Processing of Leaf Proteins into Food Ingredients

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ABSTRACT

Many process alternatives are possible. Most of them are currently being investigated by laboratory or pilot tests. The successful future of some of these processes is certainly connected to the quality of the end product vs. market requirements and consumer acceptability. The status of development of the processes does not allow any economic forecast on profitability, but it is reasonable to think that the simplest techniques to produce acceptable leaf protein isolate and sufficient control on processing conditions could be profitable. These processes need further technological development. and the end product market needs to be investigated further, but leaf proteins could be an alternative and complementary source of proteins for food ingredients in the predictable future.

It is well known that green crops can produce a higher amount of crude proteins per hectare than any other crop. Some figures from the literature are given in Table I.

However, the protein in green crops is bound to cellulosic material in such a way that it cannot be utilized directly by nonruminants and particularly by humans. Therefore, a simple technique for processing leaf proteins into food ingredients has been used for many years: the animal farming system. This process ends up with red and white proteins having good nutritional properties and acceptable flavor but, unfortunately, low processing yields. Other processing techniques able to use the high potentialities of green crops have been investigated.

Green leaf proteins are generally classified into two fractions:the chloroplastic proteins bound to the pigments with a high molecular weight (100,000), which can be recovered as a coprecipitate at a relatively low temperature (55 to 60 C), and the cytoplasmic proteins uncolored with a lower molecular weight (30 to 50,000), which can be recovered with the highest purity – over 90% proteins on dry basis (db) by high temperature precipitation.

The first approach and the simplest technology is to recover these two fractions together by high temperature heat coagulation. The protein-xanthophyll concentrate obtained is strongly colored, grass flavored, and contains around 50% proteins d.b.

Some tests in India and Pakistan show that feeding a sufficiently purified green leaf protein concentrate (LPC) to malnourished children has a favorable effect on their growth. However, because of the problems of flavor and color, the green LPC will probably not find wide spread application for humans. Furthermore, green LPC for human consumption has not been approved by the protein advisory group of the United Nations.

Processes for producing a colorless and tasteless protein concentrate or isolate consequently have to be developed. The following ways are possible and currently being investigated: heat or isoelectric fractionation and organic, polar solvent extraction.

FRACTIONATED PRECIPITATION

The fractionated precipitation is based upon the following protein characteristics: the heat precipitation of chloroplastic proteins is possible at 50 to 60 C while it needs 85 C and over for cytoplasmic proteins; the isoelectric point of chloroplastic proteins is 4.5 to 4.8, while it is 3.3 to 3.6 for cytoplasmic proteins. By varying pH and temperature, it is therefore possible to separate these two protein families.

The following alternatives have been investigated.

Pressing Stage

The green fresh crop can be pressed in order to get a green juice containing both green and white proteins. This is the way followed by the Western Regional Research Center and known as the Proxan II process. The green crop can be heated in a 55 C bath in order to coagulate the green proteins inside the leaves and then process a juice containing soluble white protein. This processing way has been patented by France-Luzerne. The green crop can be heated in a 55-60 C bath and the pH raised over 10 by a chemical addition. The pressing stage then gives a higher protein extraction rate, and the juice contains both green and white proteins soluble at this pH. Patented by France-Luzerne.

Green Protein Recovery

The green proteins can be recovered by heat precipitation or isoelectric precipitation. These different techniques are very much connected to the end use of the proteins, as their functional properties will be affected. This subject will be detailed in other lectures.

In case of green juice extraction, all protein recovery techniques can be used. However, it should be pointed out that the first precipitation step is quite difficult to perform, as a lot of different parameters have to be considered to get a satisfactorily high yield of white protein. In case of heat-treated alfalfa, most of the green proteins remain inside the leaves and therefore just a final clarification of the juice is necessary to separate the cellulose particles and some green proteins extracted together. In case of alkaline juice extraction, the green proteins will be precipitated at a pH around the neutrality and separated.

Centrifugal separators or centrifugal decanters are normally used for the green protein separation at this stage. The heat-precipitated proteins are much easier to separate while the isoelectric precipitation requires high performing centrifuges (decanters are excluded). In case of heat-treated alfalfa, vacuum filters can be used for fine clarification.

"White" Protein Recovery

The white proteins can be recovered by heat precipitation, isoelectric precipitation, or ultrafiltration. The heat precipitation is made at a temperature above 85 C. The iso-electric precipitation is performed at a pH around 3.5. Very little has been published on ultrafiltration, but obviously tubular systems should be preferred over plate systems in case of clogging risks.

TABLE I

Protein Yields of Various Crops

	Yield/kg crude proteins/ha per year
Wheat grain	550 kg
Sunflower	550 kg
Potatoe	600 kg
Rapeseed	700 kg
Field bean	800 kg
Soybean	850 kg
Mustard	1,800 - 2,000 kg
Alfalfa	2,000 - 2,500 kg

White Protein Drying

In most of the reported laboratory or pilot tests, the proteins have been freeze-dried. Spray-drying should certainly be preferred for a large scale operation. Pneumatic, or fluid-bed, drying could be used in case of heat-precipitated proteins.

Press Cake Drying and Energy Requirements

Press cake drying is effected in any case with drum driers. In the case of fresh green crop pressing (with 20% d.b. alfalfa), around 25 kg of water will have to be evaporated in the drum for 100 kg fresh crop. In the case of heated green crop, around 22 kg of water will have to be evaporated in the drum. In the case of alkaline extraction, around 35 kg of water will have to be evaporated. This last alternative means a higher protein extraction rate, but a wetter press cake, and therefore a higher energy requirement in the process.

Deproteinized Juice

For any of the above mentioned alternatives, deproteinized juice can be used as a fertilizer or evaporator. All economic studies show that evaporation should be selected, preferably with an evaporator heated by the exhaust gases from the drum drier as France-Luzerne with the Alfaprox fractionation process. This solution gives a very favorable energy balance for the whole process with 30 to 40% energy savings over conventional dehydration.

SOLVENT EXTRACTION

In the case of solvent extraction, both chloroplastic and cytoplasmic proteins should be pressed out of the green crop in a green juice. Solvent extraction can be performed at four different steps: (a) the solvent (acetone or ethanol) can be mixed with the extracted juice (Tao 1970); (b) the solvent can be mixed with the juice after ultrafiltration concentration (Trajardh 1974, isopropanol); (c) the solvent can be mixed with the heat or isoelectric-precipitated proteins; and (d) the solvent can be used on dried LPC.

Four counter current extraction stages are required to get a satisfactory decoloration with solvent/D M ratios of five to one in the case of dried LPC and more than ten to one in the case of whole juice.

Several works report that acetone and isopropanol have the most favorable effect on pigment extraction. The process parameters for protein fractionation have an influence on pigment extractability. It is nearly impossible to achieve a bland, colorless concentrate from dried proteins, while green juice solvent extracted as soon as possible will give a nearly white product. Finally, we must mention that floculating agents (polyelectrolytes) have been proposed for the chloroplastic fraction recovery, and some work has been done in Pisa University (Italy). But the utilization of polyelectrolyte vs. food regulations is unclear, and economic evaluations are still to be made.

COMPARISON OF PROCESS ALTERNATIVES

It is unrealistic to expect extensive process comparisons in this short paper. However, all these process alternatives aim to produce leaf protein concentrates or isolates to be used for food ingredients. This includes requirements on the properties of the end product, including the nutritional point of view, the functionality, and the acceptability. These properties are directly connected to processing conditions. Futhermore, the processes involved should be competitive with other sources of food ingredients and, therefore, some economic evaluation has to be done.

Protein Properties

Nutritional properties. Crude protein content of the whole LPC is around 50% d.b. It has been reported to be increased up to 65% by suitable washings. The acid-precipitated, cytoplasmic proteins have a crude protein content of around 75%, while the test heat-precipitated ones have a crude protein content over 90% (isolate). The whole LPC is limited in methionine + cystine with 3.3g/16gN, while the cytoplasmic fraction is around 3.9g/16gN. Both in vitro and in vivo studies show that the whole LPC should be supplemented with methionine to reach a protein efficiency ratio similar to that of casein, while the cytoplasmic fraction with no addition equals that of casein.

The processing technique of white LPC has a marked influence on the protein efficiency ratio.

Finally, the cytoplasmic fraction of LP shows generally a favorable nutritional value with a good amino acid balance. Processing conditions are, however, of importance for the level of antinutritional factors. Ultrafiltration and solvent extraction are reported to increase the PER. This would be explained by the elimination of antinutritional factors. Heat precipitation as compared with acid precipitation is reported to have some negative effects.

Functional properties-solubility. First of all, the heat precipitation obviously causes heat denaturation with a subsequent very low solubility over the whole range of pH. This processing should therefore be avoided each time a good solubility of the protein is required for the end use. The solubility of acid-precipitated proteins is related to the pH adjustment before drying and the drying technique. Neutralization before drying is favorable. Freeze-drying and spray-drying give the best solubilities. Solvent treatment causes denaturation and therefore decreases the solubility.

Foam stability. None of the leaf proteins processed can show comparable foaming properties with soy protein isolates (SPI) except isopropanol-extracted samples. Emulsion ability of LP is reported to be poor as compared with SPI. Processing technique has little effect. The fat absorption properties of Leaf protein isolates (LPI) are dependent upon drying technique. (Freeze-drying is reported to be the most favorable, but the drying temperature when spray-drying has certainly a very big influence as well).

Protein Yields

Little is available in the literature about leaf protein yields with different processing techniques, and it is more reasonable to draw some conclusions concerning relative yields rather than absolute figures. Absolute figures are in fact very connected to the raw material, and they are difficult to compare from very different tests. Heat treatment is reported to give yields of 2 to 3% of creamy cytoplasmic protein based on the dry matter processed. Alkaline treatment would give 5 to 6%. Solvent extraction could give 7 to 9%. Heat treatment, alkaline treatment, and solvent extraction will respectively give increased yield of cytoplasmic fraction.

Economic Comparisons of the Processes

The profitability of any LP process is dependent upon price of the raw material, market price of the different end products vs. their properties and possible use in food ingredients, processing costs, capital costs, running costs, and yield of the different outputs.

Any solvent extraction process will involve costly explosion proof equipment, which means high capital costs as well as solvent losses and high energy requirement for solvent recovery which means high processing costs. Solvent extraction, therefore, solves the color and flavor problems, but the profitability of such processes is certainly questionable.

The heat fractionation involves the simplest equipment and the lowest processing costs. It can produce a sufficiently colorless LPI to be used in some processed foods. However, the protein denaturation will always limit its uses.