MANIPULATION OF PHYSICAL PROPERTIES IN THE DRY FRACTIONATION OF PULSE SEED FLOURS

by

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MASTER OF SCIENCE

ABSTRACT

Sieving of pin-milled fababean flour yielded a fractio enriched in protein than the parent flour possibly suitabl for use in wheat/fababean composite flour. The generation o static charge between the components of the flour had larg effects on the variation in fractionation profile, particu larly in fractions on the 45 and 75 micron sieves. The genera tion of electrical charges was assumed to be responsible fo the production of agglomerates, spheres and a film or barrie of the flour over the screens, which ultimately clogged an blinded sieves causing obstruction in flow of the flour i falling through to the bottom pan on the basis of its particl size range.

Charge phenomena apparently played some role in siev analysis and it was recognized that the most highly charge material was the protein portion. Modification of charges o the protein components was influenced by introducing a posi tive charge to the shaking system by the use of an antistati agent, which , showed enhanced material flow through th sieves, possibly by the neutralization of electrical charges.

A combination of protein rich fractions of 63 and 75 μm sieves of 10 minutes run through successive sievings helped i obtaining a protein-rich fraction (Fraction A) which had a final protein content of 32%(as is basis). Fraction A, whic was further sieved over an increasing time interval, produce an additional protein enrichment in Fraction B (a final blen of the combined material from 63 and 75 μm sieves of 20 min.

run) contained 36% protein (as is) or about 40.0% on dry basis. This final blend represented about a 1.5 fold protein enrichment over the starting flour.

The biochemical characterization of fractions A and B showed no real differences in amino acid composition of any flour preparations and all the anti nutritional factors namely phytic acid, trypsin inhibitor, vicine and convicine contents were found to be concentrated with the protein.

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1. INTRODUCTION

Plant proteins are now considered to be a major source of dietary protein and energy for a large area of the world population today with an annual production in excess of 100 million metric tons. Among the plant protein sources, the legumes contain considerable protein and are an important source of food and food proteins for human beings. Food legumes are particularly important in the tropics and sub-tropics where many inhabitants have chronic protein deficiency (Kanamori et al., 1982).

In view of the cost of animal proteins or non-consumption due to religious or cultural reasons, legumes have assumed great importance in the diets based on either cereal grains or starchy foods of people in underdeveloped and undeveloped countries. Though it is well documented that most legumes have a reasonably good balance of essential amino acids but legume proteins have a relatively low biological value due to a deficiency of the essential amino acid methionine, however these are of course rich in another essential amino acid lysine, in which the cereals are deficient. Therefore, they are of good supplementary value to cereal based diets with special reference to the preparation of baked or cooked products like bread, chapatties, rice, etc. (Wankhede and Ramateke, 1982).

Legumes are expected to play an important role in filling world needs for edible protein. Hence, the increasing need for protein in the world has stimulated scientists and technologists to search for unique and sophisticated techniques to

produce protein concentrates/isolates.

Presently the production of protein concentrates/isolates is based in two methods, namely 1) Dry method - which involves physical separation of protein rich fraction and 2) Wet method - which involves the solubilization of proteins using suitable solvent systems followed by precipitation under conditions of varying pH, ionic strength and then drying. Sometimes a combination of both is employed (Sathe and Salunkhe, 1981). Extensive studies were done on the preparation of protein isolates from fababean (Patel and Johnson, 1975), navy and pintobeans (Seyam et al., 1983), great northern beans (Satterlee et al., 1975), lima beans (Maneepun et al., 1974), and mung beans (Thompson, 1977), based on precipitaion at isoelectric pH, ionic strength with different solvents. The wet technique of course, has some major drawbacks. These include low yield and the need to evaporate large quantities of water which involves high cost. The disposal of waste effluent material containing sugars, low molecular weight proteins and salts is another problem (Youngs, 1975). It has been reported that the quality of alkali-treated proteins is often reduced (DeGroot and Slump, 1969), the racemization of both L- to D- amino acids and formation of the crosslinked dipeptide, lysinoalanine(LAL) appeared to be at least partly responsible for decreased quality of alkali-treated proteins (Robbins and Ballew, 1982). Toxicity studies have shown that rats fed with alkali-treated food proteins may develop nephrocytomegaly (NC), a unique renal lesion due to the presence of lysinoalanine (Feron et

al., 1978).

On the other hand, Dry milling methods like air-classification have proven to be effective techniques for producing protein-rich and starch-rich fractions from a wide range of starchy grain legumes (Kon et al., 1977; Patel et al., 1980; Sosulski and Youngs, 1979; Tyler et al., 1981; Vose et al., 1976; and Youngs, 1975). In contrast to wet methods, this method has shown significant protein separation efficiency of legumes (Tyler et al., 1981). Moreover, the dry method avoids the high cost of drying and related labour requirements. There are no costly effluent disposal requirements; no microbial spoilage; sanitation problems are minimal and there are no by products except possibly hulls (Sosulski and Youngs, 1979).

Presently the flour milling industry, particularly in developed countries is applying air-clasiffication to tailor-make legume flours meeting specific requirements such as protein content or granularity. But in countries that are economically poor and technologically underdeveloped, the use of air-classification is not feasible, because the method involves high expense in terms of cost as well as maintenance. Hence, the poor countries are not in a position to utilize such a huge amount of foreign currency, and these countries cannot even afford to buy this kind of expensive machine, particularly for a densely populated underdeveloped country like Bangladesh.

Hence, there is a need for additional dry methods for processing legume flours to provide higher protein fraction(s)

from normal flours using relatively inexpensive and readily available equipment suitable for these countries. The simple and inexpensive method like sieving could be a useful alternative device to obtain high protein fractions from normal legume flours. Sieving is a method which is frequently used and is pertinent to measurements of particle size of flour. That particle size distribution has an effect on the quality of flour with respect to the ratio of starch to protein content has been known for many years (Gracza, 1959).

In view of a constant demand for food proteins with good nutritional and functional properties from a less expensive source and method, the many fold objectives of this study were to explore:

(I) A less expensive, simple and direct method for the production of protein-rich fraction(s) by the use of sieving for the classification of pin-milled fababean flour on the assumption that the particles falling through the different sieves during the shaking of the machine will fall according to their size into fractions which could be differentiated on the basis of chemical composition.

(II) To assess the impact of processing (sieving) on the amino acid distribution in the resulting protein-rich fractions.

(III) To obtain information on the quantitative composition of antinutritional factors in samples (fababean pin-milled flour, protein concentrate, and

starch fraction) of the cultivar, Aladin.

(IV) To determine the distribution of these factors among the sub-sieved protein enriched fraction(s). Trypsin inhibitor, phytic acid, vicine and convicine were the principal components investigated in this study.

2. REVIEW OF LITERATURE

In recent years, with a view to improving the nutritional quality and lowering the cost of legume products, considerable attention has been directed toward study and development of methods for production of protein concentrates and isolates. The production of concentrates and isolates of proteins from different sources is primarily aimed at providing a satisfactory solution for protein malnutrition/undernutrition and effective utilization of under utilized protein sources (Sathe and Salunkhe, 1981).

All the methods presently used for the production of protein concentrates and isolates are based on two categories, namely, i) wet methods or ii) Dry methods. Both wet (aqueous) and dry methods have so far been utilized for the fractionation of grain legumes.

2.1. Preparation of Protein Isolates and Concentrates from Legume Flours by Different Methods - Wet Methods.

Protein separation from the cellular structure of the seed and its flour by extracting with aqueous solvents is the easiest method from the practical point of view. The wet method of isolation of proteins involves the solubilization of the proteins followed by precipitation and drying. A protein isolate is usually prepared by first dispersing the raw material in an aqueous alkaline solution to solubilize the proteins. In the next step, the insoluble material (cell wall fibre and starch) is removed by centrifugation and washing. Then the dissolved proteins are recovered by selective precip-

itation at the apparent isoelectric point under conditions of varying pH or ionic strength. The precipitated curd constitutes the protein isolate which is commonly washed and spray dried before use. As the solubility or dispersability of proteins in aqueous solvents is very dependent on pH, varying pH conditions are best utilized in dispersing the protein for separation from the starch granules and in precipitating the protein for recovery as a protein isolate. However, in the preparation of protein isolates extreme precaution is essential to choose a suitable pH and temperature to protect against protein denaturation and darkening of the protein isolate as a result of very alkaline pH (Fan and Sosulki, 1974). Researchers have tried to extract the protein under weak alkaline conditions followed by acid precipitation at isoelectric point of the protein. However, in this process the "whey" which contains about 20% of the total protein, is discarded (Bramsnaes and Olsen, 1979). Many studies have been done on the production of protein isolates from various legumes based on precipitation at the isoelectric pH. To asses the potential of food uses of grain legumes grown in Canada, Fan and Sosulki (1974) studied the percent of nitrogen dispersibility vs. pH on both extraction and precipitation characteristics in nine legume flours. The yield of protein isolates was found to be proportional to the protein contents of the legume flours, yields were found to vary from 18g in Limabean to over 36g/100g flour for Soybean (Table 1). The extraction and precipitation curves which they obtained are reproduced in Figure

TABLE 1: Yield and Concentration of Protein Isolate from Legumes, Dry Basis

Legume flour	Protein isolate Yield g/100 gms flour	Protein isolate Yield % of total protein	% Protein N x 6.25
Soybean	36.6	78.9	93.8
Lupine	30.8	65.6	95.0
Fababean	28.2	80.2	93.1
Pea bean	28.6	74.6	81.8
Mung bean	26.9	87.6	88.1
Field pea	22.7	79.8	87.5
Lima bean	17.9	64.3	78.1
Lentil	19.0	72.8	83.1
Chick pea	18.5	74.6	85.0

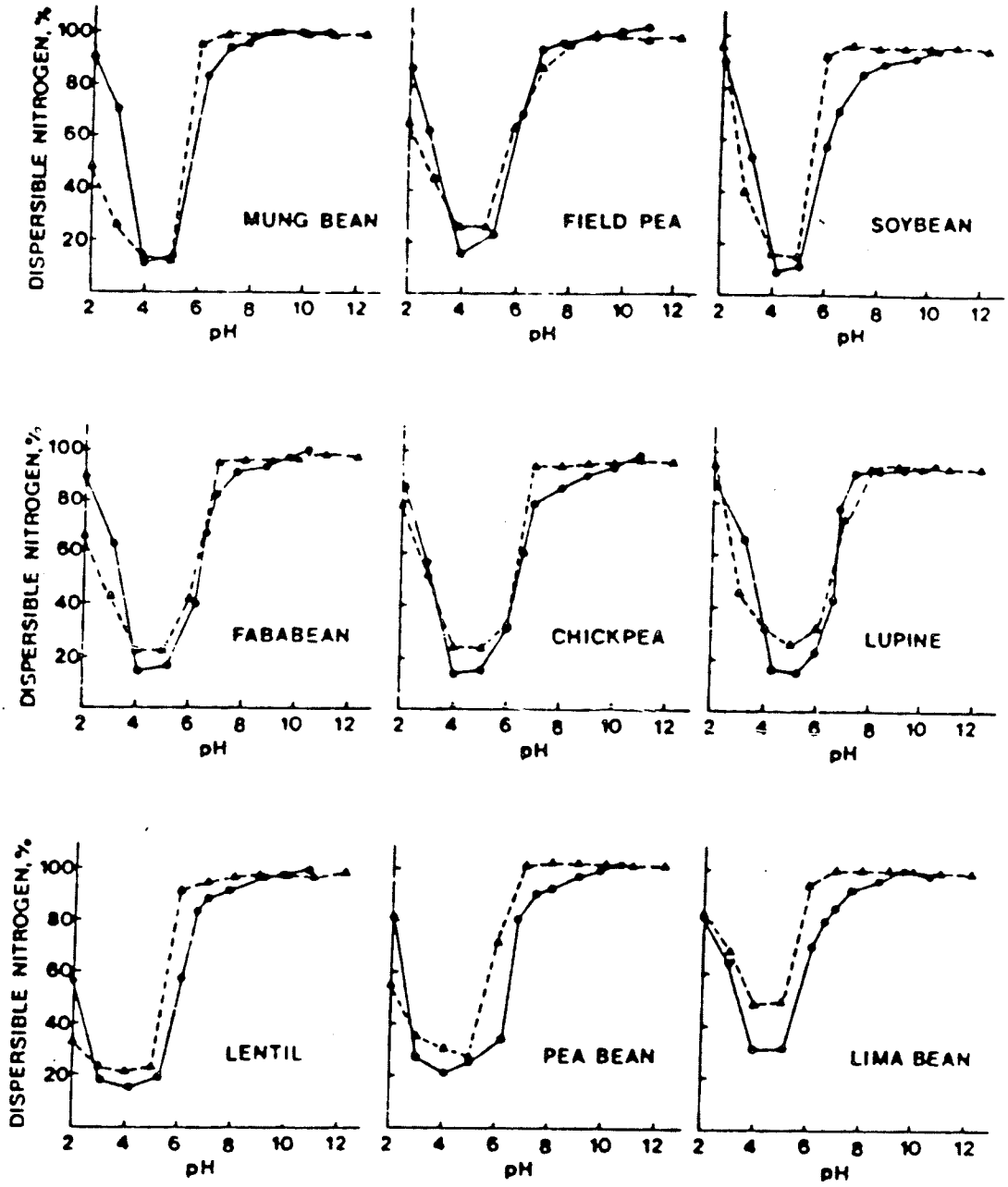


FIGURE 1: Nitrogen dispersibility of legume flours.

●——● extraction ▲ _ _ _ ▲ precipitation.

1. The proteins of all legumes except the Soybean, Chickpea and Lupine were almost completely dispersed at a pH of 8 to 10 and the minimun dispersibility or isoelectric point was observed in the range of pH of 4 to 6. Soybean, Chickpea and Lupine flours contained significant amounts of oil and so these flours were defatted to 1% level to improve nitrogen extractability and avoid oil contamination prior to preparation of the isolates. Fan and Susulski (1974) also determined the amount and concentration of protein isolated that could be obtained from each species by two consecutive extractions with 0.02% NaOH at pH 8-11 and precipitation at pH 4.5 with concentrated HCl. However, they also reported that in each protein isolate there was evidence of contamination with non-protein constituents, whey losses and influences of differences in alkali solubility. In this type of process, about 20 to 25% of the flour ends up as "whey" - a dilute solution containing sugars, low molecular weight proteins and salts which perennially poses a serious effluent disposal problem (Youngs, 1975). The isoelectric precipitation very often involves protein aggregation or denaturation, which can limit the usefulness and the functional properties of the proteins (Olsen, 1978).

Patel and Johnson (1974) also studied the isolation of fababean protein. The extraction was carried out with water or dilute $Ca(OH)_2$ solution using rapid agitation and precipitating at a pH of 4.25 with 6 N HCl. The total yields of the protein isolates varied from 16.5 to 21% based on flour weight, with protein contents of 71 to 83% (N x 6.25). They

also found that the addition of 0.25% L-ascorbic acid to the flour prevented the development of a green colour in the isolate of the alkaline extract. More efficient extraction of protein was observed with reduced particle size of the flour and similar findings were reported by Sumner et al. (1981) who developed laboratory and pilot plant processes for producing pea protein isolate as the sodium proteinate (PNaI) and isoelectric (PII) products from field peas by using the conventional alkaline extraction method and the products were dried by spray-, drum-, and freeze dry methods. The schematic flowsheet for preparation of pea protein isolate is shown in Figure 2. In this process one-half of the isoelectric protein isolate (PII) was slurried and converted to sodium proteinate by adjusting to pH 6.5 - 7.0 with 1 N NaOH. Sodium proteinate and isoelectric products obtained by alkaline extraction and precipitation at the isoelectric point contained up to 90% protein. In general, the sodium proteinate exhibited more functionality than the corresponding isoelectric protein isolates, they concluded that the properties of these two chemical forms can be varied widely by selection of the drying method and other processing conditions as shown in Table 2.

A new method has recently been developed by Murray et al. (1978) which involves protein extraction with sodium chloride solution of 0.2 to 0.8 ionic strength and precipitation of the isolate by dilution with water. The salt soluble protein isolate contains about 95% protein and is named as PMM (protein micellar mass).

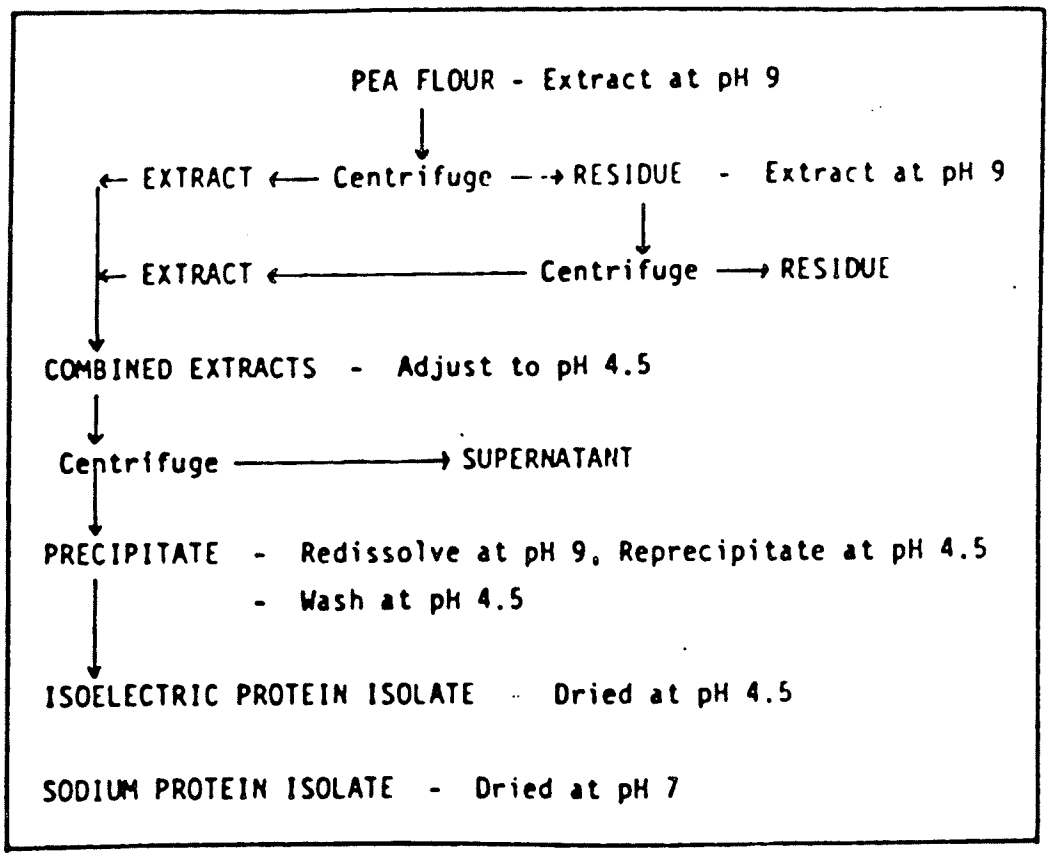


FIGURE 2: Preparation of pea protein isolate.

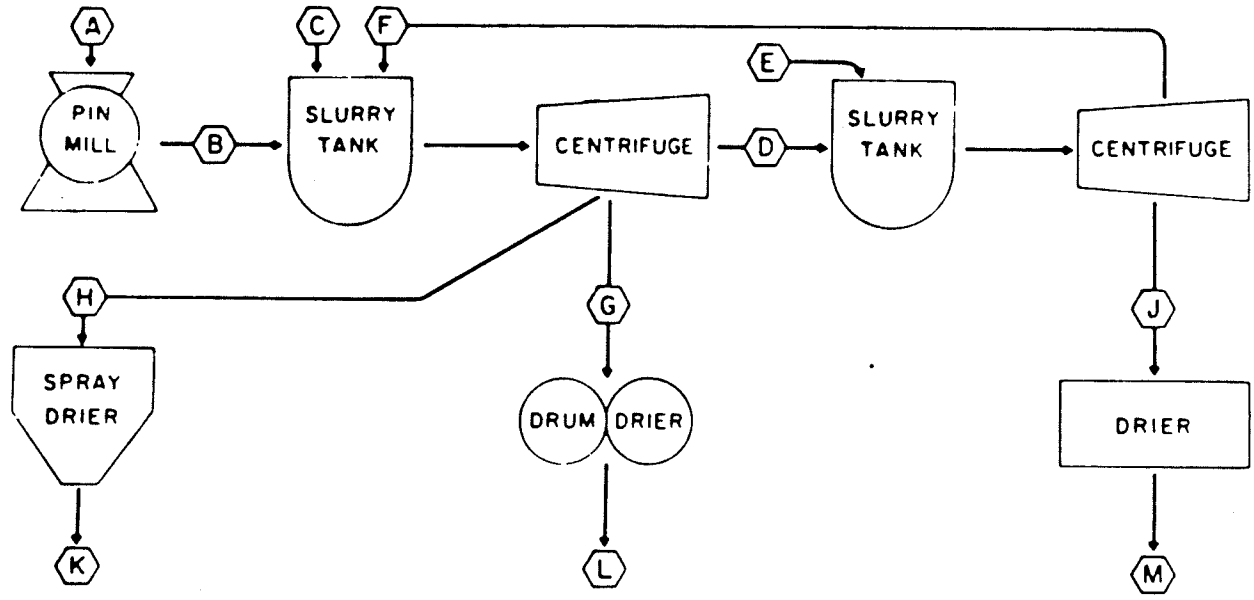
TABLE 2: Properties of Pea Isolates, Dry Basis

Product	PNa1			Pl1		
	Spray	Freeze	Drum	Spray	Freeze	Drum
pH	7.0	7.0	7.0	4.5	4.5	4.5
Moisture %	5.0	2.4	7.8	8.5	4.2	11.0
Crude protein %	85.8	83.0	83.2	88.5	90.0	85.9
Crude fat %	5.3	4.5	2.8	5.3	4.4	1.8
Crude fiber %	0.5	0.7	0.6	0.2	0.2	0.3
Ash %	5.2	5.0	5.0	2.7	2.8	3.1
NFE %	3.2	6.8	8.4	3.3	2.6	8.9
Nitrogen solubility %						
pH 3.0	53	53	14	56	56	7
pH 4.5	1	3	0	3	2	2
pH 7.0	63	64	16	72	87	3
pH 10.0	81	94	34	100	100	23
Emulsification %	28	42	52	38	38	38
Fat absorption %	104	230	204	90	122	127
Water absorption %	250	205	283	132	112	191
Foaming %	433	75	335	412	143	198
Color[a] L	87.7	74.6	74.4	82.2	62.8	62.6
a	-0.3	1.5	1.6	1.3	5.0	5.8
b	14.7	20.4	21.8	15.3	22.1	20.6
Flavor[b]	8.9	8.1	6.5	8.6	7.4	7.8

[a] L (100 white, 0 black); a (+red, -green); b(+yellow, -blue)

[b] 1 = raw pea flavor; 10 = bland

A wet process for field peas called slurry-centrifugation has been installed at the Prairie Regional Laboratory, Saskatchewan, Canada, for preparing pea protein concentrate has been reported in PFPs Bulletin No. 1 (1974). A schematic flow is shown in Figure 3. The whole or dehulled peas can be finely dry ground in a mill or wet ground with water and the flour slurried in a tank with five parts of water. The pH of the slurry is raised to around pH 9 by the addition of lime to solubilize and disperse the protein. The slurry is centrifuged to yield a high protein supernatant and starch solids. The starch fraction, containing about 6% protein, is passed to a second slurry with five parts of water and again centrifuged to yield starch solids by removing more protein. The supernatant is spray or drum dried to yield a protein concentrate. In this process 45.5 kilograms of protein concentrate with a protein content of 60% and 30.4 kilograms of starch with a protein content of about 2% were obtained. The protein recovery was very high, about 94% by this process. However, the process involved the addition of 200 L of water in the first stage and another 200 L in the second stage, which can be recycled. The wash water containing protein is usually used to slurry the next batch of flour, and concern for increasing microbial loads does not appear to be a factor. The protein concentrate obtained was pale yellow in color, and bland in flavour. The proximate analysis of wet processed pea protein concentrate is given in Table 3. This wet method involved a major problem, to evaporate large quantities of water, which is an energy



(A) 100 LBS WHOLE PEAS (21% PROTEIN)

(B) 100 LBS PEA FLOUR (21% PROTEIN)

(C) 1 LB LIME

(D) 72 5 LBS PEA STARCH (6% PROTEIN)

(E) 500 LBS WATER

(F) 500 LBS WASH WATER 5 5 LBS PEA PROTEIN (60% PROTEIN)

(G) 33 LBS PEA PROTEIN (60% PROTEIN) 433 LBS WATER

(H) 33 LBS PEA PROTEIN (60% PROTEIN) 433 LBS WATER

(J) 67 LBS PEA STARCH (1 8% PROTEIN) 67 LBS WATER

(K) 33 LBS PEA PROTEIN UNDENATURED (60 % PROTEIN)

(L) 33 LBS PEA PROTEIN - SHEETS OR FLAKES (60 % PROTEIN)

(M) 67 LBS PEA STARCH (1 8 % PROTEIN)

FIGURE 3: Schematic flowsheet for wet processing of field peas.

Table 3: Proximate Analysis of Wet Processed Pea Protein Concentrate

Moisture %	5.3
Protein (N x 6.25) %	60.8
Fat %	0.7
Fibre %	0.2
Ash %	7.8
N.F.E. %	25.2

consuming process and the disposal of large volumes of effluent material containing sugars, low molecular weight proteins and salts (Young, 1975), and is necessary to conform with present and proposed regulations with regard to pollution control (Craig, 1974).

The production of protein isolates in undenatured form during processing using ultrafiltration (UF) is a new alternative approach to conventional acid precipitation . The earliest reported attempts to produce protein concentrate by UF of legume extracts was demonstrated by Portar and Michaels, (1971) for soy and by Olsen et al., (1973) for fababean. The protein isolates were prepared by using UF including a continuous diafiltration process for fababean by Olsen (1975) and for soy and cotton by Lawhon et al., (1977). The mild operating conditions and relatively high selectivity during fractionation are the significant virtues of UF process (Nichols and Cheryan, 1981).

2.2. Preparation of protein concentrates by the dry method of air-clasification

The dry method, based on physical separation of a protein rich fraction, is a technique involving fine grinding and air-clasification, it has been applied to prepare protein concentrates from a number of cereal grains. According to Kadan et al., (1979), the studies done by Gracza (1959) as well as Schaller and Lapple (1971) showed that air-classification of solids (flours) results in a fine fraction and a coarse fraction that have overlapping particle sizes, unlike sieving that

produces two fractions with sharp particle size cut point. The particles differ also in flow dynamic characteristics, especially by their geometric measurements, density and mass. The application of flow dynamic principles in size grading takes advantage of these differences and makes it possible to concentrate protein matter and/or starch granules in specific size ranges. The fractionation of pin-milled flours by this technique using flow dynamic principles influences changes in some physical and chemical properties in fractions in the sub-sieved size range (Gracza, 1959). The air-classification method has been exploited with varying effectiveness for separating flour into fractions of different particle size. The finest fraction (below 15 microns) generally has higher protein content than the starting flour (Wu and Stringfellow, 1979). Its application to a wide variety of cereal flours like wheat (Stringfellow and Peplinski, 1964), corn, sorghum and soy (Pfeifer et al., 1960) triticale (Stringfellow et al., 1961), rice(Stringfellow et al.,1961), barley (Pomeranz et al., 1971) and grain legumes (Vose et al., 1976) has recently been reviewed by Vose (1978).

2.2.1. Air-Classification Principles and its Types and Operations

2.2.1.1. Air-Classifying Principle:

Air-classification is a process by which particles differing in density and mass are separated by centrifugal elutriation utilizing an air stream. There are two basic types of air-classifiers viz. 1) Gravity classifier, where a series of

air streams are subjected on the falling streams of material, 2) Centrifugal classifiers, where the separation of the particles is achieved by utilizing two opposing forces like centrifugal and frictional forces. The centrifugal models are found to be more common and popular among commercial millers and investigators due to their ability to make sharp separations at cut sizes as low as a few microns (Tyler, 1982). Therefore, the separating principles of the centrifugal type only will be focused on in this review. The separation of the flour in the centrifugal air classifier is achieved by concentrating protein in the fine fraction and starch in the coarser fraction on the basis of particle size and density. The separation is enhanced by the round, smooth, dense nature of the starch granules and light, jagged nature of the protein rich particles (Youngs, 1975).

2.2.1.2. Types of Centrifugal Air-Clasifiers

Among the several designs of centrifugal air-classifier two major types are widely being used in the milling industry, namely a) Rotor type and b) Spiral type. The rotor type is produced by several manufacturers including C-E Bauer, Springfield, OH, The Donaldson,Co., St. Paul, MN. and Mikropul Corp., Summit, NJ. The Bahco Elutriator and Mikroplex air-classifiers are the spiral air stream types. The Bahco classifier is a laboratory batch analyser manufactured (under licence from B.A. Hjorth & Co.) by Etablissements Neu of Lille, France. The Mikroplex developed by Alpine A.G., Augsburg, Germany is a production machine for continuous operation

in three sizes with capacities ranging from about 1 cwt to over 1 ton per hour (Jones et al., 1959). In a rotor type air-classifier, particles of diameter d and density ρ_s are exposed to a centrifugal force, F, produced by a revolving rotor and a fluid resistance or frictional force, R, whose centripetal direction is produced by the air current. These centrifugal and frictional forces are expressed in the following equation.

$$F = \frac{\pi d^3 (\rho_s - \rho_g) V_\theta^2}{r} \qquad (1)$$

$$R = 3 \pi \eta d V_r \qquad (2)$$

where η = viscosity of air, V_θ = peripheral velocity of rotor, V_r = centripetal directional velocity of the air stream, r = rotor radius, and ρ_g = density of air. The theoretical limit of particle size or cut size (d_{th}) value can be derived when F and R are in exact equilibrium from the above equations of (1) and (2):

$$d_{th} = \frac{1}{V_\theta} \left[\frac{18 \eta r V_r}{\rho_s - \rho_g} \right]^{1/2} \qquad (3)$$

The centrifugal force is predominant over the particles with diameters greater than d_{th} and particles with diameters less than d_{th} are predominated by the frictional force. Thus the larger particles travelling to the periphery fly outwards via a coarse material outlet, whilst the smaller particles are carried inward by the air stream through the rotor to a fine material outlet. The large particles and the small particles constitute the coarse and fine fractions, respectively. The same theoretical aspects also apply equally in case of spiral air classifiers. However, the two types differ only in their

methods of generating centrifugal force. In spiral classifiers, air flows inward in a spiral path (Tyler, 1982). Hence, air-classification is the separation of a mixture of powders suspended in air into two fractions, above and beneath a determined cut size. The classifying principle actually lies in the action of two opposite forces, The centrifugal force and the centripetal frictional drag of the air, on each solid particle. The classification takes place in the flat cylindrical classifying chamber, through which air or any other conveying medium flows in a spiral path (in a spiral classifier) from outside inwards, as shown in Figure 4.

2.2.1.3. Operation of Air-Classifier

Operation of one type of air-classifier is shown in Figure 5. From the top of the machine the flour is fed and that falls onto a rotating plate which deaglomerates and imparts centrifugal force to each particle. The particles are thrown outward through a stream of air which retains the fine particles by drag force, but which can not overcome the centrifugal force of the larger particles. The flour is thereby separated into a fine fraction. The cut indicates the size of particles representing the boundary between fine and the coarse fraction. The particle size cut point is dependant on the steepness of the spiral air, the velocity of peripheral component of the air stream, the angle of application of air stream, upon the size of the classifying chamber and feed rate (Stringfellow et al., 1962). Smaller classifying chambers supply lower cut size ranges (Tyler, 1982).

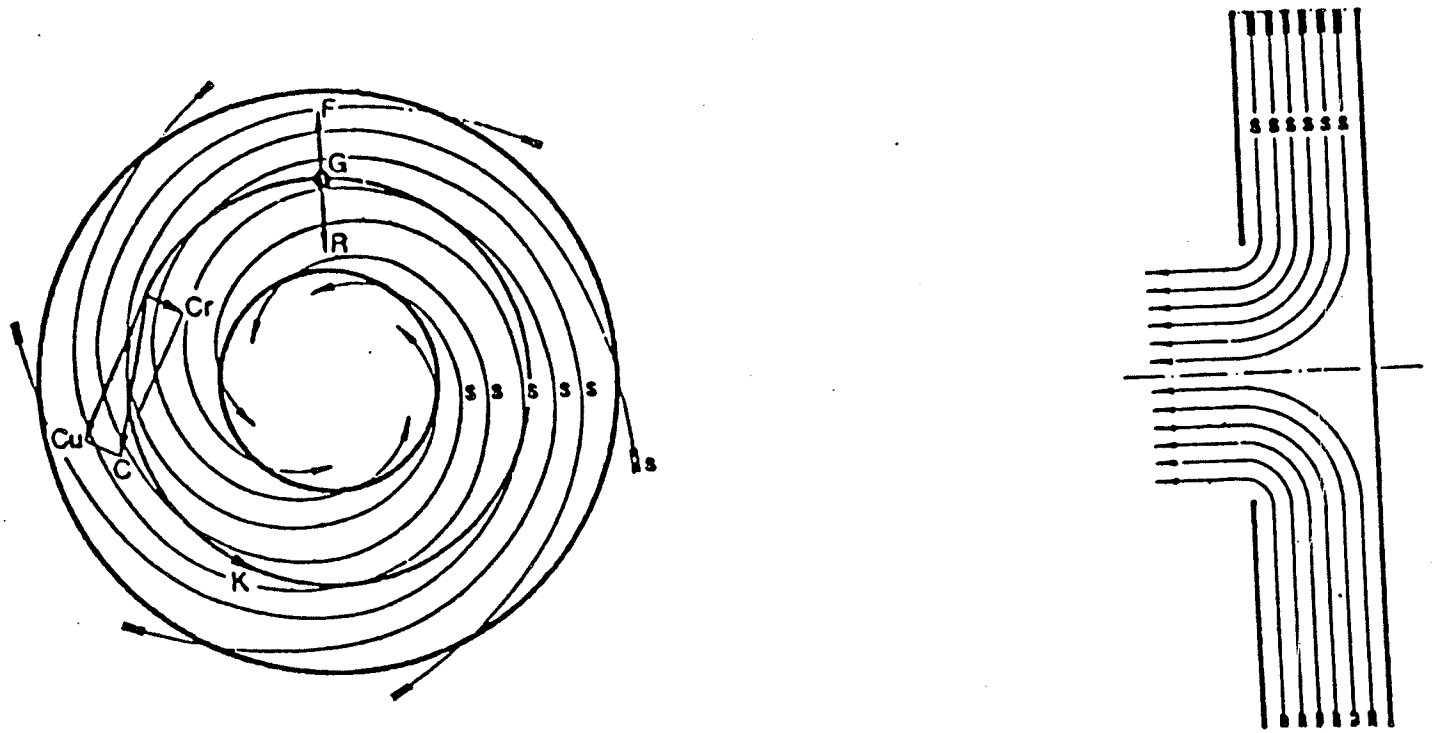


FIGURE 4: Forces in the spiral airstream of an Alpine Mikroplex air classifier. F = centrifugal force, R = frictional force, G = cut-size particle, K = circular pathe, S = spiral flow line, c = air velocity, CU = peripheral component, Cr = radial component (Tyler, 1982).

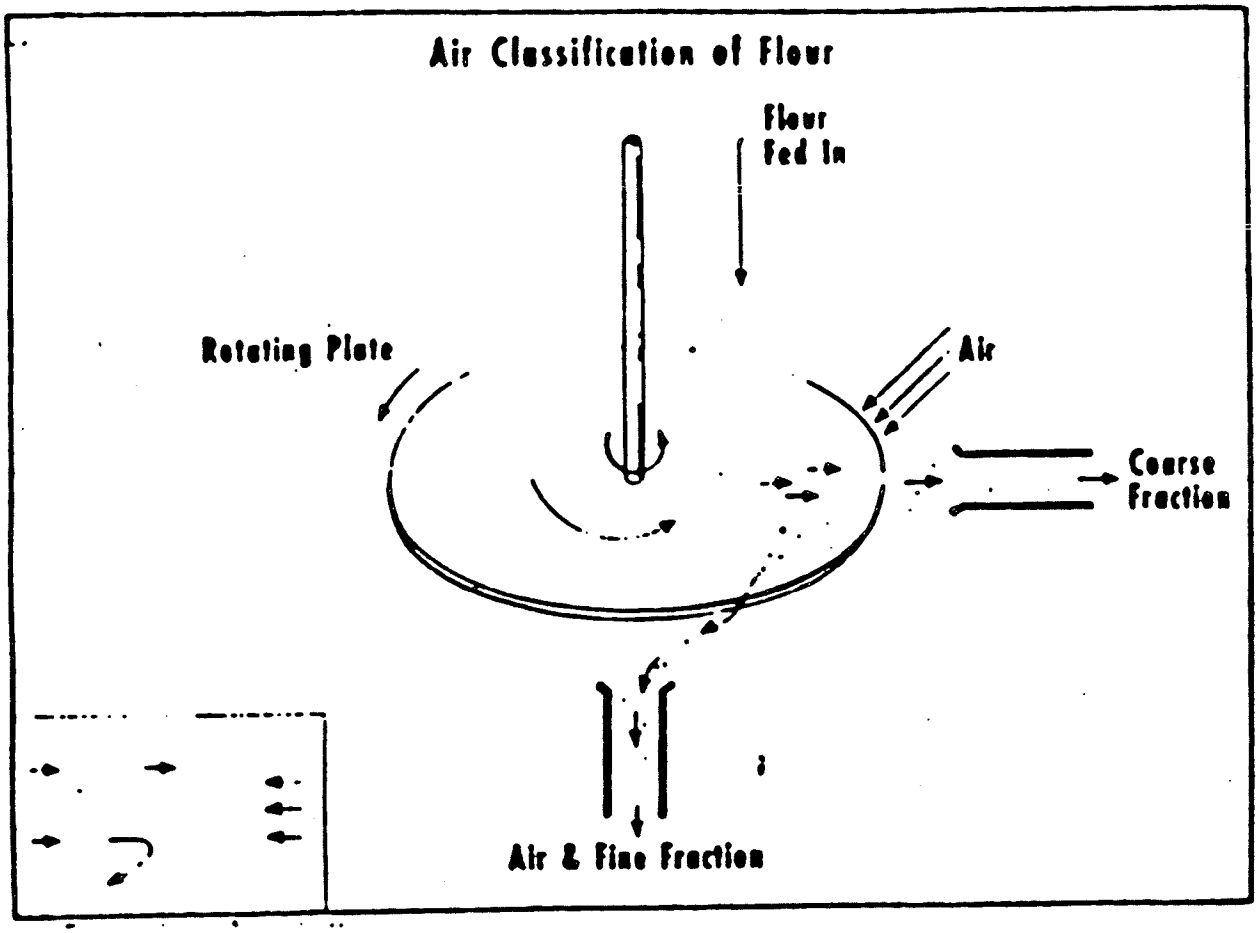


FIGURE 5: A schematic diagram of how air classification can be used to separate coarser and fine particles.

A schematic cross-section of frontal view of an Alpine air-classifier is shown in Figure 6. Heavier or more dense particles (e.g. starch granules) travel along the classifying chamber. These particles are removed from the classifying chamber via the coarse material outlet with the help of the discharge auger as they are skimmed off by the knife edge of the chamber. A spiral air stream, generated by a turbine which draws air inward through the vanes, helps the lighter or less dense particles (e.g. protein bodies) to exit via the fine material outlet of the classifier. The spiral air flow is regulated by adjusting the vane setting. At higher vane settings a wider opening is established between the guide vanes allowing a greater air flow and a steeper entrance angle giving a shorter spiral path (Reichert, 1982). The quality of the result of classification is dependent upon the uniformity of the flow conditions inside the classifying chamber, and each single particle of the grain size mixture has to be subjected to the same conditions. The physics of the flow process showed that the classifying chamber must be flat-cylindrical and the classifying walls must be made to rotate and classifying propeler has to take place in the field of centrifugal force (Anonymous, 1977).

2.3. Fractionation of Legume Flours by Air-Classification

This technique of fine grinding and air-classification has been applied to a number of cereal grains; also, Youngs (1975) reported that the application of air-classification to grain legumes (pulses) was first done by Youngs in 1970.

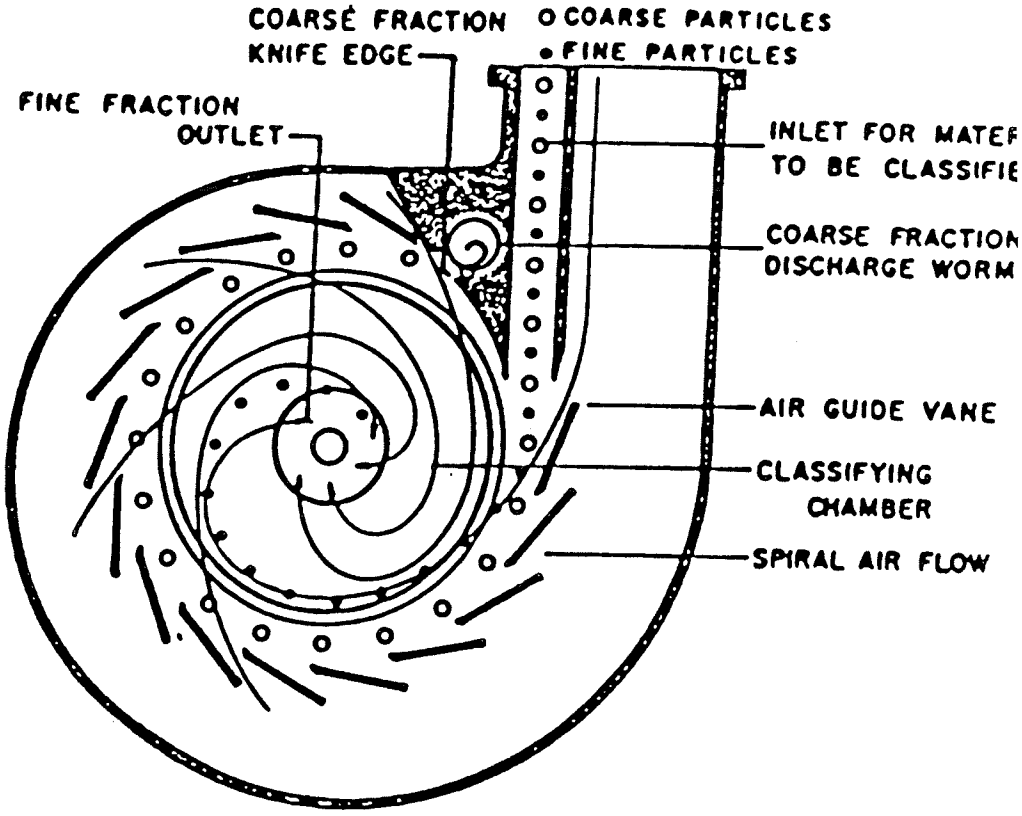
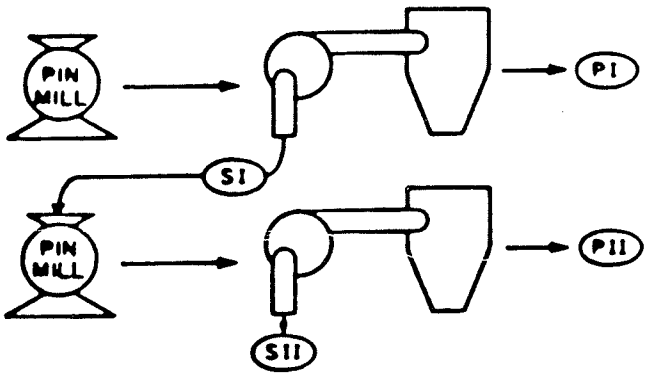


FIGURE 6: Schematic frontal view of an Alpine air classifier type 132 MP (from Alpine bulletin 31 am).

Youngs (1975) reported that a trial run at the Grain Research Laboratory in Winnipeg, using a bench top, batch, air-classifier on a pin-milled pea flour, gave 20 fractions, ranging in protein content from 60.4% to 5.8%. Obviously, this was a much greater shift in protein than had been reported so far for cereal grains and warranted further evaluation.

Since then the application of air-classification to tailor-make impact-milled legume flours meeting specific requirements (such as protein content or granularity) has proven to be an effective technique. From a number of starchy grain legumes, starch-rich and protein-rich fractions were produced using this technique by several workers Kon et al., 1977; Patel et al., 1980; Sosulski and Youngs, 1979; Vose et al., 1976; Reichert, 1982; Tyler et al., 1981; and Youngs, 1975). Many grain legumes contain a high proportion of starch, and a commercial procedure has been developed for separation and concentration of protein and starch components by fine grinding and air-classification (Sosulski, 1974; Youngs, 1975). Legumes are high in starch, so utilization of this fraction will be economically important if protein legume concentrates are used in foods (Morad et al., 1980). Studies with field peas and fababeans have shown that the enrichment of protein and starch in the light and dense components, respectively, is much greater than in cereals (Young, 1975). Youngs (1975) obtained starch and protein concentrates in the ratio 65:35, containing 2.5 and 56% protein, respectively, from pea flour containing 21% protein. Vose et al., (1976) studied smooth

peas and fababeans in order to develop a satisfactory proces technique of considerable importance to the future developmen of these crops. The whole and dehulled peas and fababeans wer pin-milled. The pin-milled flours were separated using th air-classification technique to yield a protein concentrate containing 60-70% protein in the light fraction and a crud starch fraction. The pin-milled legume flours were air-classi· fied in a spiral air stream with a cut point of about 800 mes (or 15) between the fine and coarse particles. The fraction· ation of these flours, the yield and composition of air-cla· siffied fractions are ilustrated in Figure 7. In these exper· iments a 25% yield of protein concentrate (10.2% N) and a 28 yield of protein concentrate (10.6% N) was obtained from air· classified whole peas and fababeans, respectively. The combi· nation of the two protein fractions obtained from two passe through the air classifier produced a 44% yield of a protei meal (9.1% N) from peas and a 42% yield of a protein mea (9.8% N) from fababeans. The composition of the two protei fractions and the two starch fractions obtained by two passe was also studied and the results are presented in Tables 4 an 5. It is observed from these tables that lipid, ash, sugars, crude fibre, phytic acid and most minerals were concentrate in the high protein (`light') fractions from dehulled peas an fababeans. Air-classification of course did not remove al nitrogen from the high starch (`heavy') fraction, even afte two passes. Dehulling of the seed was not required, in thi process, as the fibre is dense and was shifted into the hig



PEAS

	WHOLE SEED			DEHULLED SEED	
FLOUR	100 lbs		(25.7% protein)	100 lbs	(28.8% protein)
SI	75 lbs	"STARCH"	(13.3% protein)	69 lbs	(14.5% protein)
PI	25 lbs	"PROTEIN"	(63.5% protein)	31 lbs	(60.5% protein)
SII	56 lbs	"STARCH"	(5.6% protein)	52 lbs	(4.8% protein)
PII	19 lbs	"PROTEIN"	(48.3% protein)	17 lbs	(40.0% protein)

HORSE BEANS

	WHOLE SEED		DEHULLED SEED	
FLOUR	100 lbs	(27.9% protein)	100 lbs	(31.9% protein)
SI	72 lbs	(15.1% protein)	70 lbs	(16.5% protein)
PI	28 lbs	(66.1% protein)	30 lbs	(69.0% protein)
SII	58 lbs	(5.2% protein)	52 lbs	(4.2% protein)
PII	14 lbs	(51.1% protein)	18 lbs	(49.6% protein)

FIGURE 7: Schematic flowsheet for dry processing of field peas and fababeans. (Vose et al. 1976)

TABLE 4: Percentage Composition of the "Heavy" Fractions from Air-Classified Peas and Beans

	Field Peas				Fababeans			
	Whole		Dehulled		Whole		Dehulled	
	SI	SII	SI	SII	SI	SII	SI	SII
Protein (% N x 6.25)	13.3	5.6	14.5	4.8	15.1	5.2	16.5	4.2
Starch	60.3	73.6	63.0	78.0	58.5	69.3	61.2	76.6
Oil (a) neutral	0.48	0.14	0.46	0.17	0.7	0.4	0.6	0.2
Oil (b) polar	0.77	0.55	0.96	0.37	0.9	0.4	0.9	0.4
Ash	1.6	1.0	1.6	0.8	2.0	1.0	2.0	0.7
Sugars	...	...	5.3	3.8	...	...	5.5	4.5
Crude fiber	8.4	8.4	1.1	0.5	10.2	11.4	1.1	0.7
Phytic acid	...	...	0.2	...	...	...	0.9	...

TABLE 5: Percentage Composition of the "Light" Fractions from Air-Classified Peas and Beans

	Field Peas				Fababeans			
	Whole		Dehulled		Whole		Dehulled	
	PI	PII	PI	PII	PI	PII	PI	PII
Protein (% N x 6.25)	63.4	48.3	60.5	40.0	66.1	51.1	69.0	49.6
Starch	7.6	17.4	7.8	27.6	7.3	18.0	4.2	22.3
Oil (a) neutral	2.2	1.4	1.8	1.4	2.1	1.9	2.5	1.8
Oil (b) polar	2.7	1.7	2.3	2.6	2.2	1.4	2.2	1.3
Ash	5.8	4.5	5.6	4.4	7.0	5.3	6.7	5.3
Sugars	...	...	11.2	9.7	...	...	5.8	5..
Crude fiber	2.1	3.2	2.2	2.6	2.6	3.8	2.0	2.
Phytic acid	...	...	1.9	...	...	...	4.2	..

starch, coarse fraction, resulting in a low fibre proteir concentrate. Kon et al., (1977) following the procedure of Vose et al., (1976) produced starch and protein concentrates from turbo-milled California small white bean flours (21.73% protein) containing 43.3-45.7% protein in the fines fraction and 15.3-17.3% protein in the coarse fractions. They alsc studied the effects of air velocity on protein shift, and observed that separation of small starch granules from the light fraction was better at higher air velocity; but the lowest protein content achieved was 15.3%. Youngs (1975) and Vose (1977) have reported the presence of some starch in the protein fraction, presumably due to the presence of small and damaged starch granules in the pin-milled flour. On the other hand, a complete separation of the protein from the starch fraction could not be achieved by pin-milling and air-classification of field peas by Vose et al., (1976). Reichart and Youngs (1978) studied the possible sources and the nature of this residual protein associated with air-classified pea-starch. The protein which is bound to the air-classified starch granules has been referred to as adherent protein, while the protein between starch granules has been characterized as wedge or interstitial protein (Richert and Youngs, 1978). According to them the protein bodies, agglomerates of starch granules embedded in protein matrix, chloroplast membrane remnants and dehydrated stromal material were found to contribute to this residual protein. These were not readily separated from starch granules even after several pin-millings

and are concentrated in the starch fraction. Therefore, differences in the levels of these components containing protein among legumes would contribute to differences in protein separation efficiency (PSE) in the air classification of legumes (Tyler et al., 1981). Repeated milling and air-classification of the starch fraction (SI) from field peas has proven to be effective in disintegrating the agglomerates and in removing most of the protein bodies from the starch granules (Figure 8). Scanning electron microscopy of the starch fraction revealed protein bodies attached to the surface of starch granules, agglomerates of starch granules embedded in protein matrix and an uneven granular surface. However, repeated milling and air-classification purified the starch fraction but reduced its protein content and yield of starch fraction (Figure 9), the starch-rich fractions still contained over 2% protein after four cycles of pin-milling and air classification. Water washing of this unmillable protein of the IV fraction yielded a suspended and water-soluble fraction. Chemical, amino acid and electrophoretic analyses characterized these fractions as dehydrated stromal material and chloroplast membrane remnants (Tyler 1982). Sosulski and Youngs (1979) studied the efficiency of pin-milling and air-classification for concentration of components in the fines and coarse fractions in the starchy legume as well as the non starchy legumes, like lupine and soybean. The protein contents of soybean and lupine flours were 52.6% and 41.4%, respectively, while the starchy legumes contained 19.5 - 29.8% protein.

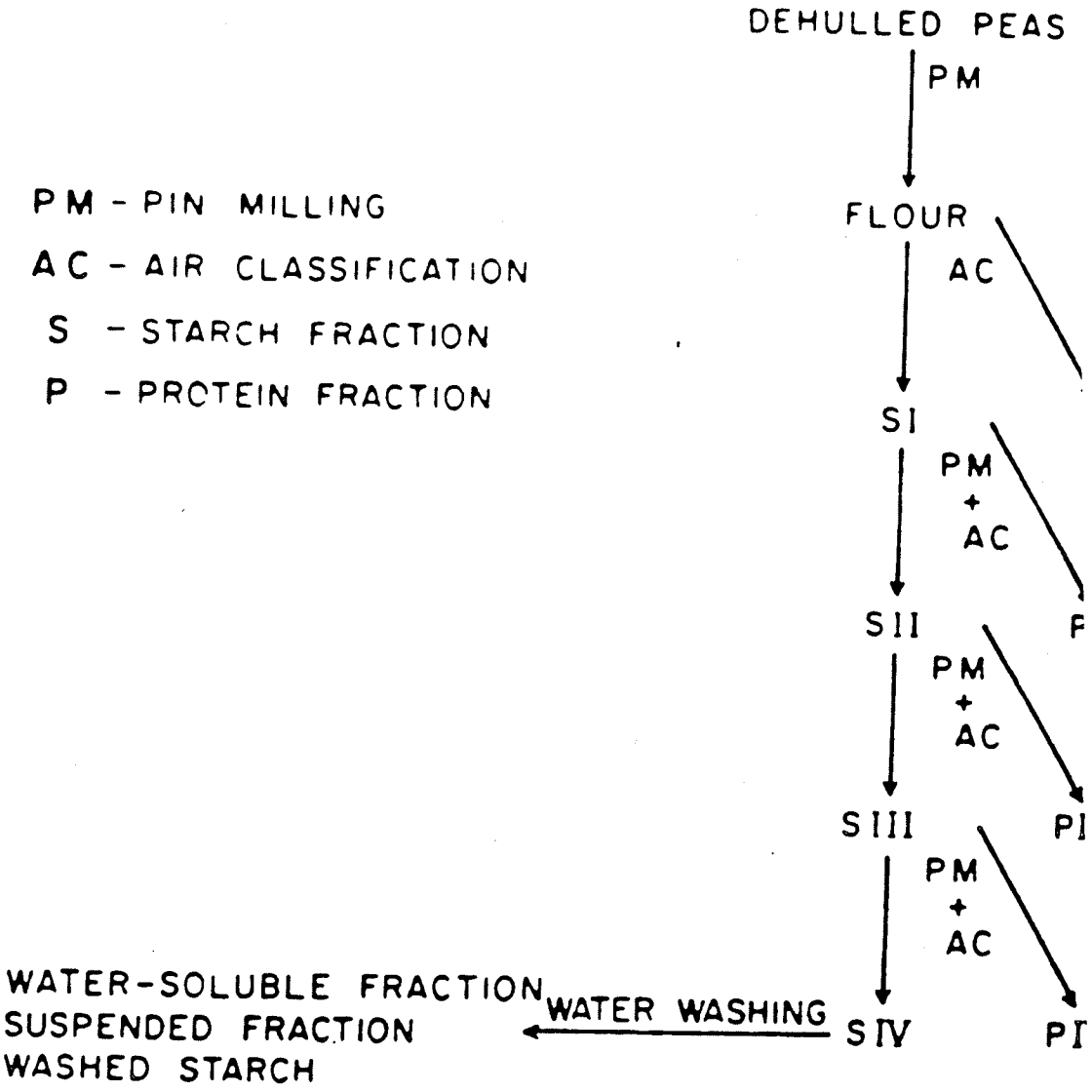


FIGURE 8: Schematic diagram of process used to prepare air classified and water-washed pea starch.

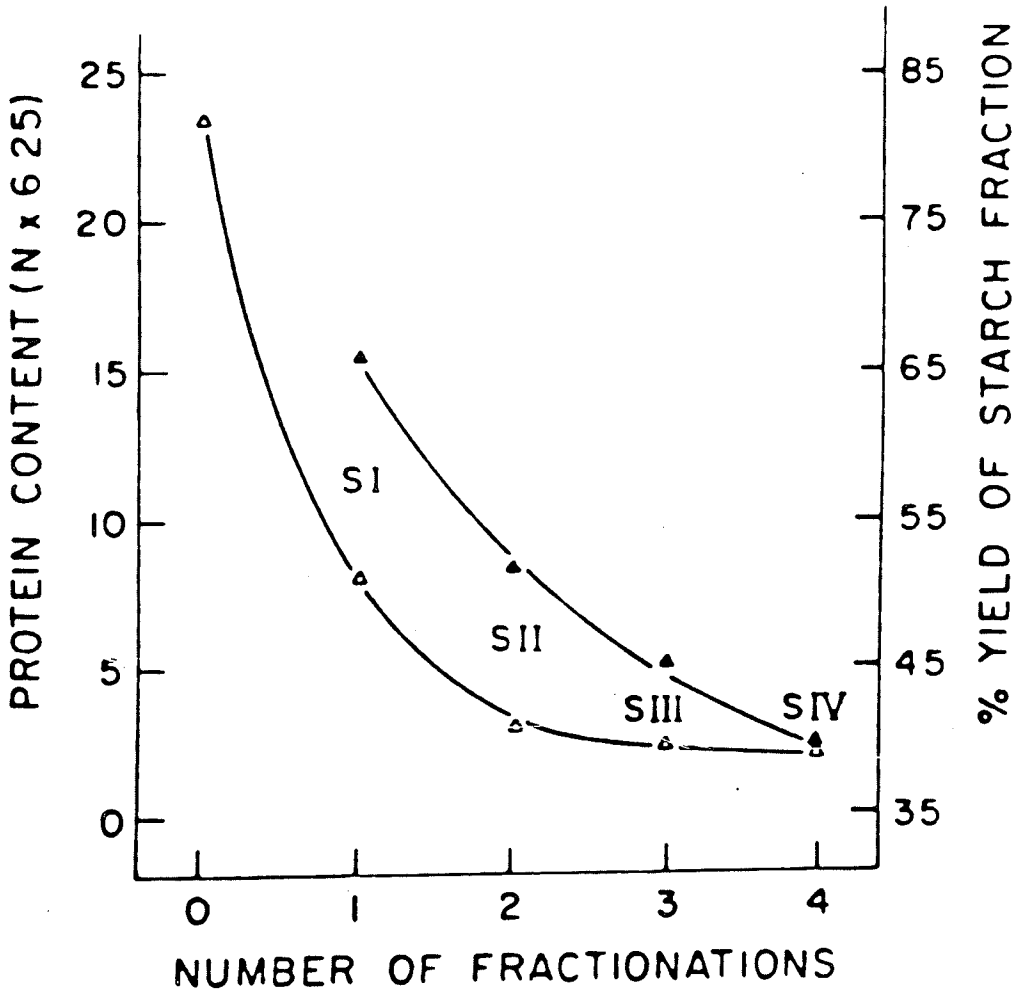


FIGURE 9: Purification of starch fraction by repeated pin milling and air classification. △ protein content; ▲ yield of starch fraction.

Air-classification of the pin-milled soybean and lupine, both low starch legumes, showed only slight enrichment of protein in the fines fractions, while much of the protein remained in the course fractions. Air-classification appeared to be of no benefit in refining these legumes. Considerably higher content of lipid in chickpeas, than the other starchy legumes, caused interference with air-classification of the pin-milled flours exhibiting low protein recovery in the fine fractions due to the tendency of flour to agglomerate.

Other cellular constituents were also fractionated by the air classification process. The fine fractions contained from 29 to 66% protein, as well as most of the lipid and ash while the coarse fraction contained 51 to 68% starch and much of the crude fiber, primarily hulls segregated with the large, dense starch particles. The flours of these legumes and their air-classified fractions also exhibited a wide range of functional properties like absorption, emulsification, foaming, whippability and gelation.

Patel et al., (1980), following the fractionation procedure of Vose et al., (1976), using an Alpine Microplex air-classifier, were able to fractionate navybeans into protein concentrate (PC) and starch residue (SR). The resulting fractions were analysed to asses the impact of processing on the amino acids and mineral distributions. The overall yields of the PC and the SR were 34.7% and 61.9% respectively, containing 61.9% protein in their protein concentrate and 10.7% protein in the satrch residue, respectively, starting from flour

with 30.4% protein. The navybean flour and its air-classified fractions had a relatively high lysine content and the low sulphur amino acid content characteristic of legume proeins. However, the fractions showed no major variation in the amino acids, of course with the exception of sulphur containing amino acids. Major shifts of S-amino acids (40%) were evident into the starch fraction. Colonna et al., (1980) fractionated dehulled broadbeans, smooth peas and wrinkled peas which included consecutively two times of grinding and air-classification for broadbeans and wrinkled peas, respectively and three times of the same for smooth peas (Figure 10). The enrichment of protein in the light fractions (L1 and L2) of broad beans and smooth peas was very similar, whereas the recovery of protein was significantly poorer for wrinkled peas due to the higher lipid content of the initial flour which prevented a good separation of particles in the air-stream and to the broad distribution to the granule size. The third run in the case of smooth peas, however, did not contribute in the improvement of the protein, compared to the yield of the light fraction. The light fractions of all three legumes were concentrated by lipids, ash, furfural generators, cellulose and lignin. Ethanol soluble sugars of broad beans and smooth peas also followed the same pattern but a small shift was noticed in the case of wrinkled peas. Sahasrabudhe et al., (1981) studied four varieties of white bean and their air-classified starch and protein fractions and reported their chemical composition and certain functional properties were

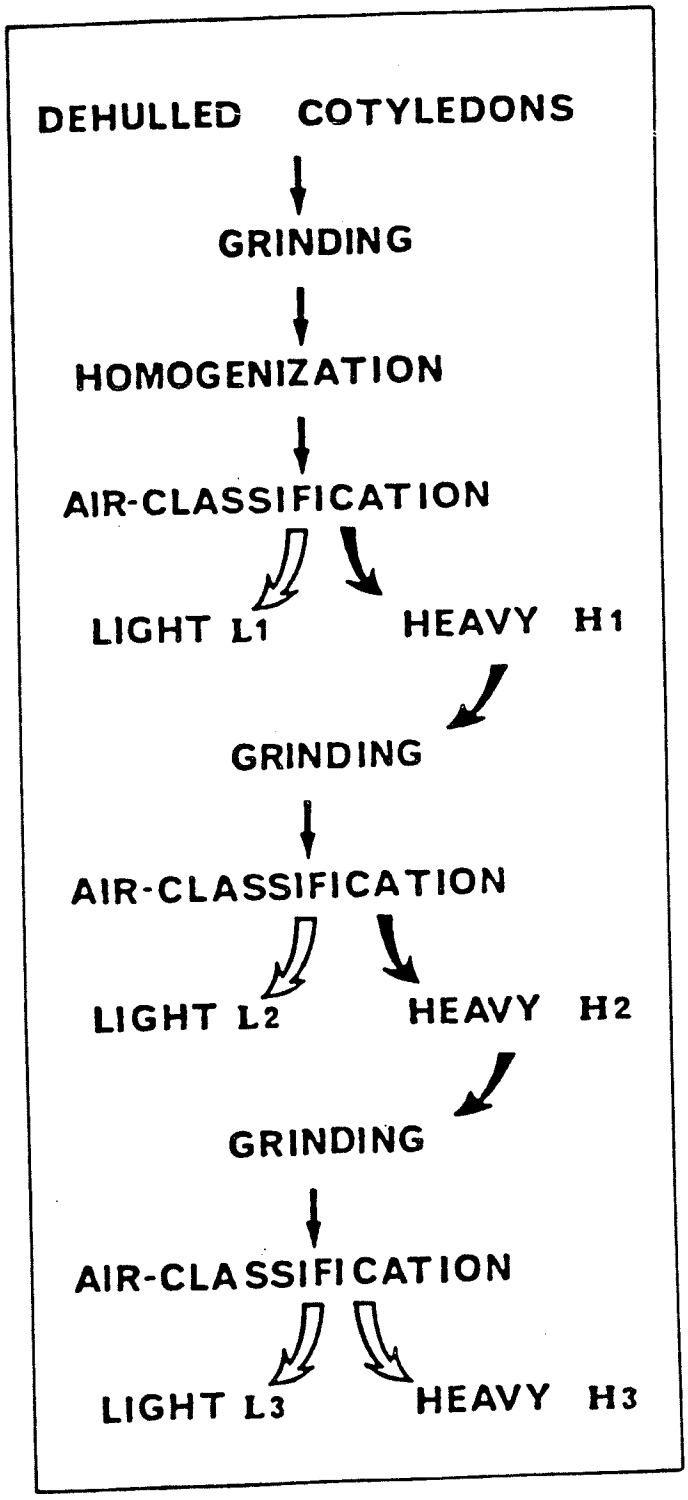


FIGURE 10: Schematic flow sheet for dry processing of legume flours.

found to be very similar to other vegetable proteins. Tyler et al., (1981) have recently fractionated dehulled samples of eight grain legumes (mungbeans, green lentils, Great Northern beans, fababeans, field peas, navy beans, lima beans, and cowpeas) under controlled conditions by pin-milling and air-classification with the double-pass procedure employed by Vose et al., (1976). The suitability of pin-milling and air-classification as a method of producing starch and protein concentrates from these legumes were assesed on the basis of protein separation efficiency (PSE) plus the starch and protein contents of the air-classified fractions of each legume. Table 6 shows the calculated values for starch separation efficiency (SSE) and protein separation efficiency for each legume. Significant differences were detected in PSE among the legumes but differences in SSE were insignificant. They reported much higher PSE values compared to the values obtained by Sosulski and Youngs (1979) by using the double-pass procedure which contributed in starch free milling of greater proportion of protein, thereby concentrating it into the protein fractions. The differences in PSE among legumes were attributed to differences in seed hardness or in the amount of residual protein that can not be separated from the starch granules during pin-milling. Significant correlations were detected between PSE and seed hardness or the amount of residual protein retained in the flour. They also obtained a linear correlation between the levels of protein, starch, fat, ash and crude fiber in air-classified fractions with levels of these con-

TABLE 6: Starch Separation Efficiency (SSE) and Protein Separation Efficiency (PSE) Achieved in Pin Milling and Air Classification of Eight Legumes

	SSE (%)			PSE (%)		
	Series			Series		
Legume	1	2	Mean[a]	1	1	Mean
Mung bean	96.7	96.0	96.4a	89.1	88.7	88.9a
Lentil	100.0	94.0	97.0a	87.1	87.2	87.2a
Northern bean	98.7	97.8	98.3a	87.6	86.4	87.0a
Faba bean	100.0	97.5	98.8a	85.2	82.9	84.1b
Field pea	99.1	99.8	99.5a	82.2	83.3	82.8bc
Navy bean	97.6	98.7	98.2a	78.9	8.16	80.3cd
Lima bean	100.0	98.2	99.1a	81.1	79.2	80.2cd
Cowpea	96.9	97.1	97.0a	78.8	77.6	78.2d

[a]Means followed by the same letter are not significantly different ($P<0.05$)

stituents in the starting flour.

Reichert (1982) studied the air-classified fractions obtained from dehulled peas varying widely in protein content to investigate the effect of variability in chemical composition of the flour on the composition of the fractions. Employing the double-pass procedure of air-classification using 25/20 vane settings, protein concentrates and starch concentrates were obtained containing 33.6 - 60.2 and 3.8 -11.3% protein, respectively, from field peas ranging from 14.5-28.5% in protein content. It was found that the starch, lipid and cell wall material contents of the flour and air-classified fractions were negatively correlated with the protein content of the peas. Positive correlation; were obtained between the yields and the protein content of the air-classified fractions and the protein content of the peas. Lower SSE values at a higher air flow were observed whereas PSE values showed the opposite trend. Air-classification at lower air flows (smaller cut sizes) resulted in protein and starch fractions containing higher levels of protein (Figure 11). However, at higher air flows (vanes at 25/20) the SSE and PSE values were found to be positively correlated with pea protein content and were increased with increasing protein content as shown in Table 7. Therefore, it was neccesary to adjust the vane setting of the air-classifiers accordingly to compensate for the variability of the protein content of the seed. Aguilera et al., (1982) confirmed a relationship between particle size distribution and protein content in high-protein fractions obtained from

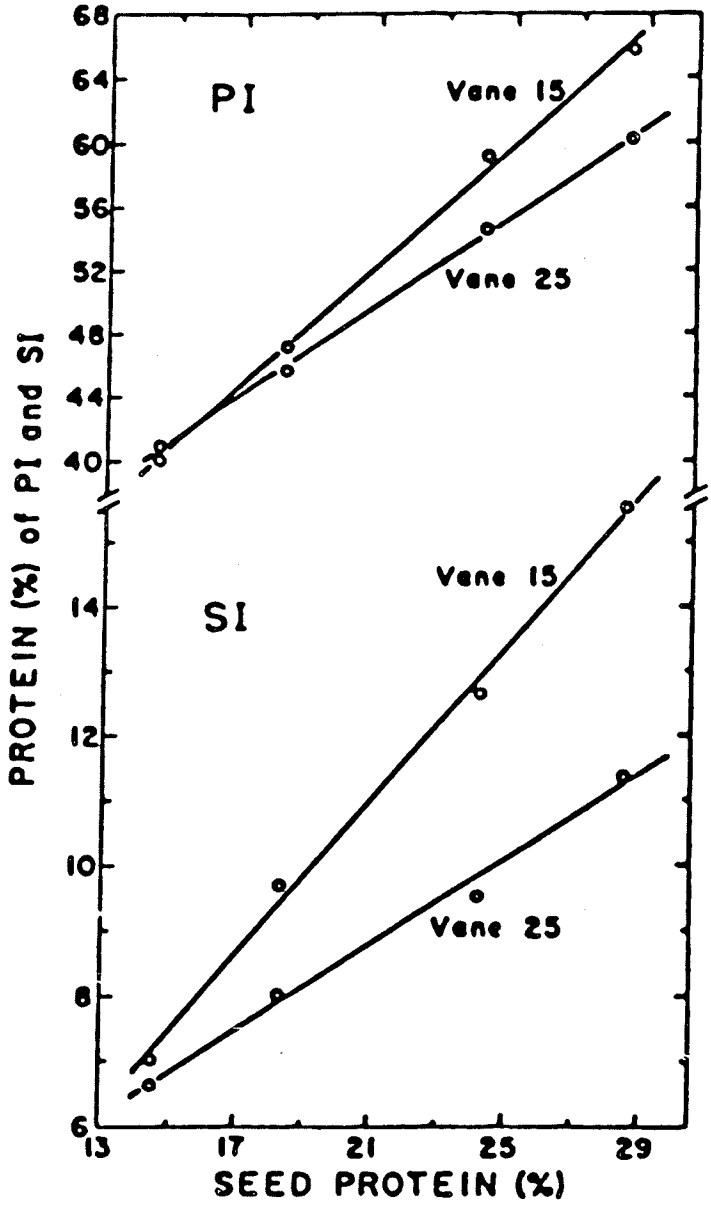


FIGURE 11: Effect of air classifier vane setting on the protein content of the PI and SI fractions prepared from dehulled peas varying in protein content.

TABLE 7: Starch Separation Efficiency (SSE) and Protein Separation Efficiency (PSE) of Peas Varying in Protein Content

Protein (%)	Vanes 25/20[a] SSE (%)	Vanes 25/20[a] PSE (%)	Vanes 15/15 SSE (%)	Vanes 15/15 PSE (%)
14.5	88.7	82.6	94.1	80.4
18.3	94.7	85.6	98.3	83.4
24.2	94.4	87.6	102.3	84.8
28.5	98.9	89.6	101.1	79.4

[a]The vane setting of the air classifier regulates the air flow in the classifying chamber.

roasted navy beans by air-classification. The finer flours having 50% cumulative mass points at lower particle sizes will contain more protein (Figure 12), which means greater protein shift may be further accomplished by finer grinding and followed by air-classification with the adjustment of the cut point.

2.4. Effect of Seed Moisture on The Air-Classification

In an early study Kent (1965) reported a distinct effect of moisture content of wheat and wheat flour on the achievement of the greater protein displacement and endosperm breakdown on the fractionation of the flours by impact milling and air-classification. The effect of seed moisture content on the air-classification of grain legumes were first reported by Tyler and Panchuk (1982). They prepared starch and protein concentrates from dehulled samples of field peas and fababeans containing 3.8-14.3% moisture by pin-milling and air-classification. Seed moisture content affected both yield and the composition of the air-classified fractions. At reduced seed moisture content there was a decline in the yield of starch fraction, the protein content of the starch and protein fractions and starch separtion efficiency. At the same time, at lower seed moisture content, increases were observed in the yield of the protein fractions, PSE, and the neutral fiber content of the protein fractions. However, a moisture content below which no significant change occurred for each of these characteristics was found.

Increased seed hardness was observed with declining mois-

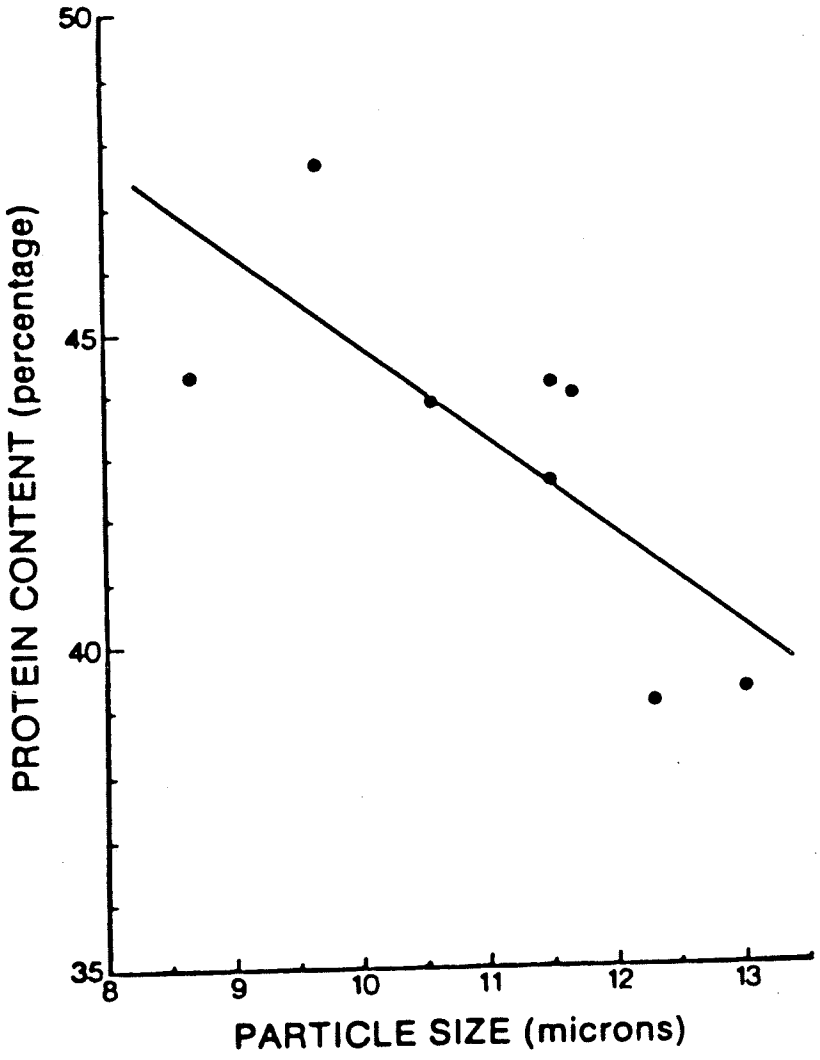


FIGURE 12: Variation of protein content with particle size in high-protein fractions at 50% cumulative mass. Cumulative mass refers to the percent mass of particles larger than a certain particle size as determined from a particle size distribution curve.

ture contents. Impact milling is more efficient as seeds become harder and more brittle, facilitating in the improvment of the yields of the protein fraction (PF) at lower seed moisture contents. Tyler and Panchuk (1982) observed lower feed rates to the air-classifier (at a particular feed-gate setting) at lower flour moistures, and thus a more complete separation of protein and starch was achieved.

2.5. Sieving Analysis: A Conventional Dry Method

It was recognised long ago by several workers that flour particles vary in size (Harris, 1955). The effect of particle size distribution exhibits marked differences in chemical and physical characteristics (Wichser and Shellenberger, 1948). The effect of particle size distribution on the quality of flour in terms of the ratio of starch to protein content, has been known for many years (Gracza, 1959). Baking quality is partially influenced by flour granulation. The subject of flour particle size and particle size distribution of flour inspired investigators to explore the possibilities of separating flour components. These studies were based on size classification. One group of investigators fractionated the flour by reducing the particle size of flour to various degrees of fineness. Their samples had progressively larger specific surfaces and progressively slightly narrower, but still not considerably different, size distribution character than their parent stock. Other investigators have divided flour into various groups according to the particle size ranges. In the latter approach, flour fractions are produced

which had specific surfaces larger and smaller than the parent flour, and narrow size ranges with considerably different size distribution characteristics (Gracza, 1959). This second approach was followed by many workers in the fractionation of flour by sieving methods based on size classification only, but not applying flow dynamic characteristics of air-clasification. Sieving is a simple method quite frequently used and is pertinent to measurement of particle size distribution of flour (Dallavalle, 1948). Irani and Fong (1961) reported that Whiteby (1954) divided the mechanics of sieving into two steps. During the first step, particles with a size much smaller than the aperture of sieve opening pass through. The second step was referred to as relatively slower, during which particles whose size is close to that of sieve openings are sieved through. In this sieving procedure the weight of material that passes a screen of one size and fails to pass a screen of smaller size is determined. Certain size distribution data, which appeared to be simple and direct, are obtained from such measurements (Hildebrand et al., 1942). In sieving, the size-distributed particles are commonly referred as 1) coarse particles (4-100 mm), 2) medium particles (0.2-4 mm), and 3) fine particles (under 0.2 mm) done by arbitrary classification on the basis of the particle size range (Tyler, 1982). Dallavalle (1948) reported certain advantages of sieving besides simplicity which include relatively low equipment cost, short analysis time and wide applicability. However, a number of short comings of sieving, like 1) sifting is slow in

the finer size ranges, 2) sieve motion, sifting time, sample load, proportion of fine material in sample, and composition of sieve material affect the efficiency of sieving, 3) the tendency of fine material to agglomerate seriously interferes with accuracy, 4) the size distribution is dependent on the shape of both the particles and the sieve openings, 5) loss of oversize material due to abrasion occuring on the sieves, and 6) dusting causes errors in the small size range are reported to be its disadvantages (Hildebrand et al., 1942; Dallavalle, 1948 and Tyler, 1982).

2.5.1. Classification of Cereals by Sieving Method

A correlation between particle size of flour and baking quality has emphasized the importance of a satisfactory method for determining the particle size distribution of flours. As such, the sieving technique has received most of the attention among the various methods used for measuring flour particle size. The particle size distribution analysis has been conducted by many workers (Markley, 1934; Kent-Jones, 1941; Hildebrand et al., 1942; Wichser and Shelenberger, 1948; Irani and Fong, 1961; Donelson and Yamazaki, 1972) using the method of sieving and compared with other methods of air flotation, sedimentation. microscopic, centrifugal and Coulter counter. The sieving method was found to be simple, reliable, reproducible and applicable to particle sizes up to 37 μm. Earlier workers used silk bolting cloth to investigate the granulation of wheat flour by sieving analysis. They experienced difficulty in making a complete separation of flour into well-de-

fined particle size groups. Most probably the mesh fineness of the silk cloth and inaccuracies in the shape and size of the aperture openings, besides the tendency of the particles to agglomerate, were the major limitations. Later, the method of wire sieve analysis was developed to overcome these difficulties. Wichser, et al., (1947) made a complete fractionation of a commercially milled wheat flour into several well defined particle size ranges with a Ro-Tap shaker equipped with metal sieves (Tyler standard sieves) nos. 115, 150, 170, 200, 250, 300 and 400 as shown in Figure 13. They determined the physical and chemical properties and baking qualities of each group. They showed a wide range in characteristics, which were related to the size of the flour particles. A decrease in the size of the flour particles to the lowest limit of approximately 38 μm was accompanied by an increase of ash, protein, water absorption, gassing power, area under mixograph curves, and loaf volume. The 0-38 μm fraction was composed largely of free starch granules and constituted approximately 27% of the whole flour. This fraction showed a low protein content, low viscosity, reduced area under mixograph curve, and low loaf volume. This same fraction showed higher values for ash content and gassing power. No significant trend for inorganic elements in distribution in the various fractions was noted related to the quantity of ash or particle size. The largest particles(105-150 μm) had the lowest protein content, and produced shell topped bread of poor volume and appearance and showed a creamy coloured crumb. This indicated a higher con-

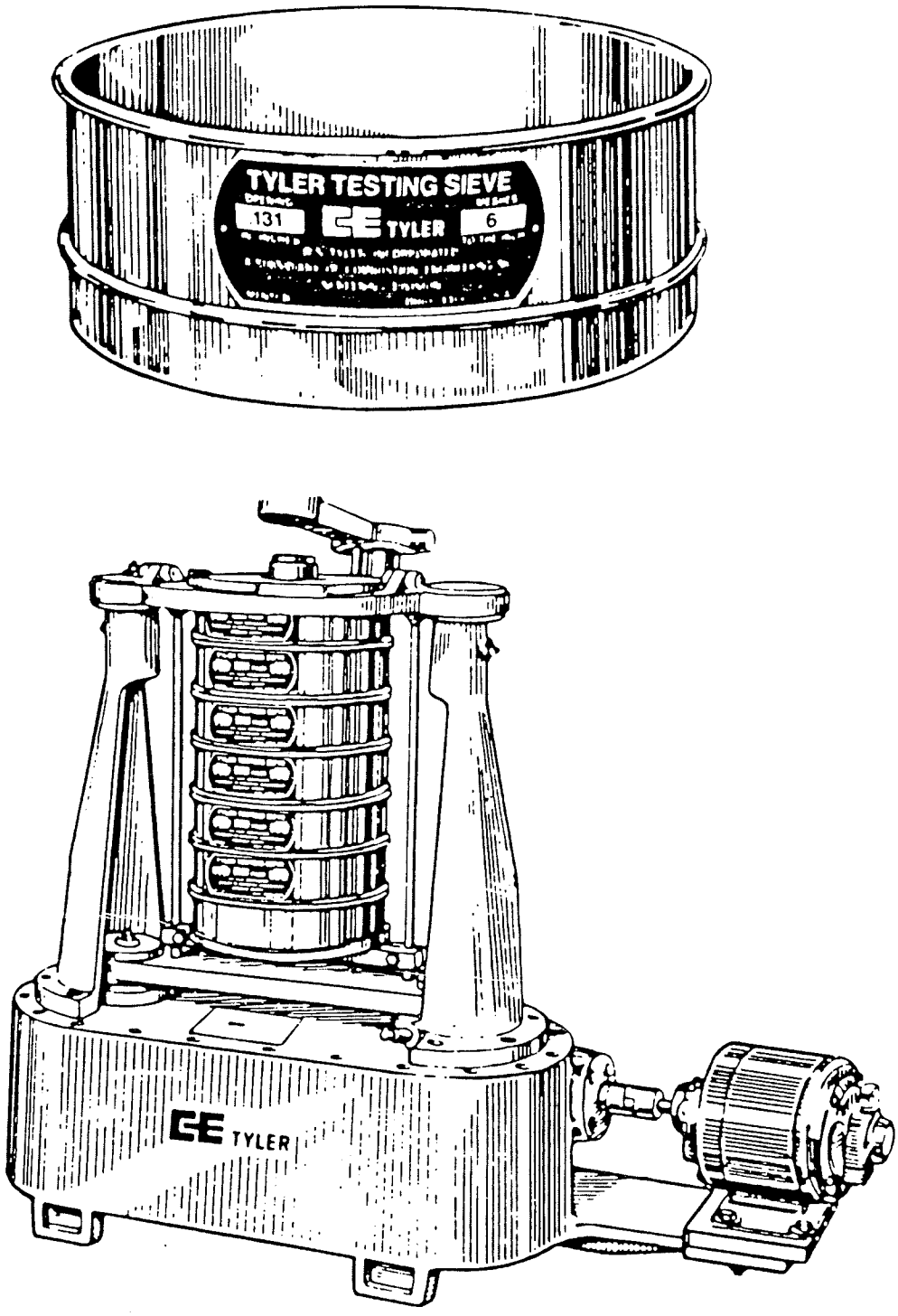


FIGURE 13: Tyler testing sieve and Ro-Tap Testing Sieve Shaker with sieves.

centration of the carotenoids, since the finer particles produced bread with a creamy white colour. The baking results of earlier investigators, despite limitations of using cloth in sieving, had an agreement with the results of Wichser et al., (1947). Kress (1929) and Pulkki (1938) also found that the coarsest and finest flour particles exerted detrimental effects on flour quality in general. Pulkki (1938), employing cloth in the sieving tests, obtained definitely better baking characteristics with the medium-fine flour than the coarser and finest flours. The coarsest and the finest materials produced smaller loaves and the crust and colour of the crumb were inferior in quality, apparently due to low protein content in the finer and coarser flours. Harris (1955) separated hard red spring wheat flour according to particle size by sieving method. The fractions were much influenced by the location of growth, and to a lesser extent by variety, the flour particle size distribution is represented in Figures 14 and 15. The interaction between sources of variation like varieties, locations, and sieve sizes were found to be very significant. Marked differences in the particle size distribution were observed in the varieties which were known to be difficult to mill from the varieties known to be satisfactory for milling. Pulkki, (1938) also reported best loaves with the fractions of the intermediate particle sizes and the smallest loaf with the fraction of the smallest particle sizes which were obtained from one hard red spring wheat by sieving. The flour ash content varied inversely with particle size as

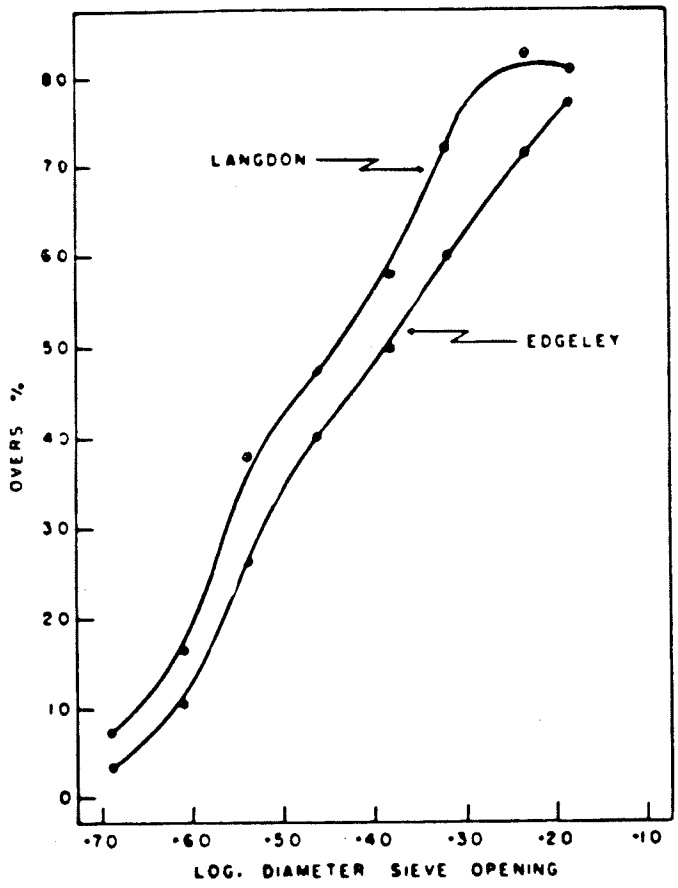


FIGURE 14: Particle size distribution curves for different hard red spring wheat flours. Broken lines indicate distribution limits for six acceptable milling varieties. Unbroken lines show the distribution for new varieties not released and of doubtful milling quality.

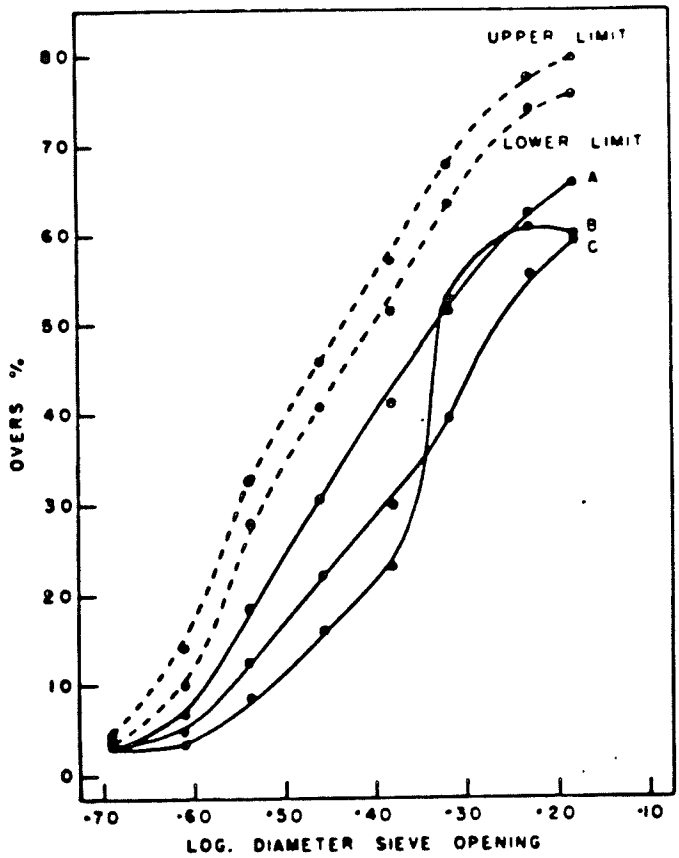


FIGURE 15: Limits of particle size distribution for flours milled from wheats grown at six locations in North Dakota.

reported by previous workers. Gardner et al., (1973) also found the same trend in the composition of that portion of the fractions which were obtained by sieving on particle size ranges from cooked defatted corn germ flakes. The fractions showed decreases in protein and sugar values as the particle size increased. Ash and phosphorous contents were strikingly increased as the particle size decreased. But starch content in the sized fractions was inversely related to ash and phosphorous. Hudson and Ogunsua (1976) reported that the sieving of cassava flour yielded a fraction suitable for use in composite flour (70:30) for bread baking. In both the processes (ADD and CBP) of bread baking, the highest loaf volume was obtained by the finest fraction and the coarsest fraction produced the lowest (1390 cc vs. 1170 cc). Unfractionated cassava flour was found to be less suitable giving low loaf volume compared to the fractionated finer fraction. Pomeranz et al., (1976) reported the fractionation of a commercial malt flour into fractions of differing particle size by sieving and subsequently by air centrifugation elutriation on a microclassifier. Initially they separated the malt flour by sieving through a Tyler sieve 250 with 63 μm sieve openings into two main fractions of overs and throughs of 250 mesh sieve. The overs were then separated into four fractions by sieving using Tyler sieves of increasing mesh size. Between these two main fractions, the coarser fraction (overs of Tyler sieve 250) was higher in protein content (12.5%), diastatic power, and α-amylase activity compared to the original malt flour and the

finer fraction (throughs of Tyler 250) containing low protein content of 10.6% and 9.1%, respectively. The malt flour sample showed a 46% yield over a 250-mesh Tyler screen. Moreover, some of the subfractions from the coarsest (>250 mesh size) fraction showed consistant increase in both protein content and enzymatic activity. Significant shifts in total ash and mineral components were evident during fractionation of malt flours. An extensive review of literature reveals no information on the fractionation of legumes on the basis of particle size range by the sieving method.

2.6. Possible Surface "Charge" Phenomena on Major Flour Components:

A computer search for literature to review failed to gather any information on the existence of surface charge or its nature and property or the occurrence of "static charge" either on flour or its major components of protein and starch. Tyler (1982) observed a tendency for flour particles to agglomerate during the process of air-classification of legume flours which interfered with a perfect separtion of coarse and fine particles. He reported briefly that generation of `static charges' during milling may cause fine particles to clump together or to adhere to coarser particles. Similarly, Pulkki (1938) reported that the use of sieving tests in the determination of the real sizes of particles of wheat flour became more uncertain with the smaller particles. Also sieving difficulties were increased with the very dry material. This later observation in conjuction with certain other facts however,

induced the assumption that electrical phenomenon can play an important, in some cases even decisive, role in the bolting process. Unfortunately, no information could be gathered about the kind of charge possessed by the protein or starch component individually, which may play a role in the creation of electrical phenomena.

2.7. Toxic or Antinutritional Factors

It is ironic that while nature has generously provided man with a liberal supply of plant protein foods, she has at the same time seen to contaminate these foods with a variety of substances which may be "toxic" to the animal body (Liener, 1966). The presence of these "toxic" or antinutritional factors can decrease the nutritional quality of a product and the availability of nutrients and may produce adverse physiological effects in people and animals. Unfortunately, legume seeds contain a wide range of these constituents which have adverse effects on enzyme activity, digestibility, nutrition and health, if not properly inactivated (Elkowicz and Sosulski, 1982). Phytic acid, trypsin inhibitors, vicine and convicine are four of these undesirable factors that will be focused in this review.

2.7.1. Phytic Acid

Phytic acid or myoinositol 1,2,3,4,5,6-hexakis dihydrogen-phosphate is a hexaorthophosphate ester of myoinositol (Figure 16), it occurs primarily in plant tissues such as seeds and grains. In plants, phytate is the principal storage compound of phosphorus and represents 60-90% of total phospho-

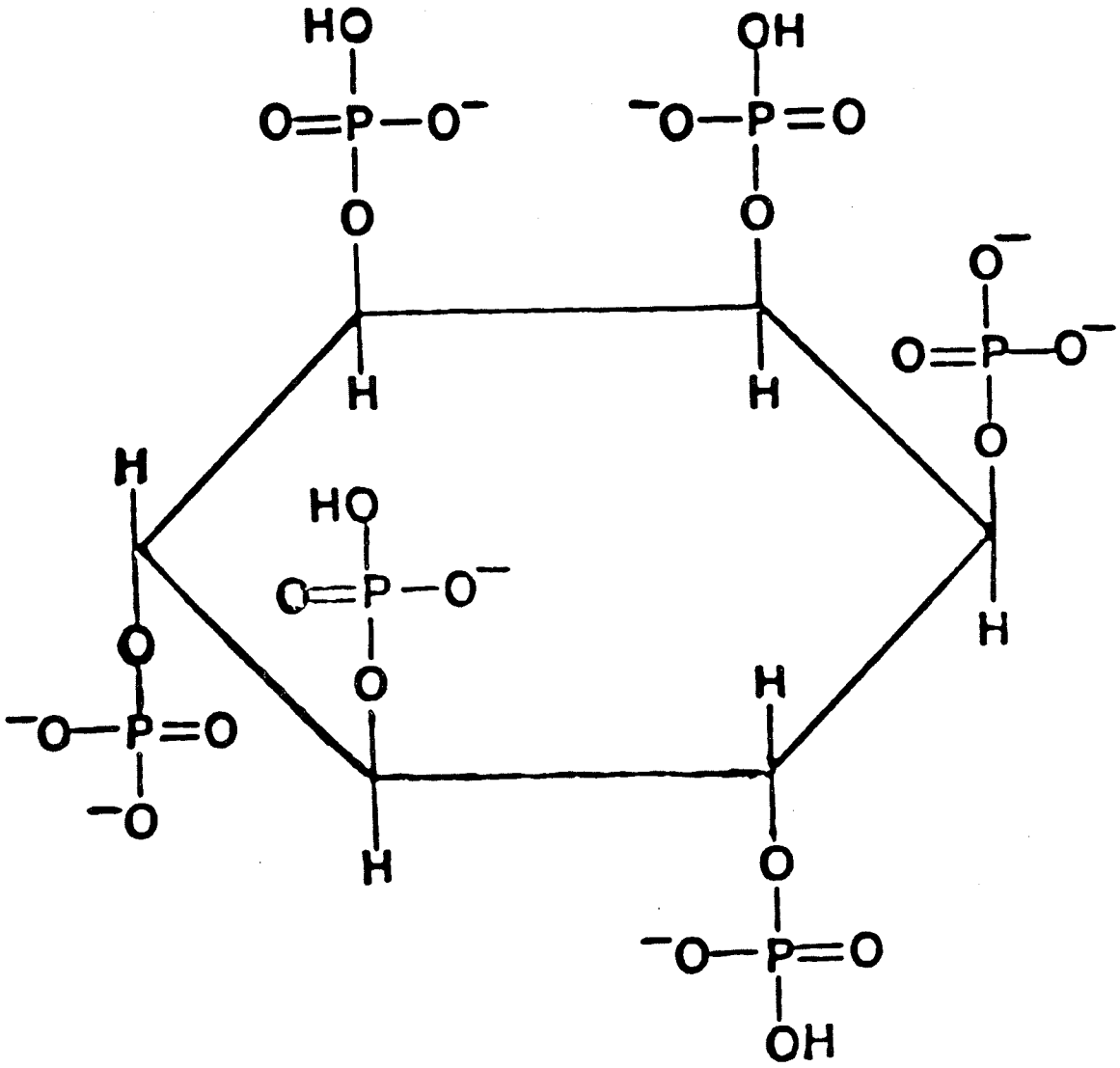


FIGURE 16: Structure of phytic acid.

rus that is gradually utilized during germination (Nahapetian and Bassiri, 1976). In grains and oilseeds, phytic acid occurs as the calcium-magnesium salt, phytin (Lolas and Markakis, 1975). It constitutes 1-5% by weight of most nutritionally important legume, cereal and oilseeds (Cheryan, 1980). The metal ion chelating properties of phytic acid has been shown to be responsible for causing antinutritional activity. Numerous studies indicate that it interferes with intestinal absorption of certain minerals, specially zinc, calcium, magnesium and iron, to make them physiologically unavailable in monogastric animals by forming an insoluble complex with di- and trivalent cations at neutral pH (Bassari and Nahapetian, 1977). In wheat, phytates are localized mostly in the bran which contributes significantly to the unavailability of minerals (Reinhold et al., 1974). Therefore, the controlling of the extraction rate in flour, according to the mineral and phytic acid ratio, might help to increase mineral availability (Dadgari-Raymond, 1980).

Lolas and Markakis (1975) determined the phytic acid level of dry beans and found a high degree of correlation between total phosphorus content and phytic acid level. They also isolated a protein-phytate complex and observed that over 99% of the total phytic acid was in a water soluble form. It is evident that phytic acid occurs to some extent in most foods in association with protein and exhibits its unique ability to reduce nitrogen solubility (Fontaine et al., 1946) and nitrogen recovery (Gillberg and Tornell, 1976) and form an

insoluble protein-phytate during the acidic precipitation of protein at its isoelectric point (O'Dell and de Boland., 1976), and Okubo et al., 1976). Barre (1956) also reported that protein-phytate complexes are less subject to proteolytic digestion than the same uncomplexed protein. O'Dell and deBoland (1976) have suggested that the bioavailability of zinc and other minerals is even less with low-digestable phytate-protein complex compared with a zinc-phytate complex alone. The interaction of phytic acid with minerals and proteins is recognized to be one of the primary factors limiting the nutritive value of cereal grains and legumes and poses a concern on the digestibility of protein and utilization of plant protein concentrates and isolates. With this point in mind, Martens (1982) has investigated the levels of phytic acid in protein concentrates and isolates prepared by physical and chemical methods. An increase was observed in phytic acid level from 1.5% present in the pin-milled fababean flour (29.9% protein) prepared by the air classification method. Under chemical recovery techniques, protein isolates were prepared by isoelectric precipitation, protein micellization, and salt solubilization/acid precipitation methods. The levels of phytic acid found in the products of these methods are reproduced in Table 8. In the isoelectric method the phytic acid showed its greater concentration with the protein while in the protein micellization method (Murray et al., 1981) the phytic acid level was found to be substantially reduced as compared to the rest of the methods. Elkowicz and Sosulski (1982) also

TABLE 8: Phytic Acid Levels in Isolates Prepared by Three Different Methods

Method of Preparation	Phytic Acid (%)a	Protein (%)b
Isoelectric Precipitation pH 8.0/pH 4.5	3.0±.1	73.3±.3
Protein Micellization	0.39±.05	89±1
Salt Solubilization/ acid precipitation	1.32±.05	78±2

a. Latta and Eskin (1980)

b. N x 5.7

have reported the proportions of phytic acid present in the air classified protein fractions containing the protein contents ranging from 49-75% (prepared from eleven legumes) were nearly three times that of the original flour. In a recent study, Satterlee and Kadir (1983) observed that the effect of the phytate content of plant proteins on the protein nutritional quality is different for different protein sources. The prepared low phytate content soy isolate did not produce any significant improvment in that proteins nutritional quality in either the rat bioassay or in-vitro digestibility assay. However, low phytate containing high protein bran flour (HPBF) prepared from wheat bran resulted in a significant improvement of the nutritional quality of that protein. This improvment was evident in PER, (c-PER), percent protein digestibility, biological value, and total weight gain by the rats consuming this low phytate HPBF diet.

The presence of phytase, an enzyme capable of hydrolyzing phytates into inositol and free orthophosphate, has been reported in seeds (Chang, 1967; Mandal et al., 1972; Mager et al., 1980 plus Lolas and Makakis, 1977). Eskin and Wiebe (1983) studied the presence of phytase in several cultivars of fababean which showed a marked increase in phytase activity over a ten-day period of germination with a concomitant reduction in phytate.

2.7.2. Trypsin Inhibitors

Trypsin inhibitors are widespread in nature, being present in many plants and animals. These naturally occurring pro-

teins, having the ability to inhibit the proteolytic activity of digestive enzymes are common constituents of legumes and are found to be responsible for reduced protein digestibility, depressed growth and pancreatic hypertrophy (Liener, 1976). The protease inhibitors are the best known, of all the antinutritional factors present among the legumes and have been studied most extensively. Trypsin inhibitors in plant materials were recognized first by Read and Haas(1938). Whitaker and Feeney (1973), in a review on enzyme inhibitors in foods, reported that the structure and properties of trypsin inhibitors of different sources vary widely. Kakade et al., (1969) reported that several of these inhibitors pose dual detrimental effects to the utilization of soybean protein by reducing the protein digestibility i.e. they are capable of inhibiting both trypsin and chymotrypsin and also carry away a significant portion of cystine in the form of inhibitor-enzyme complex, which is resistant to enzymatic attack. In view of varying characteristics and pathological significance of trypsin inhibitors, it was felt necessary to study the individual trypsin inhibitors occurring in different sources. Therefore, the inhibitory effects on the proteins have been investigated in soybean seed (Ham and Sandstedt, 1944; Kunitz, 1947; Birk, 1961; Kakade et al., 1969); limabean (Fraenkel et al., 1952); navybeans (Wagner and Riehm, 1967; Gomes et al., 1979); blackgram (Padhye and Salunkhe, 1980); kidneybeans (Pusztai and Palmer, 1977); and chickpeas (Belew et al., 1975). Trypsin inhibitors in Lathyrus sativus seeds were partially purified,

characterized and the biological properties were reported by Roy and Rao (1971). Bowman (1947) differentiated the trypsin inhibitors of soybean and navybean on the basis of solubility and inhibitory activity. However, most information on trypsin inhibitors of plant origin has been obtained from soybean, which contains five or possibly six trypsin inhibitors (Liener and Kakade, 1969).

The trypsin inhibitors of legumes are low-molecular weight proteins having molecular weights in the range of 8,000 to 23,000 daltons (Liener and Kakade, 1969). The ocurrence of trypsin inhibitors in fababeans (Vicia faba minor) has been reported by a number of workers (Wilson et al., 1972; Warsy et al., 1974; Bhatty, 1977 and 1979). Nitsan (1971) reported the existance of trypsin inhibitors in fababeans which were resistant to heat at 120°C for 20 minutes. In contrast, the trypsin inhibitors were inactivated completely by Wilson et al., (1972) following similar treatments. Similarly Al-Bakir et al., (1982) also found that antitryptic activity was decreased in broadbeans, chickpeas, lentils and mungbeans after soaking at room temperature overnight. When the soaked legumes when heated at 121°C for 30 minutes the inhibitory activities were completely eliminated. Warsy et al., (1974) isolated trypsin inhibitors from fababeans which were found to be resistant to heat at 100°C for an hour in acidic pH but lost activity at 70°C and above. Marquardt et al., (1975) reported that trypsin inhibitor levels were much lower in the cotyledon than in the hull of the seed. The antitryptic activity was found to

be partially responsible for reduced performance in livestock and poultry when fed a fababean containing diet. Bhatty (1975) observed a shift in the level of trypsin inhibitor activity in fababeans during the period of germination. It is reported that fababean contains compartively low trypsin inhibitor activity. This level was observed to be one seventh to one ninth of the level of trypsin inhibitors found in soybean (Bhatty, 1977); however, the extraction solvent may have affected this ratio. These investigations have also reported that the efficiency of extraction is influenced by the solvent used (Bhatty, 1979); El-Mahdy et al., 1981). Conflicting reports regarding the number of individual trypsin inhibitors occurring in fababeans indicate that the matter is not yet resolved. Warsy et al., (1974) isolated four trypsin inhibitors from fababeans, two of this were purified and their properties were studied. In another occasion, Bhatty (1979) obtained three trypsin inhibitor fractions by ammonium sulphate precipitation and column chromatography. However, both acid and alkali electrophoresis of each fraction revealed that they are highly heterogeneous in nature. Upon ultracentrifugation these three inhibitors each showed a single peak having sedimentation coefficients of 1.2 S, 1.5 S and 1.7 S for fractions I, II and III, respectively. The amino acid composition of each fraction was determined, each showed high levels of cystine and low levels of isoleucine while methionine was present in trace quantities. In contrast, El-Mahdy et al., (1981) obtained as many as six fractions containing

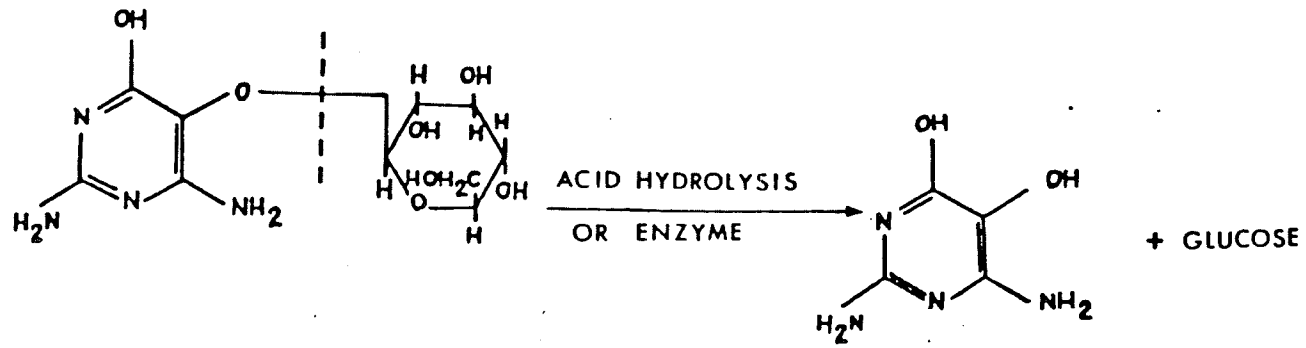
trypsin inhibitors employing DEAE-cellulose. However, these fractions were not analysed for their homogeneity and thus the information concerning the number of trypsin inhibitors present in fababeans remained in question. Further research was initiated to investigate the fate of trypsin inhibitors during processing of legumes, in particular fababeans, as food ingredients. In all the cases it was observed that the trypsin inhibitors always tend to concentrate mainly with the protein fractions. Marquardt et al., (1975) reported higher levels of trypsin inhibitors in the protein concentrates prepared from fababean flour by air classification and also showed a considerable variation in the levels of trypsin inhibitors among cultivars. Elkowicz and Sosulski (1982) also reported the same migration pattern of trypsin inhibitors with the protein fractions in all the legumes under investigation and were almost nearly two to three times those of the original flours. Gueguen et al., (1980) found that protein isolates prepared by alkaline extraction followed by isoelectric precipitation retained only one third of the trypsin inhibitor activity of the original flour.

Processes used for the preparation of protein concentrates and isolates, employing either dry or wet methods, are mainly aimed at achieving higher protein concentrations and yields, the fate of trypsin inhibitors has not had extensive consideration in these attempts. In a recent study Aguilera et al., (1982), considering the fate of those inhibitors, has made an attempt to prepare high protein fractions to be used

as food ingredients by reducing the levels of trypsin inhibitors to a significant extent by roasting navybeans prior to milling and air-classification. They reported that the residual trypsin inhibitor activity of high protein fractions obtained from roasted navybeans by air-classification exhibited reduced antitrypsin activity ranging from 25-108 TIA/mg protein, down from 116 units/mg protein of raw flour.

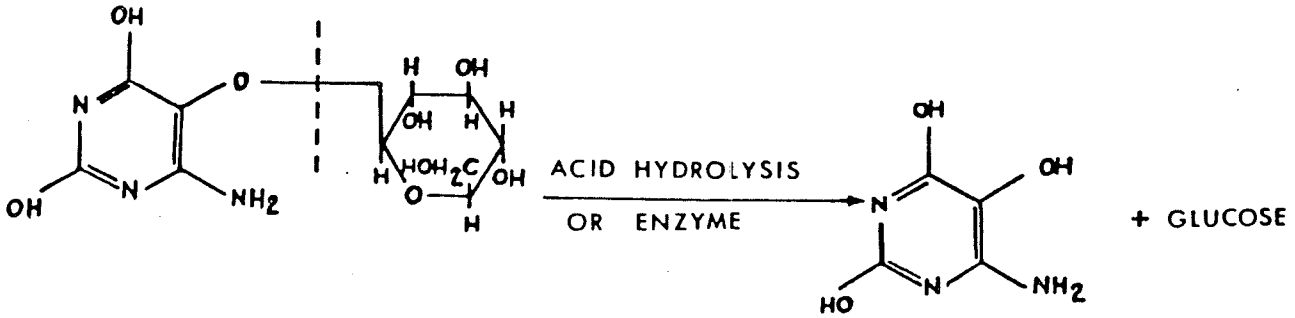
2.7.3. Vicine and Convicine

Vicine {2,6 diamino-4,5-dihydroxy pyrimidine, 5-(β-glucopyranoside)} and convicine {2,4,5-trihydroxy-6-amino pyrimidine, 5-(β-D-glucopyranoside)} are two naturally ocurring toxins present in fababeans (Mager et al., 1965; Bendich and Clements, 1953). The aglycones of these compounds have been implicated as the causative factors for an acute hemolytic disease known as favism in some susceptible individuals following ingestion of the bean (Mager et al., 1965; Lin and Ling, 1962a and 1962b). The aglycones of vicine and convicine, known as divicine and isouramil, respectively (Fig. 17), have a marked destructive effect on reduced glutathione (GSH) levels of glucose-6-phosphate dehydrogenase deficient erythrocytes, a characteristic feature of favism (Mager et al., 1965; Jamalian et al., 1977). The reduction in the level of GSH consequently affects the structural integrity of the cell membranes and causes hemolysis of red blood cells (Liener, 1979). Pitz et al., (1980) studied the occurrence and distribution of vicine and convicine in a number of legumes including beet root using gas-liquid chromatography. They observed the pres-



VICINE (β-GLYCOSIDE)

DIVICINE(AGLYCONE)



CONVICINE (β-GLYCOSIDE)

ISOURAMIL(AGLYCONE)

FIGURE 17: Divicine and isouramil, the hydrolysed products from vicine and convicine, respectively.

ence of vicine and convicine only in Vicia species. These findings were inconsistant with others (Jamalian et al., 1977; Pitz and Sosulski, 1979) that the occurrence of favism is strictly restricted with the ingestion of Vicia faba and the incidence of favism is more obvious with ingestion of fresh green beans than dry beans Pitz et al., (1980). Olsen and Anderson (1978) and Pitz et al., (1980) further revealed that these glycosides were mainly concentrated in the protein rich fraction produced by pin-milling and air-classification of dry milled fababeans. But the protein isolates obtained by isoelectric precipitation contained considerably lower contents of vicine and convicine. However, protein isolates produced by direct ultrafiltration were almost free of vicine and convicine (Olsen and Anderson, 1978). Pitz and Sosulski, (1979) reported that environmental and varietal differences could affect the vicine and convicine level and total glycoside contents in the mature seed. Obviously these findings will be useful in designing succesful breeding and selection programs of non-toxic strains of fababean. Olaboro et al., (1981a-c) and Maduuli et al., (1981,1982) have demonstrated that vicine and convicine are two thermostable antinutritional compounds which have influenced in animal metabolism. These compounds when fed to laying hens showed reduced egg size, weight, production rate, fertility and hatchability of egg, increased yolk fragility, occurance of blood spots in the egg yolks, a marked increase in liver and plasma lipid concentration, erythrocyte hemolysis in-vitro and deprssed vitamin E level in

blood plasma. D'Aquino et al.,(1981) in an attempt to investigate the presence of haemolytic factors vicine and convicine in fresh dry fababeans, utilised a biological model involving erythrocytes of riboflavin and tochopherol deficient rats. The values obtained with the "biological model" were compared as vicine and convicine equivalents with the values for vicine and convicine with the chemical analysis. The results of this comparison have shown that (1) all the oxidative factors with the biological model potentially haemolytic are present in fababeans and proteins derivatives as free or bound glucosides (2) the only toxic glucosides present in fababeans were vicine and convicine and (3) besides these, other oxidative factors were also present in fresh beans. Most recently Higazy and Marquardt (1983) developed a procedure of removal of vicine and convicine almost completely from dehulled fababeans by extracting with 1% glacial acetic acid at 40°C for 24 hours compared to water or 0.5% Na_2CO_3 solution. The low pH of the acid solution prevented microbial spoilage that occured in the case of water treatment. In contrast, germination did not markedly reduce the concentration of vicine and convicine in fababeans. The literature reports several methods for analysis of vicine and convicine which include 1) Spectrophotometric methods (Higazi and Read,1974; Collier,1976 and Kim et al.,1982) 2) Paper chromatography procedure (Brown and Roberts, 1972, 3) Thin-layer chromatography procedure (Jamalian et al., 1977 and Olsen and Anderson, 1978, and Hoehn et al., 1980) 4) Gas-liquid chromatography procedure

(Pitz and Sosulski, 1979 and Pitz et al., 1980) and 5) High pressure liquid chromatography procedure (Marquardt and Frohlich, 1981 and Quemener et al., 1982). Marquardt et al., (1983) have recently developed an effective isolation procedure with substantial yields of vicine and convicine from fababean protein concentrates with some modifications of an earlier method developed by Olaboro et al., (1981). This presently developed method of Marquardt et al., (1983) is simple to carry out, rapid and yields relatively large quantities of pure vicine and convicine in crystallized form. They have also indicated that this procedure certainly facilitates the research of metabolic effects of vicine and convicine following the ingestion of fababeans by animals.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Raw Materials

The fababean flour (Vicia faba L., Var., Minor, Aladin) of 1982 crop year used in this study was provided by the Glenlea Research Farm, University of Manitoba. The clean whole fababean seeds were ground in an Alpine pin-mill. From this pin-milled flour, a protein concentrate (PC) and starch concentrate (SC) were prepared by air-classification according to the method described by Vose et al., (1976). Other flour samples(used in some of the studies of sieve analysis before obtaining the flour, PC and SC of the Aladin Variety of 1982 crop year) included, pin-milled fababean flour, air-classified protein concentrate and starch concentrate obtained from Glenlea Research Farm of University of Manitoba, 1978 crop (Var. Diana). The protein contents of the flour, PC and SC were 29%, 58% and 20%, respectively.

The materials (pin-milled flour, air-classified protein concentrate and starch concentrate) of Aladin Variety (1982 crop) were only used in the study of proximate analysis, amino acid analysis and the determination of antinutritional factors. This pin-milled flour was fractionated using the sieving technique to yield a protein concentrate.

3.1.2. Chemicals

Reagent grade chemicals were used in this study unless stated otherwise.

3.1.2.1. Phytic Acid determination:

- Phytic acid in the form of sodium phytate was purchased from Sigma Chemical Co. (St. Louis, Mo), the acid was extracted from corn. Purity of sodium-phytate was 97% (d.b.), it contained 15% moisture by weight and had 12 sodium atoms per mole. Analytical grade, 200-400 mesh AG1-x8 anion exchange resin in a chloride form was purchased from Bio-Rad Laboratories (Richmond, CA).
- Ferric chloride ($FeCl_3.6H_2O$) and sulfosalicylic acid were purchased from Sigma Chemical Co. and Fisher Scientific Company, Fair Lawn, N.J. respectively.

3.1.2.2. Trypsin inhibitor assay:

- Trypsin (Twice crystallized, type II, salt free) from bovine pancreas and substrate BAPNA (α-N-Benzoyl-DL-Arginine-p-nitroanilide HCl) were both purchased from Sigma Chemical Company.

3.1.2.3. Vicine and Convicine determination:

- Reagent grade phosphoric acid, ammonium hydroxide, perchloric acid (PCA), and methanol were purchased from Fisher Scientific Company.
- Purified reference samples of vicine and convicine were gererously donated by Dr. R.R. Marquardt of the Department of Animal Science, University of Manitoba.

3.2. Methods

3.2.1. Sieving studies

The starting material (flour, protein concentrate or starch concentrate) was fractionated at different timings with different sample sizes to the different particle size groups using a Ro-Tap test shaker equipped with W.S. Tyler standard wire sieves of 20 cm diameter (Table 9). The sieves were placed one above the other on the sieve shaker in order of increasing sieve openings. The coarsest sieve was placed on top. Initially 200g flour (this was reduced finally to 50g) were placed on the upper most of a chosen nest of sieves. The Tyler Ro-Tap test shaker was operated for different time periods (75, 60, 45, 30, 20, and 10 minutes) to prepare fractions of different particle sizes.

In all the runs seven fractions of different particle sizes were collected:

Fraction # I: material retained on 100 mesh;

Fraction # II: material passing 100 mesh but retained on 140 mesh;

Fraction # III: material passing 140 mesh but retained on 170 mesh;

Fraction # IV: material passing 170 mesh but retained on 200 mesh;

Fraction # V: material passing 200 mesh but retained on 230 mesh;

Fraction # VI: material passing 230 mesh but retained on 325 mesh;

TABLE 9: Tyler Rotap Sieve Stack
Used for Fractionation

U.S. Equivalent	Tyler Sieve	Opening
100 mesh	100 mesh	150 µm
140	150	106
170	170	90
200	200	75
230	250	63
325	325	45
pan	pan	< 45

Fraction # VII: material passing 325 mesh but retained on pan.

After each run of fixed timing, the sieves from the top were carefully removed and weighed.

This procedure was used throughout the study with necessary alterations in sample size, sieving time, etc. and by combining the fraction or fractions of interest of the study from first series of sievings of each 10 minutes of run and then sieving for 20 minutes of the combined fraction to produce a protein-rich fraction.

Methods used to influence or change the electrical charge on flour and concentrate materials included:

(a) Grounding the unit: A long copper wire used simply by attaching its one end to one of the sieves and the other end grounded to the earth via a connection to plumbing.

(b) The use of a Zerostat Anti-Static Instrument: This ion gun designed to eliminate the static build-up, was used by aiming the device at the problem area and slowly squeezing and then slowly releasing the trigger action. Squeezing the trigger gave a flow of positively charged ions over a spread of about 400mm and releasing the trigger slowly produced negatively charged ions.

(c) The addition of Anti-Static Agent: A type of material used to modify the generated charge on fababean flour and concentrate particles was a synthetic tissue-wipe

sheet impregnated with specific chemicals designed to modify surface charges. Commercially this material was known as "Bounce". Samples of flour and protein concentrate were sieved by affixing a number of pieces (2x5 cm in size) within the inner walls of the screens.

(d) A piece of fur: Used to modify the generated charges during sieving.

3.2.2. Determination of Phytic Acid

The Latta and Eskin (1980) procedure was followed for analysis of phytic acid using the following steps.

A. Sample extraction:

i) Extract 5.00g of sample into a 150 ml beaker with 100 ml 2.4% V/V HCl (54 ml conc. HCl/1000 ml) placing it on a magnetic stirrer.

ii) Mix for 1 h at room temperature.

iii) Centrifuge a portion of the sample at 20,000xg in 50 ml centrifuge tubes for 10 min.

iv) Remove 5-10 ml of clear supernatant and store in a vial in the refrigerator until ready for next step.

B. Column Chromatography:

i) Use an ion exchange column approximately of 0.7 cmx 27 cm packed with a piece of glass wool at the bottom and 0.5g of 200-400 mesh AG1-x8 chloride anion exchange resin.

ii) Rinse column with 15.0 ml of 5% W/V HCl followed by 20 ml deionized water.

iii) Dilute sample 1/25 in a 25 ml volumetric flask and pipet 10.0 ml onto the column. When the phytic acid is 1%, dilute 1/5 instead of 1/25.

iv) After sample has passed through the column add 15 ml of 0.1 M NaCl.

v) After (iv) has passed through the column discard the

eluant. Place a 25 ml volumetric flask under the column.

vi) Add 15 ml of 0.7 M NaCl to the column and collect the eluant.

vii) Fill the volumetric flask up to the mark and pour into large test tubes.

C. Phytic Acid Standard:

Prepare stock solution containing 200 g/ml of phytic acid. Sodium phytate represents 59% phytic acid, therefore 33.9 mg sodium phytate/100 ml will give 200 g/ml phytic acid. Prepare a phytic acid solution to contain 10, 20, 30 and 40 g/ml for analyses.

D. Colour Test:

Phytic acid can be assayed by using the wade reagent.

a) The wade reagent is prepared by combining 0.15g hydrated ferric chloride ($FeCl_3.6H_2O$) and 1.50g sulphosalicylic acid in water and diluting to 500 ml.

b) Pipet 3 ml of blank (water), standards and samples into 15 ml conical centrifuge tubes.

c) Add exactly 1.0 ml of Wade Reagent and mix thoroghly on a vortex type mixer.

d) Centrifuge for 10.0 minutes at full speed on a bench top centrifuge to precipitate phytic acid.

e) Read absorbance at 500 nm on a colorimeter using water to zero the instrument.

E. Calculations:

a) Absorbance readings for samples and standards are subtracted from the absorbance reading to the blank to get a final reading.

b) A standard curve is constructed by plotting these final readings against concentration.

c) Final readings from the samples are applied to the standard curve to determine the concentration.

d) The value obtained is divided by eight (accounting for

all dilutions in the procedure) to obtain an answer in% phytic acid in the original sample.

e) If the dilution is 1/5 instead of 1/25, divide the resulting phytic acid by five.

f) The percent phytic acid on the samples should be reported on the dry basis.

3.2.3. Determination of Trypsin Inhibitor Activity

The trypsin inhibitor activity (TIA) was assayed according to Kakade et al. (1974).

A. Extraction of Inhibitors

The extraction of trypsin inhibitors from the sample was carried out by following the procedure outlined by Bhatty (1979) with some modifications. The sample (25.0 g) was extracted at room temperature with 250 ml of 1% trichloro acetic acid (T.C.A.) at pH 1.5. The mixing was done by an Omnimixer for 1 min and then the mixing procedure was repeated three times with 5 min interval. The extract was centrifuged at 10,400xg for 25 min. The supernatant was removed by filtering it through a Whatman #1 filter paper into a 500 ml volumetric flask. The pellet (sediment) was re-extracted with another 250 ml of 1% TCA, as before. The two extracts were combined in a 500 ml volumetric flask and this represented the crude extract.

The substrate solution was prepared by dissolving 60 mg of BAPNA in 1.5 ml dimethyl sulfoxide and diluted to 100 ml Tris buffer pre-warmed to 37°C. The trypsin solution was made by dissolving 6 mg trypsin in 200 ml of 0.001 M HCl. This solution can be stored in the refrigerator for 2-3 weeks without appreciable loss in activity.

B. The TIA Assay:

The TIA assay contained 5.0 ml of the substrate solution, 2.0 ml of trypsin solution and 2.0 ml of water and aliquots containing 0.4, 0.8, 1.2, 1.6 ml (diluted extract in combination with varying amount of water to give a total of 2 ml mixture) as outlined in Table 10. The assay was carried out in a water bath at 37°C. The reaction was stopped after 10 minutes by adding 1.0 ml of 30% acetic acid. The absorbance of p-nitroaniline, the assay product, was read at 410 nm. Appropiate reagent blanks were also used in which 1 ml of 30% acetic acid was added to terminate the reaction before adding the 5 ml substrate (BAPNA) solution.

C. Expression of activity:

One unit of TIA is defined as an increase of 0.01 absorbance unit at 410 nm per 10 ml of the reaction mixture. The TIA is defined as the number of trypsin units inhibited (TUI) and was obtained by substracting trypsin units obtained in the presence of the inhibitor from units in the absence (standard) of the inhibitor.

3.2.4. Determination of Vicine and Convicine

Determination of vicine and convicine content in the samples were performed according to the method outlined by Marquardt and Frohlich (1981) using a reverse phase HPLC system. The chromatograph consisted of a Beckman Model 110 A pump (Beckman, Irvine, CA) equipped with a ISCO Model UA-5 multiwavelength absorbance detector (ISCO, Lincoln, NE) The sample was injected by a syringe via Beckman Model 210 injector valve

TABLE 10: Trypsin Inhibitor Assay Procedure

	Test Tube Number											
Step 1 Prepare 12 test tubes	1	2	3	4	5	6	7	8	9	10	11	12
Step 2 Pipet given ml of diluted sample extract into test tube	0	.4	.8	1.2	1.6	0	0	.4	.8	1.2	1.6	0
Step 3 Add given ml of H_2O	2	1.6	1.2	.8	.4	2	2	1.6	1.2	.8	.4	2
Step 4 Add Trypsin solution	2	2	2	2	2	2	2	2	2	2	2	2
Step 5 Add 5 ml of BAPNA soln. at the following times (min.) then incubate for 10 min.	0	1	2	3	4	10.3*	5	6	7	8	9	10.3*
Step 6 Stop reaction with 1 ml of 30% acetic acid at the following times	10	11	12	13	14	10*	15	16	17	18	19	10*
Step 7 Mix thoroughly												
Step 8 Read absorbance at 410 nm against reagent blank (test tube #6 and #12)												

*Note difference for reagent blanks (#6 and #12): add 1 ml acetic acid, and then 5 ml BAPNA and incubate for 10 minutes.

containing a 20- l sample loop. The HPLC stainless-steel column (4.6 mm (I.D)x250 mm) was prepacked with ultrasphere ODS (mean particle diameter 5 μm, Beckman) by the manufacturer. A guard pre-column(4.6 mm I.D.x40 mm) was packed with Co:Pell ODS (mean particle diameter 30 μm, Whatman, Maidstone, Great Britain). The absorbance of vicine and convicine was monitored at 280 nm, peak heights and areas were determined.

A. Sample preparation

The finally ground sample (0.5 g) was diluted with 60 ml of 5% PCA. The sample was stirred in a beaker using a magnetic stirrer at room temperature for 30 minutes. The extracts were centrifuged at 13,000xg for 10 min and the supernatant was filtered through a Millipore Syringe filter (13 mm cellulose acetate, 0.45 μm pore size. Millipore, Bedford, MA) before injecting 20 l into the liquid chromatograph. The supernatants were diluted as required with 5% PCA and stored at 2-6°C until analysed. The samples were analysed at the Animal Science Department, University of Manitoba, within 6 h of preparation.

B. Preparation of reagents:

The standard solutions of vicine and convicine were prepared by disolving 32.8 mg of vicine and 15.0 mg of convicine in 100 ml of water, respectively. The stock standard solutions of vicine (1.08 mM) and convicine (0.49 mM) were stored at 2°C for one week. Prior to the analysis, the stock solutions were diluted to the appropiate concentrations with 5% PCA. The diluted stock solutions were prepared each day

before the analysis and were stored at temperatures of between 2 and 6°C to minimize hydrolysis of vicine and convicine.

The stock eluting solutions of 5.0 M concentration was prepared from concentrated phosphoric acid and distilled-deionized water and adjusted the pH of 1.44 by titrating with ammonium hydroxide. The stock eluting solution was stable for several weeks at 2°C. This stock eluting solution was diluted 10 fold with water and then used as the eluting solution (0.05 M) with a final pH of 2.0. This eluting solution (pH 2.0) was stable at 23°C for one week. At the end of each day's analysis, the column was washed with water, a mixture of methanol-water (70:30) and if necessary with methanol. Prior to using, the column was flushed with water and was equilibrated with the eluting buffer. All aqueous solutions were filtered through a 0.45 μm membrane filter (Millipore). Polytetrafluoro ethylene filter (5 μm pore size) was used to filter the methanol. The eluents were degassed by filtration or in an ultrasonic bath (Mettler Electronic Corp. F.R.G).

3.2.5. Proximate Analysis

Total nitrogen content of the samples was determined by the microkjeldahl method (A.O.A.C., 1975), and the crude protein content was calculated by using a conversion factor of 5.70. Moisture determinations were performed under vacuum oven, using a method described by the Official Method of Analysis (A.O.A.C., 1975). Ash and fat contents of the sam-

ples were determined by the AOAC methods (1975).

3.2.6. Amino Acid Analyses

The amino acid compositions were determined on a Beckman Model 119C automatic amino acid analyser, using the standard hydrolysis procedure (6 N HCl; vacuum; 24 hours, 110°C) at the Animal Science Department, University of Manitoba. The Beckman modification of the single column procedure of Spackman et al. (1958) was applied. Cysteine and Methionine were determined according to the method of Hirs (1967).

3.2.7. Microscopic Studies

Microscopic observations of the flour and sieve fractionated fractions were carried out by using an Olympus light microscope (model EH). A small quantity of the dry sample was placed directly on a microscope glass slide, this was covered with a cover slip and examined at suitable magnifications using both normal and polarized light.

4. RESULTS AND DISCUSSION

4.1. Characteristics of Pin-milled Fababean Flour and its Major Components

In general, pin-milled fababean flour may be regarded as a proteinaceous material (containing 24.5% protein) and 75.5% non-proteinaceous components, with the most common component being starch granules (Table 11). The size range of starch granules is known to vary from 10-40 μm (Gracza, 1959) while the protein rich particles are mainly less than 6 μm in size (McEween et al., 1974). Although it is well known that density differences of the protein and starch structures are exploited during air-classification, no specific values for fababean materials could be found but general values for starch of 1.50g/cm^3 and for protein of 1.35g/cm^3 reported by Gracza (1959), would probably be typical of fababeans. Even less information is available on surface charge values for starch and protein at low moisture levels (e.g. 10.5%), although Wankhede and Ramteke (1982) have reported that legume starch is non-ionic and hence no charge would be expected on the starch granules from fababeans. Aqueous biochemical methods have reported an isoelectric point for fababeans storage proteins of about pH 4.7, hence at the neutral pH (about pH 6.2) in fababean flours some negative surface charge would be expected, assuming a degree of similarity between high and low moisture systems. It would therefore seem that real differences in size, shape, density and charge do exist between two of the major components in fababean flour.

Table 11: Typical Composition of Fababean Flour Mostly Used in the Study

Components		% Composition
Protein content*		24.5
Non-protein content		
% carbohydrate**	60.25	
% Lipid	1.75	
% Ash	3.00	75.5
% Moisture	10.50	

* % N x 5.70

** Determined by difference.

4.2 Fractionation of Pin-milled Fababean Flour by Sieving

Initial experimentation involved the sieving of pin-milled fababean flour in a laboratory Ro Tap test shaker using a typical selection of sieves employed to characterise dry/powdered material; sieve openings were 150, 106, 90, 75, 63 and 45 micrometers. With this range all of the free protein pieces and starch granules would be expected to be found ultimately on the bottom collection pan.

When a 50g sample of fababean flour was subjected to sieving for 30 minutes it was found that only 20.0% of the total sample was in the bottom pan (i.e. under 45 μm) with 34.8% of the sample material held on the 75 μm sieve (Table 12). In other words 80% of a pin-milled (very fine) flour which should have passed through the 45 μm sieve failed to do so.

Examination of the fractions obtained at 30 min sieving under the light microscope are summerized in Table 12. The coarser fractions mainly consisted of hull pieces and vascular tissues and no endosperm tissues. In the finer fractions (i.e. 45 and under 45 μm size particles) showed no presence of fibrous material. Mainly inner endosperm starch granules of various shapes ranging from round to oval to irregular were present. This irregularity in shape is very common in many legume starch-granules (Greenwood, 1976). Hillum and lamella were visible in the large starch granules. Studies under microscope showed that the field bean, lima bean and great northern bean hillums usually varied in shapes and lengths (Salunkhe and

TABLE 12: Fractionation[a] of Fababean Flour*
After Shaking for 30 Minutes

Sieve Size (μm)	Part of Total (%)	Microscopic Observations
150	3.0	Many large pieces of hull
106	3.6	Many pieces of hull, some endosperm chunks
90	1.0	Less proportion of fibrous material, mainly endosperm pieces
75	34.8	Endosperm pieces, many small and large starch granules, maltease crosses visibile under polarized light
63	4.0	No chunks of endosperm cells, starch granules showed maltease cross under polarized light
45	30.8	No presence of hull pieces, only starch of various shapes ranging from oval to irregular
Pan*	20.0	Finest material, free from fibrous material mainly inner endosperm starch granules
Total	97.2%	

[a]Values are the average of two determinations expressed on as is moisture basis.

*The flour contained 24.5% protein.

Pollard, 1955; Salimath and Tharanathan, 1982 and Sathe and Salunkhe, 1981).

Changes in shaking times for both longer and shorter periods, produced similar results in that a significant amount of flour particles failed to pass through the 45 μm sieve (Table 13). In addition to this considerable variation was encountered with some of the finer opening sieves, specially the 75 and 45 μm units. Although every effort was made to reduce variable conditions, this variation in fractionation profile could not be eliminated. In addition to monitoring very closely sample size and shaking time, such factors under existing conditions, as pilot plant, temperature and atmospheric relative humidity were also observed from run to run. No apparent relationships between these variables and sieveing results could be detected. However, one problem that did arise was blinding or clogging of the screen with flour materials. In this situation, the flour tended to agglomerate which helped to form a film or barrier and prevented the passage of particles through the sieves. This blinding effect appeared to vary for different runs, no readily apparent reasons for this effect could be suggested. However, it is generally recognized that sieve blinding is one of the major weaknesses in using the Ro Tap sieving procedure. One possible reason for this effect was considered to be moisture of the flour sample.

4.3. Effect of Moisture Content of the Flour

An attempt was made to determine the influence of varying degrees of moisture content on flour sieving and to find to

TABLE 13: Fractionation of Fababean Flour* at Various Shaking Times

Sieve Size (μm)	Fraction** of flour after shaking for 5 min (%)	10 min (%)	20 min (%)	60 min (%)
150	3.8	3.2	3.4	3.6
106	4.2	3.0	3.5	3.8
90	1.4	1.8	1.7	1.0
75	32.4	37.0	25.5	19.4
63	3.4	8.4	6.3	4.0
45	26.0	31.4	29.7	25.6
pan	26.8	14.8	29.3	42.0
Total:	98.0%	99.6%	99.4%	98.4%

*The flour contained 24.5% protein.
**The values are the average of two determinations expressed on as is moisture basis.

what extent this might facilitate the reproducible fraction of flour. Three different moisture ranges were used, namely the regular starting flour at 10.5% moisture. Flour dried at 55°C for 24 h (final moisture content 8.0%) and flour spread thin (i.e. maximum surface area) and held at 4°C for 24 hours in a sealed chamber (dessicator) containing water. The final moisture content of this latter flour sample was 11.25%.

Sieving of these flour preparations (Table 14) indicates that the higher moisture content (11.25%) did not impede the passage of flour particles through the 45 μm sieve, as might have been expected, indeed this sample had the highest amount of material passing through to the collection pan. On the other hand, the low moisture sample (8%) showed even less material passing through the 45 μm sieve, once again a reversal of what might be expected with decreasing moisture. This latter observation is in agreement with observations of Pulkki (1938) who reported great difficulty in sieving of dry materials. Moisture effects were not pursued further as it seemed that electrical charge effects could be an other possible explanation for the variation in results and the peculiar moisture effects.

4.4 Attempts to Manipulate Electrical Charge

In any experimental arrangement involving the shaking of a dry flour in a metal apparatus, the build up of some static charge would be expected. Recognizing this situation various attempts were made to influence the gross charge on the shaking apparatus during particular sieving trials. Initially, the

TABLE 14: Effect of Changes in Flour Moisture in Sieving Fractions* (all samples were 50 g and Sieved for 30 min.)

Sieve Size (μm)	Moisture content of starting sample		
	8.0%	10.5%	11.2%
150	4.0	3.2	3.2
106	7.0	3.0	3.0
90	22.61	1.8	1.6
75	36.6	37.0	24.6
63	1.8	8.4	3.2
45	20.4	31.4	29.2
pan	5.8	14.0	33.6
Total:	98.21%	98.8%	98.4%

*Values are the average of two determinations.

unit was simply grounded by attaching a copper wire to the bottom collection pan and to copper plumbing drain. An apparatus (electrometer) for measuring voltage down to the picovolt range was also wired into the grounding connection. Despite assistance from electrical engineering staff, this unit failed to detect any electrical charges, therefore it could be assumed that grounding reduced electrical charge, this assumption could not be confirmed nor quantitated. The use of the grounding wire produced a definite effect, which was quite reproducible, in that more material passed through the 75 μm than encountered in any previous sieving experiment, although there was significant accumulation on the 45 μm sieve(Table 15). This build up seemed to be more related to sample size and time required to pass through the final sieve as sieve blinding did not seem to be a problem i.e. flour particles did not form barrier at this final sieve.

A further attempt to manipulate charge by attaching a piece of fur to the bottom of the collection pan, so that a continuous frictional contact could be made between the shaking sieve and the frame of the shaker. No grounding wire was used in this experiment. An even greater change in sieving results was observed (Table 15) with less than 14% of the material being held on the 75 μm sieve and over 50% of the flour particles passing through the 45 μm sieve. Once again attempts to quantitate any electrical charge differences were not successful. However, it is well known that when two materials having dry surfaces are rubbed together they may

TABLE 15: Attempts to Manipulate Electrical Charge on Shaking Apparatus (All Shaking times were 60 min and sample sizes were 50g)

Sieve Size (μm)	Flour* Fractionation: Ungrounded Unit (%)	Grounded Unit (%)	Fur attachment (%)
150	1.14	1.17	1.21
106	4.86	3.02	2.82
90	1.90	1.58	2.07
75	42.44	23.52	13.92
63	5.40	7.85	3.35
45	13.60	40.85	19.66
Pan	27.24	18.46	53.10
Total:	96.58%	96.45%	96.13%

*The flour (11.5% moisture) contained 29.0% protein.

acquire equal and opposite charges. Various materials may be arranged in a series (Table 16) indicating qualitatively the type of charge build-up (Bulluck, 1941). This series would indicate that a negative charge potential could be established on the shaking sieves. If this were the case, and if the dry protein does carry a net negative charge then a more repulsive, non-adhering situation would be expected.

4.5. Effect of Varying Protein Levels upon Sieving Characteristics

With results to this point indicating that charge played some role in sieve analysis and recognizing that the most highly charged, major component in fababean flour was the protein portion, attempts were made to investigate the influence of protein particles on sieving characteristics. In addition to the regular pin-milled fababean flour, an air-classified protein preparation (57.03% protein, Nx5.70) and an air-classified starch stream (protein 20.0%) were used. All these materials were sieved on a non grounded apparatus. Surprisingly the protein preparation could not be sieved (Table 17), this material agglomerated very rapidly forming spheres varying in size from 2 to 8 mm. Spheres collected from the coarse sieves are shown in Figure 18. In addition to the spheres, a layer of coating of flour particles as developed on the surface of all the screens (fine and coarse). The formation of these blocking layers was very rapid, suggesting that the first material through a screen quickly formed a barrier allowing little free material to pass.

TABLE 16: Positive-Negative Series of Common Materials[a] (Bullock, 1941)

1	Fur	8	Paraffin Wax
2	Flannel	9	Ebonite
3	Ivory	10	The hand
4	Glass	11	Metals
5	Cotton	12	Sulfur
6	Paper	13	Celluoid
7	Silk	14	Rubber tubing

[a]The list is so arranged that if any two materials are placed in rubbing contact and separated, the first on the list will become positively charged while the other will be negative.

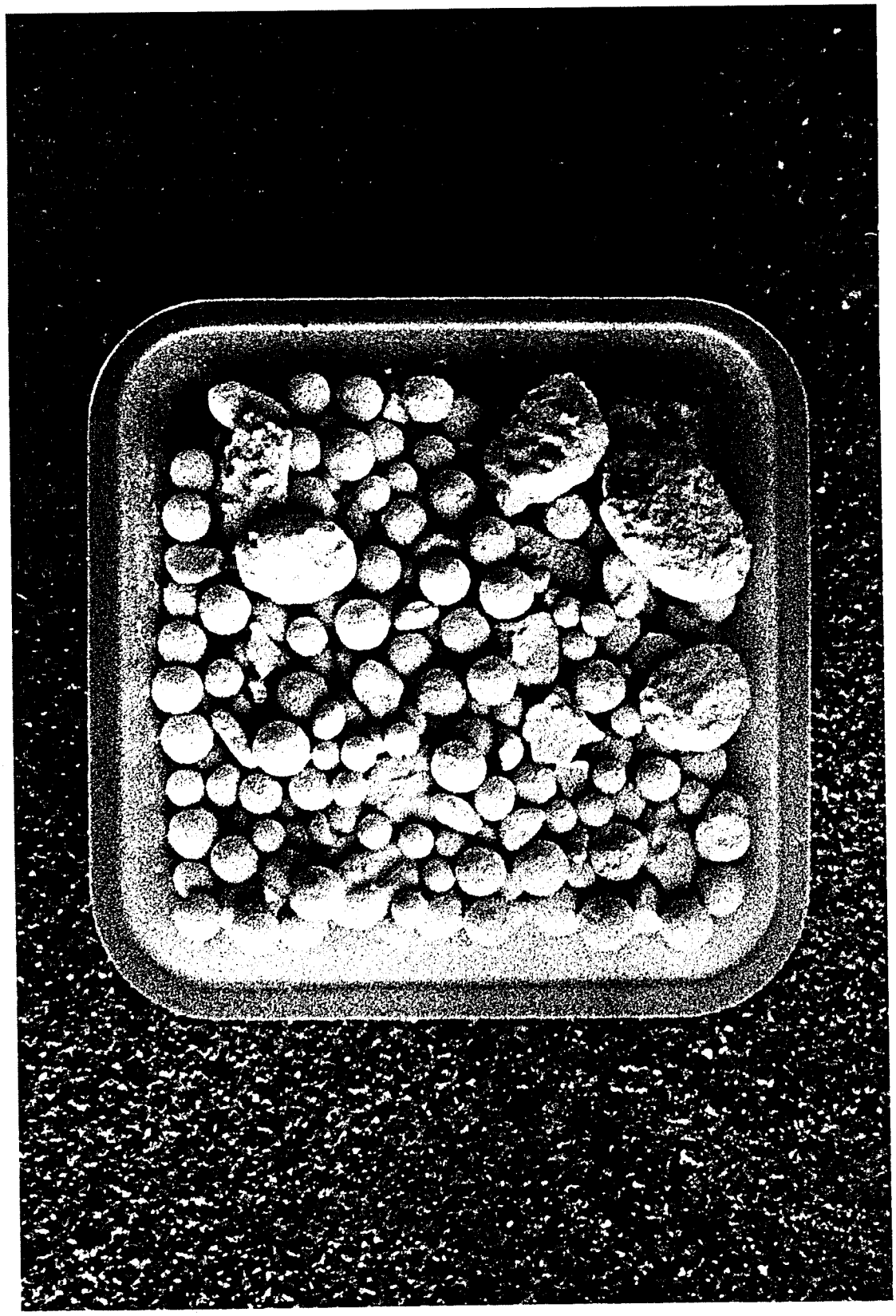
TABLE 17: Effect of Varying Protein Levels Upon Sieving Characteristics

Sieve Size (μm)	Fababean Sample[a] Protein stream* (%)	Starch stream* (%)	Pin-milled Flour (%)
150	sieving was not possible	0.8	3.0
106		1.6	3.6
90		1.2	1.0
75		2.8	34.0
63		1.6	4.0
45		5.8	30.8
Pan		85.0	20.0
	Total:	99.0%	97.2%

[a]50g samples were sieved for 30 minutes periods, values are the average of two determinations expressed on as is moisture basis.

*From air-classification.

FIGURE 18: Spheres Varying in Size Collected from the Coarse Sieves in the Sieving of Protein Concentrate.



Interestingly, a quite opposite effect was encountered with the starch stream in that no sphere formation was encountered nor was there any evidence of a blocking barrier effect. Indeed, the data (Table 17) showed that over 80% of this material passed through a 45 μm sieve, this being close to the theoretical expected value and certainly far closer that any result obtained thus far. Clearly, fababean flour proteins played a key role in sieving characteristics by apparently causing adherence of flour particles and forming either blocking barriers or large spherical artifacts. In either case, sieve efficiency was reduced severely.

A flour with an intermediate protein level (34%) was made by combining the regular pin-milled flour with material from the air-classified protein stream. Upon sieving this blend also exhibited barrier and sphere formation, but to a lesser extent than the protein stream alone. When the moisture content of this blend was reduced to 6.8% by drying at 60°C for 24 hours, a deterioration of sieving characteristics was encountered, similar to the situation observed with dried flour. Also, the protein content in various fractions (Table 18) suggested that protein was being held back on the sieves, in addition to general sample retardation. Material passing through the 45 μm sieve contained 10% less protein than the starting sample, while material retained on the coarser sieves contain 4 to 6% more protein than the starting material.

4.6. The Involvement of Proteins in Possible Charge Agglomeration Effects

TABLE 18: Sieving Characteristics of the 34% Protein Blend, (50g samples were sieved for 30 min. periods)

Sieve Size (μm)	As is Sample Yield (%)	As is Sample Protein (%)	Oven-dried Sample Yield (%)	Oven-dried Sample Protein (%)
150	2.4	N.D.	3.8	N.D.
106	41.6	N.D.	65.8	39.9
90	20.0	39.2	11.4	38.3
75	18.2	40.1	8.0	N.D.
63	4.8	N.D.	1.6	N.D.
45	3.0	N.D.	2.0	N.D.
Pan	8.0	25.2	5.7	24.0
Total:	98.0%		98.3%	

N.D. = Not determined.

Experimentation in the previous section indicated that protein concentration was a significant factor in sieving efficiency while carbohydrate (starch) level was not. Also there was a suggestion that agglomerated material had a higher protein level than non agglomerated starting material. To pursue this line of thinking a series of blended flours was prepared by mixing different proportions of flour with the air-classified protein stream. Samples were prepared containing 26, 28, 30, 32, 34, 38 and 42% protein. Since sphere formation was very rapid on the shaking sieves, aliquots of these blends were shaken for 10-15 seconds in hand held containers. Sphere formation was observed to begin with the 30% protein sample (Table 19) and increase with increasing protein level. The spheres began to form very quickly (in less than 15 seconds) and varied in size with the protein content of the blend (Figure 19a, b and c). On the other hand in starch concentration none of these phenomenon encountered as can be seen in Figure 20, but flour tended to agglomerate (Figure 21).

Initial sphere formation was assessed using plastic dishes (6 cm diameter and 3 cm deep) similar size dishes made of styrofoam, paper, glass, porcelain, earthenware, tin, brass and aluminium were tested with these flour blends, in all cases sphere formation was similar to that obtained in a plastic container, i.e. rapid and directly related to protein concentration.

Sieving of the samples in the increasing protein series which showed agglomeration (50g samples for 30 min) showed

TABLE 19: Sphere Formation in Flour Blends at Different Protein Levels

Protein Level (%)	Sphere Formation
24.5	Nil
26.0	Nil
28.0	Nil
30.0	±
32.0	+1
34.0	+2
36.0	+3
38.0	+4
42.0	+5

Visual assessment at sphere formation was estimated as follows:

± low level of spheres, just started to form
+1 some small spheres present
+2 many small spheres present
+3 many small to medium sized spheres
+4 very many small to medium sized spheres
+5 many medium to large sized spheres

FIGURE 19: Rate of Formation and Variation of Size of Spheres with the increase of the Protein Content of the Blend. A) sphere formation starts at 30%. B) increased with 32% and (C) further increased with 34%.

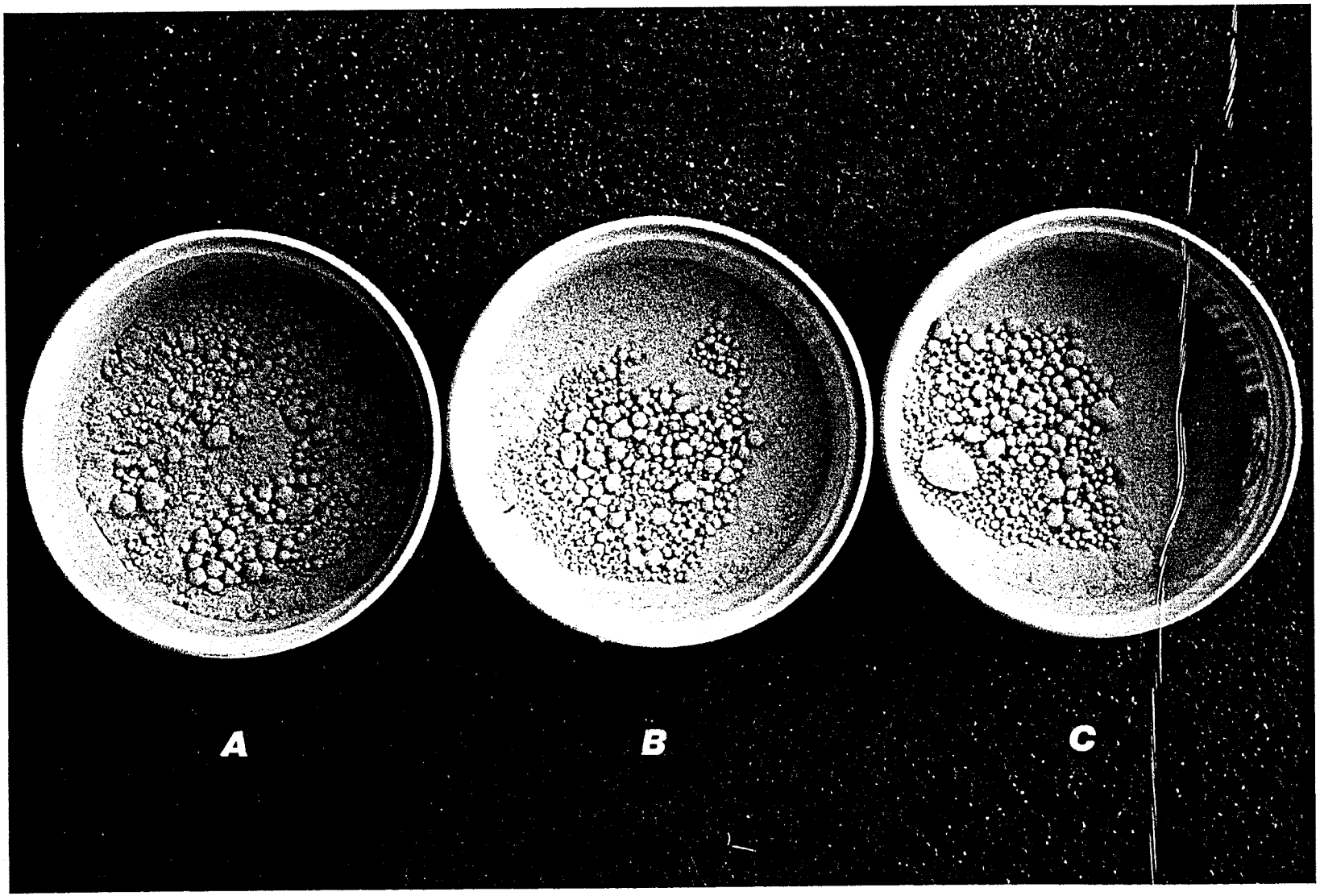

A
B
C

FIGURE 20: No Formation of Spheres in Starch Stream.

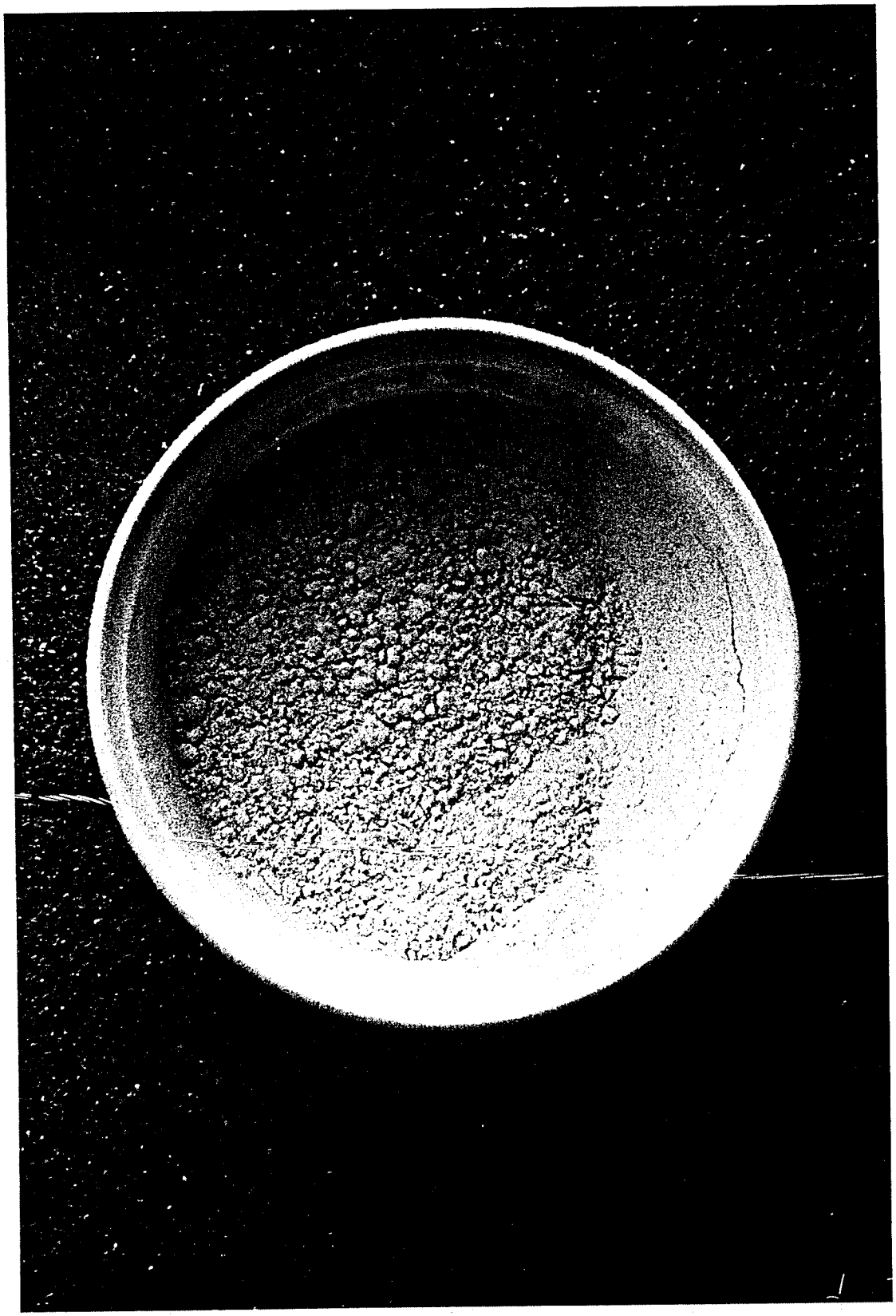
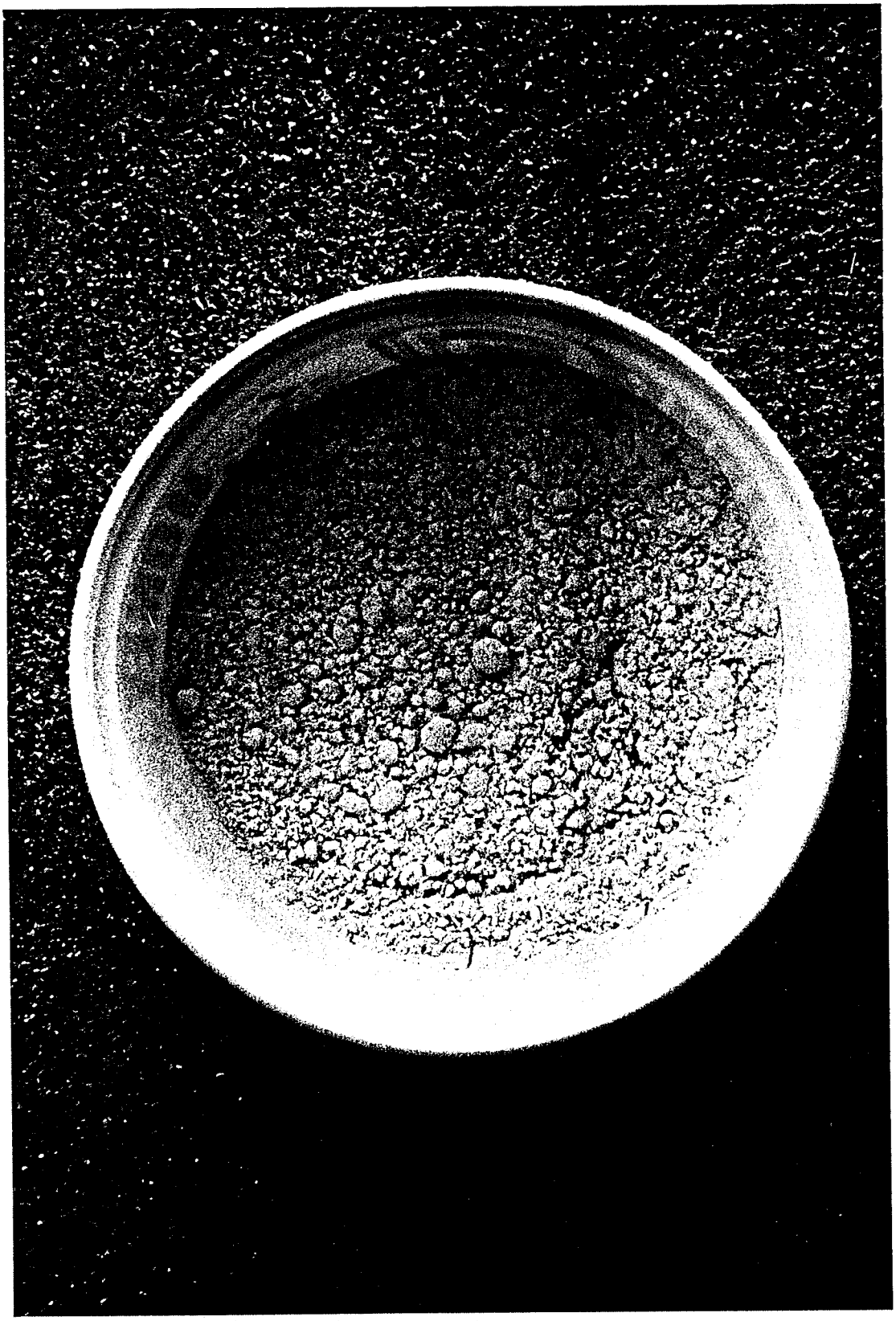


FIGURE 21: Pin-milled Flour Tended to Agglomerate.



effects on the sieves similar to those encountered with the shaking dishes. In all the trials complete sieving was not achieved (Table 20), with total material passing through the 45 μm sieve decreasing significantly with increasing protein levels. Protein determination on specific samples indicated a protein enrichment of 6 to 16% over the starting blend, while protein levels in pan samples (45 μm through) were in all cases less than the starting material.

Protein determination on individual spheres taken from the 38 and 42% systems contained 43 and 48% protein, respectively. Enrichments here were interesting but not of practical significance.

From all of these findings, it would appear that the sieves become electrically charged during the sieving operation. Grounding serves to reduce this charge effect and permit more material to pass through the sieves, probably by allowing the system to become less electronegative (Halliday and Resnick,1974). The negatively charged material on the sieves appeared to be neutralized by the influence of opposite charges developed by the fur rubbing on the metallic parts of the shaker, this also allowed more material to flow through to the collection pan.

A consideration of the components in legume flours indicates that the proteins are the most readily chargeable material present in relatively high concentration. The glutamic and aspartic residues in legume protein, which constitute about 32% of the total amino acids, may be readily

TABLE 20: Sieving Characteristics of Blends Showing Agglomeration Effects (50g sample, 30 minutes shaking)

Sieve Size (μm)	Protein Blended Material							
	30%		34%		38%		42%	
	Yield* (%)	Protein (%)	Yield* (%)	Protein (%)	Yield* (%)	Protein (%)	Yield* (%)	Protein (%)
150	3.0	N.D.	2.4	N.D.	2.0	N.D.	17.2	N.D.
106	5.6	25.7	41.6	39.2	43.6	43.2	53.6	29.06
90	29.4	37.7	20.0	40.2	24.6	43.3	23.4	58.0
75	29.2	N.D.	18.6	N.D.	21.8	N.D.	2.0	N.D.
63	3.0	N.D.	3.8	N.D.	2.0	N.D.	0.2	N.D.
45	6.4	N.D.	3.8	N.D.	1.0	N.D.	0.2	N.D.
Pan	22.2	27.8	8.0	25.2	3.0	26.2	0.6	26.4
Total:	98.8%		98.2%		98.0%		97.2%	

* Values are the average of two determinations expressed on as is moisture basis.

N.D. = Not determined.

charged at certain pH conditions. Although almost all protein chemistry is worked out in high aqueous systems, it would seem that even at low aqueous levels(eg. 6-12% water) the charge effects of proteins are operative. Indirect evidence for this is obtained when the starch stream(ie. low protein content) was sieved with no agglomeration, clumping or sphere formation. These effects were all shown to correlate with increasing protein levels. Since the effects also occurred in containers that were non-conductors of electrical charge, this further implicated protein components in electrostatic effects.

Therefore, protein surface charge appeared to be a significant force in sieving efficiency and particle interaction. The physical effect of rubbing or shaking caused a very rapid formation of charge artifacts. Under conditions pursued in this study, the formation of artifacts could not be exploited to yield protein enrichment procedures containing significant levels of protein.

4.7. Attempts to Reduce Total Charge in Sieving Systems

As it appeared that fababean proteins were very much involved in the agglomeration phenomenon, and it was assumed that these proteins carried a net negative charge, attempts were made to manipulate these charges in a dry environment by introducing a positively charged force. This was done by using a tissue type material impregnated with a quaternary ammonium compound, hence the positive charge addition. This material is available as an anti-static agent (ASA) and known commercially

as "Bounce".

With a view to modifying the charge environment during sieving, pieces of the ASA sheets (2x5 cm) were taped onto the inside of various sieves. With an increasing number of sheets per sieving tray there was a decreasing amount of flour material held on the 75 μm sieve (Table 21) and an increase in material passing through the 45 μm sieve. If a charge neutralization is assumed here with the increasing ASA sheets, then it would appear that sieving efficiency (i.e. agglomerate suppression) is increased with reducing charge. This being similar to the suggestion obtained with a grounded shaker and also with samples low in protein. Position of the ASA sheets at various screen locations (Table 22) did not appear to have as great an influence on sieveing properties as did the total amount of positive charge introduced to a shaking system. Once again the suggestion being that electrical charge neutralization enhanced material flow through the sieves.

Two further attempts were made to influence the total charge in sieving systems. One involved the addition of varying levels of crystalline sodium chloride to the flour sample while the other used an ion gun. Sodium chloride levels up to 8% failed to produce any positive results involving either sieving efficiency or protein localization. The ion gun, designed to release positive charges when discharged towards an object, failed to show effects when ion treated flour samples were sieved. If an electrical neutralization were occurring here, the extent was not sufficient to yield the expected

TABLE 21: Effect of ASA on Sieving* Characteristics of Fababean Flour** (50 g flour sieved for 60 minutes)

Sieve Size (μm)	No ASA (%)	ASA taped into each sieve: 1 pc. on all Sieves (%)	2 pcs. on all Sieves (%)	3 pcs. on all Sieves (%)
150	1.35	1.26	1.33	1.26
106	3.69	3.39	3.85	3.14
90	3.23	2.53	2.38	2.44
75	60.86	49.99	36.84	24.76
63	8.40	10.74	3.99	3.51
45	10.90	10.70	9.22	11.66
Pan	9.83	18.94	41.03	51.36
Total:	98.26%	97.55%	98.64%	98.13%

** The flour (11.5% moisture) contained 29.0% protein.

* Values are the average of two determinations expressed on as is moisture basis.

TABLE 22: Effect of ASA Location and Quantity on Sieving Characteristics (50 g Flour* for 60 minutes)

Sieve Size (μm)	Four pieces of ASA taped into screen with 'A' beside yield value			
	Sample yield[a] (%)	Sample yield[a] (%)	Sample yield[a] (%)	Sample yield[a] (%)
150	1.15	1.21	1.24A	1.26A
106	3.39	3.28	3.67A	3.14A
90	3.92	3.22	3.28A	3.44A
75	38.85A	42.33	21.69A	22.76A
63	5.53A	7.95A	21.65	3.51A
45	23.85	17.74A	4.96	10.66A
Pan	22.23	23.96	41.68	54.06A
Total:	98.89%	99.69%	981.7%	98.83%

[a]Values are the average of two determinations expressed on as is moisture basis.

*The flour (11.5% moisture) contained 29.0% protein.

result.

4.8. Sieving Rates of Different Flour Particles

Although protein analyses were done routinely on many sieved fractions, and some fractions did indeed contain more protein than the starting flour, a combination of increased protein level and major fraction size was never achieved.

One indicator that did occur many times, was that starch granules (possessing little or no charge) appear to pass through the sieves more rapidly than charged lower density protein pieces, despite the fact that the starch granules were the larger structure. Recognizing this situation, flour samples were sieved for varying time intervals. After 10 minutes of sieving the shaker was stopped and the pan materials (i.e. under 45 μm in size) were collected, the empty pan was replaced and the remaining materials on the sieves were shaken for another 10 minutes. The pan fraction was again collected and then another 10 minutes of sieving produced a third pan fraction. Protein analysis on these samples (Table 23) indicated that the large non-proteinaceous materials (mainly starch) did pass through the screens more rapidly than the smaller protein particles. This approach was pursued as approximately 60% of the starting sample was held back on the coarser screens and it was presumed that this delayed material contained considerable protein.

Successive sieving trials were done next where a flour sample was shaken for seven consecutive runs for 2.5 minutes each time. After each short shaking period the bottom pan was

TABLE 23: Effect of Increasing Sieving Time on Fababean Flour (24.5% protein, 50 g sample)

Sample*	Sample weight (g)	Protein Content (%)	Non-protein Content** (%)
A - after 10 min. sieving	6.39	18.0	82.0
B - after 20 min. sieving	6.59	22.6	77.4
C - after 30 min. sieving	6.39	24.4	74.5

* material through a 45 μm sieve.

** by difference.

removed and the sample was collected. All the other material remaining on the sieves was collected and remixed thoroughly by shaking in an inflated plstic bag. The re-mix was subjected to a second sieving for 2.5 min. and the pan fraction removed. This operation was repeated a total of seven times. The amount of pan material collected was 18.19g (Table 24) as well as protein contents determined. Interestingly, although the sample size decreased with each successive trial (and only 36% of the total sample passed through the 45 μm sieve) each sample had essentially the same percent of protein in it, in all cases less than the starting flour, and only about 25% of the total protein in the starting sample was collected, i.e. 75% remained on the upper sieves. When this upper sieve material was collected it contained 27.5% protein, i.e. only 3% more protein than the starting flour. Although some protein enrichment occured, thus supporting earlier observations, the actual extent was not of practical significance.

Further attempts to manipulate sieving conditions involved shaking pin-milled flour for 5, 10, and 15 minutes. By microscopic assessment, material retained on the 150, 106 and 90 μm sieves (about 9% of the total sample) contained many hull pieces, vascular tissue and some endosperm chuncks. Removal of this low protein material only slightly increased the total protein level in the remaining flour. Fractions retained on 63 and 75 μm sieves (Table 25) after 10 minutes contained 7-9% more protein than the starting flour. This low level of enrichment at 10 minutes, was repeated nine times and

TABLE 24: Effect of Successive Sieving Trials for Short Time Intervals (2.5 min each), 50 g flour sample* at 24.5% protein

	Characteristics of Fraction Under 45 μm		
Sieving Trial	Sample wt. (g)	Protein Content (%)	Protein in Fraction (g)
1	6.5	18.25	1.18
2	4.1	17.25	0.70
3	2.5	18.15	0.45
4	2.0	18.00	0.36
5	1.5	18.01	0.27
6	1.2	18.99	0.22
7	0.3	20.28	0.04
Total:	18.19g	$\bar{x}$ 18.41	Total: 3.24g

* Total protein in sample 12.25g.

TABLE 25: Protein Content of Pin-milled Flour for Varying Time Intervals (50 g samples at 24.5% protein)

	Sieving Time Periods					
	5 min.		10 min.		15 min.	
Sieve Size (μm)	Sample Yield (%)	Protein (%)	Sample Yield (%)	Protein (%)	Sample Yield (%)	Protein (%)
150	3.8	N.D.	3.6	N.D.	3.6	N.D.
106	4.2	N.D.	4.0	N.D.	3.8	N.D.
90	1.4	N.D.	1.6	N.D.	1.0	N.D.
75	32.4	27.56	14.0	32.05	12.4	30.95
63	3.4	33.82	4.2	33.51	4.0	33.47
45	27.0	24.80	28.0	26.97	25.6	26.29
Pan	26.8	26.50	44.0	25.94	48.0	26.70
	99.0%		99.4%		98.4%	

N.D. = Not determined.

all 63 and 75 μm fractions were recovered. The average protein content for these fractions was 30.88% (75 μm sample) and 33.20% (63 m sample). These materials were combined to produce fraction A which had a final protein content of 32% as is or 35.57% dry basis.

Fraction A was further sieved over an increasing time interval to produce an additional protein enrichment on the 63 and 75 μm sieves (Table 26). Combining the material from these two sieves produced a final blend (Fraction B) containing 36% protein as is or about 40.0% dry basis. This final blend represented about a 1.5 fold protein enrichment over the starting flour. Fractions A and B were used for further biochemical characterization.

4.9. Biochemical Characterization

The amino acid values presented in Table 27 are characteristic of the legume proteins with a high lysine and low sulfur containing amino acids content. No real differences were observed in specific amino acid composition of any flour preparations. A comparison to FAO reference values (Table 28) indicated a uniform deficiency in sulfur containing amino acids typical of legume proteins with no readily apparent variations amongst samples. The values for the rest of the essential amino acids considerably exceeded that of the FAO reference pattern (1973).

When phytic acid, trypsin inhibitor, vicine and convicine were determined in various preparations (Table 29) it was found that all tended to concentrate with the protein, i.e.

TABLE 26: Protein Content of Material* Sieved for Varying Time Intervals (50 g samples at 32.0% protein)

	Sieving Time Periods							
	5 min.		10 min.		15 min.		20. min.	
Sieve Size (μm)	Sample Yield (%)	Protein (%)	Sample Yield (%)	Protein (%)	Sample Yield (%)	Protein (%)	Sample Yield (%)	Protein (%)
150	0.25	N.D.	0.20	N.D.	0.20	N.D.	0.0	N.D.
106	3.6	31.5	0.40	N.D.	0.40	N.D.	0.2	N.D.
90	54.2	30.88	37.20	30.86	4.60	29.80	2.4	32.84
75	28.8	30.60	37.4	31.09	38.30	31.90	22.4	35.25
63	4.2	31.72	5.8	31.80	14.0	34.50	14.6	36.55
45	5.6	25.40	15.6	26.57	30.4	28.37	43.0	28.40
Pan	1.4	21.40	2.4	23.38	11.4	24.82	16.2	25.40
	98.05%		99.0%		99.3%		98.8%	

* Fraction A

N.D. = Not determined.

TABLE 27: Amino acid compositions[a] of fababean flour (FBF), protein concentrate (PC), starch concentrate (SC) and sieve-classified fraction A and B

Amonioacid*	FBF	PC	SC	Fr. A	Fr. B
Lysine	6.82	6.46	6.28	6.88	6.77
Histidine	2.66	2.78	2.60	2.71	2.63
Arginine	10.60	12.95	11.60	11.29	10.74
Aspartic acid	10.53	8.53	10.01	10.81	10.28
Threonine	3.44	2.80	3.21	3.59	3.60
Serine	4.31	4.09	4.29	4.39	4.16
Glutamic acid	16.13	18.53	17.29	16.30	15.11
Proline	4.70	6.17	5.27	4.88	4.66
Glycine	4.39	4.19	4.31	4.43	4.27
Alanine	4.40	4.25	4.30	4.58	4.30
Cystine*	1.60	1.52	1.58	1.71	1.70
Methionine*	0.82	0.97	0.88	0.87	0.88
Valine	5.17	5.30	5.12	5.21	4.98
Isoleucine	4.44	4.64	4.37	4.54	4.40
Leucine	7.50	7.16	7.48	7.58	7.30
Tyrosine	2.44	3.08	2.60	2.97	2.95
Phenylalanine	4.43	4.58	4.37	4.48	4.29

[a]Grams of amino acid per 16 g of nitrogen.

Values are the average of two determinations expressed on dry basis.

* Cystine and methionine by oxidation.

Tryptophan was not assayed.

TABLE 28: Comparison of the Essential Amino Acids Composition* of Fababean Flour (FBF), Protein Concentrate (PC), Starch Concentrate (SC) and Fraction A (Fr. A), Fraction B (Fr. B) and HRS wheat flour

Amino Acid	Amino Acid Composition g a.a per 16 gN						
	FBF	PC	SC	Fr. A	Fr. B	FAO Reference Protein[a]	HRS wheat flour**
Lysine	6.82	6.46	6.28	6.88	6.77	4.3	1.9
Isoleucine	4.44	4.64	4.37	4.54	4.40	4.0	3.9
Leucine	7.50	7.16	7.48	7.58	7.30	7.0	6.8
Threonine	3.44	2.80	3.21	3.59	3.60	2.9	2.6
Tryptophan	--	--	--	--	--	1.4	0.9
Valine	5.16	5.30	5.12	5.21	5.0	4.3	4.3
Cystine/methionine	2.42	2.49	2.46	2.58	2.58	4.3	3.7
Phenylalanine/ Tyrosine	6.87	7.66	6.97	7.45	7.25	6.0	7.9

[a] FAO/WHO (1973)

* Values are the average of two determinations expressed on dry basis.

** from Tkachuk, 1966.

TABLE 29: Composition of Protein* (%) and its ratio with the Composition of Phytic acid, Trypsin inhibitor Activity, Vicine and Convicine Contents[a] of Pin-milled Fababean Flour, Air-classified Protein Concentrate, Air-classified starch concentrate and Sieve-classified fractions A and B

	Components	Pin Milled Flour	A/C Protein Stream	A/C Starch Stream	Fraction A	Fraction B
A	Protein (%)	27.46	51.20	24.89	35.517	40.0
B	Phytic Acid (%)	1.45	2.57	0.88	1.70	1.88
C	Ratio B:A	1:19	1:20	1:28	1:20	1:21
D	TIA (U^b/mg)	16.37	16.58	7.70	20.01	22.20
E	Ratio D:A	1:1.7	1:3.1	1:3.2	1:1.7	1:1.8
F	Vicine (%)	0.89	1.04	0.49	1.04	1.27
G	Ratio F:A	1:31	1:49	1:51	1:33	1:31
H	Convicine (%)	0.27	0.56	0.23	0.33	0.38
I	Ratio H:A	1:101	1:91	1:108	1:107	1:105

[a] Values are the average of three determinations expressed on dry basis.

[b] Trypsin units inhibited.

enrichment by sieving techniques indicated similar trends as did enrichment by air-classification.

5. CONCLUSIONS AND RECOMENDATIONS

The results obtained in this study demonstrate the feasibility of conversion of pin-milled fababean flour into protein rich fraction(s) suitable for incorporation into processed foods by fractionation involving a physical method of sieving which could be simple, rapid as well as economical. The concentration of the proteins from fababean pin-milled flour by mechanical sieving method appeared to be possible (besides the differences in size, shape and density of the starch and protein particles) on the basis of the manipulation of ionic properties (net surface charge) of the protein particles. It was clearly observed that a charge phenomenon is operative here, but to be addressed further, electronic skills and techniques are required. Electrostatic separation on the basis of the difference in charges or field strength could be used in the food industries for separating components from a powder mixture i.e. flour. The separation by an electrostatic method of the materials dealt with, being independent of specific gravity and shape of the particles, will therefore be according to the polarity of their charges. The knowledge of precise distribution of proteins and ionic properties in low moisture systems will require further study to obtain better concentration of protein within the subsieved ranged fractions by the simple method of sieving. The procedure should significantly reduce the production cost of protein concentrate compared to the expensive method of air-classification with no significant loss in nutrient quality of the protein concentrate compared

to protein concentrate prepared by air-classification method.

Therefore, future research objectives could focus on obtaining more concentrates by this method, exploring any suitable way or device to identify the precise nature of these charges and establishing the mechanism to modify these "static charges" in order to manipulate the flow patterns of the components either by retaining the protein particles suspended on the sieves or by discarding the starch granules.

Finally, possible electrical engineering knowledge and methods may eventually be helpful in order to produce more protein enriched fraction(s) by the manipulation of these flour components on the basis of their electrical property.

The other objective of this study was to evaluate the nutritional and antinutritional contents present in the developed protein by sieving and to compare with the contents of the PC, SC and original starting material. The findings, however, indicated that the antinutritional factors, namely phytic acid, trypsin inhibitors, vicine and convicine were present in relatively higher amount than the starting material flour and these factors follow the same migration pattern with the concentration of proteins as happened with air-classification method. The separated fractions were enriched with more protein than the flour as well were either increased or maintained the levels of amino acids in the sieved fractions A and B, respectively. The nutritional quality of proteins is usually related to the amino acid content. So, higher levels of amino acid, particularly higher levels of S-containing amino

acids in the fractions A and B should be of considerable value along with the high lysine content if these fractions are used in conjuction with the cereal proteins which are normally low. Therefore it could be recommended that before the utilization of these protein enriched fraction(s) in food products, it would be necessary to inactivate, eliminate or reduce these antinutritional factors to a large extent employing an appropriate degree of heating, roasting, or soaking the beans or by any other appropriate measures without destroying nutritional properties. It would be further necessary to evaluate the degree of residual antinutritional effects of the beans prior to milling them in order to prepare protein concentrates from the flours.

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MANIPULATION OF PHYSICAL PROPERTIES IN THE DRY FRACTIONATION OF PULSE SEED FLOURS

BY
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