobacco is grown on every continent except Antarctica, and is found in both hemispheres at latitudes from the Equator to 55°. World demand for tobacco is enormous and, despite smoking's adverse effects on health, the demand continues to grow. At least 4 trillion cigarettes were consumed in 1981. About 2.5 million hectares (6,2 million acres) of land worldwide are devoted to tobacco plants that are used exclusively for smoking and chewing. China uses nearly a million hectares for growing tobacco, and the United States nearly 400,000 hectares, followed by smaller areas in nearly every other country in the world.

Some people believe that the future of tobacco is in jeopardy, at least in Western countries where per capita tobacco consumption is declining. Tobacco is being attacked increasingly by health authorities, as medical evidence accumulates on the adverse effects of smoking on health. However, this situation could change because a simple process that extracts high-grade proteins suitable for human consumption from tobacco plants has been developed (9). A byproduct of the extraction process is a deproteinized cigarette tobacco (10) considerably reduced in components hazardous to smokers' health,

● Retired, Leaf Protein International, Inc.

Potential Uses of Major Extraction Products

The extraction process perfected to a pilot plant stage by Leaf Proteins, Inc. (LPI) yields the following six potentially useful products.

1. **Crystalline Fraction 1 protein.** This product constitutes more than 30 percent of the total protein in tobacco leaves. It is soluble in water, is tasteless and odorless, and is composed entirely of amino acids of high nutritional quality. When fed to rats, crystalline Fraction 1 protein exhibited a somewhat higher protein efficiency ratio (PER) than casein, the standard commonly used for comparing the nutritional quality of proteins (4).

Crystalline Fraction 1 protein has unique properties that might be used in medicine. When washed free of sodium and potassium, the crystals might be used in patients with kidney failure to meet their amino acid requirement without taxing the kidney's inability to eliminate cations. Crystalline Fraction 1 might also be used to meet the entire protein requirement of patients with acute gastrointestinal problems or patients recovering from surgery of the alimentary canal. The amino acid mixtures used in patients with kidney disease are expensive and they taste, at best, vile. Since crystalline Fraction 1 protein is tasteless, it could be incorporated into appetizing diets and is potentially more economical than amino acid mixtures now used.

Properties of crystalline Fraction 1 protein render it potentially useful to the package food industry. Because it is tasteless and odorless, it could be added to food without altering the food's taste and smell. As a dry, almost white powder, it could be mixed with cereal grains to improve their protein content and quality. It might be used as a milk substitute in countries that lack a dairy industry. In its soluble form, it could be added to soft drinks, removing them from the junk food category. Similar to egg albumin, Fraction 1 protein irreversibly gels when heated above 80° C (176° F). Its func-

tional properties, such as "heat set," are similar to egg albumin or casein that are widely used throughout the package food industry. The amino acid composition, protein efficiency ratio (PER) data, and data concerning functionality properties of Fraction 1 protein are given in appendix 1.

2. **Fraction 2 protein.** This product also is water-soluble and of high nutritional value. Its main use could be as a supplement to upgrade the nutritional quality of cereals and other food products. The functional properties of Fraction 2 protein are less desirable than those of Fraction 1 protein, but improvements in extraction procedures may eliminate this difference. The amino acid composition of Fraction 2 protein is shown in appendix 2.

3. **Green sludge.** This product consists mainly of water insoluble proteins and starch. Because it also contains beta carotene and xanthophyll, green sludge could be marketed as a supplemental feed for poultry and monogastric animals. By solvent extraction, green sludge can be converted into a material similar in properties and amino acid composition to defatted soybean meal. The decolonized material could be used in the food industry for the same purposes as soybean meal. The essential amino acid compositions of green sludge protein and of soy flour protein are compared in appendix 3.

4. **Green residue.** This product is a fibrous material whose solids are composed of more than 50 percent cellulose and hemicellulose and about 13 percent protein. Its nutritional value is similar to that of alfalfa hay. By solvent extraction, green residue is dehydrated and converted into white fibers that can be used for manufacturing deproteinized cigarette tobacco. The chemical composition of dried green residue and a comparison of the composition of white fibers and wheat bran are given in appendix 4.

5. **Pigments and other bio-organic compounds.** These are products of organic solvent extraction of green residue and green sludge. The material can be separated further into chlorophyll, carotenes and carotenoids, solanesol, and other compounds of lipoidal nature. Poultry producers use large quantities of purified carotenoids.

6. Low **molecular weight compounds.** This product is composed of water soluble sugars, amino acids, vitamins, salts, and other compounds. When concentrated by evaporation of water to about 10 percent solids, the product probably could be used as a fermentation liquor. The product is used as a flavoring and coloring material which, when added to the white fibers, produces a deproteinized cigarette tobacco low in components thought to be harmful to health. The apparent tar and nicotine composition of LPI deproteinized tobacco and conventional cigarette tobacco are compared in appendix 5.

Processing Technique

Operation of a pilot plant in North Carolina during 1981 and 1982 demonstrated the commercial feasibility of the LPI process for obtaining crystalline Fraction 1 protein and the other raw materials from tobacco. The processing technique is diagramed in figure 1. There are six steps of the LPI process as described below,

1, **Disintegration of plants.** The aerial portions of fresh tobacco plants are placed on a conveyor (fig, 2) for transport to the mouth of a Rietz Disin-

Figure 1.—Schematic of the LPI Process for Obtaining Proteins and Other Products From Tobacco and Other Plants

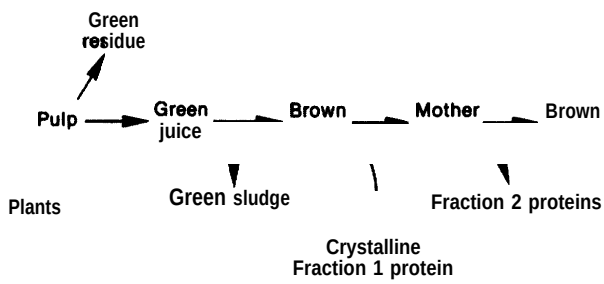
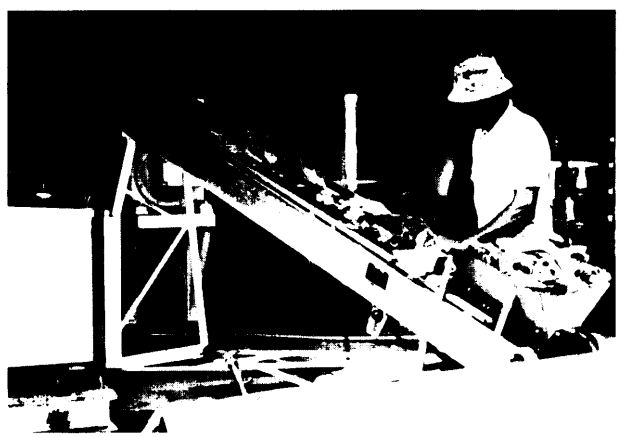


Figure 2.–Conveyor Transporting About 15 lb of Tobacco Plants per Minute at Beginning of LPI Process



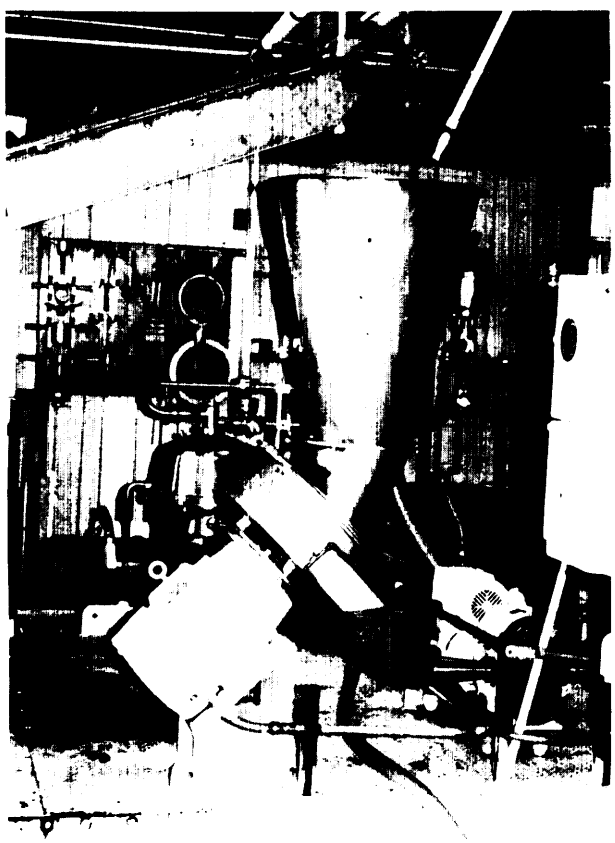
tegrator (fig. 3), where the plants are sprayed with a 0.5 percent solution of sodium metabisulfite in water as they fall into the pulping device. Sodium metabisulfite, listed as GRAS, is used widely in the food industry. It is the only exogenous chemical added during the extraction process.

z. **Extraction of green juice from disintegrated plants.** The pulp emerging from the disintegrator is pumped into a Rietz Rotary Press (fig. 4) where pressure from a mechanical screw forces out a green juice through a fine-mesh screen while retaining the green residue until it is discharged into a wheelbarrow (fig, 5) at the other end of the press.

3. **Removal of green sludge from green juice.** The green juice emerging from the press is pumped through a heat exchanger so that the juice is heated rapidly to about 1250 F, then cooled rapidly to room temperature. The abrupt temperature change aids in particulate matter aggregation to which the green pigments and other lipoidal compounds are attached. The juice then enters a Westphalia SB-7 Continuous Flow Centrifuge (fig. 6) which removes all of the starch and about 85 percent of the green particulate material which is discharged periodically as green sludge (fig. 7). A partially clarified brown juice emerges from the centrifuge.

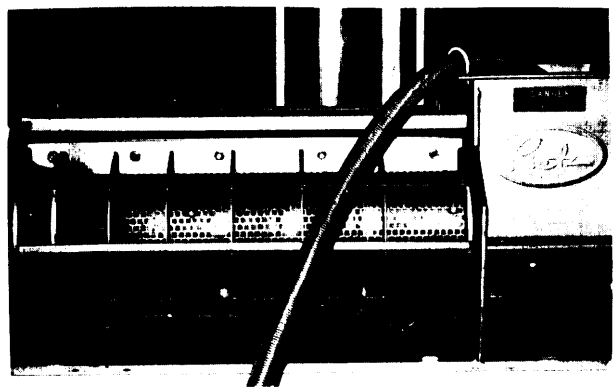
4. **Filtration of brown juice.** The partially clarified brown juice containing soluble proteins is pumped to a Rotary Vacuum Filter (fig. 8) whose filter is made out of diatomaceous earth. The filter removes the last traces of green sludge. The clear brown juice (fig. 9) emerging from the filter (fig. 10) is sent to a storage tank where crystallization of

Figure 3.—Plants Falling Off Conveyor Into Mouth of a Rietz Disintegrator



White pipe at 60° angle at top right is fitted with a nozzle to spray plants with 0.5 percent sodium metabisulfite solution as they pass into pulping chamber. Pulp is pumped (lower right) to the press shown in figure 4

Figure 4.—Hose Containing Pulp Being Conveyed to Opening of a Rietz Rotary Press



Green juice is squeezed out through fine-mesh, stainless steel screen in center of press and drips into sump beneath. Green residue is forced by rotating screw to left end of press for discharge

Figure 5.—Wheelbarrow Located Beneath Press To Catch Discharged Green Residue With Moisture Content of About 65 Percent

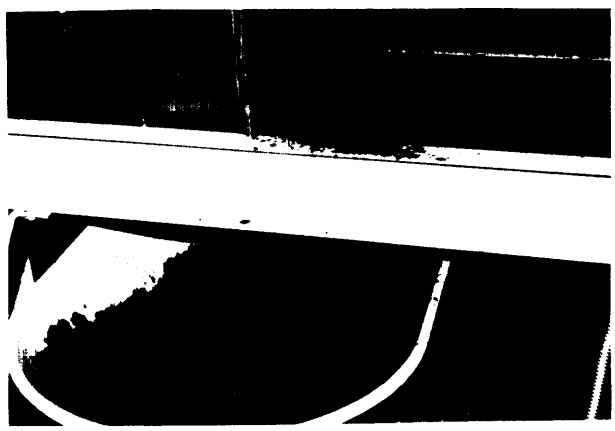
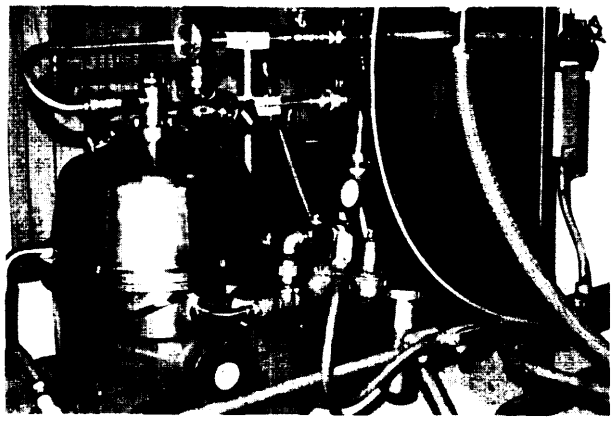


Figure 6.—Westphalia Disk Type Centrifuge



Used to remove about 65 percent of green sludge from green juice coming from press and after having been heated momentarily to 150° F and cooled to ambient temperature on the way. Green juice enters centrifuge at top and exits via plastic hose partially hidden by dial (center of photo) on way to rotary press shown in figure 7

F gu e 7 Green S udge A e D scha ge From Cen fuge

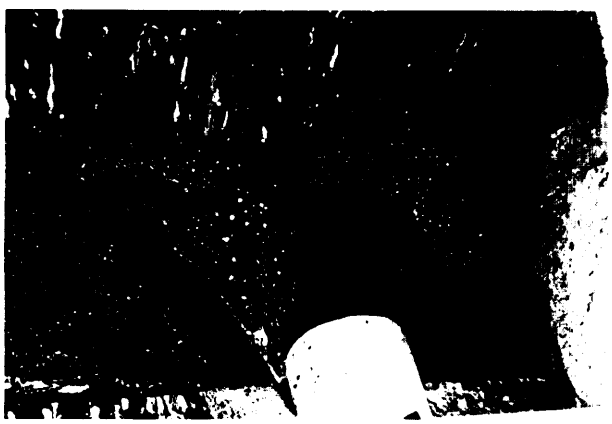
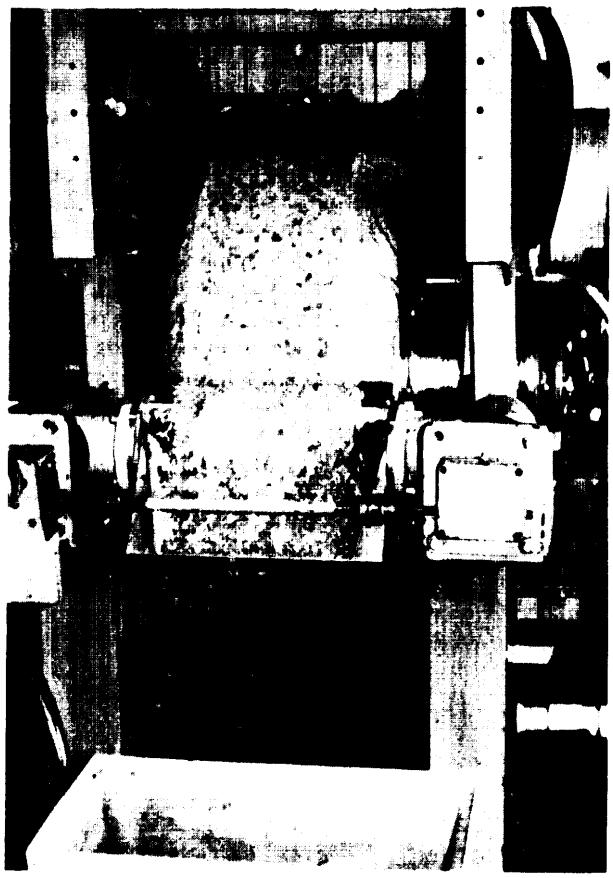


Figure 8.- Rotary Vacuum Filter Whose Surface is Covered by a Thick Layer of Diatomaceous Earth Which Dips Into a Reservoir of Plant Juice (obscured In photo) as it Rotates



Green sludge not previously removed by centrifuge In figure 6 is caught on surface of filter while brown juice containing soluble proteins is sucked through the pores of the filter cake by the vacuum. Green sludge "blinds" the filter so that the surface must be constantly renewed by the knife seen removing very thin layer of filter cake so that a renewed filter surface reenters the reservoir of plant juice at bottom of filter

Fraction 1 protein begins within 3 to 6 hours of storage and is completed within 3 to 4 hours after the first crystals appear. If left undisturbed, the crystals will settle into a layer at the bottom of the vessel.

5. **Collecting and washing Fraction 1 protein crystals.** Because funding limitations have precluded purchase of equipment for a more efficient washing method, the crystals are collected by passage through the SB-7 centrifuge and the mother liquor is sent to a storage tank. The crystals are resuspended in tap water followed by recentrifugation and a second washing cycle. After the crystals are discharged from the centrifuge and allowed to settle, they compact into a nearly colorless suspension of about 10 percent protein in water (fig. 11).

6. **Collecting and washing Fraction 2 protein.** While not a desirable method because of denaturation of proteins, limited resources necessitated adding acid to the mother liquor to precipitate the Fraction 2 proteins at pH 4. The Fraction 2 protein precipitate is collected by centrifugation, and the brown liquid containing the low molecular weight compounds is sent to a storage tank. The precipitate then is resuspended in water and washed in the same manner as the Fraction 1 protein crystals.

Figure 9.—Brown Juice Coming From Rotary Vacuum Filter at About 4 L/rein (1 gal/min) Entering Storage Tank Where Crystallization of Fraction 1 Protein Will Occur

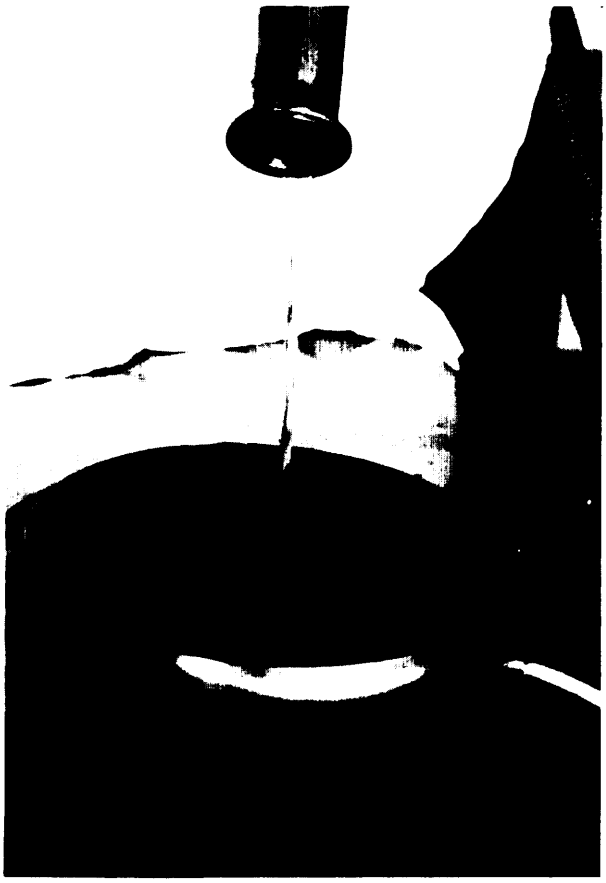


Figure 10.—Appearance of Brown Juice Before Crystallization of Fraction 1 Protein Has Occurred Showing To Be Devoid of Green Sludge

Level of Development of Technology

LPI, a California corporation, developed and patented its simple method for obtaining crystalline Fraction 1 protein and developed and patented a method for converting green residue into a deproteinized tobacco suitable for cigarettes, To explore the commercial possibilities of the protein extraction process, LPI formed a joint venture with the North Carolina Farm Bureau Federation which, together with General Foods Corp., provided funds and equipment to construct a pilot plant at Lucama, N. C., in the heart of the flue-cured tobacco region. The pilot plant was operated for the first time in August 1980, but was unable to produce Fraction 1 protein of desired purity during the remaining 2 months of the growing season. The following summer, acceptable Fraction 1 protein was made in a few experiments, but unusual drought conditions destroyed much of the plant material designated for the pilot plant program. Each experiment required a minimum of 600 lbs (272 kg) of fresh plants. Not until July 1982 could the pilot plant consistently produce crystalline Fraction 1 protein of high purity. As of September 1982, the pilot plant has operated without failure on 14 occasions to produce Fraction 1 protein crystals. The plants used in these experiments were harvested from various fields, at various stages of growth, and after exposure to various climatic conditions. The success of the pilot plant operations conducted during 1982 demonstrates the feasibility of the LPI process as a method of producing pure Fraction 1 protein and its byproducts on a commercial scale.

The existing pilot plant is capable of processing about 1 gallon (3.785 l) of plant juice per minute or about 1 ton of fresh tobacco plants during a 6-hour run. After 6 hours, the process flow must be interrupted for 3 to 4 hours to clean up and remake the

Figure 11.—Appearance of a Suspension of Washed Fraction 1 Protein Crystals

Crystals obtained by processing about 1 ton of tobacco" plants by the LPI process. Crystals have settled below the 60 L mark on the vessel, the layer of crystals consisting of about 10 percent Fraction 1 protein (dry basis)

filter cake. With another rotary filter, the pilot plant could be operated continuously. With larger capacity filters, the process rate could be doubled or tripled without changing the other equipment. With a larger capacity disintegrator, the process could handle 5 gallons (19 l) of juice per minute.

According to laboratory determination, the yield of raw products from 1,000 kg of fresh tobacco plants is as follows:

- 20 kg of Fraction 1 protein crystals (80 percent water);
- 10 kg of Fraction 2 protein precipitate (80 percent water);
- 250 kg green residue (64 percent water) containing raw material for cigarette tobacco;
- 100 kg ton green sludge (70 percent water) containing insoluble proteins and starch; and
- 1,060 1 (280 gal) of brown liquid containing 2 percent low molecular weight, water soluble, solids.

Wet Fraction 1 protein crystals were shipped to a custom, spray-drying company which found that spray-drying produced a powder of Fraction 1 protein that was stable at room temperature. The powder readily dissolved in water to produce a Fraction 1 protein solution with properties equivalent to those before drying.

Table 1 shows the expected yield of dry Fraction 1 protein and its finished byproducts per 60 tons of fresh tobacco. This is the average biomass of closely grown tobacco plants expected per acre per growing season in a climate like that of North Carolina. The figures in column 3 of table 1 are based on existing pilot plant yields, but for realization would require additional spray-drying capacity of 1.3 gal/hr (4.9 l/hr), water evaporation capacity of

Table 1.—Yield and Estimated Value of Finished Products From Tobacco

Finished product	Minimum estimate of wholesale value	At 60 ton plant/acre/ growing season	
		Yield (dry)	Value
White fibers for cigarette tobacco	30¢/lb	5.3 ton	*$3,500*
Decolonized insoluble protein + starch (equivalent to soybean meal)	$150/ton	1.8 ton	290
Carotenoids for poultry	$7/02 (25¢/gram)	13.2 lb (6 kg)	1,500
Fraction 1 protein (equivalent to egg white)	$1.81/lb ($4/kg)	529 lb (240 kg)	980
Fraction 2 protein (equivalent to soy protein)	45¢/lb ($ l/kg)	265 lb (120 kg)	120
Condensed brown liquid	No estimate	1.4 ton	No estimate
			$6,370/acre

13 gal/hr, and organic solvent extraction and recovery capacity of 5.3 gal/rein. Column 4 shows the estimated dollar value of the products based on the minimum estimate of wholesale value in column 2.

State of Art; Additional Research and Development

As mentioned earlier, the SB-7 centrifuge was used for collecting and washing Fraction 1 protein crystals. Preliminary studies indicate that washing Fraction 1 protein crystals by ultrafiltration or reverse osmosis could be more efficient. Additional comparative experiments need to be made before deciding on which equipment to install for continuous operation.

Preliminary experiments on a laboratory scale suggest the possible advantage of simultaneous concentration and washing of Fraction 2 protein by ultrafiltration, a procedure that would avoid the denaturation now caused by acid precipitation. Further experiments are needed before deciding on the best equipment to install for continuous production of the Fraction 2 protein product.

More work needs to be done on solvent extraction of green residue and green sludge and on methods for recovering the solvent and refining the carotenoids and other compounds extracted by the organic solvent. Preliminary work need not be on a scale to keep up with the green juice process stream, but should be at a level sufficient to produce 5 to 10 lb batches of white fibers needed for evaluation by tobacco manufacturers.

Other Sources of the Extracttion Product

About 40 years ago, N. W. Pirie (8) of England advanced the idea of using leaf proteins for human food and developed a technology for extracting them from various plant species, While nutritious, the protein preparations were green colored and had an odor, taste, and texture that made them largely unacceptable to the human palate,

1. **Pro-Xan process for obtaining alfalfa proteins.** A group at the USDA Western Regional Research Laboratory under the leadership of Dr. G. O. Kohler modified and enlarged the Pirie process to where 10 tons/day of alfalfa leaf protein known as "Pro-Xan" could be produced for animal feed (5), A plant of this capacity consumed 40 tons/hr of green chopped alfalfa. In 1977, the cost of such a plant was estimated by USDA to be $3,334,477 (3). The annual operating cost, for 22 hr/day and 130 days/yr, was estimated to be $401,796, Field chopped alfalfa is treated with ammonia while being crushed to express a green plant juice whose proteins then are coagulated by heat. When dried, the green Pro-Xan contains 57 percent protein, 9 percent lipoidal material, and 100 mg xanthophyll per kg. Xanthophyll, a yellow pigment, is valuable for the color it imparts to chicken flesh and egg yolks. Industrial plants were in operation until the market for dehydrated alfalfa disappeared in the United States. A Pro-Xan II process evolved to the pilot plant stage whereby a Pro-Xan type of product containing less protein and more lipid was removed before the remaining soluble proteins were coagulated by heat to produce, when dry, a bland, off-white material containing 90 percent protein (2). The off-white protein was greatly improved in the organoleptic properties that prevented acceptance of the original Pro-Xan by humans. Table 2 shows some of the differences in quality of the products produced by the Pro-Xan II process from alfalfa compared with the products produced by the LPI process from tobacco.

2. **LPI process for alfalfa and other plants.** The products enumerated in table 1 have also been obtained on a laboratory scale by the LPI process from the aerial portions of alfalfa, soybean, clover, and

Table 2.—Quality of Products Obtained From Alfalfa Plants by the Pro-Xan II Process Compared With Those From Tobacco Plants by the LPI Process

Product	Alfalfa	Tobacco
Green residue	Can be used only for animal feed	Used for deproteinized cigarette tobacco and for animal feed
Green sludge.	Same as tobacco	Same as alfalfa
Fraction 1 protein. . . .	Denatured together with Fraction 2 protein to yield water insoluble material containing 90°/0 protein	Obtained as crystals containing more than 99°/0 protein which redissolves in water
	2% of total solids	4% of total solids
	No functionality	Functional properties similar to egg white
	Some taste and color	Odorless and tasteless
Fraction 2 protein. . . .	Denatured and mixed with Fraction 1 protein	Contains no Fraction 1 protein
		2°/0 of total solids

sugar beets, and probably could be obtained from other plants with succulent leaves such as cotton, tomato, eggplant, spinach, etc.

3. **USDA method for obtaining tobacco proteins as byproducts of the homogenized leaf curing process,** USDA has been developing a process known as homogenized leaf curing (HLC) that was conceived by Dr. T. C. T'so and associates as a means to provide a simplified industrial method for producing safer tobacco. Donald De Jong and Jesse Lam, Jr. (1) set up a pilot plant at Oxford, N. C., for recovering edible proteins from the plant juice released during the pulping process at the beginning of the HLC process. After squeezing the green juice from the residue, green sludge is removed from the juice by centrifugation. The soluble proteins then are precipitated by acid and the deproteinized liquid is added back to the green juice. The mixture is allowed to incubate at ambient temperature before being dried and made into deproteinized, reconstituted tobacco sheet for cigarette manufacture. The acid coagulated proteins are less valuable than crystalline Fraction 1 protein from tobacco because acid denaturation destroys the functional properties of the protein. However, it would be relatively simple to modify the Oxford pilot plant to produce crystalline Fraction 1 protein by the LPI process.

Use of Technology

LPI was founded in 1979 with the goal of transforming tobacco into a commodity used on a large scale for human food as well as for smoking. Private capital supported the pilot plant operation. A great opportunity exists for the private sector to participate in developing the pilot plant process into a profitable commercial venture. The possible role for government is twofold. First, it could have a short-term role in providing financial aid to perfect the process so that large enough quantities of the products can be made for long-term animal feeding tests. These tests are needed to obtain FDA approval before Fraction 1, Fraction 2, and the insoluble proteins can be marketed for human consumption. Second, government could support testing of the health effects of deproteinized tobacco relative to conventional tobacco. If deproteinized tobacco prepared by the LPI process is less hazardous, governmental involvement with this product is justified by national health considerations.

Agronomic Considerations and Requirements

Agricultural Scales and Systems

1. **Biomass in relation to protein yield.** The agronomic factors that affect smoking quality and now determine the value of conventional tobacco are not so important when tobacco plants are to be used for proteins and deproteinized tobacco. During processing of the green residue into reconstituted cigarette tobacco, flavorants can be added to make the product acceptable to smokers, Thus, the most important agronomic factor affecting commercial development of the LPI process will be maximizing production of plant biomass per unit of land per growing season at minimum cost.

Production of biomass is a direct function of photosynthesis. The amount of photosynthesis is related to the total area of green leaves exposed to the sun (7). When the bottom leaves are shaded from sun and turn yellow, the photosynthate accumulated in the form of starch, proteins, sugars, and other compounds is destroyed in a secondary process of supplying nutrients to the roots and topmost portion of the plant where rapid growth is occurring. A balance is reached where further growth in the biomass will not increase the protein yield per unit of biomass. This balance exists when the canopy of plant leaves completely covers the ground and the plants have grown until the leaves nearest the ground start to turn yellow. At this point, the plants should be harvested and processed for maximum yield of proteins.

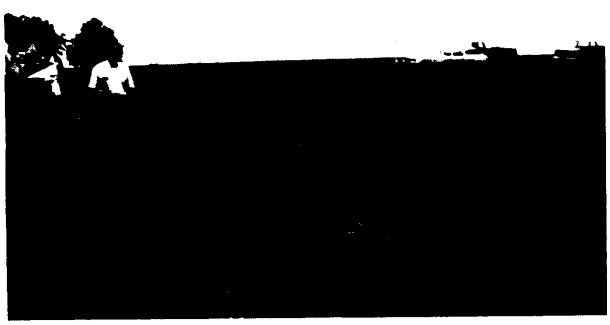
2. **Multiple harvests of densely spaced plants.** Tobacco has tiny seeds, so plantlets arising from seeds also are small and vulnerable at the beginning of the growth period. With ideal conditions of moisture, soil fertility, temperature, duration of sunshine, and freedom from insect and parasite attack, in 5 to 6 weeks the seedling could grow to a height of 18 to 20 inches with its leaves covering an area of 1 square foot or more. Under these conditions, a planting density of 44,000 plants per acre would produce a closed canopy 5 to 6 weeks after seeding. Periods of drought, inclement weather, etc. would lengthen the time until the canopy is closed. One or two weeks of additional growth after the canopy closes brings the biomass to the balance point at which it should be harvested.

During harvest when the stalks of tobacco plants are cut off about 4 inches above the ground, each stalk will regenerate two to three new aerial shoots. If soil fertility and moisture are maintained, the new shoots will grow rapidly and form new leaves, soon growing to a size close to that of the previously harvested shoot. The leaf canopy of closely spaced plants with regenerated shoots will close in about 5 weeks, so that a second harvest of biomass can be made about 6 weeks after the first harvest. By this process of "rattooning," four biomass harvests have been obtained from the same plants in the 5-month growing season in North Carolina. The appearance of densely grown plants ready for a first harvest in early June 1982 is shown in figure 12.

3. **Dense spacing by transplanting.** Because of their small size, tobacco seeds are usually germinated in plastic-covered seed beds whose soil has been fumigated with methyl bromide to eliminate diseases and, most importantly, to prevent weeds from germinating and crowding out the smaller tobacco plants. The tobacco seeds are sown at a density of about 100 seeds per square foot, and about 2 months after germination are ready for transplanting. With existing farm equipment, transplants for protein production could be spaced at about 30,000 plants per acre so that closing the canopy might require 7 to 8 weeks with a similar time interval between rattoon harvests.

4. **Close-spacing by direct sowing of seeds.** It is likely that the need for transplanting can be avoided in growing tobacco for protein production. For example, each year S. P. Willingham of Florida grows 160 acres of directly seeded, closely spaced tobacco seedlings, which he sells for transplants to other tobacco farmers. To avoid fumigation he clears new palmetto scrubland each year and prepares the soil in 6-foot-wide beds raised 6 to 8 inches above the surrounding soil. The region is frost-free and irrigation keeps the small plantlets moist during their vulnerable period immediately after germination. There is no need to cover the beds with plastic. Even when sown in the cool period during the last week of December, in 60 days the plants will have grown to harvestable size for protein production. The disadvantage of the system is the requirement for new land each year.

Figure 12.—Close-Grown Tobacco Plants in North Carolina Ready for a First Harvest for Protein and Deproteinized Tobacco in June 1982



Chemical growth retardants that kill grass-like plants without harming tobacco are available. Experiments in Florida and North Carolina indicate that directly sown tobacco seeds could be grown in dense spacing on a raised bed system such as Willingham's. Weed control would be accomplished by proper cultivation of the soil prior to sowing and by use of selective growth retardants after germination of tobacco seeds. As in Florida, success of the system would depend on a reliable irrigation system.

Tobacco seed can be pelleted, making it possible to sow the seed in rows. Curtis Griffin of Florida has developed machinery that could be modified to sow seed in dense spacing within a row.

5. **Cultivars of tobacco.** Conventional tobacco agriculture uses the species *Nicotiana tabacum* almost exclusively. Different cultivars of N. *tabacum* are used to obtain desirable flavor or other qualities. Examples are the NC 95 cultivar grown extensively in the flue-cured region of North Carolina and the KY 14 cultivar grown in the burley producing States. Cultivars not in use now may prove more desirable for protein and deproteinized tobacco production. Some cultivars produce less than 10 percent of the nicotine ordinarily found in tobacco. Other cultivars do not produce flowers until late in the growing season, which could increase the biomass yield. Professor R. C. Long, of the Crops Research Department of North Carolina State University, has compared biomass yield of different cultivars commonly used for conventional tobacco agriculture but grown at dense spacing. In 1981, the annual biomass yield ranged from 59 tons/acre for a TI 174 cultivar to 38 tons/acre for a V 174 cultivar. The V 174 cultivar also had a 30 percent less protein yield than TI 174. Selection of the proper cultivar for a particular geographical region evidently will be an important factor in attaining maximum yield of biomass.

To keep ahead of disease problems, new cultivars of tobacco are constantly being developed by plant breeders. In North Carolina, a new cultivar is released from experiment stations to growers about

every 5 years, Genes for resistance to diseases found in some cultivars, or even in different species of *Nicotiana*, are transferred by hybridization to cultivars with desirable traits for smoking tobacco. The process of transferring a desirable gene may require several successive hybridizations to eliminate undesirable genes accompanying genes for disease resistance. Some intermediate hybrid cultivars of no use in conventional tobacco agriculture could have characteristics valuable for protein production, such as hybrid vigor and disease resistance.

Inputs Required for Implementation of Technology

Professor Long proposed a system for growing closely spaced tobacco for protein production using conventional nursery practices. A l-acre nursery would yield enough plants to transplant 9 acres at a density of 84,000 plants per acre while leaving a similar density of plants in the nursery. The biomass from the 10 acres would be harvested four times. Long (6) estimated the following inputs:

1. **Preparation of nursery seed bed. Materials:** Seed, **6.8** oz/acre; fertilizer, 3,400 lb/acre; fungicide and insecticide, 90 lb/acre; herbicide, 13 lb/acre; fumigation, plastic cover, and irrigation ($1,960/acre). **Labor:** fumigation, plowing, fertilizing, sowing seed, etc. (82 hr/acre at minimum wage). **Machinery and Overhead:** tractor, truck, spraying, rolling plastic cover, irrigation (32 hours at average cost of $14.20/hr).

2. **Preparation of land for transplants. Materials:** fertilizer, 1,681 lb/acre ($151/acre); nematicide, herbicide, insecticide ($86/acre). **Labor:** land preparation, spraying, fertilizing (3 hr/acre); transplanting (143 hr/acre at minimum wage). **Machinery and Overhead:** tractor, truck, sprayer, irrigation (36.3 hrs at average cost of $23/hr).

3. **Four harvests of biomass and inputs for three periods of regrowth. Materials:** fertilizer (3,200 lb/acre or $213/acre); herbicide, insecticide ($20/acre). **Labor:** harvesting, hauling, spraying, irrigating (11.7 hr/acre at minimum wage). **Machinery and Overhead:** harvester, tractor, truck, sprayer, irrigation (10.9 hr/acre at average cost of $23/hr).

Adding land rent at $75/acre, irrigation at $120/acre, interest on investment (at 20 percent for 6 months) at $160/acre, and profit at $200/acre, total input cost for the North Carolina transplant system was estimated to be $2,275/acre.

With a direct seeding system of the type used in Florida, input cost was estimated to be $1,900/acre; because of the longer growing season this figure includes five harvests with an annual yield of 75 tons of biomass/acre.

Scientific, Environmental, Economic, Cultural, and Political Aspects of Development and Implementation of Technology

Engineering. As stated earlier, there appear to be no unsolved engineering problems to constrain large-scale expansion of the LPI process. Plants capable of processing 40 tons of biomass per hour have already been used to extract the green juice from alfalfa. For a yield of 60 tons/acre in North Carolina, a 40 ton/hr plant could process the biomass grown on 2,432 acres. The problems remaining before designing such a plant are of a development nature. Additional information is needed to make proper choices among the various types of equipment available to minimize operating costs for labor, power, water, maintenance, and overhead.

Environmental. In the LPI process, no aerial portions of the plants are discarded as waste except the water which comprises 85 to 90 percent of the biomass. The water is removed by evaporation of the brown liquid residue and could be condensed and used again, if economical. To be economically viable, solvent extraction of green residue and green sludge would have to recover the solvent as efficiently as factories engaged in hexane extraction of oil from soybeans. Solvents used in the LPI process are less volatile and have higher flash points than hexane.

Economic. The finished products (table 1) produced from tobacco biomass by the LPI process are tobacco for smoking and food for human consumption. Private industry with manufacturing and marketing expertise in food products may be uncomfortable with the prospect of entering into the manufacture and marketing of a smoking product of equal or higher value than the food product. This constraint could be overcome by organizing a company to construct and operate the factories that make the finished products in table 1, all of which could be stored at ambient temperature. The decolorized white fibers and condensed brown liquid would be supplied to tobacco manufacturers for further processing into smoking material. The proteins would be supplied to food processing manufacturers and pharmaceutical companies. The solvent-extracted pigments could be supplied as raw stock to manufacturers that would refine and mar-

ket the chlorophyll, beta carotene, carotenoids, solanesol, and other compounds.

Consumer Acceptance. A significant barrier to commercial exploitation of this technology is the public's image of tobacco as a poisonous plant. Tobacco does indeed contain nicotine. However, repeated analyses have been performed on crystalline Fraction 1 protein from tobacco grown to different stages at close spacing and after different rattoon harvests, Nicotine contamination was in the parts per **billion** range, detectable only by the most sophisticated methods of analysis. The contamination was far below the level of 2 parts per **million** (ppm) that the Food and Drug Administration (FDA) considers safe in poultry meat which gets contaminated by nicotine-containing insecticides used in poultry farms, The contamination of Fraction 1 protein is also far below the 2 to 3 ppm nicotine found naturally in tomatoes, eggplant, green peppers, and tea.

Constant reminders of the link between smoking and cancer and other debilitating diseases further exacerbate the negative image of tobacco. Nicotine is not oncogenic. Tobacco has to be burned to produce the oncogenic compounds found in smoke. Smoke from burning leaves or charcoal-broiled steaks also contains oncogenic compounds, but they constitute no significant hazard to health because of the infrequency of inhaling such smoke. So it is not the nicotine nor the possible presence of cancer-inducing chemicals in tobacco proteins that constitute the problem with public acceptance. The problem is tobacco's negative image that is constantly being reinforced by reminders of the harmful effects of smoking, Thus, a food company contemplating the market for products containing tobacco proteins could be faced with an inability to overcome the negative image of tobacco.

If those who see tobacco agriculture and cigarette manufacture as a declining industry in the United States are correct, the best interests of the tobacco industry might be served by educating the public on the virtues of tobacco as food and actively supporting efforts to produce less harmful tobacco for smoking. However, the fact that the tobacco industry steadfastly refuses to accept as proven that smoking is harmful to health makes their promotion of tobacco as food unlikely.

Government could play a decisive role in overcoming the problem of public resistance to the use of tobacco for food. Existing government laboratories could perform the extensive testing of the tobacco proteins required for FDA approval of their use as a food additive. There is scientific evidence indicating that tobacco proteins will receive a clean bill of health after such testing. Then government could exercise a publicity function to ensure widespread dissemination of unbiased data. In this function, the Office of the Surgeon General could be as effective in changing the negative image of tobacco as it has been in educating the Nation on the perils of smoking.

Cultural. As with other crops in the United States, production of the conventional tobacco crop has become increasingly more mechanized as new types of farm equipment have become available. Intensive labor is required only for brief periods during transplanting, harvesting, and curing the crop. The North Carolina transplant system to grow tobacco biomass for the LPI process would require somewhat more labor for transplanting but less for harvesting than the conventional system. Labor requirements would remain about the same so that adoption of the new form of agriculture would have little impact on existing cultural patterns. As direct seeding of tobacco becomes perfected to replace transplantation, tobacco grown for biomass would become as mechanized and capital intensive as corn, soybeans, alfalfa, and other crops.

Political. In the United States, conventional tobacco is grown and marketed under a Government regulated allotment program that limits total acreage in return for a basic support price for cured tobacco. By leasing allotments, some farmers grow 100 or more acres of tobacco using mechanized equipment as much as possible. Because efficient farmers derive high income from tobacco, it is natural that the lessors and lessees of allotments will zealously guard a program so favorable to their welfare. Since the individual allotment is usually less than 3 acres, the number of individuals owning allotments is large, considering that almost 1 million acres of tobacco are grown in the United States. Their votes translate into a significant political force.

Balanced against the political clout of the allotment holders are the public health concerns over the hazards associated with the use of smoking tobacco. These concerns have left their mark; this year Congress required the allotment program to operate without Government subsidies. The program has to be renewed annually by Congress, and each successive renewal vote has become more uncertain.

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Impacts on Developed and Developing Countries

Implementation of tobacco as a food crop in European countries would provide an alternate source of protein and decrease the present need to import soybeans that cannot be grown in many parts of Europe. In Japan, which cannot grow enough soybeans to meet its domestic requirement, tobacco would become an additional source of protein to replace some soybean imports. Agriculture in developing countries in the Tropics is notable for lack of crops of high protein content. Soybeans cannot be grown in tropical climates, Tobacco, however, is grown extensively in the Tropics and could become a source of protein not previously available because foreign exchange was not available to import soybeans. Tobacco protein could have tremendous impact by helping to alleviate malnutrition in these countries.

High technology equipment is not required to use tobacco for protein production, and analogs of the equipment used by LPI in the United States are readily available in all industrialized nations. Easy availability of the equipment would help reduce the cost of initiating production of the new food in developing countries. Less developed countries would not have to import scientific know-how because the LPI process is simple. Both domestic and foreign patents of the process have been issued so that the scientific information is available in the patent literature. LPI views a logical development of the technology as starting with a demonstration factory in the United States which could train personnel in the agricultural and processing aspects of tobacco for protein and the other finished products listed in table 1.

When tobacco is grown for protein, the agricultural practice will conform to the existing cultural and social patterns of the country in which it is grown. In the United States, great emphasis will be placed on mechanization. In countries with high population density and land scarcity, cultivation of tobacco for food may remain highly labor intensive. Plants would be transplanted by hand at even closer spacing than can be accomplished by transplanting machines. Fertility, weed control, and harvesting would be managed by people instead of costly herbicides, insecticides, and harvesting machinery, and biomass production per unit of land could exceed that produced by a high degree of mechanization. Labor intensive cultivation of tobacco for protein could also have an impact by reducing the acreage used for smoking tobacco and making the land available to grow other kinds of food crops. For example, China uses about 2.5 million acres for smoking tobacco. If only 30 percent of the LPI deproteinized tobacco were combined with 70 percent conventional tobacco in Chinese cigarettes, about 400,000 acres now used for conventional tobacco could be released because the yield of deproteinized tobacco per acre is about five times greater than conventional tobacco. In addition, enough crystalline Fraction 1 protein would be produced as a byproduct of deproteinized tobacco to satisfy the total annual protein requirement for 2.4 million people, to say nothing of the Fraction 2 protein and insoluble protein produced at the same time. At 100 percent deproteinized tobacco in cigarettes, 2 million acres could be taken out of smoking tobacco and used for much needed food crops.

As in other parts of the world, the Chinese Government has become alarmed by the health hazards caused by smoking tobacco. The health hazards associated with "tar" in burning tobacco have been reduced in Western countries by filters and sophisticated techniques for diluting the smoke with air before it enters the lungs, * but the materials required add appreciably to the cost of cigarettes, Thus, another advantage that could accrue from deproteinized tobacco in developing countries is that a significant reduction in tar producing ingredients would have occurred during processing so that the extra cost of filtration and dilution devices might be avoided.

References

1. De Jong, D. W., and Lam, J. J., Jr., "Protein Content of Tobacco, " *Proc. of Amer. Chem. Soc. Symposium, 1977, pp. 78-103,*
2. Edwards, R. H., et al., "Pilot Plant Production of Edible White Fraction Leaf Protein Concentrate From Alfalfa," *J. Agr. Food Chem. 23: 620-626, 1975.*
3. Enochian, R. V., et al., "Producing Pro-Xan (Leaf Protein Concentrate) From Alfalfa: Economics of an Emerging Technology," *USDA Agricultural) Economic Report No, 445, 1980.*
4. Ershoff, B. H., et al., "Biological Evaluation of Crystalline Fraction 1 Protein From Tobacco," *Proc. Soc. Expt. Biol. Med. 157: 626-630, 1978.*
5. Knuckles, B. E., et al., "Pro-Xan Process: Methods of Increasing Protein Recovery From Alfalfa, " *J. Agr. Food Chem.* 20: 1055-1057, 1972.

*OTA footnote: Readers interested **in characteristics of low-yield cigarette use are referred to N. L. Benowitz, et al., "smokers of Low-Yield Cigarettes Do Not Consume Less Nicotine, "** *The New England Journal of Medicine, vol. 309, No, 3,* **July 21. 1983, pp. 139-142. The art** icle **appeared in print while this proceedin, was in press,**

6. Long, R. C., "Estimates of the Cost of Producing Tobacco for Protein, " unpublished report made available to Leaf Protein International, Inc., Raleigh, N. C., January 1980.
7. Peterson, R. B., and Zelitch, I., "Relationship Between Net CO, Assimilation and Dry Weight Accumulation in Field-Grown Tobacco, " *Plant Physiol. 70: 677-685, 1982.*
8. Pirie, N. W., "The Direct Use of Leaf Protein in Human Nutrition," *Chem. & Ind.* 61: 4-48, 1942.
9. Wildman, S. G., and Kwanyuen, P., "Process for Isolation of Ribulose I-5 Diphosphate Carboxylase From Plant Leaves," U.S. Patent No. 4,268,632, 1981.
10. Wildman, S. G. and Sheen, S. J., "Process for Obtaining Deproteinized Tobacco Freed of Nicotine and Green Pigment, for Use as a Smoking Product," U.S. Patent No. *4,289,147, 1981,*

Appendix 1

Fraction 1 Protein

Also known as ribulose-1,5-bisphosphate carboxylase-oxygenase, the enzyme that catalyzes the assimilation of carbon dioxide during photosynthesis, Crystals of Fraction 1 protein are insoluble in water even though 80 percent water is an intrinsic part of their structure. Crystals rapidly dissolve in water containing traces of sodium, potassium, or ammonium ions. When dried, the crystalline protein contains no nucleic acid, lipoids, or carbohydrates, and more than 99 percent of its composition is composed of amino acids with the composition shown below:

Amino Acid Composition of Fraction 1 Protein

Amino acid	*g/100 g protein*	*AA*	*g/100 g*	*AA*	*g/100 g*
Ile	4.3	Thr	5,2	Asp	8.5
Leu	8.8	Try	1.5	Ser	3.3
Lys	5.8	Val	7,2	Glu	11.2
Phe	4.4	His	2.2	Pro	4.6
Tyr	4.9	Cys	3.0	*Gly*	9.2
Met	1.6	Arg	6.1	Ala	7.0

High nutritional value of Fraction 1 protein shown by feeding rats with the PER results (4) summarized below:

Protein Efficiency Ratio (Weight Gained/Protein Consumed) of Fraction 1 Protein Compared to Casein as Standard

	Days after feeding			
	7	14	21	28
Casein	2.73	3.17	2.88	2.83
Fraction 1 protein	3.40	3.44	3.10	3.01

Exposed to trypsin, one of the digestive enzymes of the human stomach, Fraction 1 protein rapidly breaks down into about 80 tryptic peptides of small molecular weight demonstrating its high degree of digestibility. The native protein contains many sulfhydryl groups but no disulfide linkages.

Functionality studies were performed with three different forms of crystalline Fraction 1 protein: **Crystals,** washed free of salts by water, were spray-dried before testing; **pH** 8.5, washed crystals were redissolved with NaOH to pH 8.5 before spray-drying the redissolved protein; **pH 3.0,** washed crystals were redissolved with NaOH to pH 10.5, then acidified to pH 3,0 with phosphoric acid before drying.

Foaming Capacity and stability

Sample	*Foaming capacity (ml) after ½ min*	*Foam stability (ml) after 10 min*	*30 min*	*1hr*	*2 hr*
Fraction 1 protein (crystal)	75	48	42	30	24
Fraction 1 protein (pH 8.5)	77	30	14	6	6
Fraction I protein (@ 3.0)	82	60	60	58	58
Soy protein*	53	6	6	4	4
Eggwhite	62	18	14	14	10

• A product of Natural Sales Co , Pittsburgh, Pa , All Star, 95 percent protein containing lecithin and papain.

Water and Oil Absorption

Sample	*Water (pH 5 acetate buffer) Percent weight increase*	*Crisco oil Percent weight increase*
Fraction 1 protein (crystal)	180.7	105.8
Fraction 1 protein (pH 8.5)	319.0	304.5
Fraction 1 protein (pH 3.0)	393.3	375.5
Soy protein	272.1	187.5
Eggwhite	466.9	144.4

Whipping property

Sample	*Volume increase after whipping Before add'n of sugar*	*After add'n of sugar*
Fraction 1 protein (crystal)	6730/o	7800/o
Fraction 1 protein (pH 8.5)	570	640
Fraction 1 protein (pH 3.0)	633	670
Soy protein	none	none
Eggwhite	600	633

Emulsifying Property

Sample	Protein concentration 1%	20A	4%	6%
1. 40% oil				
Fraction 1 protein (crystal)	18 cps	26 cps	45 cps	97 cps
Fraction 1 protein (pH 8.5)	30	33	117	284
Fraction 1 protein (pH 3.0)	44	535	10379	too thick
Soy protein	40	140	978	18915
2. 40% oil + 1% each of NaCl and starch				
Fraction 1 protein (crystal)	21	41	166	690
Fraction 1 protein (pH 8.5)	55	126	343	681
Fraction 1 protein (pH 3.0)	26	30	112	146
Soy protein	30	52	151	481
3. 20% oil				
Fraction 1 protein (crystal)	8	8	12	25
Fraction 1 protein (pH 8.5)	6	22	45	99
Fraction 1 protein (pH 3.0)	43	281	539	5941
Soy protein	35	127	442	696
4. 20% oil + 1% each of NaCl and starch				
Fraction 1 protein (Crystal)	8	28	28	55
Fraction 1 protein (pH 8.5)	28	55	133	159
Fraction 1 protein (pH 3.0)	4	9	11	139
Soy protein	5	10	29	55

Appendix 2

Fraction 2 Proteins

Amino Acid Composition

Amino acid	g/100 g protein	AA	g/100 g	AA	g/100 g
ASP	6.1	ALA	5.1	PHE	8.4
THR	4.6	VAL	10.5	LYS	3,9
SER	4.0	MET	4,1	HIS	5.0
PRO	8.2	ILE	3.8	ARG	10.4
GLU	8.9	LEU	4.1	CYS	0.8
GLY	3.5	TYR	8.8		

Appendix 3

Green Sludge

Essential Amino Acid Composition of Green Sludge Protein Compared With Soyflour Protein

Amino acid	g/100 g Protein: Soyflour protein	Tobacco insoluble protein
THR	3.6	4.4
MET	1.0	1.8
ILE	3.5	4.1
LEU	6.4	4.2
TYR	2.1	9.6
PHE	4.7	8.8
LYS	5.1	3.9

Appendix 4

Green Residue

Chemical Composition of Dried Green Residue From Tobacco (Average of Four Different Preparations)

mg/g	mg/g	% of ash
Protein	148	
Ash	53	
K	0.6	
Ma	0.2	
Starch	30	
Cellulose	291	
Ca.	0.6	
S	0,2	
Lipids	20	
Hemicellulose	413	
Mg	0.15	
Lignin	45	

Comparison in Composition Between **Green Residue** Decolonized by Solvent Extraction and Wheat Bran

Constituent	Percent total solids: White tobacco fibers	Wheat bran
Cellulose	29	9
Hemicellulose	33	40
Lignin	6	4
Starch	13	17
Protein	13	18
Oil	trace	4
Ash	7	8

Appendix 5

Low Molecular Weight Compounds

Protein, Nicotine, and Apparent Tar Content of LPI Deproteinized Cigarette Tobacco Compared With Two Kinds of Conventional Cigarette Tobacco

	Percent dry weight		
Product	***Protein N***	***Nicotine***	***Tar***
Conventional flue-cured tobacco, variety NC 95 . . . . , ., , ...	2.25	2.0	6.77
Deproteinized NC 95	1.84	0.6	0.70
Conventional burley tobacco, variety KY 14 . . . . . , ... ,	4.15	3.0	6.07
Deproteinized KY 14	3.00	0.7	0.82