THE ISOLATION AND CHARACTERISATION OF PROTEINS FROM PHASEOLUS BEANS

A Thesis

by

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ABSTRACT

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Proteins were isolated from two varieties of Phaseolus vulgaris (white kidney beans and navy beans) and one variety of Phaseolus lunatus (lima beans). The method of isolation of the proteins involved the use of a dilute organic acid solution as extractant. The proteins were recovered by cooling the solutions at 5°C. The effects of pH (pH 2.5 to pH 5.5) and normality (0.0N to 0.8N) of extractants (citric acid solution and DL-malic acid solution) on protein yield were studied. In addition the effects of temperature (27°C. to 50°C.), meal particle size (0.25 mm. to 2.00 mm. diameter) and extraction time (5 mins to 45 mins) on protein yield, were investigated. The crystalline and non-crystalline proteins which were obtained from the three types of beans were examined by polyacrylamide-gel disc electrophoresis. The protein preparations were also analyzed for their amino acid contents.

RÉSUMÉ

Extraction et caractérisation des protéines de fevès du genre Phaseolus

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Les protéines de deux variétés de Phaseolus vulgaris (fevès blanches et fevès bleues marine) et d'une variété de Phaseolus lunatus (fevès de lima) ont été isolées. Dans la méthode d'extraction utilisée, l'extracteur était composé d'une solution acide et organique diluée. Les protéines ont été récupérées en refroidissant les solutions à 5°C. Les effet de pH (2.5 à 5.5) et de normalité (0.0N à 0.8N) des extracteurs (solution d'acide citrique et solution d'acide DL-malique) ont été étudiés quant à leur rendement en protéines. De plus, les effets de température (27°C. à 50°C.), de dimension des particules alimentaires (0.25 mm. à 2.00 mm. de diamètre) et la durée de l'extraction (5 minutes à 45 minutes) ont aussi été examinés quant au rendement protéinique. Les protéines, sous forme cristallisée ou non-cristallisée, venant des trois types de fevès ont été étudiées au moyen de l'électrophorèse sur disque de gel polyacrylamide. Les protéines ont aussi été analysées quant à leur contenu en acides aminés.

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GENERAL INTRODUCTION

Beans belong to the family Leguminosae. They are sometimes called pulses and one of their characteristics is that they develop their seeds in "pods". The seeds are the propagating organs of these plants; they contain the embryo and a relatively large amount of storage protein. The seeds of legumes are an important source of food for man. They are unique in their ability to produce protein from nitrogen absorbed from the soil or the atmosphere, via their root nodules. Furthermore, some of the nitrogen they "fix" can be recovered by crops grown in association with, or immediately following a leguminous crop. Leguminous plants are therefore, important in crop rotation.

With the exception of soybean, leguminous seeds are not used to any extent in the commercial production of protein isolates. Soybean has the advantage of having a relatively higher protein content (40 to 50 per cent) compared with most other leguminous seeds (20 to 30 per cent). In addition, soybean is a very important source of edible oil. Although none of the other cultivated leguminous seeds has been used to the same degree as soybean, nevertheless, it is possible that proteins isolated from the beans might find many uses especially in areas where climatic conditions are not

favourable for the cultivation of soybean.

An interesting development concerning protein isolates from beans is the work of Melynchyn (1969). He claims that a crystalline protein isolated from beans is soluble in acid solutions having pH values within the range of the isoelectric points of the proteins. Casein and soybean protein, the major proteins of commerce, are insoluble in the pH range mentioned above. These proteins are prepared by isoelectric precipitation. It is possible that the bean protein isolates might be used in the preparation of acid foods and beverages. Fan and Sosulski (1974) noted that about 50 per cent of the alkali-extracted proteins of lima beans (Phaseolus lunatus) remains in solution after isoelectric precipitation of the protein. This, they suggested could be concentrated for use in acidic beverages.

Although considerable research has been done on the proteins of leguminous seeds, it is evident that a great deal more research must be done before the commercial production of the proteins becomes practical. As recent as 1970, Hang et al (1970) reported that there is very little available information on practical methods for the extraction of proteins from kidney beans (Phaseolus vulgaris). Burrowes et al (1972) claimed that the same comment could be applied to other species of Phaseolus beans.

The purpose of this study was to isolate crystalline protein from three species of Phaseolus beans and to characterise the isolated proteins.

PART 1

ISOLATION OF PROTEINS FROM PHASEOLUS BEANS

I. REVIEW OF LITERATURE

1. General Methods for Extraction of Proteins from Seeds

An ideal solvent for the extraction of proteins from seeds should remove all the proteins from the seeds without changing the properties or structure of the original protein. Strong acid or base will dissolve virtually all the proteinaceous material but the proteins may be changed profoundly. The extracting solvents which have been widely used are water, sodium chloride solution, 70 per cent alcohol solution and dilute alkali and acid. Other solvents such as urea, glycol, detergents such as sodium dodecyl sulphate and sodium salycilate have also been used (Brohult and Sandegren 1954). Salt solutions other than sodium chloride, such as potassium sulphate, ammonium sulphate, magnesium chloride and sulphate have also been used.

As early as 1728, Beccari (1745) attempted to separate proteins from wheat flour by simply washing out the soluble carbohydrate material with water. He called the residue "gluticosum". Einhof (1806) used water to extract proteins from peas and beans. He showed that leguminous seeds contained a type of protein which was insoluble in water or alcohol. Braconnot (1827) extracted proteins from peas and beans with water and precipitated the proteins by the addition of acid

to the water extract. Bibra (1860) used cold water to extract albumins from oats; he precipitated the proteins by boiling the water extract. He called this precipitate an albumin fraction; the yield was 1.24 per cent of the total meal. Ritthausen (1884) obtained a protein from white beans (Phaseolus vulgaris) by extraction of the meal with water. Osborne (1891) extracted relatively large quantities of protein from oats with water; he extracted five pounds of oats with six litres of water. He removed the supernatant, re-extracted the residual meal with another six litres of water and precipitated the proteins by saturating the combined extracts with ammonium sulphate.

Osborne used water to extract proteins from several seeds. He extracted flaxseed meal (1892,b) with water and obtained protein yield of 10.5 per cent of the weight of the meal; he extracted barley proteins using water (1895), and Osborne and Campbell (1896) used water to extract proteins from vetch; they reported a globulin yield of 1.04 per cent of the original meal. Osborne (1907) obtained a yield of 9.3 per cent protein from oil-free wheat germ meal by water extraction and precipitation of the proteins by heating the water extract to 65°C.

Several other workers investigated the use of water for the extraction of protein from seeds. Jones and Johns (1916) extracted 15.0 per cent of the proteins from the jack bean using distilled water. Two years later Johns and

Chernoff (1918) reported that distilled water extracted 2.5 per cent of the protein from buck wheat at room temperature. Working with the lima bean (Phaseolus lunatus), Jones et al (1922) extracted proteins which accounted for 15.13 per cent of the original meal. Jones and Gersdorff (1923) extracted proteins from wheat bran with water at 1.2°C. and precipitated the proteins by dialysis.

Water has been used as an extraction medium in investigations of the effect of various factors on nitrogen solubilization from seeds. Nagel et al (1938) used water as the extractant for studies on the effect of meal particle size, temperature and extraction time, on the dispersion of soybean proteins. Smith et al (1938), Fontaine and Burnett (1944), Smith and Johnson (1948), and Patel and Johnson (1974) also carried out various studies on protein extraction from seeds using water.

Henning (1947) patented a process for the isolation with water, of proteins from oleaginous protein bearing seeds such as soybean, peanut and cottonseed. In this process cold water is used to extract the proteins which are then precipitated by the addition of dilute acetic acid. Two years later Erkko (1949) also patented a method for the commercial extraction of proteins from soybean using water and isoelectric precipitation of the proteins by the addition of sulphuric acid.

Aqueous solutions of acids and bases have also been used for the isolation of proteins from seeds. Ritthausen

(1862) extracted proteins from seeds with dilute alkaline solutions. This author (1880) extracted oil-free peanut meal with solutions of potassium, calcium and barium hydroxides. Perov (1931) extracted seeds of Phaseolus vulgaris with dilute sodium hydroxide solutions and precipitated the protein by addition of dilute acetic acid. He called the isolated protein "proto-acid". Morse (1943) patented a process for the extraction of vegetable protein by solubilization of the protein material with aqueous solution at pH 7.0 and precipitation of the protein at pH 4.6.

Several workers have shown that seed protein may be solubilized by acid solutions. Smith and Circle (1938), Olcott and Fontaine (1939), Fontaine and Burnett (1944), Fontaine et al (1946,a), Fontaine et al (1946,b), Painter and Nesbitt (1946), Smith et al (1946), Evans et al (1948), Bourdillon (1951), Djang et al (1952; 1953), Smith and Rackis (1957), Smith et al (1959), Powrie (1961), Evans and Kerr (1963), Cagampang et al (1966), Gheyasuddin et al (1970), Patel and Johnson (1974), and Fan and Sosulski (1974) have all used acidic and or basic solutions in investigations on the solubility characteristics of seed proteins.

Melynchyn (1969) published a process for the isolation of crystalline proteins from beans (Phaseolus vulgaris) using solutions of organic acids.

Alcohol has also been used as an extractant for seed proteins. Einhof (1806) showed that leguminous seeds contain a type of protein which is insoluble in water or alcohol.

Gorham (1821) used alcohol to extract proteins from corn and obtained a yellow wax-like substance which he called "zein". Ribra (1860) used hot alcohol to extract proteins from ground oats; on cooling the hot extract a solid product separated out which accounted for 0.15 to 0.17 per cent of the proteins. He claimed that about 3 per cent of the proteins was extracted by alcohol. Ritthausen (1872) also extracted a "maize fibrin" from corn using alcohol. Osborne (1895) used alcohol to extract proteins from barley. Hoagland (1911) studied the effect of alcohol concentration on the yield of protein obtained from wheat flour. He found a gradual increase in extractable nitrogen as the alcohol concentration increased (10 to 50 per cent). Extractable nitrogen decreased when the alcohol concentration was greater than 50 per cent. Greaves (1911) used alcohol to extract protein from wheat flour and showed that when the extraction time was increased from twenty-four hours to forty-eight hours, the percentage of protein that was extracted did not increase. Bishop (1929) used hot aqueous alcohol (70 per cent) to extract protein from ground barley meal; he called the extracted protein, hordein.

Alcohol has been used more for the extraction of protein from wheat flour than from any other type of seed. Blish and Sandstedt (1925), Blish and Sandstedt (1929) and Sharp and Herrington (1927) investigated the use of alcohol for the extraction of proteins from wheat.

Powrie (1961) used aqueous alcohol (70 per cent) and also aqueous alcohol (70 per cent) containing sodium acetate to extract proteins from navy bean (Phaseolus vulgaris). The author showed that the alcohol-acetate mixture extracted slightly more protein than did the alcohol alone.

Solutions of sodium chloride have been used extensively for the extraction of protein from seeds. This was mainly because of the work of Denis (1858) who showed that many proteins are soluble in neutral saline solutions. Hoppe-Seyler (1866-1871) confirmed the findings of Denis. Weyl (1876-1877) used sodium chloride solutions to extract proteins from a number of seeds. He found that all the seeds he studied contained protein that was soluble in sodium chloride solution. Ritthausen (1880) extracted oil-free peanut meal and sunflower seed meal with sodium chloride solution (10 per cent). He precipitated the protein by dilution of the extract with water and then saturation of the resultant solution with carbon dioxide. The same worker extracted white bean (Phaseolus vulgaris) with sodium chloride solution (2 per cent) and obtained a protein yield of 14.45 per cent based on the original bean meal.

Osborne (1891-1892) extracted the proteins from oats and from kidney bean meal (Phaseolus vulgaris), (Osborne 1894), with sodium chloride solution (10 per cent). He showed that the kidney bean meal contained phaseolin (15 per cent), phaselin (2 per cent), and an alkali-soluble protein (20.6 per cent).

The author (1897) used the same solution to extract the proteins from maize. He obtained three globulins which together accounted for 24.07 per cent of the total nitrogen of the meal. Osborne and Campbell (1896; 1897, b; 1898) extracted proteins from various leguminous seeds (pea, lentil, horse-bean, vetch and adzuki bean) with sodium chloride solution (10 per cent) and precipitated the proteins by saturation of the extracts with ammonium sulphate. Osborne and Campbell (1897) used sodium chloride solution (10 per cent) to extract the proteins from oil-free sunflower seed meal. They precipitated the proteins by dilution of the extract with water followed by dialysis or saturation with sodium chloride solution. Osborne (1907) extracted wheat germ with sodium chloride solution (3 per cent) and heated (60°-63°C.) the filtered extract to precipitate the proteins. The author also extracted wheat with sodium chloride solution (10 per cent) and isolated the proteins by saturation of the extract with ammonium sulphate.

Ladd (1909) used sodium chloride solution (1 per cent) to extract proteins from wheat and other cereals and precipitated the proteins by use of phosphotungstic acid. Bailey and Blish (1915) used sodium chloride solutions to extract the protein from wheat flour and showed that 48.3 per cent of the protein nitrogen was extracted by 1 per cent sodium chloride solution whereas 83.1 per cent of the protein nitrogen was extracted by 10 per cent sodium chloride solution.

Other workers (Johns and Jones, 1916; Jones and Johns, 1916;

Johns and Chernoff, 1919) have used sodium chloride solution for the extraction of proteins from peanut meal, jack bean and buck wheat. Davies (1927) observed that 25 per cent of the total proteins of rapeseed could be extracted by use of sodium chloride solution (10 per cent).

Sodium chloride solutions have been used in studies on the effect of such factors as meal particle size, extraction time, temperature and pH, on the extraction of proteins from different seeds. Smith et al (1938) studied the effect of particle size on solvent extraction of proteins from soybean. Olcott and Fontaine (1939) used 0.5N sodium chloride solution as extraction medium in their studies on the effect of pH on nitrogen solubility in cotton-seed meal. Painter and Nesbitt (1946) studied the effect of the particle size of the meal and the pH of the solvent on the solubilization of proteins in flax-seed. Djang et al (1953) used sodium chloride solution in studies on the effect of meal to solvent ratio, meal particle size, pH of extractant, extraction time and temperature on the yield of protein from the mung bean (Phaseolus aureus).

Chamberlain (1906) found that potassium sulphate solution (5 per cent) extracted almost the same amount of protein from wheat flour as did sodium chloride solution (10 per cent). Bishop (1929) and Hofman-Bang (1930) used potassium sulphate (5 per cent) solutions to study the proteins of barley. Osborne and Harris (1903; 1907), Wetter and McCalla (1949) used ammonium sulphate to fractionate seed proteins.

Straker and Gortner (1931) used several different types of salts and twenty-eight different types of seeds in their studies on the peptization of proteins. Gortner et al (1929) made similiar studies on wheat proteins.

In addition to salt solutions buffered salt solutions have been used to extract proteins from seeds. Danielsson (1949) extracted pea proteins using buffered sodium chloride solution (1.0M; pH 7.0); Wetter and McCalla (1949) extracted pea protein with a phosphate buffer (pH 7.8) and obtained a yield of 65 per cent.

2. Crystalline Proteins in Seeds

The existence of crystalline proteins in seeds was discovered over a century ago.

Hartig (1855) claimed that a large part of the reserve protein in seeds was present in the cells in the form of crystals and grains of more of less definite structure.

Maschke (1858) claimed that he crystallised the protein of Brazil-nut which Hartig (1855) had shown to be present in the seed in the form of rhombohedral crystals.

Schmiedeberg (1877) obtained crystals of the globulin of Brazil-nut by treatment of protein solution with magnesia followed by slow evaporation of the solution. He considered the crystals to be the magnesium salt of the proteins.

Ritthausen (1881), Grübler (1881) and Osborne (1891) isolated crystalline globulins from hemp-seed, squash seed and from the oat kernel respectively.

Osborne (1892,a) obtained crystalline globular proteins from Brazil-nut by dialysis of slightly acidic saline solutions of the protein. He concluded that the crystals were formed by reaction of the globulins with the acid rather than with bases as suggested by Schmiedeberg (1877).

Osborne (1901) obtained crystalline edestin from sodium chloride solution and from sodium sulphate solution

of hemp-seed protein. The author showed that the crystalline products which were obtained by use of sodium chloride and sodium sulphate were combined with hydrochloric acid and sulphuric acid respectively.

Osborne (1924) claimed that a number of vegetable globulins could be prepared in the crystal form; nearly all those which did not crystallise were obtained in the form of minute spheroids. The author prepared phaseolin from P. vulgaris, by dialysis of protein solutions; the phaseolin was in the form of minute spheroids mixed with a few octahedral crystals.

Perov and Lisitzuin (1932) claimed that proteins were present in the seeds of Phaseolus vulgaris as calcium proteinates which were combined with calcium phosphate. The complexes could be decomposed with dilute acetic acid to yield the free proteins.

Bailey (1949) prepared a number of different types of crystals from different seeds. He showed that the spheroidal globulin prepared from castor bean, could be converted to perfect octahedral crystals by cooling solutions of the spheroidal globulin containing sodium chloride to 15°C. Lugg (1949) claimed that crystalline proteins could be seen under the microscope in the cells of certain oil-bearing seeds and that crystalline proteins having similiar appearance could be prepared from saline extracts of these seeds.

Bourdillon (1951) obtained two different types of crystalline material from beans; one was a phytic acid-protein

complex ; the other was protein alone.

Fruton and Simmonds (1953) stated that crystallinity does not necessarily indicate purity or homogeneity of the protein. They claimed that many crystalline proteins have been found to be mixtures of several components.

Danielsson (1956) claimed that crystalline proteins occur in the aleurone layer of certain cereals. He stated that the crystallinity of a protein preparation is not a criterion of homogeneity.

Melynchyn (1969) prepared crystalline proteins from beans by extraction of the ground seed with sodium salts of dilute organic acids. He suggested that the crystalline protein preparations which were obtained were substantially homogenous even though they might exist as complexes.

Pant and Tulsiani (1969) suggested that the crystallinity of a globular type protein preparation tempts one to suspect that the protein is pure.

3. Effect of pH of Extraction Medium on Protein Yield

The basic units of protein molecules are amino acids. The summation of the ionic charges of the component amino acids of a given protein gives the molecule electrical properties. Any change in the electric conditions of the environment will change profoundly the ionic charge and hence, the characteristics of the protein. One such characteristic is called the isoelectric point of the protein; it is the pH of the environment at which the ionic units comprising the protein molecules, is not influenced by the presence of an electric field. Proteins generally show minimal solubility at their isoelectric points. Fan and Sosulski (1974) stated that the point of minimal solubility of proteins of several leguminous seeds corresponds to the isoelectric point of the proteins. As the pH is increased or lowered relative to the isoelectric point, the solubility of the protein increases. This has been the basis of methods for the extraction of proteins from seeds. It has been customary to use solutions having pH values far removed from the isoelectric point of the protein. Melynchyn (1969) showed, however, that appreciable quantities of crystalline protein can be extracted from beans using solutions with pH values which are close to the isoelectric point of the protein.

Rich (1936) showed that the amount of nitrogen which was extracted from wheat flour with distilled water was affected by the pH at which the extraction was done while the amount of proteins which was extracted by magnesium sulphate solution (0.5N) was influenced only slightly by the presence of dilute acid or alkali.

Smith and Circle (1938) studied the extraction of nitrogenous constituents from oil-free soybean meal by the use of acids and bases. Curves showing the relationship between the pH of the extractant and the quantity of nitrogen extracted indicated that hydrochloric, sulphuric, oxalic and phosphoric acids all gave minimal dispersion (8 per cent) of nitrogen at pH 4.1 to 4.2 and maximal dispersion (83 per cent) at pH 1.8; at pH values below 1.8 there was a rapid decrease in the amount of nitrogen that was dispersed. On the basic side the dispersion of nitrogen reached a maximum (96 per cent) at pH 12.0. They also showed that with dilute calcium chloride solution (0.1N) the effect of pH on the salt dispersion of the protein was greater than with more concentrated salt solution (0.5N). At this relatively high salt concentration the effect of pH practically disappeared.

Olcott and Fontaine (1939) extracted oil-free cotton-seed meal with water containing sulphuric acid; they showed that there was a minimal quantity (2.9 per cent) of nitrogen extracted at about pH 3.0. On opposite sides of this minimum pH value the percent nitrogen which was extracted reached high

values of 23.6 per cent at pH 1.0 and 24.9 per cent at pH 6.0. On the basic side, the percent nitrogen extracted increased from 32.9 per cent at pH 7.6 to 90.9 per cent at pH 12.0. Extraction with sodium chloride solution (0.5N) instead of with water gave values ranging from 78.2 per cent nitrogen extracted at pH 6.4 to 22.9 per cent extracted at pH 1.5. There was no point of minimal nitrogen solubility at an isoelectric point. On the basic side of the pH scale there was only a slight increase in the percent nitrogen extracted when the pH was increased from 6.9 (78.4 per cent) to 11.5 (82.9 per cent). Table I gives the results of the authors' work on the effect of pH on the extraction of protein from cotton-seed meal.

Fontaine and Burnett (1944) extracted nitrogenous constituents of oil-free peanut meal and found that more than 80 per cent of nitrogen was solubilized by water at pH 6.6. Minimal solubility occurred in the range pH 3.5 to pH 5.0. More than 80 per cent of the total meal nitrogen was solubilized at pH 1.5 and more than 90 per cent at pH 7.2. They showed that the peptizing action of solutions (0.25N to 1.0N) of calcium, magnesium and barium chloride was pH dependent, especially at lower salt concentrations. Effective peptization by these salts occurred between pH 5.0 and 6.0. The authors emphasized the importance of pH in the evaluation of the peptizing agents of nitrogenous material.

TABLE I

EFFECT OF NaOH AND H_2SO_4 ON THE EXTRACTION OF PROTEINS FROM COTTON-SEED MEAL[+]

	% of Total N Extracted			
	Ether extracted meal extracted with H_2O		Ether extracted meal extracted with 0.5N NaCl	
meq. H_2SO_4	pH	(%)	pH	(%)
3.60	1.9	23.6	1.5	22.9
1.80	2.3	18.1	2.0	24.7
1.17	2.9	12.9	2.9	27.1
0.80	3.8	13.6	3.8	28.9
0.45	4.8	18.1	4.7	38.8
0.18	5.8	22.8	5.6	72.1
0.0	6.9	24.9	6.4	78.2
meq. NaOH				
0.10	7.6	32.9	6.9	78.4
0.21	8.7	73.8	7.5	78.7
0.31	9.6	85.6	8.8	78.4
0.41	10.4	90.0	9.1	78.7
0.62	10.7	91.4	9.9	79.5
0.82	11.1	93.1	10.5	78.4
1.03	11.4	92.8	10.8	78.0
3.09	11.9	92.6	11.5	82.9
4.12	12.0	90.9		

[+]Olcott and Fontaine (1939).

Fontaine et al (1946,a) compared the solubility of the proteins of cotton-seed meal, the proteins of the corresponding dialysed meal and the isolated proteins. They suggested that the presence of naturally occurring substances in cotton-seed meal decreased the peptization of protein under certain conditions of acid reaction but seemed to have no effect under conditions of alkaline reaction.

Fontaine et al (1946,b) suggested that the low solubility of peanut and cotton-seed protein at pH 3.66 was the result of the formation of a protein-phytic acid complex. They suggested that the optimum reaction for the formation of this complex was pH 3.5. They concluded that the phytic acid in the meals caused a reduction in the solubility of the seed proteins at pH values below the isoelectric point of the proteins. The authors also suggested that the low protein solubility at pH 5.28 was the result of intermolecular attraction of proteins in the pH range of their isoelectric point since there was little reaction between the protein and phytic acid in this pH range.

Painter and Nesbitt (1946) showed that 85 to 90 per cent of the total nitrogen in flaxseed could be extracted at pH 11, 15 to 21 per cent at pH 3.5 to 4.0 and 53 to 65 per cent at pH 1.0 to 1.6. The authors found that formic acid dispersed 96 per cent to 99 per cent of the total nitrogen in flaxseed.

Evans et al (1948) performed experiments on the peptization of the nitrogenous constituents of dry peas and on methods

for the precipitation of the extracted protein solutions by adjusting of pH. They showed that about 70 per cent of the nitrogen could be peptized with 0.06N HCl (pH 1.7-2.0) and that maximum precipitation occurred at around pH 4.5 to 5.0. The authors also showed that the amount of nitrogen that was dispersed with sodium chloride solution (1.0N) depended on the pH of the solution. Approximately 70 per cent of nitrogen was dispersed at pH 5.8 to 8.6 while nearly 80 per cent was dispersed at pH 10.8.

Smith and Johnson (1948) showed that water at pH 6.6 dispersed only 20 per cent of the total nitrogen from sunflower seed while at the same pH, water extracted about 85 per cent to 90 per cent of the total nitrogen from soybean, peanut and flaxseed. The water extract from sunflower seed contained very little protein which was precipitated by acid. The nitrogen from sunflower seed, however, was almost completely dispersed at pH 10.0. The authors suggested that the low water solubility of sunflower seed proteins might be attributed to the presence of chlorogenic acid which is a tannin-like compound widely distributed in plants.

Bourdillon (1951) used sodium chloride solution (1.0N) to extract proteins from beans and precipitated the proteins at pH 4.6 and pH 4.2. He succeeded in the preparation of two types of crystalline protein from the proteins which precipitated at pH 4.2. One was in the form of an-isotropic dodecahedral crystals which the author regarded as a

protein-phytic acid complex. The other was a mixture of spheroids and bi-pyramidal crystals which the author regarded as protein alone.

Djang et al (1953) found that the hydrogen ion activity of aqueous solutions used for the extraction of nitrogenous material from mung beans (Phaseolus aureus) had a greater effect in the absence than in the presence of salt in the extractant.

Smith and Rackis (1957) suggested that the proteins in soybean meal formed complexes with phytin; the composition of the complexes varied with the pH of the extractant. Removal of phytin from the complex raised the isoelectric point of the acid precipitated proteins by 0.8 unit.

Smith et al (1959) studied the solubility of nitrogenous material in radish seed over a wide range of pH values. They showed that there was a sharp increase in the amount of nitrogen that was solubilized when the reaction of the extract was changed from pH 8 to pH 12.

Powrie (1961) studied the extraction of nitrogenous material from the navy bean (Phaseolus vulgaris) with hydrochloric acid. He showed that in the presence of sodium chloride, the percent of total nitrogen that was extracted decreased from 24.1 per cent at pH 5.38 to 16.6 per cent at pH 3.66 then increased to 29.5 per cent at pH 2.82. Sodium chloride caused a repression of the solubility of the alkali soluble proteins.

Evans and Kerr (1963) showed that maximal levels of

nitrogen were extracted from navy bean (Phaseolus vulgaris) with hydrochloric acid at pH 1.5, with sodium hydroxide at pH above 7.0 or with dilute sodium chloride solution while minimal quantities were extracted with solutions of pH 3.8. They suggested that the proteins which were extracted at pH 3.8 were probably albumins.

Pusztai (1965) carried out studies on the extraction of nitrogenous material from kidney bean (Phaseolus vulgaris). He found that the dispersion of protein was incomplete below pH 7.0 and that the exact amount of protein which could be extracted depended on the pH and ionic strength of the solvent. The author found that the extraction of proteins was practically complete in the pH range 7 to 9 but that the relative amounts of the individual proteins which were obtained depended on pH.

Cagampang et al (1966) found that alkaline solvents were generally better than acid solvents for the extraction of proteins from rice. They showed that lactic acid was superior to any of the other acid solvents for the extraction of rice proteins.

Melynchyn (1969) described a method for the preparation of crystalline proteins from beans using dilute aqueous solutions of salts of mono-, di- or tri-carboxylic acid. He obtained the most satisfactory result when the reaction of the extraction was between pH 3.5 and pH 5.0. The author reported yields of approximately 30 per cent of crystalline protein.

Pant and Tulsiani (1969) studied the effect of pH on the extraction of proteins from leguminous seeds. They found that for four species of Phaseolus, minimal extraction (15-22 per cent) occurred between pH 2.1 and pH 3.38. Highest yields of protein were obtained when the reaction of the extract was about pH 1 or above pH 7. For three species of the Phaseolus the maximum nitrogen extracted was about 80 per cent.

Gheyasuddin et al (1970) claimed that protein preparations isolated from sunflower seed by conventional method of extraction at high pH followed by precipitation at the isoelectric point were always green in colour. The author believed that the colour was the result of the oxidation of chlorogenic acid.

Patel and Johnson (1974) obtained maximal precipitation of horse-bean proteins from meal extraction between pH 4.0 and pH 4.5. They concluded that the dependence of protein solubility on pH was about the same with horse-bean protein as with other legume and oilseed proteins. They also noted that the pH of extraction and precipitation affected the colour of the protein precipitate.

Fan and Sosulski (1974) studied nitrogen extraction and protein precipitation using nine different leguminous seeds. They found that the range of minimal solubility of the proteins were within the apparent isoelectric range of the proteins. The alkali-extracted protein from lima beans

(*Phaseolus lunatus*) was shown to have relatively high solubility at the apparent isoelectric range. They suggested that non-protein nitrogen, including peptides, free amino acids and nucleic acids would constitute a substantial portion of the soluble nitrogen extracted at the pH's of minimal solubility.

4. Effect of Concentration of Extracting Medium on Protein Yield

Since the amino acids of proteins are ionic species they have a hydrophillic interaction with polar water molecules. Any substance which changes the ionic properties of a protein solution will therefore, affect the solubility of the proteins. Classical theory suggests that neutral salts such as ammonium sulphate at low ionic strengths increase protein solubility. High concentrations of these salts, however, inhibit solubility. This is the result of the competition of the protein and salt for water molecules for hydration of their ionic species. At high salt concentration the salt attracts water molecules to hydrate its ions and the protein is precipitated. In instances where the salt solution is acidic or alkaline the reaction (pH) of the solution becomes the decisive factor that determines the solubility of the protein. It is also possible that the nature of the ionic species of the salt will affect the solubility of the protein.

Gortner et al (1929) studied the peptization of the proteins in wheat flour by inorganic salt solutions. They found that the degree of peptization of the proteins decreased as the salt concentration increased. Rich (1936) used solutions of sodium chloride and sodium and magnesium sulphates at eleven

different normalities (0 to 6N) to extract proteins from wheat flour. Solutions of 0.25N gave maximal protein extraction; as the salt concentration was increased above 0.25N the amount of protein that was extracted decreased.

O'Hara and Saunders (1937) used thirteen different concentrations (0 to 6N) of sodium chloride solutions in their studies on the peptization of the proteins in flaxseed, orange seed, peanut and rye. Table II summaries the results of their studies. In studies on the extraction of proteins from soybeans, Smith and Circle (1938) extracted 68.9 per cent of the total nitrogen with sodium chloride solution, (0.01N) 39.9 per cent with 0.1N solution and 73.8 per cent with 0.5N sodium chloride solution. Smith et al (1938) determined the effect of twelve different salts, each at seventeen different normalities (0.001N to 3.0N) on the peptization of the nitrogenous constituents of soybean meal. The experiments with univalent metal salts demonstrated that at low concentrations (less than 0.01N) there was high nitrogen extraction. There was a minimal level of extraction at about 0.01N and a maximal level of extraction at about 1.0N. For the divalent metal salts, the general pattern was similiar to that of the univalent metal salts except that the point of minimal extraction appeared at about 0.0175N. The authors also studied the effect of concentration (0.10N to 0.5N) of sodium chloride solution on the extraction of nitrogen from several different types of seeds (barley, wheat, rye, flaxseed, soybean and terpary bean).

TABLE II

EFFECT OF CONCENTRATION OF EXTRACTANT ON THE AMOUNT OF NITROGEN EXTRACTED FROM DIFFERENT SEEDS[+]

Normality of NaCl solution	% of Total Nitrogen extracted from seeds			
	Flaxseed	Orange seed	Peanut	Rye
0	24.77	32.24	52.86	14.81
0.125	41.73	35.59	71.15	20.32
0.25	58.63	51.10	91.76	32.45
0.375	64.59	58.12	94.85	34.75
0.50	64.50	65.54	94.55	34.15
0.75	64.48	70.22	93.88	26.69
1.0	64.59	73.34	94.42	25.00
2.0	65.19	72.38	95.37	17.03
3.0	63.75	71.59	94.14	18.57
4.0	61.71	67.62	95.89	14.49
5.0	56.29	67.82	92.26	11.23
6.0	50.12	65.84	87.68	7.65
Saturated	45.53	64.92	81.23	7.65

[+] O'Hara and Saunders (1937).

Nagel et al (1938) showed that when the alcohol (ethyl alcohol, methyl alcohol) concentrations in aqueous solutions were increased there was a decrease in the solubility of soybean proteins. They suggested that this was the result of the denaturation of soybean proteins.

Olcott and Fontaine (1939) studied the effect of salt concentration (0.25N to 1.0N) on peptization of cotton-seed proteins. They used twenty-three different types of salts, including the sodium salts of acetic acid, citric acid, oxalic acid and tartaric acid. Their results showed that in general there was an increase in protein solubility as the salt concentration increased.

Fontaine and Burnett (1944) used twenty-seven different salts as peptizing agents for peanut proteins. They showed that sodium and potassium salts (except flourides and acetates) were good peptizing agents at a concentration of 1.0N. There was an increase in protein peptization with solutions of barium chloride, magnesium chloride and magnesium sulphate as the concentration was increased from 0.01N to 0.1N. At concentrations between 0.02N and 0.04N the amount of nitrogen that was peptized was approximately the same as that peptized by water at the same pH. They showed that for most of the other salts which were used, there was a definite increase in percent nitrogen peptized as the salt concentration was increased from 0.75N to 1.0N.

Fontaine et al (1946,a) studied the effect of different

acids on the peptization of the proteins in peanut and cotton-seed. Trichloroacetic acid produced a marked increase in protein peptized as the concentration was increased from 0 to 5N. Phosphoric acid gave a similiar effect with cotton-seed but the opposite effect with peanuts. Increasing the concentration of acetic acid increased markedly the peptization of peanut protein but had only a slight effect on cotton-seed proteins. When the concentration of hydrochloric acid and sulphuric acid was increased from 0 to 5.0N it was accompanied by little change in the peptization of the seed proteins.

Djang et al (1953) studied some factors which might affect the solubilization of nitrogenous constituents of mung bean (Phaseolus aureus). When the concentration of sodium chloride solution was increased from 0.05M to 0.40M there resulted an increase in nitrogen solubilized from 30 per cent to 60 per cent.

Smith et al (1959) demonstrated that the solubility of the nitrogenous constituents in soybean dropped from 90 per cent to 50 per cent as the normality of the extractant (sodium chloride solution) was increased from 0 to 0.1M. The solubility of the nitrogenous constituents of radish seed under the same conditions increased from 20 per cent to 50 per cent.

Baker et al (1961) studied the effect of concentration of extractant (0 to 1N sodium chloride solution) on the peptization of the proteins in five different pulses. For all the pulses there was minimal nitrogen solubility in the

concentration range 0.050N to 0.075N range. Above 0.075N there was an increase in nitrogen peptized with increasing normality.

Evans and Kerr (1963) investigated the extraction of proteins from the navy bean (Phaseolus vulgaris). Solutions of sodium, potassium, calcium and copper chlorides and copper sulphate gave about the same degree of protein extraction over the range of concentration 0.25N to 2.0N. They concluded from their studies that the nature of the salt which was used had a greater effect on the percent of the total nitrogen that was extracted than did the concentration of the salt. Pusztai (1965) also studied the extraction of protein from Phaseolus vulgaris. His results showed that the dispersibility of nitrogenous constituents depended on the pH of the extracting solution.

Zarkadas (1964) showed that the amount of protein extracted from pea meal varied with the concentration of sodium chloride solution. He obtained maximal peptization (85 per cent of total nitrogen) with 1.0N sodium chloride solution and minimal peptization between concentrations of 0.04N and 0.08N.

Melynchyn (1969) succeeded in the isolation of crystalline proteins from Phaseolus beans using organic acid solutions with concentrations between 0.05N and 0.8N. He obtained more satisfactory result with the di- and tri-basic acids at low concentration than with the mono-basic acids at higher concentration.

Pant and Tulsiani (1969) studied the extraction of seed protein using different salts at concentrations of 0.25N, 0.50N and 1.0N. They found that different salts, at the same concentration extracted different amounts of nitrogen from the same seed while the same salt, at the same concentration extracted different amounts of nitrogen from different seeds. Their results showed that there was no general relationship between the concentration of the salt solution and amount of nitrogen that was extracted from the different types of seeds.

Hang et al (1970) made similiar studies using three varieties of Phaseolus beans. Sodium chloride solution gave minimal extraction of protein from mung bean (Phaseolus aureus) at a concentration of 0.05M and minimal extraction of protein from pea bean (Phaseolus vulgaris) and red kidney bean (Phaseolus vulgaris) at concentration of 0.025M.

5. Effect of Particle Size of Seed Meal on Protein Yield

The classical view on the effect of particle size on protein solubilization from seeds is that decreasing particle size up to a certain limit, results in an increase in solubilization of proteins. It has been suggested (a) that grinding of the seeds results in rupture of cellular and subcellular membranes surrounding protein bodies and hence, allows the solubilizing agent to come in contact with the protein, and (b) that extremely fine grinding causes denaturation of the protein and loss of protein dispersibility.

A review of the literature has revealed that the first published work on the effect of particle size on the solubilization of seed protein was that of Bishop (1929). He reported that barley flour which was ground to pass through a 1.0 mm sieve yielded 25.6 per cent of its nitrogen on extraction with salt solution whereas, 31.2 per cent was extracted from barley flour which was ground to pass through a 0.50 mm sieve. Hofman-Bang (1930) confirmed Bishop's findings.

Nagel et al (1938) studied the solubilization of soybean proteins in water. Their results showed that meal which was ground to pass through a 100 mesh screen (0.147 mm openings) yielded slightly more protein than did the meal which passed through a 200 mesh screen (0.074 mm openings). They explained

that the long period of grinding in the "Ball mill" which was necessary to produce the very fine (200 mesh) meal resulted in the denaturation of the proteins.

Smith et al (1938) also found that particle size had a marked effect on the peptization of soy protein with water or salt solutions. Soybean meal prepared with a "Pebble mill" gave lower yields of protein than that prepared with a "Wiley mill" despite the fact that particle size of the former was smaller than that of the latter. The authors attributed this to the difference in the grinding action of the two mills.

Becker et al (1940) studied the effect of particle size on the extraction of non-protein nitrogen from soybean meal using trichloro-acetic acid (0.8N). Their results reported in Table III show that the authors obtained a slight increase in the quantity of non-protein nitrogen that was extracted as the particle size decreased.

TABLE III

EFFECT OF MEAL PARTICLE SIZE ON NON-PROTEIN NITROGEN EXTRACTED FROM SOYBEAN[+]

Meal Grind	mgm. of N extracted per gm. meal
flakes	3.03
2.0 mm. screen (Wiley mill)	3.00
1.0 mm. " " "	3.09
0.5 mm. " " "	3.14
100 mesh (Ball mill)	3.19

[+]Becker et al (1940)

Nagy et al (1941) established that the amount of protein that could be extracted from corn with sodium chloride solution increased by about 2 per cent when the particle size was decreased (60 mesh to 100 mesh).

Painter and Nesbitt (1946) studied the peptization of the protein in flaxseed using sodium chloride and magnesium chloride solutions. Their results demonstrated that little is gained by grinding the seed in a "Ball mill" for more than six hours. They suggested that some of the cystein and tryptophane were lost during the grinding operation.

Djang et al (1953) investigated the effect of particle size on the extraction of protein from mung beans (Phaseolus vulgaris) using sodium chloride solution (0.4N). These workers ground their seed material to pass through mesh size numbers 20, 40 and 60. They concluded that solubilization of nitrogenous constituents was essentially complete with meal which passed through a 60 mesh sieve.

Zarkadas (1964) investigated the peptization of the protein in peas. He contended that the quantity of protein that remained in the residue after extraction was governed at least in part, by the efficiency of the grinding operations. He suggested that some protein material was rendered insoluble by denaturation during the grinding of the peas.

Cagampang et al (1966) investigated the extraction of protein from rice by a percolation method and showed that a decrease (40 to 60 mesh) in the particle size of the meal

resulted in a substantial increase in protein yield.

Patel and Johnson (1974) extracted proteins from horsebean with water and with dilute calcium hydroxide solution. They obtained a yield (percent of total nitrogen) of 86.4 percent with coarser (particle size - 360μ) flour and a yield of 94.2 per cent with the finer (particle size - 130μ) flour. They suggested that the more efficient extraction with the finer flour resulted from the more complete rupture of cellular membrane which surrounded the protein bodies.

6. Effect of Extraction Time on Yield of Protein

O'Hara and Saunders (1937) investigated the effect of extraction time on the amount of nitrogenous material that was extracted from flaxseed meal. They found that an increase in extraction time produced little change in protein yield.

Nagel et al (1938) showed that the length of the extraction period was not a critical factor in the dispersion of proteins from soybean. They made three successive extractions on the same sample and they found that one minute extraction period for each of the three extractions dispersed as much protein as did three hour extraction periods.

Olcott and Fontaine (1939) used potassium iodide solution to extract proteins from cotton-seed meal and found that almost complete extraction took place after a fifteen minute extraction period even when no mechanical agitation was used (Table IV).

Smith et al (1952) worked with three different varieties of soybean and showed that the effect of extraction time on protein yield differed from one variety of soybean to another.

Djang et al (1953) found that an increase in extraction time produced only slight increase in protein yield from the mung bean (Phaseolus aureus). They also found that mechanical shaking had little advantage over hand stirring.

TABLE IV

EFFECT OF TIME OF SHAKING ON AMOUNT OF NITROGEN EXTRACTED FROM COTTON-SEED[+]

Time of Shaking (minutes)	% of Total Nitrogen Extracted by 0.5N KI Solution
15	80.4
30	81.0
60	81.5
120	81.9
1440	81.9

[+]Olcott and Fontaine (1939)

Cagampang et al (1966) investigated the conditions for the extraction of proteins from rice and reported that the period of extraction for maximal yield varied between three and six hours.

Melynchyn (1969) in his work on the isolation of crystalline proteins from beans, allowed the ground meal to stand in the extractant with continuous or intermittent agitation for a period of thirty minutes.

Patel and Johnson (1974) found that the yield of proteins from horse-bean was affected by the temperature of the extraction. For example, at 23°C., when the period of extraction

was increased from five to fifteen minutes the percentage of protein extracted increased from 84.4 per cent to 95.8 per cent. No further solubilization occurred when the period of extraction was increased beyond fifteen minutes. When the period of extraction was increased from one hour to four hours at 9°C. the percentage of protein extracted increased by about 2.6 per cent. They suggested that the low yield of protein which was obtained when the extraction was carried out at low temperature could be increased by increasing the period of extraction or by increasing the agitation.

7. Effect of Temperature of Extraction on Protein Yield

Johns and Jones (1916) found that the amount of protein which was extracted from oil-free peanut meal at 40°C. was about the same as that extracted at room temperature.

Rich (1936) showed that the amount of protein that could be extracted from wheat flour with water, sodium chloride solution (0.25N) and sodium and magnesium sulphate solutions (0.25N) was affected only very slightly by increasing the temperature of the reaction from 5°C. to 60°C. Table Va lists the results of the author's experiments.

O'Hara and Saunders (1937) extracted proteins from orange seed meal at 2.5°C., 26°C. and 39.7°C. They obtained yields (percent of total nitrogen) of approximately 36, 41 and 49 per cent respectively.

Nagel et al (1938) extracted soybean proteins with water at temperatures ranging between 1.5°C. and 45°C. The results which they obtained are listed in Table Vb.

Olcott and Fontaine (1939) studied the extraction of nitrogenous constituents from cotton-seed using water and sodium chloride solution (0.5N). They found that the percent nitrogen which was extracted with the sodium chloride solution increased appreciably when the temperature was increased from 0°C. to 25°C. On the other hand, when the temperature was

increased from 25°C. to 50°C. no appreciable increase was noted.

TABLE Va

EFFECT OF TEMPERATURE ON THE AMOUNT OF PROTEIN EXTRACTED FROM WHEAT FLOUR[+]

Dispersing Agent	Amount of Protein Extracted as % of Wheat Flour				
	TEMPERATURE				
	5°C.	10°C.	20°C.	40°C.	60°C.
	%	%	%	%	%
Water	3.2	3.2	3.2	3.1	3.1
NaCl Solution (0.25N)	2.6	2.6	2.7	2.8	2.7
$MgSO_4$ Solution (0.25N)	2.2	2.3	2.4	2.3	2.4
Na_2SO_4 Solution (0.25N)	1.8	2.0	1.8	2.2	2.0

[+]Rich (1936.

Smith et al (1952) showed that temperature as well as method of agitation were major factors affecting the amount of nitrogenous material which could be extracted from soybean. Their results showed that mild agitation (mechanical stirring) at 50°C. gave slightly higher values than were obtained with intense agitation (lightning-type stirrer) at 25°C.

TABLE Vb

PERCENT NITROGEN EXTRACTED FROM SOYBEAN AT DIFFERENT TEMPERATURES[+]

Temperature °C.	% Nitrogen
1.5	71.4
14.0	76.3
28.0	80.0
35.0	81.6
45.0	83.1

[+]Nagel et al (1938)

Djang et al (1953) studied the effects of temperature (25°C., 45°C. and 55°C.) on the extraction of proteins from mung bean (Phaseolus aureus). They showed that when salt solution of low concentration was used the amount of protein that was solubilized decreased as the temperature was increased above 45°C. They attributed this to the denaturation of the proteins brought about by the elevated temperature. They also showed that lipid-free samples gave slightly higher yields than did non-lipid-free samples.

Cagampang et al (1966) showed that the efficiency of sodium hydroxide solution (0.1N) for the extraction of proteins from rice flour was about the same at 4°C. as it was at room temperature.

Patel and Johnson (1974) extracted proteins from horsebean with calcium hydroxide solution at 23°C. and at 9°C. Higher yields were obtained at the higher temperatures. Increasing the period of extraction and the degree of agitation, however, increased appreciably the yield which was obtained at the low temperature.

II. EXPERIMENTAL, RESULTS AND DISCUSSION

1. Materials

a. Source of Beans

White kidney beans (Phaseolus vulgaris) were purchased from Lancia Food Co., St. Laurent, P. Q.

Lima beans (Phaseolus lunatus) were purchased from Steinberg Supermarket, Dorval, P. Q.

Navy beans (Phaseolus vulgaris) were supplied by Dr. P. Melynchyn of FBI Foods Ltd., Beloeil, P. Q.

b. Moisture and Protein Contents of Beans

The samples of beans were ground in the Mikro Samplemill (Pulverising Machinery Co., Summit, N.J., U.S.A.). The mill was fitted with a 2 mm. diameter mesh. The moisture and crude protein contents of the meal were determined by the methods given in the Methods of Analysis of the Association of Official Agricultural Chemists, Eleventh Edition (1970). The results of the analyses are given in Table VI.

TABLE VI

MOISTURE AND PROTEIN CONTENT OF BEAN SAMPLES

	White Kidney bean (*P. vulgaris*)	Navy bean (*P. vulgaris*)	Lima bean (*P. lunatus*)
% Protein (%N x 6.25)	20.1	21.0	22.2
% Moisture	10.5	8.9	8.8

2. Preliminary Experiments

The beans were ground in the Mikro Samplemill (Pulverising Machinery Co., Summit, N.J., U.S.A.) to pass through a 2 mm. mesh. A sample (10 g) of the ground material was placed in a centrifuge bottle (250 ml) along with citric acid solution (100 ml; 0.2N adjusted to pH 4.0). The mixture was allowed to stand for thirty minutes with intermittent stirring and then centrifuged (International Refrigerated Centrifuge, Model PR-1, International Equipment Co.) for ten minutes (1200g).

The supernatant was filtered through fine glass wool and the filtrate was stored at 5°C. for eighteen hours. The precipitate was observed under the microscope and then isolated by centrifugation. The product was washed twice with distilled water and then freeze-dried.

The above procedure was used for the preparation of samples of protein from white kidney beans (Phaseolus vulgaris), navy beans (Phaseolus vulgaris) and lima beans (Phaseolus lunatus).

The products were analysed for total nitrogen by the Micro-Kjeldahl method described in A.O.A.C. (1970).

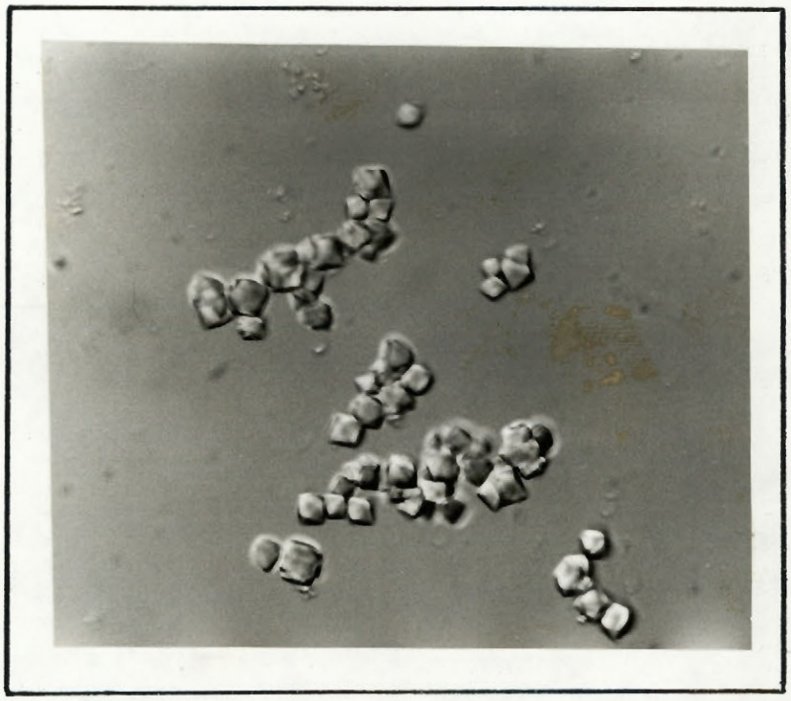
White kidney beans (Phaseolus vulgaris) and navy beans (Phaseolus vulgaris) gave protein preparations which under the microscope, appeared as bi-pyramidal crystalline material which

was similiar to proteins described by Melynchyn (1969), (Figure I). Lima bean (Phaseolus lunatus) gave a protein preparation, the particles of which were spheroidal.

FIGURE I

Crystalline Protein Isolated from the Navy Bean (*Phaseolus vulgaris*).

Magnification x 40



3. Effect of pH and Normality of Extractant on Yield of Protein and Protein Crystallinity

a. Materials and Method

Solutions of citric acid and DL-malic acid of different normalities and pH values were prepared by dissolution of the crystalline acid in water followed by the adjustment of the pH of the solution to a given value by the use of sodium hydroxide solution (50 per cent). Table VII shows the composition of the solutions that were prepared.

The extraction procedure was similiar to that described on page 45. Samples (10 g) of ground beans (white kidney beans, navy beans and lima beans) were placed in centrifuge bottles (250 ml) along with extraction solutions (100 ml). The mixtures were allowed to stand for thirty minutes with intermittent stirring and then centrifuged (International Refrigerated Centrifuge, Model PR-1, International Equipment Co.) for ten minutes (1200g).

The supernatants were filtered through fine glass wool and the filtrates were stored at 5°C. for eighteen hours. The precipitates were observed under the microscope and then isolated by centrifugation. The products were washed twice with distilled water and then freeze-dried.

The above experiment was done on each type of bean using

the extractants listed in Table VII. The nitrogen contents of the different types of precipitates (indicated by microscopic examination) were determined by the Micro-Kjeldahl method described in A.O.A.C. (1970).

b. Results and Discussion

The yields of protein which were obtained from the three beans with the various solutions are shown in Tables VIII to XIII; yields of crystalline protein are underlined. The results are shown in graphic form in Figures II to V.

TABLE VII

SOLUTIONS PREPARED FOR EXTRACTION OF PROTEINS FROM BEANS

Reagent	Solution Nos.	Normality	pH
Citric Acid	1-7	0.05	2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5[+]
"	8-14	0.08	" " " " " " "
"	15-21	0.10	" " " " " " "
"	22-28	0.20	" " " " " " "
"	29-35	0.40	" " " " " " "
"	36-42	0.50	" " " " " " "
"	43-49	0.60	" " " " " " "
"	50-56	0.80	" " " " " " "
DL-Malic Acid	57-62	0.05	2.5, 3.0, 3.5, 4.0, 4.5, 5.0
"	63-68	0.08	" " " " " "
"	69-74	0.10	" " " " " "
"	75-80	0.15	" " " " " "
"	81-86	0.20	" " " " " "
"	87-92	0.40	" " " " " "
"	93-98	0.60	" " " " " "
"	99-104	0.80	" " " " " "
Water	105-112		2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5[+]

[+] Used to extract proteins from White Kidney bean and Navy bean only.

TABLE VIII

Extraction of Protein from Lima Beans with Citric Acid Solutions

pH	Percentage Yield of Protein $\left(\frac{\text{total N in isolated protein}}{\text{total N in meal}} \times 100\right)$								
	0.0N[a]	0.05N	0.08N	0.10N	0.20N	0.40N	0.50N	0.60N	0.80N
	%	%	%	%	%	%	%	%	%
2.5	6.7	6.0	8.0	3.4	0.0	0.0	0.0	0.0	0.0
3.0	0.0	3.2	6.3	10.0	0.9	0.0	0.0	0.0	0.0
3.5	0.0	2.7	7.4	10.6	10.0	0.0	0.0	0.0	0.0
4.0	0.0	7.5	8.5	9.7	0.7	0.0	0.0	0.0	0.0
4.5	0.0	1.3	1.5	2.1	2.7	0.0	0.0	0.0	0.0
5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Underlined values indicate yield of crystalline protein.

[a]Distilled water.

TABLE IX

Extraction of Protein from Lima Beans with DL-Malic Acid Solutions

pH	Percentage Yield of Protein $\left(\frac{\text{total N in isolated protein}}{\text{total N in meal}} \times 100\right)$								
	0.0N[a]	0.05N	0.08N	0.10N	0.15N	0.20N	0.40N	0.60N	0.80N
	%	%	%	%	%	%	%	%	%
2.5	6.7	9.2	6.8	4.4	1.1	1.4	0.0	0.0	0.0
3.0	0.0	15.7	12.2	7.0	2.4	0.9	0.0	0.0	0.0
3.5	0.0	5.5	9.7	15.5	9.3	0.0	0.0	0.0	0.0
4.0	0.0	10.9	13.2	12.5	4.8	1.1	0.0	0.0	0.0
4.5	0.0	15.1	10.8	9.3	2.4	0.0	0.0	0.0	0.0
5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Underlined values indicate yield of crystalline protein.

[a]Distilled water.

TABLE X

Extraction of Protein from White Kidney Beans with Citric Acid Solutions

pH	Percentage Yield of Protein $\left(\frac{\text{total N in isolated protein}}{\text{total N in meal}} \times 100\right)$								
	0.0N[a]	0.05N	0.08N	0.10N	0.20N	0.40N	0.50N	0.60N	0.80N
	%	%	%	%	%	%	%	%	%
2.5	1.0	0.0	0.0	0.0	0.0	5.4	9.0	13.1	13.9
3.0	2.3	0.0	0.0	0.0	0.0	4.5	7.9	20.6	18.3
3.5	4.5	0.0	0.0	0.0	0.0	11.4	13.3	13.0	8.7
4.0	4.2	0.0	0.0	0.0	1.5	14.0	16.0	15.8	6.9
4.5	3.2	0.0	1.0	2.7	4.4	17.3	17.3	11.2	1.5
5.0	4.4	1.3	4.7	4.6	16.0	18.0	6.8	0.0	0.0
5.5	4.7	29.8	32.9	32.6	29.3	8.2	0.0	0.0	0.0

Underlined values indicate yield of crystalline protein.

[a]Distilled water.

TABLE XI

Extraction of Protein from White Kidney Beans with DL-Malic Acid Solutions

pH	Percentage Yield of Protein $\left(\frac{\text{total N in isolated protein}}{\text{total N in meal}} \times 100\right)$								
	0.0N[a]	0.05N	0.08N	0.10N	0.15N	0.20N	0.40N	0.60N	0.80N
	%	%	%	%	%	%	%	%	%
2.5	1.0	0.0	0.0	0.0	0.0	1.3	3.3	7.0	14.0
3.0	2.3	0.0	0.0	0.0	0.0	1.0	2.5	7.3	17.5
3.5	4.5	0.0	0.0	0.0	0.0	2.4	4.6	24.7	39.9
4.0	4.2	0.0	0.6	0.5	1.5	5.2	5.1	1.8	0.0
4.5	3.2	0.0	1.7	1.0	6.3	16.4	2.2	0.0	0.0
5.0	4.4	2.7	3.6	6.0	11.5	14.5	0.0	0.0	0.0

Underlined values indicate yield of crystalline protein.

[a]Distilled water.

TABLE XII

Extraction of Protein from Navy Beans with Citric Acid Solutions

pH	Percentage Yield of Protein $\left(\frac{\text{total N in isolated protein}}{\text{total N in meal}} \times 100\right)$								
	0.0N[a]	0.05N	0.08N	0.10N	0.20N	0.40N	0.50N	0.60N	0.80N
	%	%	%	%	%	%	%	%	%
2.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.45	1.5
3.0	6.9	0.0	0.0	0.0	0.0	0.9	1.8	6.2	18.3
3.5	6.6	0.0	0.0	0.0	0.0	5.0	4.9	15.3	0.0
4.0	5.3	0.3	0.6	0.9	4.5	10.7	7.2	1.7	0.0
4.5	4.2	0.7	2.8	5.6	12.7	3.8	2.1	0.0	0.0
5.0	4.2	2.8	9.2	12.5	12.9	0.81	0.0	0.0	0.0
5.5	3.7	8.9	8.1	4.8	7.3	3.0	2.2	0.0	0.0

Underlined values indicate yield of crystalline protein.

[a]Distilled water.

TABLE XIII

Extraction of Protein from Navy Beans with DL-Malic Acid Solutions

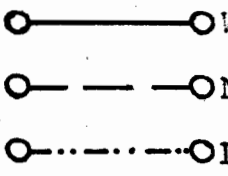
pH	Percentage Yield of Protein $\left(\frac{\text{total N in isolated protein}}{\text{total N in meal}} \times 100\right)$								
	0.0N[a]	0.05N	0.08N	0.10N	0.15N	0.20N	0.40N	0.60N	0.80N
	%	%	%	%	%	%	%	%	%
2.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.97	4.8
3.0	6.9	0.0	0.0	0.0	0.0	0.0	0.0	3.0	10.7
3.5	6.6	0.0	0.0	0.0	0.0	0.0	2.9	19.6	30.9
4.0	5.3	0.0	0.0	1.3	6.8	12.4	3.0	1.0	0.0
4.5	4.2	0.0	1.3	2.1	12.1	7.2	9.6	0.0	0.0
5.0	4.2	5.8	10.6	13.9	4.0	2.8	0.0	0.0	0.0

Underlined values indicate yield of crystalline protein.

[a]Distilled water.

FIGURE IIa, IIb, IIc.

Effect of pH of Citric Acid Solution on Yield of Protein from White Kidney Bean, Navy Bean and Lima Bean.



$$\% \text{ of Total Protein} = \frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$$

Fig IIa

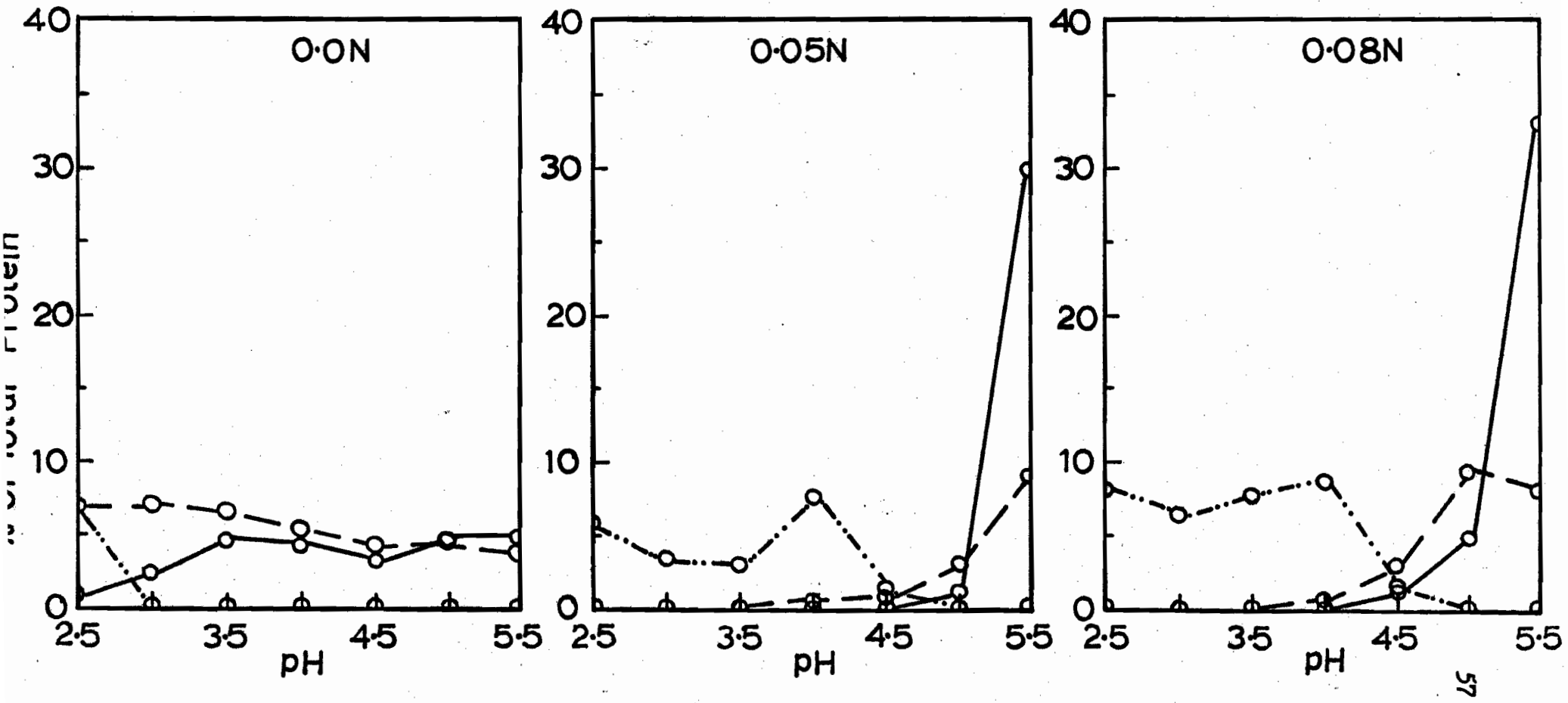


Fig IIb

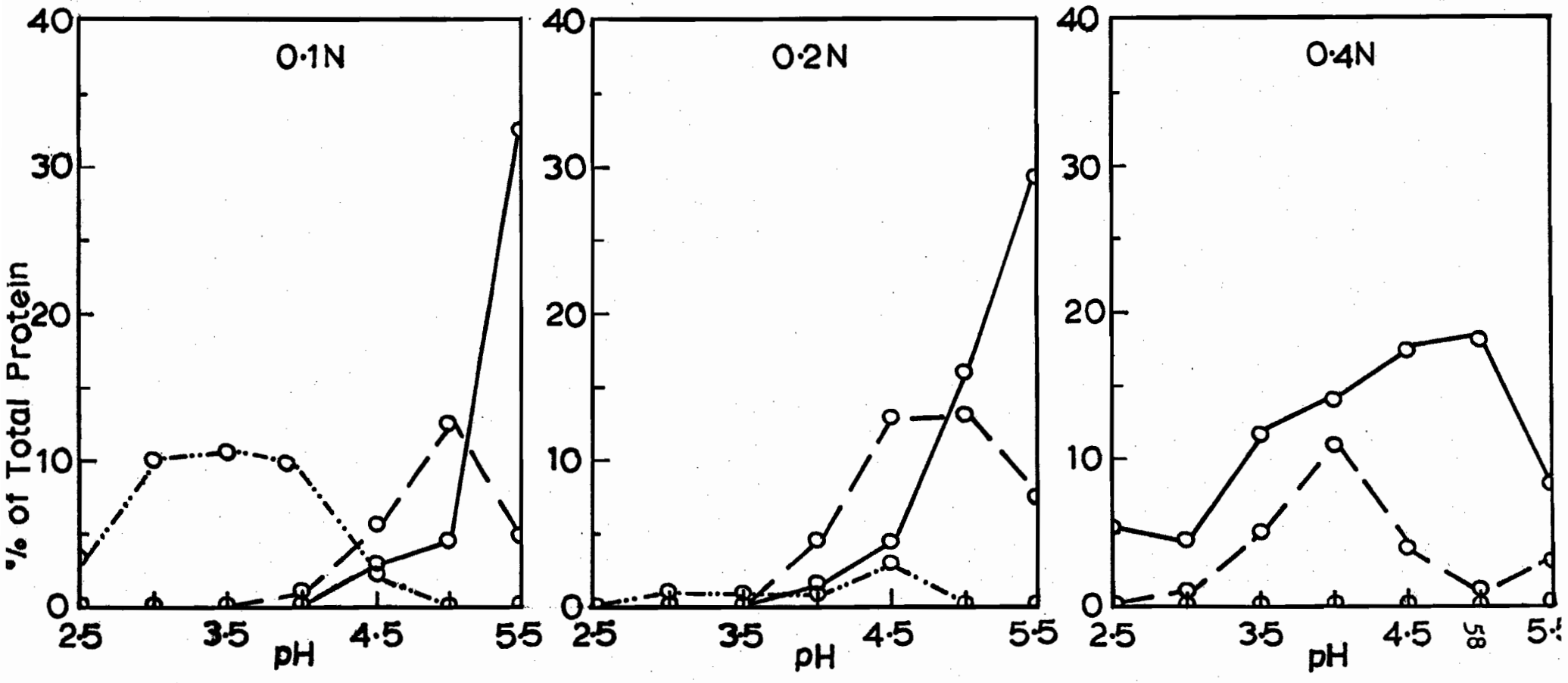


Fig IIc

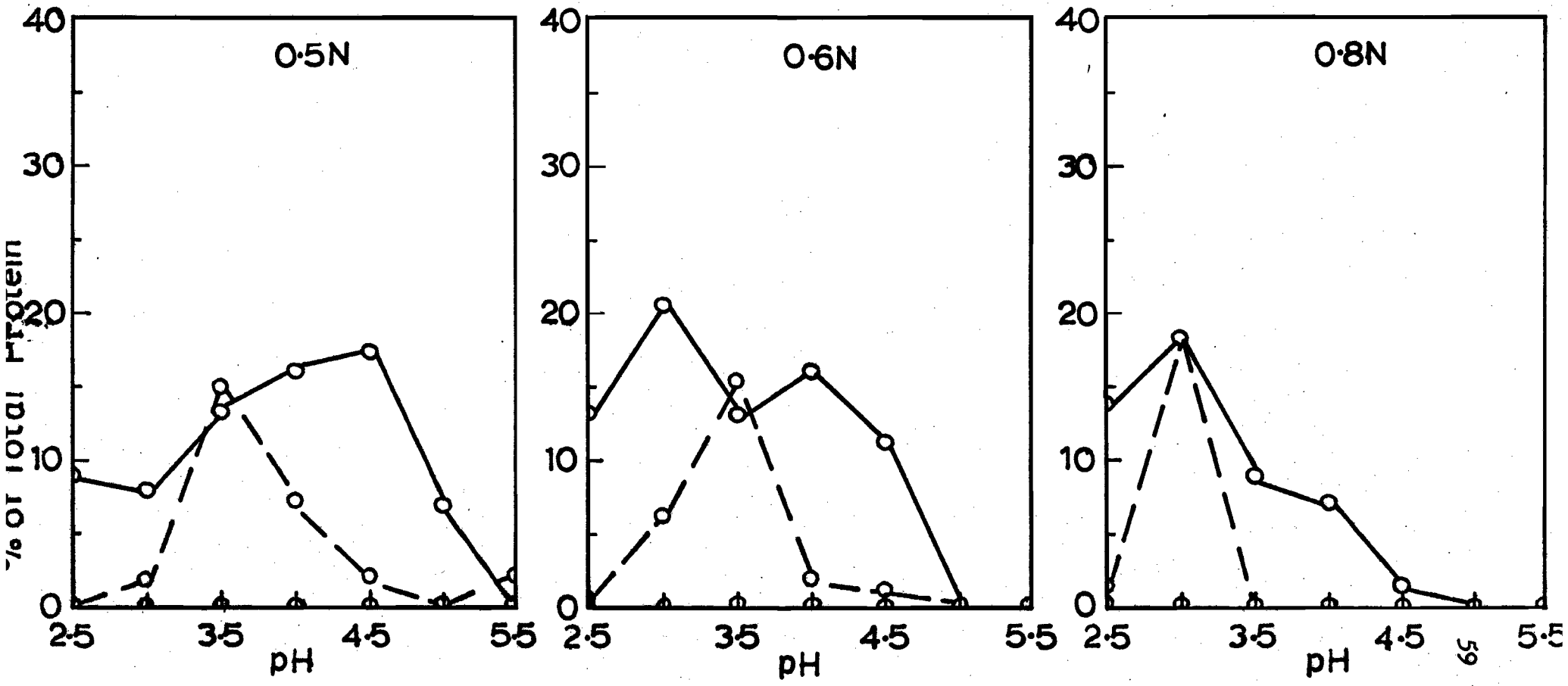
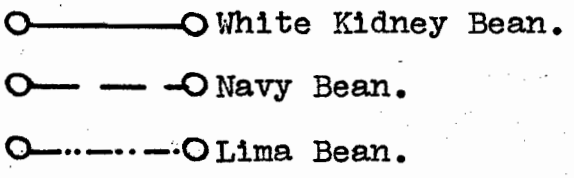


FIGURE IIIa, IIIb, IIIc.

Effect of pH of DL-Malic Acid Solution on Yield of Protein from White Kidney Bean, Navy Bean and Lima Bean.



$$\% \text{ of Total Protein} = \frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$$

Fig IIIa

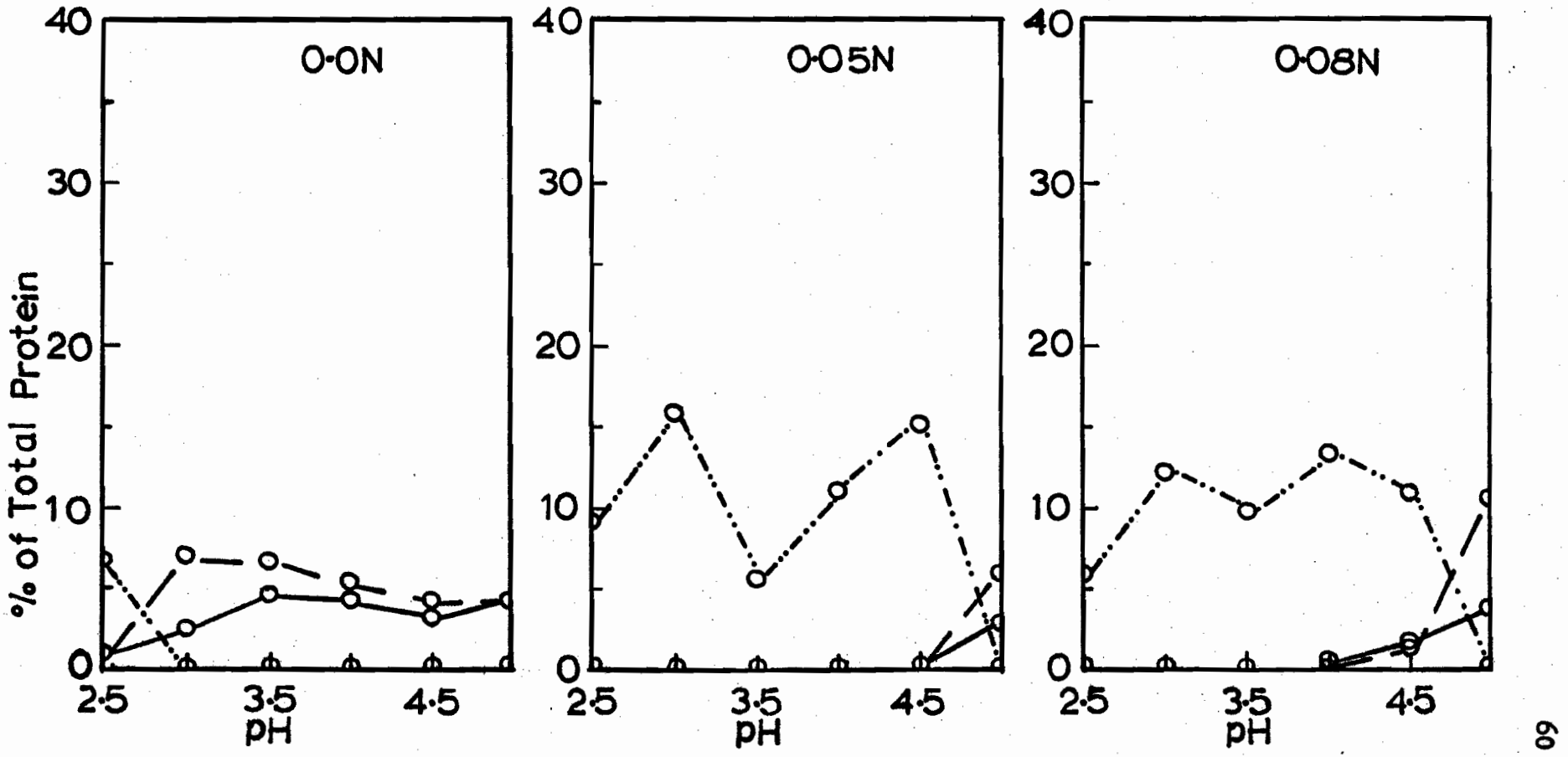


Fig IIIb

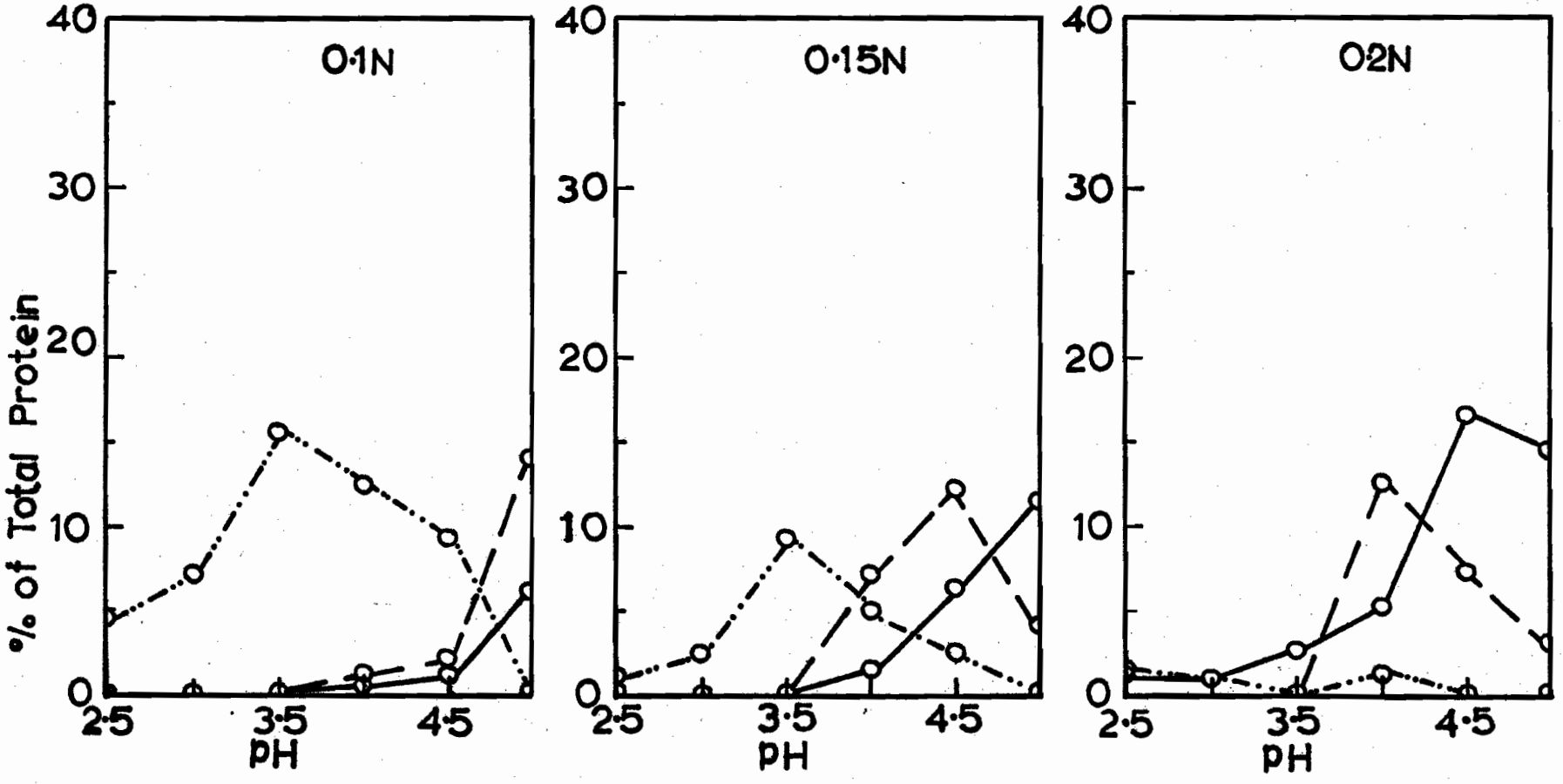


Fig IIIc

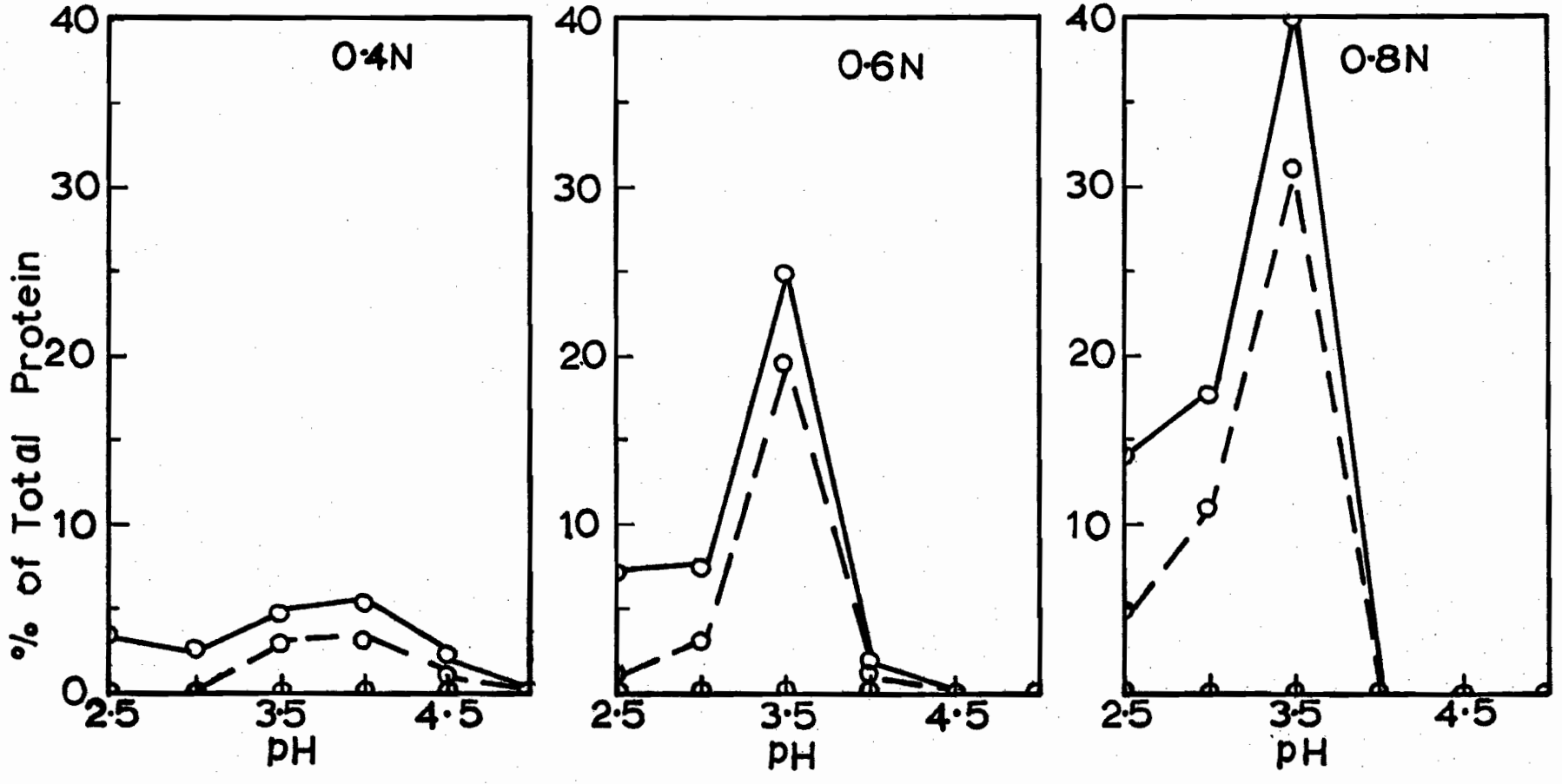


Fig IVa

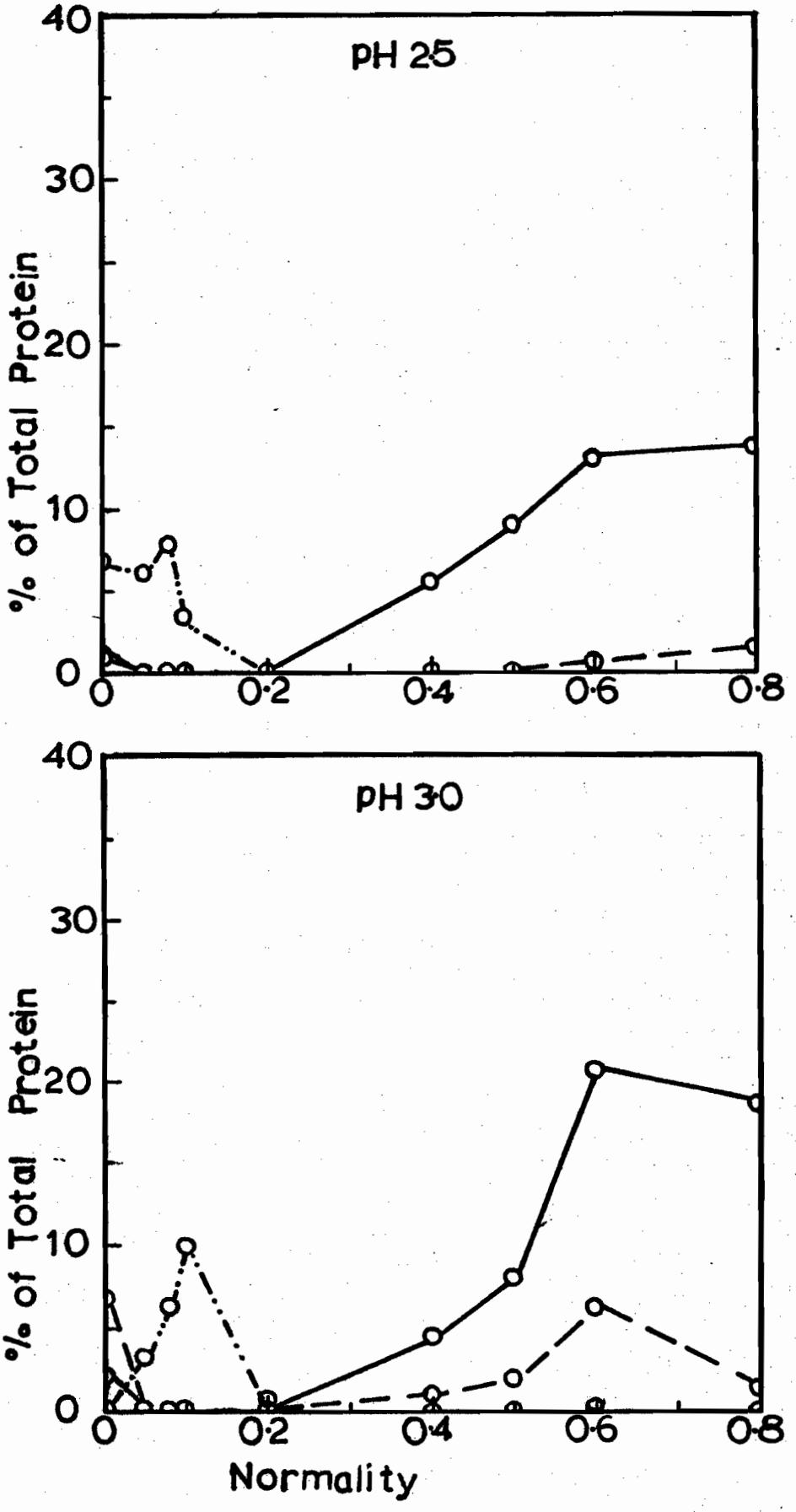


Fig IVb

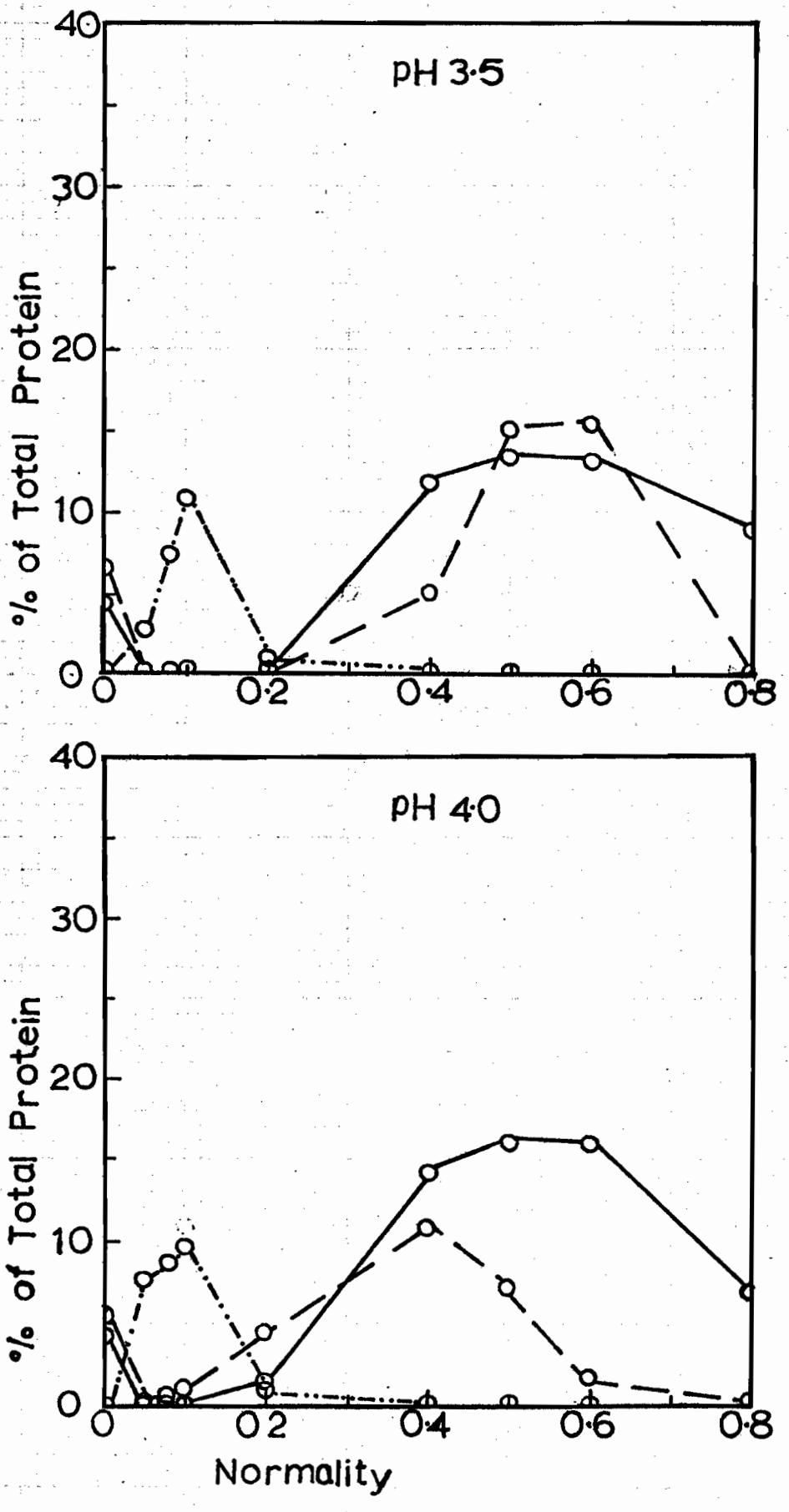


Fig IVc

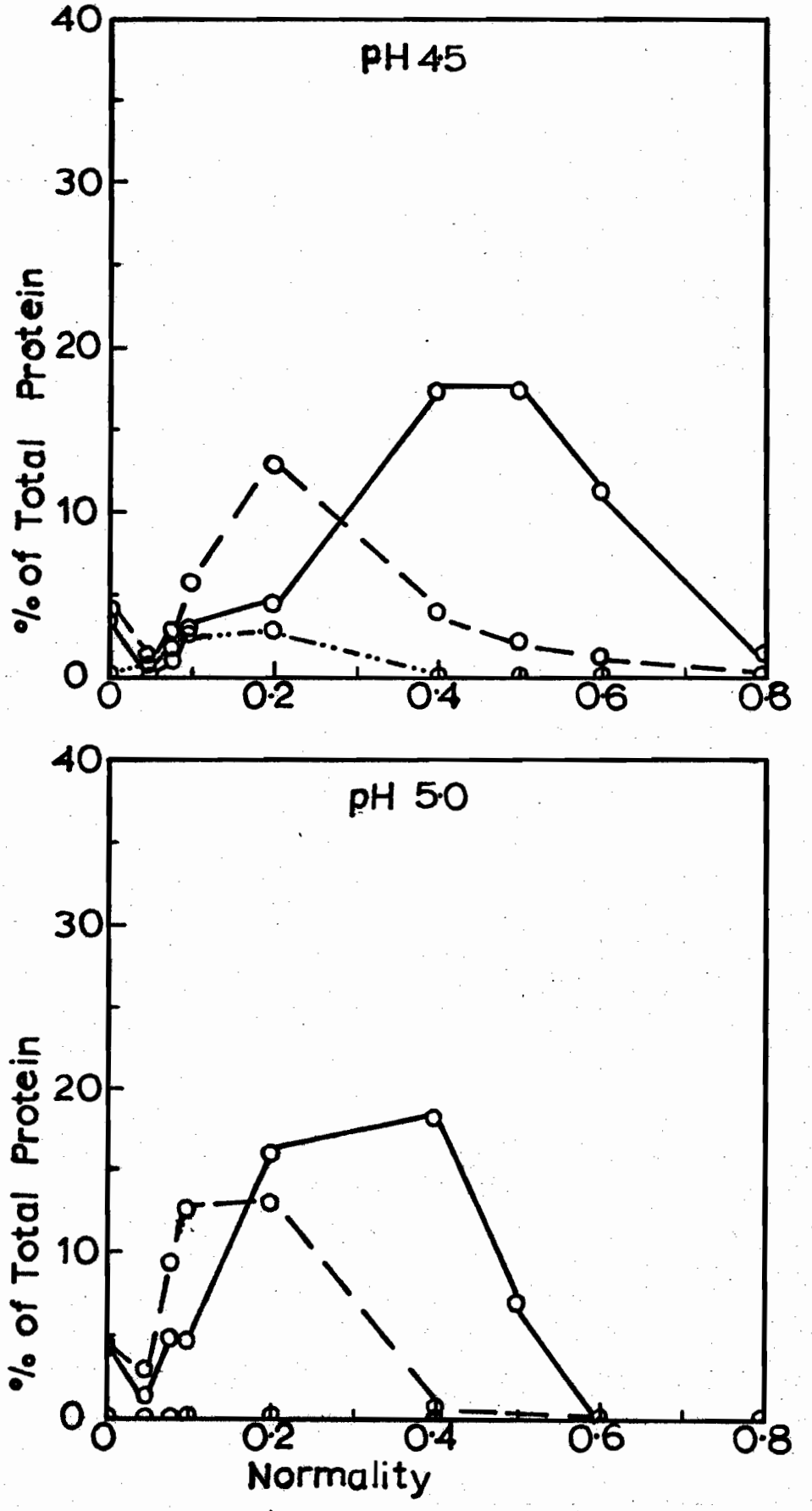


Fig IVd

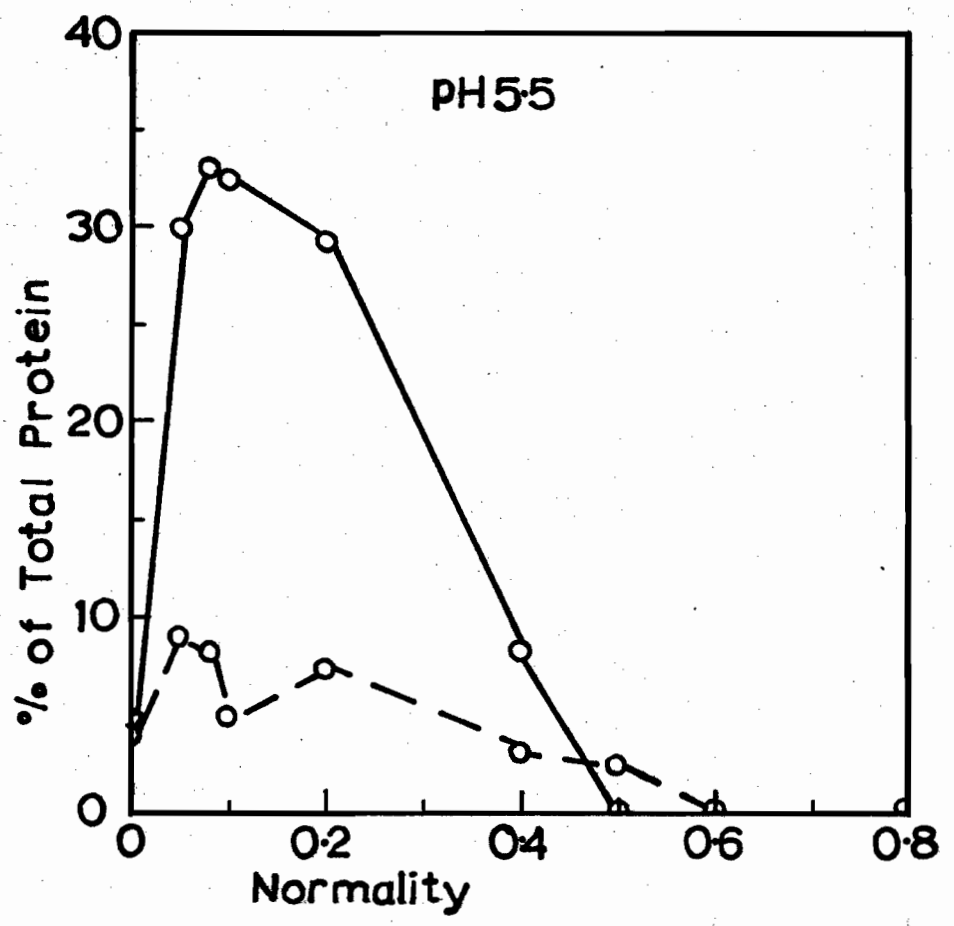
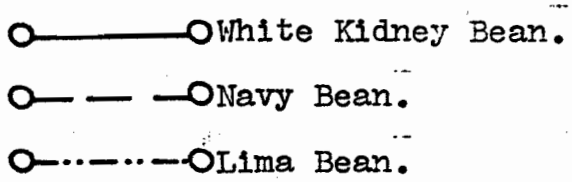


FIGURE Va, Vb, Vc.

Effect of Normality of DL-Malic Acid Solution on Yield of Protein from White Kidney Bean, Navy Bean and Lima Bean.



$$\% \text{ of Total Protein} = \frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$$

Fig Va

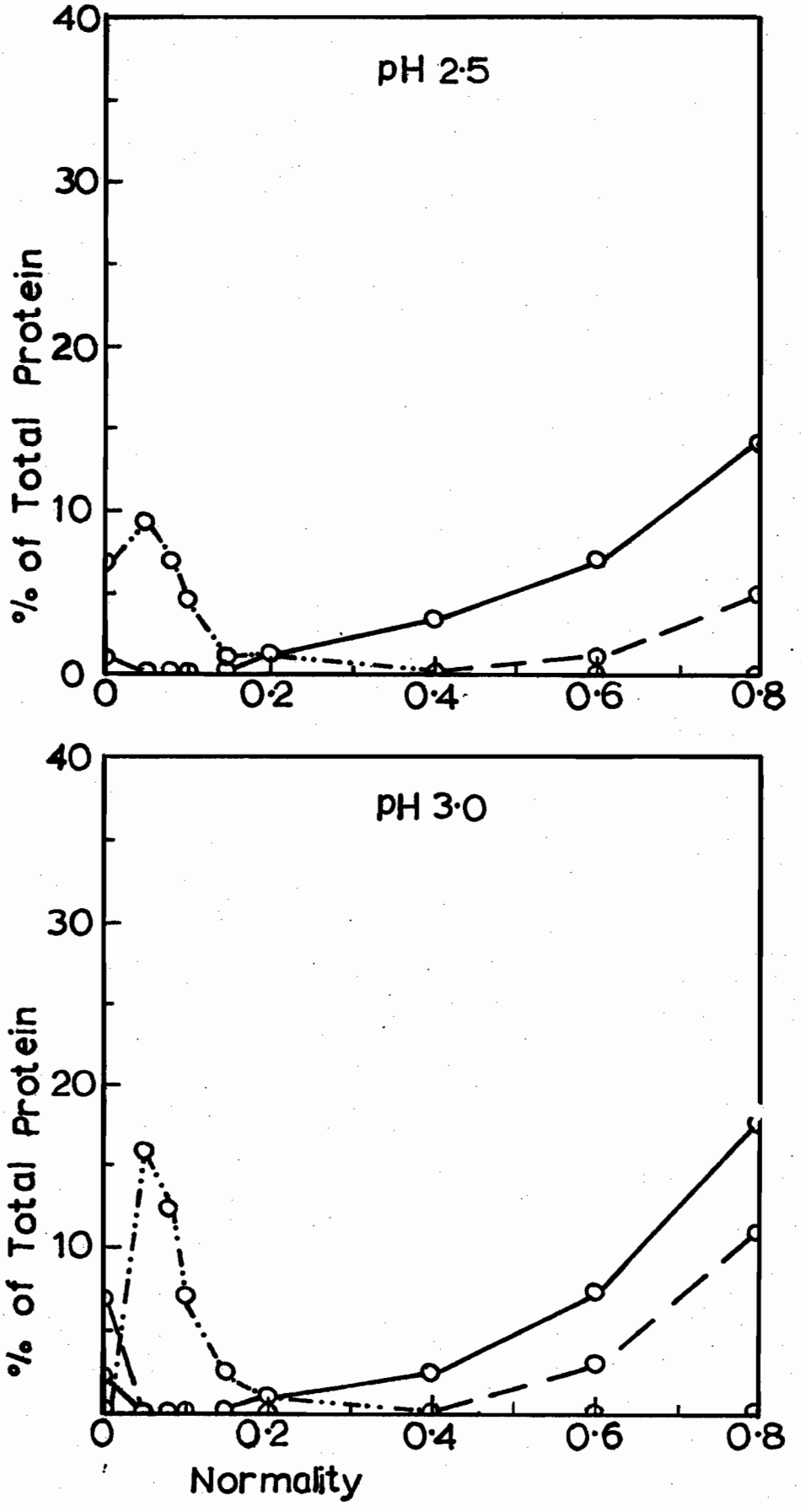


Fig Vb

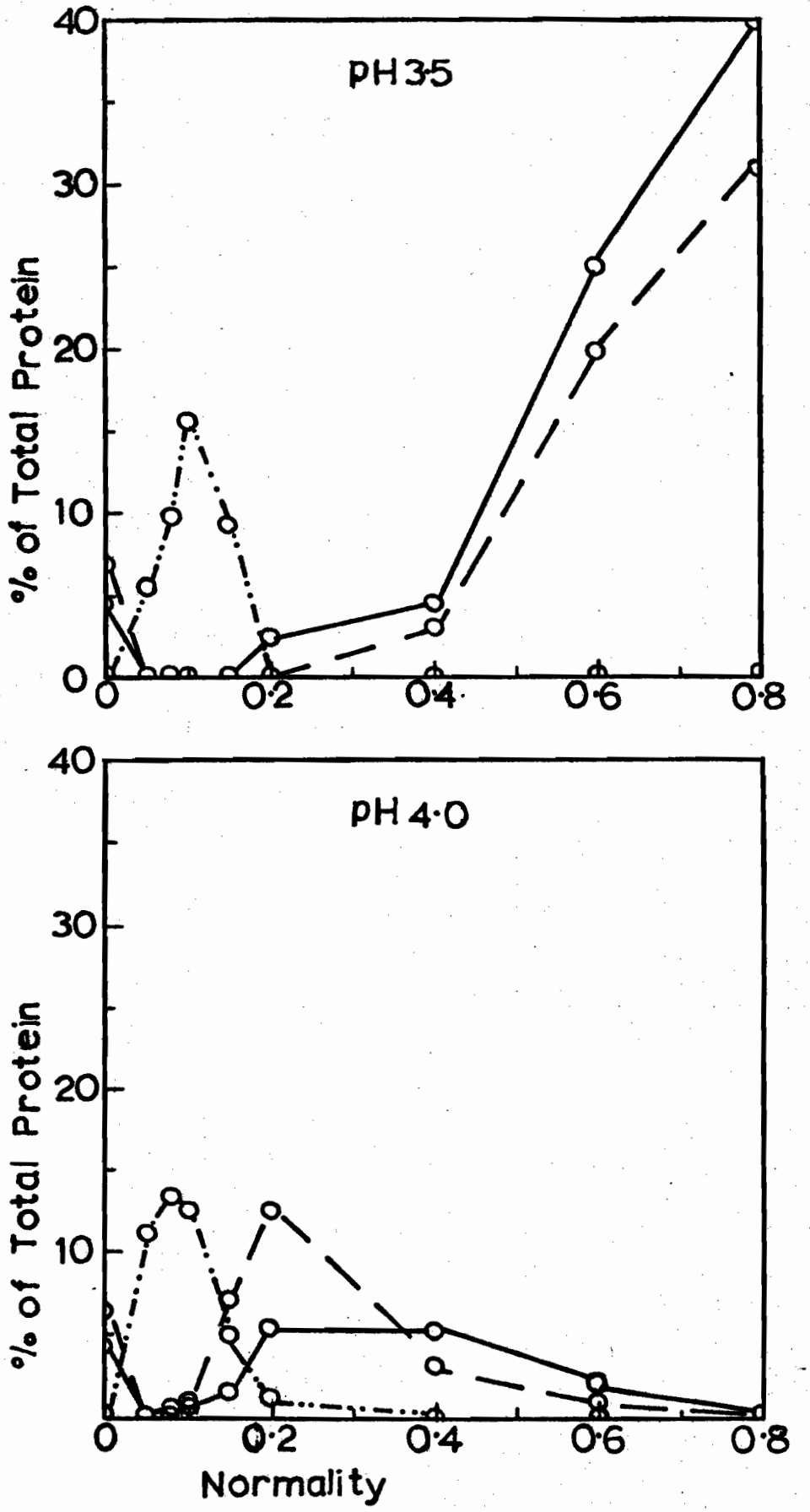
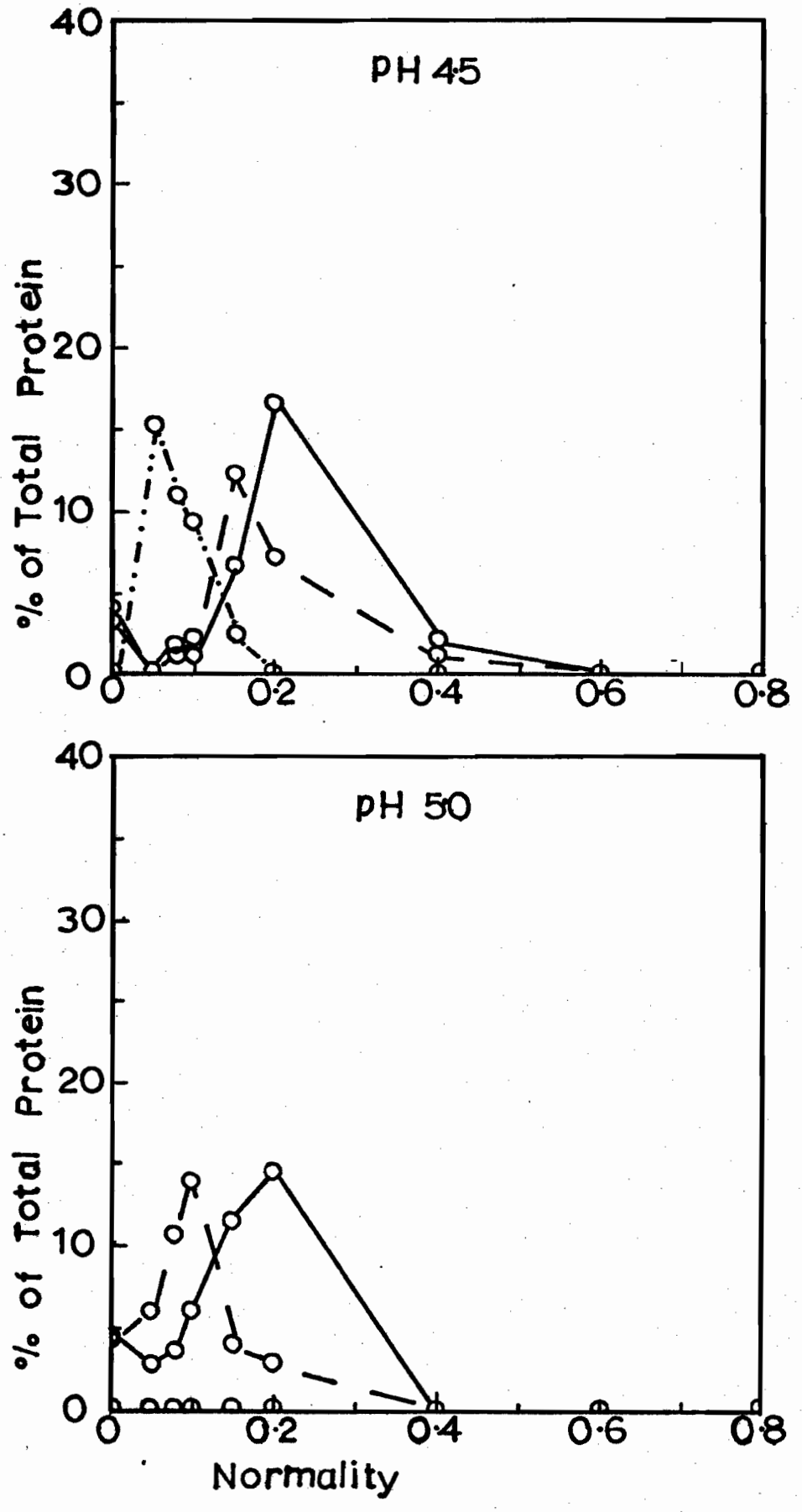

pH 3·5
pH 4·0
% of Total Protein
% of Total Protein
0
10
20
30
40
0
0·2
0·4
0·6
0·8
Normality

Fig Vc



It will be noted that citric acid gave highest yield of protein from lima bean when the normality of the extractant was 0.10N and the pH was 3.5. No protein was isolated with extractants having normalities above 0.4 and pH values greater than 4.0. Malic acid gave highest yield of protein at 0.05N when the pH of the extractant was 3.0. As was the case with citric acid, no protein was isolated with malic acid solutions having normalities of 0.4 or greater and pH values greater than 4.0. Figures II to V show that 0.05N and 0.08N solutions of citric acid and DL-malic acid gave two maxima on the curves which show the relationship between pH and yield of protein from the lima bean. It will be noted that 0.1N solutions of the acids gave increased yields of protein when the reaction was increased up to pH 3.5; this pH was also the point of maximum yield obtained with 0.15N malic acid solution and 0.20N citric acid solution.

It can be concluded that there is no general relationship between pH of extractant and protein yield from lima bean using citric acid and DL-malic acid.

Figures II to V also show that extractants (citric acid and malic acid) having relatively low pH values (less than pH 4.0) give very little protein from white kidney bean when the normality of the extractants is relatively low (less than 0.2N). When the normality of the extractant is high (greater than 0.2N), very little protein is isolated as the pH of the extractant is increased (above pH 4.0). The curves

showing the relationship between pH of extractant (citric acid and malic acid) and yield of protein from white kidney bean indicate that the yield of protein increased to a maximum as the pH of the reaction was increased. The pH at which maximal yield was obtained decreased as the normality of the extractant was lowered.

The curves giving the relationship between pH and yield of protein from white kidney bean and navy bean (Figures II to V) are similiar in shape. A striking contrast between these two beans is the amount of protein isolated when the extraction was done with citric acid (0.05N to 0.2N) at pH 5.5.

An interesting feature of the results is the yield of protein obtained from the white kidney bean and the navy bean when extraction was done with 0.80N DL-malic acid solution (pH 3.5). A relatively high yield of about 40 per cent and 30 per cent of the total protein was obtained from the white kidney bean and the navy bean respectively, by extraction of the beans with the solvent and precipitation of the proteins by diluting with distilled water. Melynchyn (1969) isolated proteins from beans in a similiar manner.

The writer's results do not show pH's of minimal yields of protein isolated from the white kidney bean and the navy bean to indicate isoelectric points of these proteins. On the other hand, points of minimal yields were obtained from the lima bean when extraction of the protein was done using 0.05N and 0.08N malic acid solutions (pH 3.5). Olcott and Fontaine

(1939) found that there was no minimal nitrogen solubility to indicate an isoelectric point of the proteins, in their extraction studies on cotton-seed meal with sodium chloride solution (0.5N). They showed, however, that extraction of cotton-seed proteins with water gave minimal nitrogen solubility when the reaction was done at about pH 3.0. Smith and Circle (1938) found that the dispersion of soy protein by salt solution was dependent on the pH as well as the concentration of the extractant. The writer's results are in accordance with this finding.

The writer's results demonstrate that proteins were isolated from the white kidney bean and the navy bean (two species of Phaseolus vulgaris) when the pH and normality of the extractants (citric acid and DL-malic acid solutions) were similiar. Under the same conditions very little protein was isolated from the lima bean (Phaseolus lunatus). This suggests a relationship between the phylogeny of the beans and the conditions for the isolation of their proteins.

Figures IV and V show in graphic form, the relationship between the normality of the extractants (citric acid and DL-malic acid) and the yield of protein. For the lima bean higher yields of protein were obtained at relatively low normalities of extractants. The normality at which maximal yield was obtained was different when the pH of the extractant was varied. Hence, a specificity of normality and pH of extractant was established for maximal yield of protein.

The curves (Figures IV and V) show that the relationship between the normality of the extractants (citric acid and DL-malic acid) and the yield of protein from the white kidney bean and the navy bean are similiar. In all instances, the amount of protein that was isolated from both beans increased to a maximum as the normality of the extractant was increased. The normality at which maximal yield was obtained was different when the pH of the reaction was varied.

The writer's results demonstrate that higher normalities of extractants (citric acid and DL-malic acid) are required for the isolation of proteins from the two Phaseolus vulgaris beans (white kidney bean and navy bean) than is required for isolation of proteins from Phaseolus lunatus (lima bean).

The writer's results agree with those of Pusztai (1965) who found that the effect of concentration on the nitrogen dispersibility of Phaseolus vulgaris beans depended on the pH of the extractant. Melynchyn (1969) claimed that a particular concentration of extractant gave maximal yield of crystalline proteins from beans; this concentration depended on the extraction medium and the type of bean. The writer's results are in accordance with this finding.

The results (Table VIII to XIII) demonstrate that crystalline proteins are isolated from the white kidney bean and the navy bean (two varieties of Phaseolus vulgaris) under somewhat similiar conditions of pH and normality of extractants. The lima bean (Phaseolus lunatus) yieldscrystalline protein

under totally different conditions of pH and normality of extractant, when compared with the two Phaseolus vulgaris beans. This suggest a relationship between phylogeny of the beans and conditions for the isolation of crystalline proteins.

Microscopic examinations of the crystalline protein isolates of the three beans indicated that there was an increase in the size of the protein crystals when the normality of the extractant was increased. In some instances (proteins isolated from white kidney bean using 0.15N and 0.40N malic acid solution, pH 4.5 and pH 4.0 respectively) the protein precipitate consisted of a mixture of crystalline and spheroidal particles. Osborne and Clapp (1907) isolated proteins from kidney bean and found that the isolate consisted of a mixture of crystalline and spheroidal material.

The writer also investigated the use of DL-tartaric acid as an extractant for the isolation of crystalline proteins from the white kidney bean, the navy bean and the lima bean. The results obtained indicated that at certain normalities and pH values, DL-tartaric acid solutions re-crystallise under refrigeration. It is suspected that the precipitate obtained by extraction of the beans with these solutions contained re-crystallised tartaric acid.

4. Effect of Particle Size of Meal on Protein Yield

a. Materials and Method

Samples of dry white kidney beans, navy beans and lima beans were ground in a Mikro Samplemill of Pulverising Machinery Co., Summit, N.J., U.S.A. The ground beans were then sieved using mesh screens of Endecotts (Filters) Ltd. Mesh numbers were 10, 14, 18, 35 and 60 which correspond to particle sizes 2.0, 1.41, 1.00, 0.50 and 0.25 mm. diameter respectively. The fractions which were separated from 400 g of ground meal are given in Table XIV.

In this experiment, different extractants were used to isolate the proteins from the three beans. The extractants which were used are given in Table XV.

A sample (10 g) of each particle size fraction of the bean meal was placed in a centrifuge bottle (250 ml) along with the extractant (100 ml). The mixture was allowed to stand, with intermittent stirring, for thirty minutes and then centrifuged (International Refrigerated Centrifuge, Model PR-1, International Equipment Co.) for ten minutes (1200g).

The supernatant was filtered through fine glass wool and then stored at 5°C. for eighteen hours. The precipitate was isolated by centrifugation. The product was washed twice with distilled water then freeze-dried.

TABLE XIV

FRACTIONS OF DIFFERENT PARTICLE SIZE OBTAINED FROM BEAN SAMPLES

Endecotts Mesh No.	Weight of Fractions (g) White Kidney Bean	Navy Bean	Lima Bean
10 (2.00 mm.)	24	0	0
14 (1.41 mm.)	74	6	7
18 (1.00 mm.)	194	271	200
35 (0.50 mm.)	90	106	140
60 (0.25 mm.)	14	14	50
Amount unrecovered	4	3	3
Total:	400 g	400 g	400 g

TABLE XV

EXTRACTANTS USED TO ISOLATE PROTEINS FROM DIFFERENT PARTICLE SIZE FRACTIONS OF BEAN

Bean	Extractant
White Kidney Bean	0.1N Citric Acid Solution (pH 5.5)
Navy Bean	0.8N Citric Acid Solution (pH 3.0)
Lima Bean	0.05N DL-Malic Acid Solution (pH 3.0)

b. Results and Discussion

The results obtained in this experiment are given in Table XVI. They are given in graphic form in Figure VI.

TABLE XVI

YIELDS OF PROTEIN FROM MEAL FRACTIONS

(PARTICLE SIZE 0.25 mm. TO 2.00 mm.)

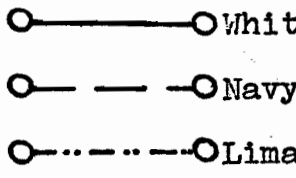
Endecotts Mesh No.	Yield of Protein (% of Total Protein in Meal)+		
	White Kidney Bean	Navy Bean	Lima Bean
60 (0.25 mm.)	17.4	12.6	8.9
35 (0.50 mm.)	32.9	14.2	12.4
18 (1.00 mm.)	33.9	16.3	13.2
14 (1.41 mm.)	23.3	5.4	4.4
10 (2.00 mm.)	9.8	-	-

$$+\% \text{ of Total Protein} = \frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$$

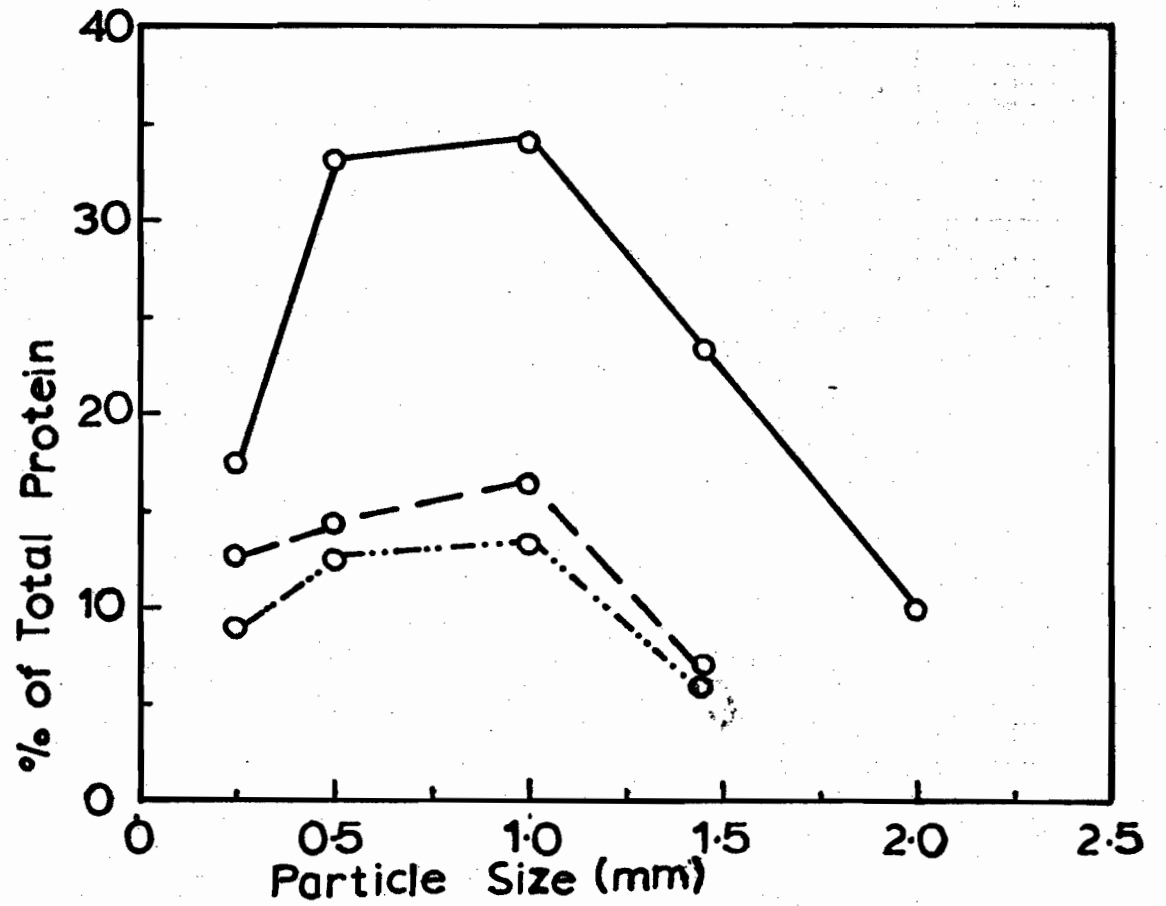
The results show that for all three beans, regardless of the extracting medium, there was a marked increase in protein yield as the particle size of the meal decreased from 1.41 mm. to 1.00 mm. Further decrease in particle size from 1.00 mm. to 0.50 mm. resulted in a slight decrease in protein yield while further decrease to 0.25 mm. particle size produced a more marked decrease. The results tend to suggest that the

FIGURE VI.

Yield of Protein from Different Particle Size Fractions of Meal of White Kidney Bean, Navy Bean and Lima Bean.



$$\% \text{ of Total Protein} = \frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$$


40
30
20
10
0
% of Total Protein
0
0·5
1·0
1·5
2·0
2·5
Particle Size (mm)

most satisfactory particle size for isolation of proteins from lima beans, navy beans and white kidney beans with solutions (acid pH) of citric acid and DL-malic acid is 1.00 mm. (No. 18 mesh).

The writer's results show general agreement with the findings of previous workers. Hofman-Bang (1930), Smith et al (1938) and Nagel et al (1938) found that a decrease in particle size of meal, up to a certain limit, produced an increase in the amount of protein extracted from barley and soybean. Further reduction in the size of the particle produced a decrease in the protein yield. Saunders (1931) emphasised that it was the meal fraction which passed through the No. 40 mesh, but not through the No. 60 mesh that produced crystalline proteins (pomelin) from orange seed while Rotha and Saunders (1932) used the meal fractions passing through the No. 20 to No. 40 mesh to produce pomelin from the same seed.

5. Effect of Extraction Time on Protein Yield

a. Materials and Method

Samples (10 g) of ground white kidney beans, navy beans and lima beans were placed in centrifuge bottles (250 ml). Citric acid solution (100 ml, 0.1N, pH 5.5) was added to the white kidney bean, DL-malic acid solution (100 ml, 0.05N, pH 3.0) was added to the lima bean and citric acid solution (100 ml, 0.80N, pH 3.0) was added to the navy bean. The mixtures were shaken using the Mechanical Shaker of Eberbach, Ann Arbor, Michigan, U.S.A. The periods of extraction were 5, 10, 15, 20, 30 and 45 minutes. The mixtures were then centrifuged (International Refrigerated Centrifuge, Model PR-1, International Equipment Co.) for ten minutes (1200g).

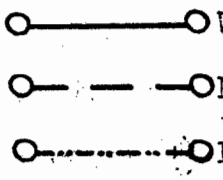
The supernatants were filtered through fine glass wool and then stored at 5°C. for eighteen hours. The precipitates were isolated by centrifugation. The products were washed twice with distilled water then freeze-dried.

b. Results and Discussion

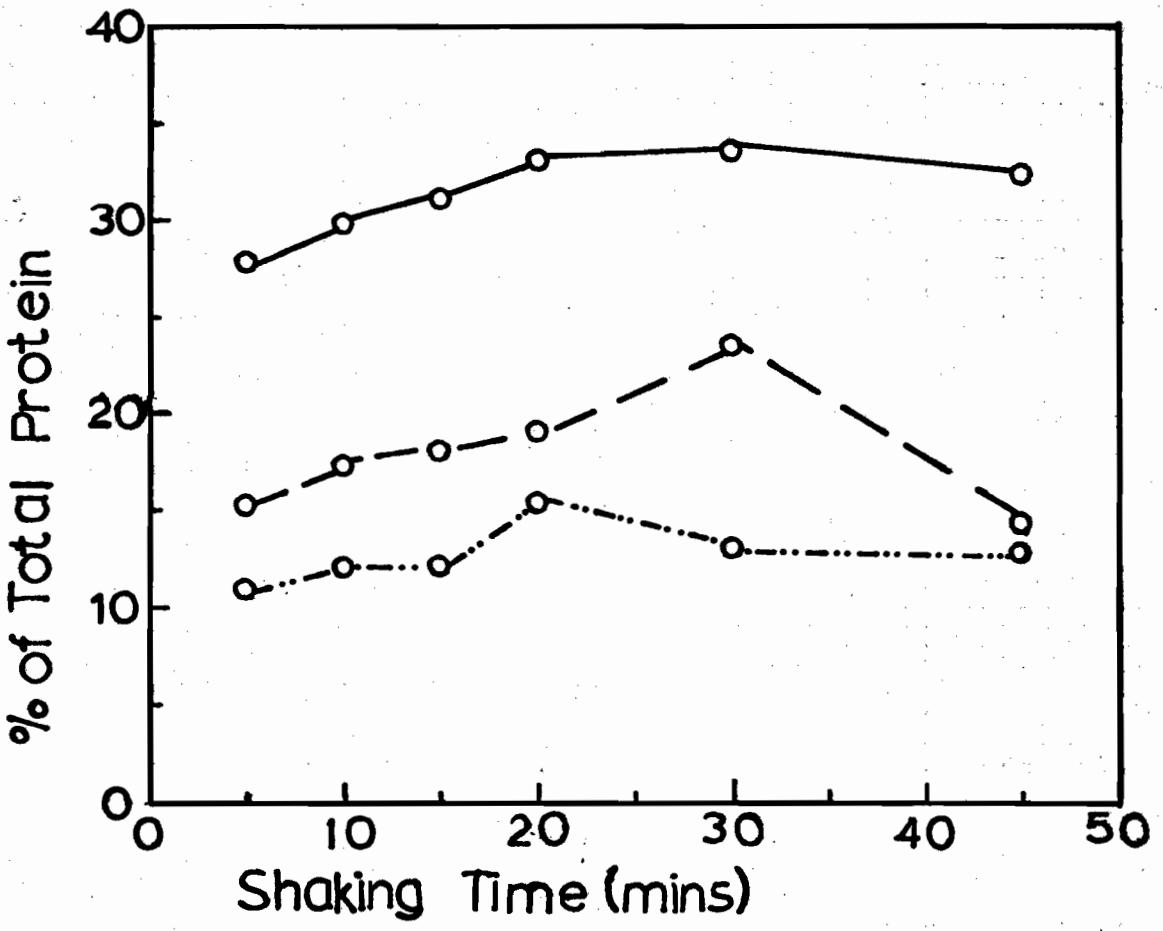
The results which were obtained in this experiment are given in Table XVII. They are given in graphic form in Figure VII.

FIGURE VII

Effect of Period of Extraction on Yield of Protein from White Kidney Bean, Navy Bean and Lima Bean.



$$\% \text{ of Total Protein} = \frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$$


40
30
20
10
0
% of Total Protein
0
10
20
30
40
50
Shaking Time (mins)

was not a critical factor in the extraction of proteins from flaxseed and soybean while Smith *et al* (1952) demonstrated that the yield of protein from soybean was affected by the variety of the soybean and the conditions of extraction.

The results of the present work are in agreement with those of Djang *et al* (1953) who showed that there was an increase in protein yield from mung beans (*Phaseolus aureus*) as the extraction time was increased and with those of Melynchyn (1969) who showed that an extraction time of thirty minutes with agitation, was ideal for the isolation of crystalline proteins from *Phaseolus* beans.

6. Effect of Temperature of Extraction on Protein Yield

a. Materials and Method

Samples (10 g) of ground white kidney beans, navy beans and lima beans were placed in centrifuge bottles (250 ml). Citric acid solution (100 ml, 0.1N, pH 5.5) was added to the white kidney bean, DL-malic acid solution (100 ml, 0.05N, pH 3.0) was added to the lima bean and citric acid solution (100 ml, 0.80N, pH 3.0) was added to the navy bean. The mixtures were allowed to stand with intermittent stirring, for thirty minutes. The extractions were carried out at the following temperatures:- Room temperature (27°C.), 35°C, 38°C, 40°C, 45°C. and 50°C. The mixtures were centrifuged (International Refrigerated Centrifuge, Model PR-1, International Equipment Co.) for ten minutes (1200g).

The supernatants were filtered through fine glass wool and then stored at 5°C. for eighteen hours. The precipitates were isolated by centrifugation. The products were washed twice with distilled water and then freeze-dried.

b. Results and Discussion

The results obtained in this experiment are given in Table XVIII. They are given in graphic form in Figure VIII.

TABLE XVIII

EFFECT OF EXTRACTION TEMPERATURE ON PROTEIN YIELD

Extraction Temperature (°C.)	Yield of Protein (% of Total Protein in Meal)+		
	White Kidney Bean	Navy Bean	Lima Bean
27°	33.0	19.8	12.6
35°	34.9	23.2	19.2
38°	34.7	24.3	18.9
40°	36.7	28.8	19.1
45°	39.4	26.6	21.3
50°	35.3	25.0	14.5

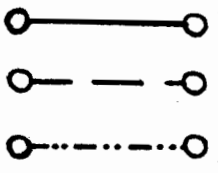
+% of Total Protein = $\frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$

The results reported in Table XVIII and Figure VIII demonstrate that the yield of protein from the white kidney bean and the lima bean increased as the temperature of the extraction was increased from 27°C. to 45°C. The yield of protein from the navy bean reached a maximum at 40°C.

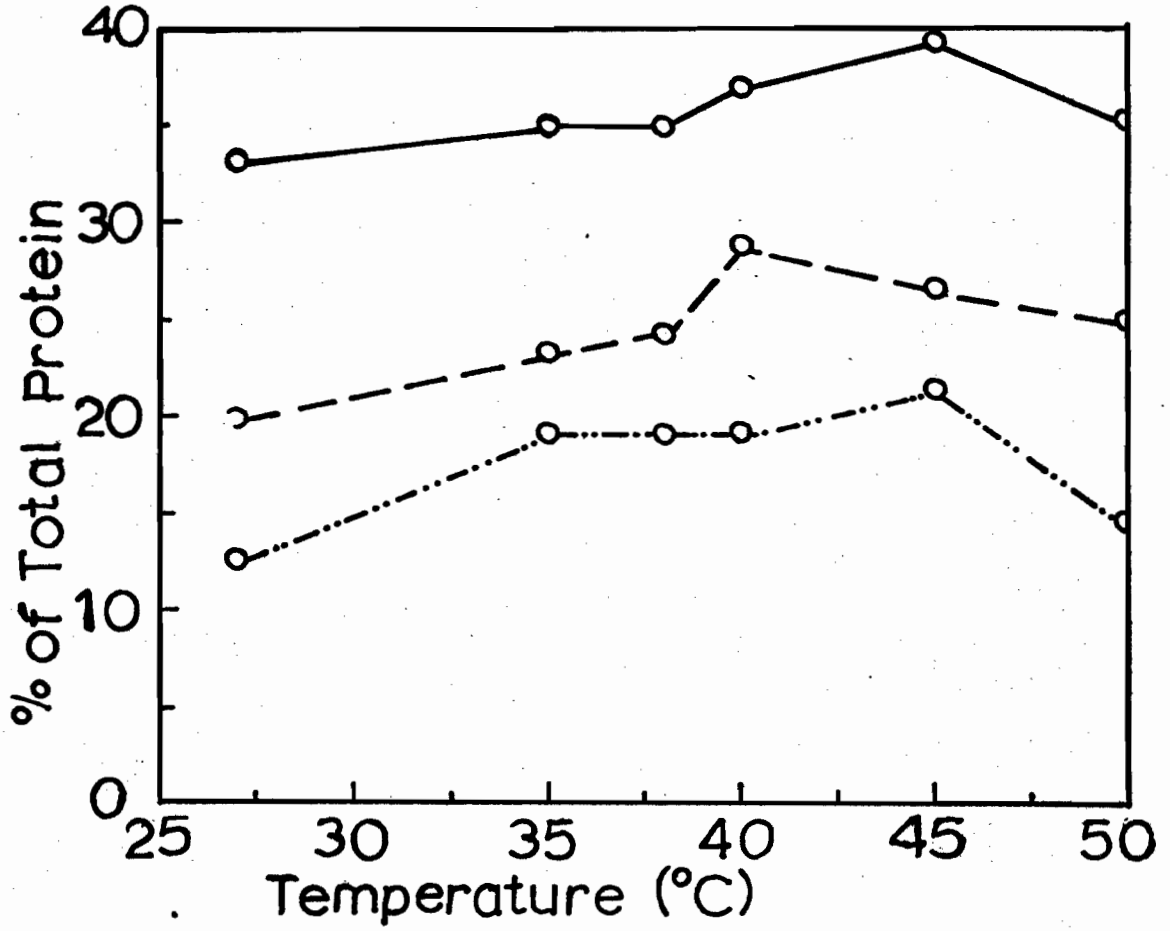
Johns and Jones (1916) found that increasing the temperature of extraction from room temperature to 45° to 50°C. did not increase the yield of protein from peanut meal. Rich (1936) also found that increasing the temperature of extraction from

FIGURE VIII

Effect of Extraction Temperature on Yield of Protein from White Kidney Bean, Navy Bean and Lima Bean.



$$\% \text{ of Total Protein} = \frac{\text{Total N in Protein}}{\text{Total N in Meal}} \times 100$$


40
30
20
10
0
% of Total Protein
25
30
35
40
45
50
Temperature (°C)

5°C. to 60°C. did not increase appreciably the yield of protein from wheat flour. Olcott and Fontaine (1939) on the other hand, found that increasing the temperature of extraction from 0°C. to 25°C. increased appreciably, the amount of nitrogen extracted from cotton-seed and O'Hara and Saunders (1937), Nagel et al (1938) and Djang et al (1953) showed that the yield of protein from orange seed, soybeans and mung beans (Phaseolus aureus) was increased by increasing the temperature of extraction.

PART II

CHARACTERISATION OF ISOLATED PROTEINS

I. ELECTROPHORESIS OF PROTEIN ISOLATES

1. Review of Literature

Most of the early work on the characterisation of proteins from seeds was based on the use of ammonium sulphate as precipitant. More recently, electrophoresis has been used extensively for protein characterisation.

Ritthausen (1872) isolated proteins from beans and called the protein preparation legumin. Osborne (1894) isolated two protein fractions from kidney beans (Phaseolus vulgaris) using fractional precipitation with ammonium sulphate solutions. One fraction was a globulin which he called phaseolin and the other fraction was an albumin which he called phaselin. Osborne and Campbell (1898) stated that albumins were present in all leguminous seeds except the navy bean (Phaseolus vulgaris) and the lupin. Osborne and Clapp (1907) prepared phaseolin from kidney bean (Phaseolus vulgaris) using ammonium sulphate precipitation.

Jones and Johns (1916) fractionated the globulins of the jack bean and called the fractions canavalin and concanavalin. Johns and Waterman (1920,a) isolated two globulin fractions from the Georgia velvet bean and called the fractions α- and β- globulins. The authors (1920,b) fractionated the globulin of the mung bean (Phaseolus aureus) and obtained the α- and β-

globulins. Jones et al (1922,a) and Jones et al (1922,b) isolated the α- and β - globulins from the adzuki bean (Phaseolus angularis) and from the lima bean (Phaseolus lunatus). The authors showed that the corresponding globulins from the different Phaseolus species were similiar. Waterman et al (1923) isolated a globulin fraction from navy beans (Phaseolus vulgaris) which they called conphaseolin. They found that their conphaseolin preparation was similiar to the α- globulin of other species of Phaseolus. Their β - globulin was similiar to phaseolin isolated by Osborne (1894).

Djang et al (1952) demonstrated by electrophoresis, that the globulin fraction obtained from navy beans (Phaseolus aureus) comprised four different fractions. They called two of these fractions α- globulins and the other two, β - globulins.

Ghetie (1959) fractionated the globulins of Phaseolus vulgaris into three components which he called fractions A, B and C. Each fraction showed different amino acid composition and different polarographic behaviour. The author concluded that the three fractions exist as well defined entities within the seed.

Klotaz (1962) showed that only some of the corresponding proteins of seeds and seedlings of Phaseolus vulgaris, Phaseolus coccineus, Phaseolus lunatus and Phaseolus aureus were similiar. Cotyledons of Phaseolus vulgaris and Phaseolus coccineus contained a similiar type of protein (phaseolin).

Evans and Kerr (1963) found that protein isolated from

navy beans (Phaseolus vulgaris) by (1) extraction at pH 7.0 and precipitation at pH 3.8 and (2) extraction with sodium chloride solution (2 per cent) and precipitation by dialysis were essentially the same as indicated by paper electrophoresis and DEAE-cellulose column chromatography.

Zarkadas (1964) from his electrophoretic analysis of the proteins of mung beans (Phaseolus aureus) showed that one globulin fraction separated to give six components on paper electrophoresis. He concluded that the mung bean globulin fraction comprises nine different fractions.

Fox et al (1964) demonstrated by means of polyacrylamide-gel electrophoresis that the albumin from leguminous seeds of species from closely related genera were similiar. They commented on the importance of correlating protein electrophoretic patterns with taxonomic position.

Sayanova (1968) investigated, serologically, bean proteins and showed that there was a taxonomic relationship between the proteins of Phaseolus vulgaris and Phaseolus multiflorus and a lack of relationship between the proteins of Phaseolus lunatus and Phaseolus aureus.

Pant and Tulsiani (1969) claimed that the albumin and globulin fractions of four species of Phaseolus gave only one band on paper electrophoresis. The authors used acetate buffer at pH 5.0, citrate buffer at pH 6.0 and phosphate buffer at pH 8.0.

Adriaanse and Robbers (1970) carried out electrophoretic and fractionation studies on the proteins of different cultivars

of Phaseolus vulgaris. They found that the electrophoretic patterns of the corresponding globulin fractions of the different cultivars were different.

Hall (1970) found by means of disc electrophoresis that the proteins of seven different cultivars of Phaseolus vulgaris were similiar.

McLeester et al (1973) stated that the globulin fraction of Phaseolus vulgaris is usually considered to include two major components (legumin and vicilin). The authors showed by disc electrophoresis that the total globulin fraction was not equivalent to the combination of the legumin and vicilin fractions.

Ishino and Ortega (1975) demonstrated that the globulin fraction of Phaseolus vulgaris consisted of four major components namely: α-, β-, γ- and δ- globulins, designated in order of decreasing electrophoretic mobility. The fractions accounted for 50, 19, 10 and 12 per cent of the total globulin fraction respectively. These authors showed that the α-component was deficient in the sulphur containing amino acids and was not dissociated with 8M urea or with 0.2M 2- mercaptoethanol.

2. Experimental, Results and Discussion

a. Materials and Methods

The method used for polyacrylamide-gel disc electrophoresis was essentially that of Ornstein and Davis as modified by Mauer (1971). The solutions used for the preparation of the polyacrylamide gels (without urea) are given in Table XIX; the mixing ratios of these solutions are given in Table XX. The other solutions prepared for use in the electrophoretic analysis of the isolated proteins are given in Table XXI.

Eight glass tubes (length = 70 mm; external diameter = 7 mm; internal diameter = 5 mm) were cleaned (chromic acid cleaning solution) and dried. One end of each tube was sealed securely with parafilm. The tubes were then placed vertically on a rack with the sealed end at the bottom. Separation-gel solution(1.2 ml) was placed in each tube with the aid of a syringe. Water was placed on the top of the separation gel to give a layer of approximately 2 mm, care being taken to avoid mixing of the gel solution and the water. The gels were left to polymerize for one hour.

The water layer was removed by means of small filter paper wicks. Spacer-gel solution (0.2 ml) was placed on top of the separation gel with the aid of a syringe. The tubes

TABLE XIX

SOLUTIONS USED IN THE PREPARATION OF POLYACRYLAMIDE GELS

(WITHOUT UREA)

Reagents for Preparation of Separation Gel.		Reagents for Preparation of Spacer Gel.	
I		IV	
1N HCl	48.0 ml	1N HCl	48.0 ml
Tris[a]	36.6 g	Tris[a]	5.98 g
TEMED[b]	0.23 ml	TEMED[b]	0.46 ml
Water to (pH 8.9)	100 ml	Water to (pH 6.7)	100 ml
II		V	
Acrylamide	30.0 g	Acrylamide	12.0 g
Bis[c]	0.8 g	Bis[c]	3.0 g
Water to	100 ml	Water to	100 ml
III[d]		VI	
Per[e]	0.14 g	Riboflavin	4.0 g
Water to	100 ml	Water to	100 ml
		VII	
		Sucrose	40 g
		Water to	100 ml

[a]Tris: tris (hydroxymethyl) aminomethane.
[b]TEMED: N,N,N',N'-tetramethylethylenediamine.
[c]Bis: N,N-methylenebisacrylamide.
[d]III: prepared immediately prior to use.
[e]Per: ammonium persulphate.

TABLE XX

MIXING RATIOS FOR PREPARATION OF GELS (WITHOUT UREA)[a]

Separation Gel	Spacer Gel
1 part[b] (I)	1 part (IV)
2 parts (II)	2 parts (V)
1 part H_2O	1 part (VI)
4 parts (III)	4 parts (VII)

[a] Number in brackets refer to solutions listed in Table XIX.

[b] Part by volume.

TABLE XXI

SOLUTIONS PREPARED FOR ELECTROPHORESIS OF PROTEINS

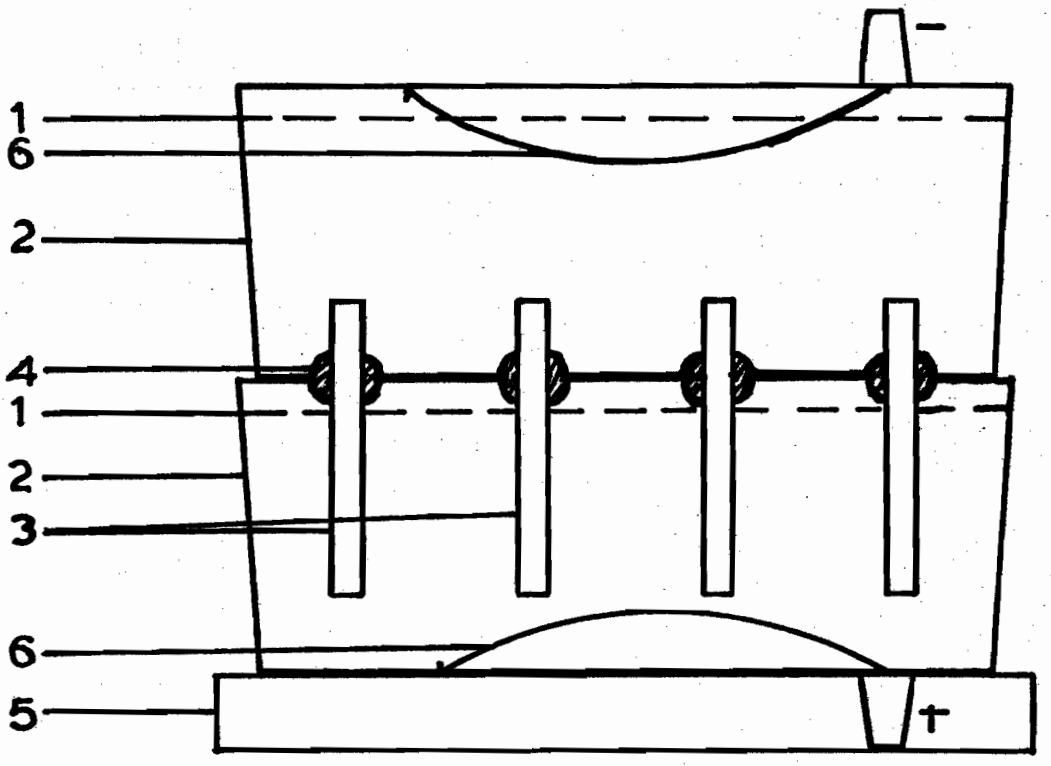
Solution	Composition
Electrode buffer solution (pH 8.3)	Tris[a] (6.0 g), Glycine (28.8 g), Distilled water to 1,000 ml, Dilute ten times (with distilled water) before use.
Indicator solution	Bromophenol Blue (1 mg), Distilled water to 1,000 ml.
Fixative solution	Trichloroacetic acid (12.5 g), Distilled water to 100 ml.
Staining solution	(a) Commassie Brilliant Blue (1 g), Distilled water to 100 ml (b) Trichloroacetic acid (12.5 g), Distilled water to 100 ml. Mixture of 1 volume of solution (a) and 19 volumes of solution (b).
Destaining solution	Trichloroacetic acid (10 g), Distilled water to 100 ml.
Storage solution	Glacial acetic acid (7 ml), Distilled water to 100 ml.

[a]Tris: tris (hydroxymethyl) aminomethane.

were then placed under a fluorescent lamp for twenty minutes to allow photo-polymerization of the spacer gel. A sample-gel solution was prepared in exactly the same manner as the spacer-gel solution except that 10 mg of protein sample was added per 5 ml of gel solution. A sample (50 µl) of this solution was placed on top of the spacer gel. The tubes were placed under the fluorescent lamp for twenty minutes for photo-polymerisation of the sample gel to take place.

The tubes were removed from the rack and the parafilm at the end of the tubes was carefully removed. They were inserted into the rubber grommets of the upper buffer reservoir of the electrophoretic apparatus which was assembled as shown in Figure IX. Buffer solution was added to the upper and lower reservoirs so as to completely cover the electrodes. One millilitre of 0.001 per cent bromophenol blue solution was added to the buffer solution contained in the upper reservoir. The electrodes were connected to the power supply (anode, lower reservoir; cathode, upper reservoir) and the current (D.C.) was turned on. The current was adjusted to one milliamp per tube for the first two minutes and then increased to four milliamp per tube for the remainder of the electrophoretic run. Electrophoresis was stopped when the bromophenol blue indicator reached the bottom of the tube; this took approximately one hour.

The gels were removed from each tube with the aid of a flexible needle which was passed between the gel and the



1-Buffer level
2-Buffer reservoir
3-Gel tubes
4-Rubber grommet
5-Stand
6-Electrode

inner wall of the tube.

The gels were placed in beakers (600 ml) containing trichloroacetic acid fixative solution (50 ml, 12.5 per cent) for half an hour. The fixative solution was then replaced by Commassie Blue staining solution (50 ml). The gles were allowed to stand in the staining solution for about eighteen hours. The staining solution was then replaced by trichloroacetic acid (50 ml, 10 per cent) which acted as destaining solution. The gels were kept in the destaining solution for several days with frequent changes of destaining solution. Finally, the gels were transferred to petri dishes containing acetic acid (50 ml, 7 per cent) as storage solution. The gels were photographed as soon as possible.

The solutions used for the preparation of polyacrylamide gels (with urea) are given in Table XXII; the mixing ratios of these solutions are given in Table XXIII. The other solutions prepared for use in the electrophoresis with urea are similiar to those prepared for electrophoresis without urea (Table XXI) except for the following modifications:-

(1) The working electrode buffer solution contained 24 g urea per litre.

(2) The fixative solution was modified to contain trichloroacetic acid solution (5 per cent) and 5-sulfosalicylic acid solution (5 per cent).

TABLE XXII

SOLUTIONS USED IN POLYACRYLAMIDE GEL DISC ELECTROPHORESIS

(WITH UREA)

Reagents for Preparation of Separation Gel.			Reagents for Preparation of Spacer Gel.		
	I			IV	
1N HCl		48.0 ml	1N HCl		48.0 ml
Tris[a]		36.6 g	Tris[a]		5.98 g
TEMED[b]		0.23 ml	TEMED[b]		0.46 ml
Urea		24 g	Urea		24 g
Water	to (pH 8.9)	100 ml	Water	to (pH 6.7)	100 ml
	II			VI	
Acrylamide		30.0 g	Acrylamide		12.0 g
Bis[c]		0.8 g	Bis[c]		3.0 g
Urea		24 g	Urea		24 g
Water	to	100 ml	Water	to	100 ml
	III			VII	
Per[e]		0.14 g	Riboflavin		4.0 g
Urea		24 g	Urea		24 g
Water	to	100 ml	Water		100 ml
	IV			VIII	
Urea		24 g	Urea		24 g
Water	to	100 ml	Sucrose		40 g
			Water	to	100 ml

[a]Tris: tris (hydroxymethyl) aminomethane.
[b]TEMED: N,N,N',N'-tetramethylethylenediamine.
[c]Bis: N,N-methylenebisacrylamide.
[d]III: prepared immediately prior to use.
[e]Per: ammonium persulphate.

TABLE XXIII

MIXING RATIOS FOR PREPARATION OF GELS (WITH UREA)[a]

Separation Gel	Spacer Gel
1 part[b] (I)	1 part (V)
2 parts (II)	2 parts (VI)
4 parts (III)	1 part (VII)
1 part (IV)	4 parts (VIII)

[a]Numbers in brackets refer to solutions listed in Table XXII.

[b]Part by volume.

The following protein preparations were subjected to electrophoresis.

(a) White Kidney Bean Isolates

Isolate A - crystalline preparation extracted with citric acid solution (0.1N, pH 5.5).

Isolate B - spheroidal preparation extracted with citric acid solution(0.5N, pH 3.5).

Isolate C - precipitate extracted with DL-malic acid solution (0.8N, pH 3.5) and precipitated by dilution with water.

Isolate D - isolate extracted with dilute alkali and precipitated with acid.

Isolate E - crystalline preparation extracted from isolate D with citric acid solution (0.1N, pH 5.5).

(b) Navy Bean Isolates

Isolate F - crystalline preparation extracted with citric acid solution (0.2N, pH 4.5).

Isolate G - spheroidal preparation extracted with citric acid solution (0.4N, pH 3.5).

Isolate H - isolate extracted with dilute alkali and precipitated with acid.

Isolate I - needle-like precipitate extracted with citric acid solution (0.8N, pH 3.0).

(c) Lima Bean Isolates

Isolate J - crystalline preparation extracted with citric acid solution (0.08N, pH 4.0).

Isolate K - spheroidal preparation extracted with citric acid solution (0.08N, pH 4.5).

Isolate L - isolate extracted with dilute alkali and precipitated with acid.

Isolates D, H and L were prepared for purposes of comparison with the other isolates prepared. They were isolated by a standard method for the isolation of proteins from beans. Samples (10 g) of ground white kidney beans, navy beans and lima beans were weighed out into centrifuge bottles (250 ml) and sodium hydroxide solution (100 ml, 0.02 per cent) was added. The mixtures were allowed to stand, with intermittent stirring for one hour. They were then centrifuged for ten minutes (1200g) and the supernatants were filtered through fine glass wool. The pH of the supernatants was adjusted to pH 4.5 with hydrochloric acid (conc.) to precipitate the proteins. The precipitates were isolated by centrifugation, washed twice with distilled water and then freeze-dried.

The precipitate obtained from the white kidney bean when treated with citric acid solution (0.1N, pH 5.5) and stored at 5°C. for at least eighteen hours gave a crystalline product. This product was named Isolate E.

b. Results and Discussion

Photographs of the electropherograms which were prepared with and without urea are given in Figures X to XV.

(i) White Kidney Bean Protein Isolates

a. Without Urea (Fig. X)

Isolates A and B each gave electropherograms which had at least six bands, designated the α_1-, α_2-, α-, β-, γ- and δ- bands in order of decreasing electrophoretic mobility. The α_1-, and α_2- bands are diffused. The α_1- band corresponds to the α- zone of Zarkadas (1964) who showed that the globulin fractions from mung bean (Phaseolus aureus) gave nine bands on paper electrophoresis. The α- band is the broadest and most intense and corresponds to the α- globulin of Ishino and Ortega (1975) who found four major bands in the globulin fraction of Phaseolus vulgaris. The β-, γ- and δ- bands correspond to the β-, γ- and δ- bands of Ishino and Ortega (1975).

The electropherograms of isolates C and E are similiar. Both show three bands corresponding to the α-, β- and γ- bands of isolates A and B.

FIGURE X

Electropherograms of Protein Isolates from White Kidney Bean - Electrophoresis without Urea.

A - Crystalline isolate extracted with citric acid solution (0.1N, pH 5.5).

B - Spheroidal isolate extracted with citric acid solution (0.5N, pH 3.5).

C - Malic acid extract precipitated by dilution with H_2O.

D - Alkali extract, precipitated with acid.

E - Crystalline isolate extracted from D with citric acid solution (0.1N, pH 5.5).

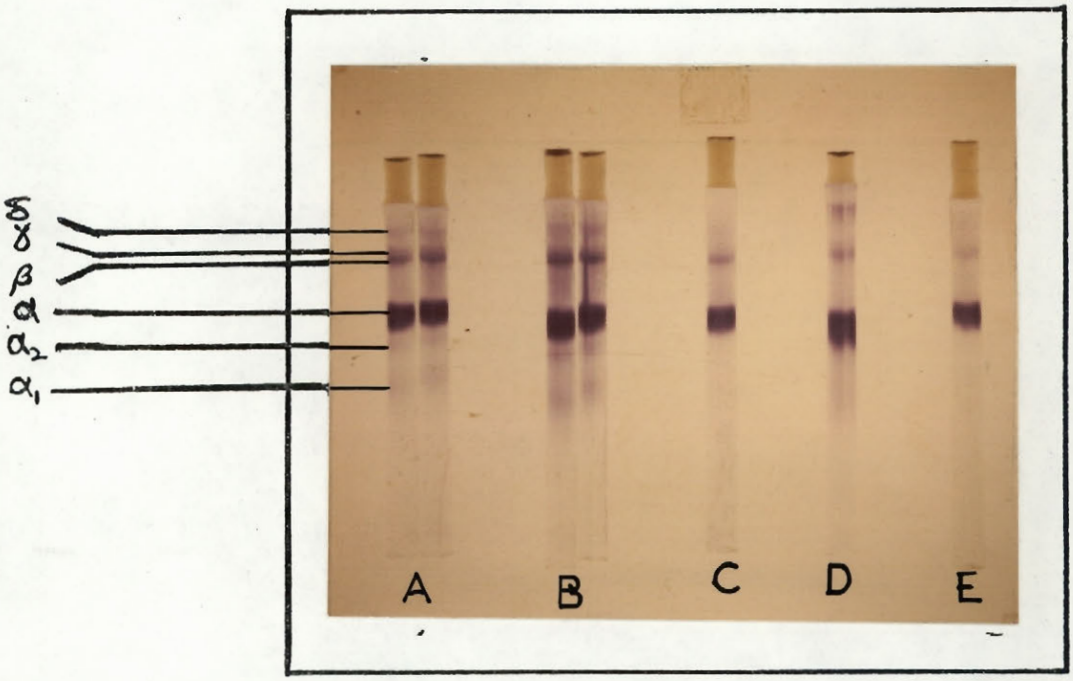

γ
β
α
α2
α1
A
B
C
D
E

FIGURE XI

Electropherograms of Protein Isolates from White Kidney Bean - Electrophoresis with Urea.

A - Crystalline isolate extracted with citric acid solution (0.1N, pH 5.5).

B - Spheroidal isolate extracted with citric acid solution (0.5N, pH 3.5).

C - Malic acid extract precipitated by dilution with H_2O.

D - Alkali extract, precipitated with acid.

E - Crystalline isolate extracted from D with citric acid solution (0.1N, pH 5.5).


A
B
C
D
E

FIGURE XII.

Electropherograms of Protein Isolates from Navy Bean - Electrophoresis without Urea.

F - Crystalline isolate extracted with citric acid solution (0.2N, pH 4.5).

G - Spheroidal isolate extracted with citric acid solution (0.4N, pH 3.5).

H - Alkali extract, precipitated with acid.

I - Needle-like precipitate extracted by citric acid solution (0.8N, pH 3.0).

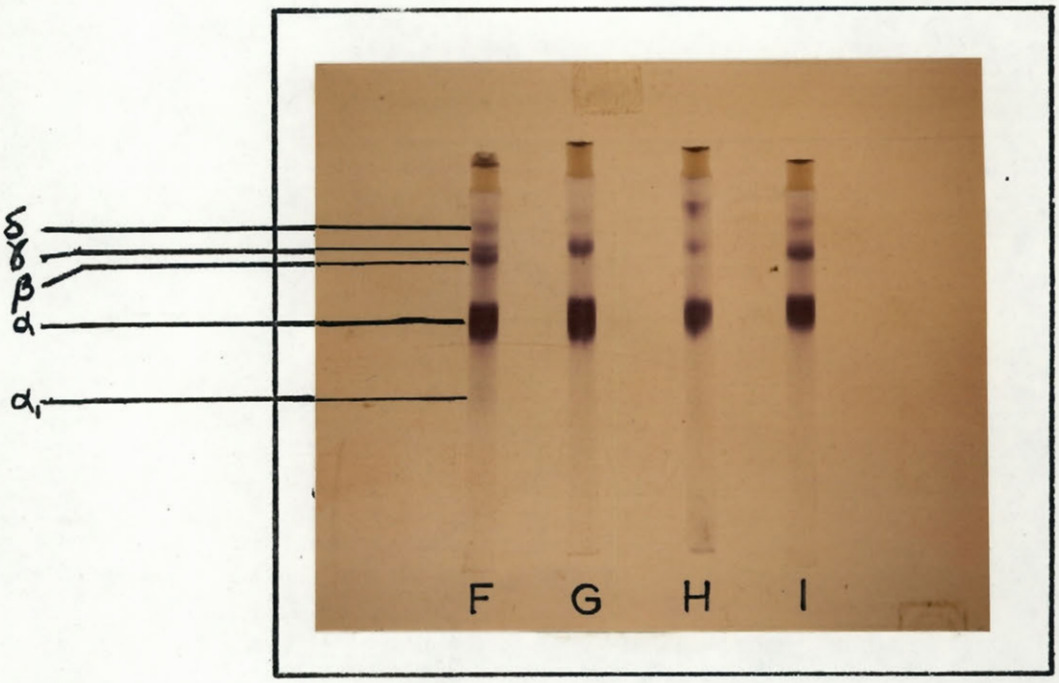

δ
γ
β
α
α1
F
G
H
I

FIGURE XIII

Electropherograms of Protein Isolates from Navy Bean - Electrophoresis with Urea.

F - Crystalline isolate extracted with citric acid solution (0.2N, pH 4.5).

G - Spheroidal isolate extracted with citric acid solution (0.4N, pH 3.5).

H - Alkali extract, precipitated with acid.

I - Needle-like precipitate extracted by citric acid solution (0.8N, pH 3.0).

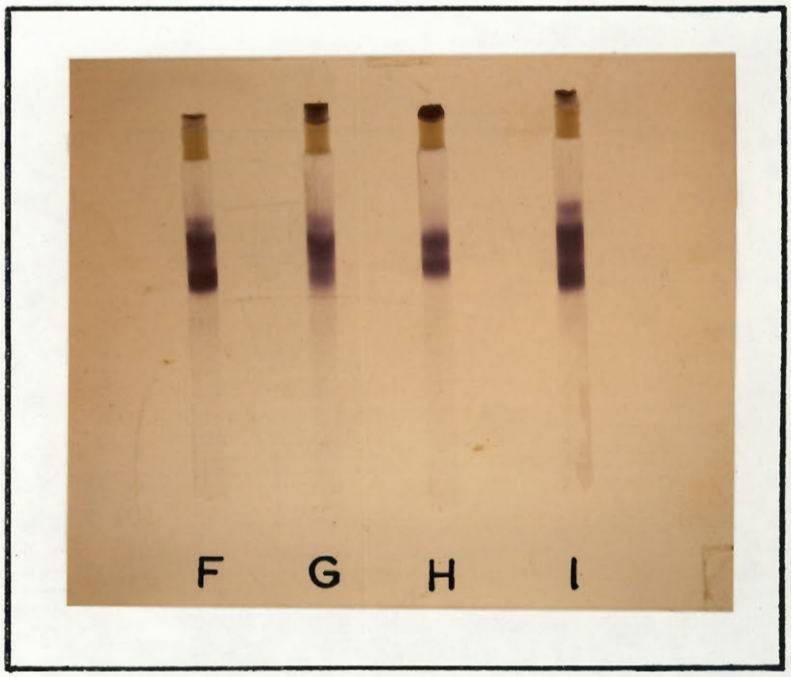

F
G
H
I

FIGURE XIV

Electropherograms of Protein Isolates from Lima Bean - Electrophoresis without Urea.

J - Crystalline isolate extracted with citric acid solution (0.08N, pH 4.0).

K - Spheroidal isolate extracted with citric acid solution (0.08N, pH 4.5).

L - Alkali extract, precipitated with acid.

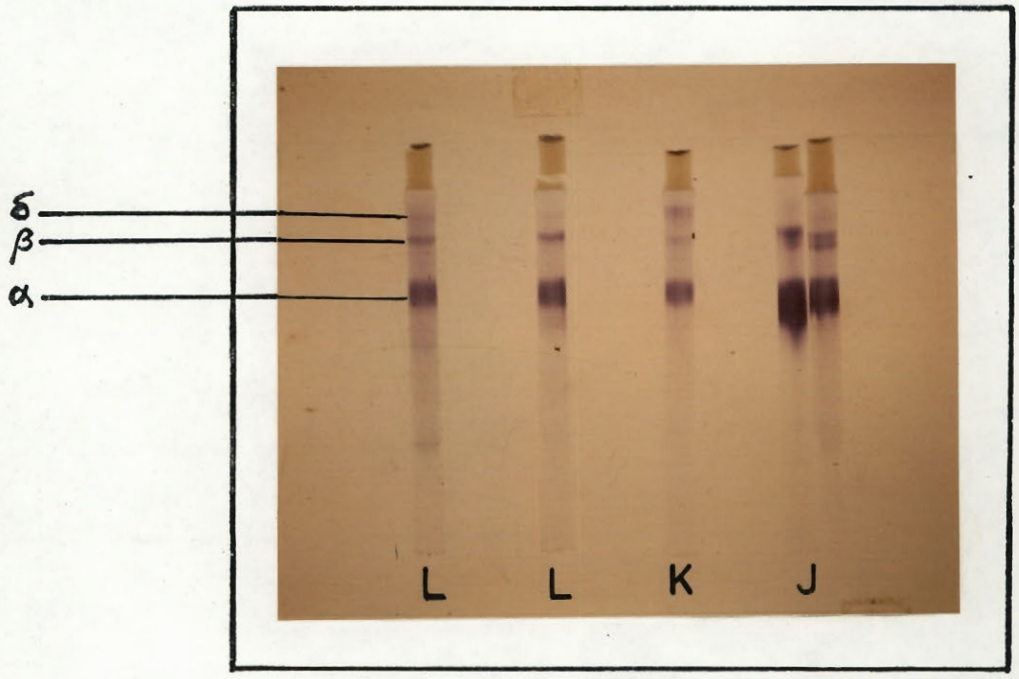

δ
β
α
L
L
K
J

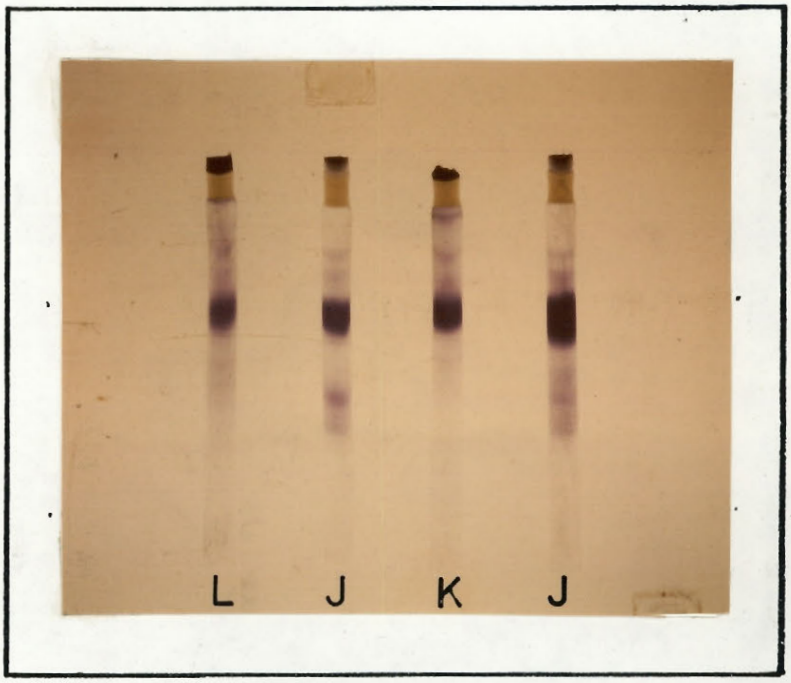
FIGURE XV

Electropherograms of Protein Isolates from Lima Bean - Electrophoresis with Urea.

J - Crystalline isolate extracted with citric acid solution (0.08N, pH 4.0).

K - Spheroidal isolate extracted with citric acid solution (0.08N, pH 4.5).

L - Alkali extract, precipitated with acid.


L
J
K
J

b. With Urea (Fig. XI)

Electrophoresis with urea gave somewhat similiar patterns for all five isolates. There is a faint band which has the greatest electrophoretic mobility and a grouping together of at least three other bands. This pattern could have resulted from the polymerization of the protein molecules. Gill and Tung (1976) stated that electrophoresis of rapeseed proteins in the presence of urea solution (10M) resulted in molecular fragments which did not enter the separation gel. It was noted in the present work that some material did not enter the spacer gel.

(ii) Navy Bean Protein Isolates

a. Without Urea (Fig. XII)

The electropherograms of isolate F is similiar to the corresponding isolate (isolate A) of the white kidney bean, except that the α_2- band is not apparent. The α_1-, α-, β-, γ- and δ- bands are all present and they have comparable electrophoretic mobilities as does isolate A of the kidney bean. Isolate G gave four bands corresponding to the α-, β-, γ- and δ- bands of isolate F. The γ- and δ- bands, however, are relatively faint. Isolate H gave only three bands corresponding to the α-, β- and δ- bands of isolate F. Isolate I gave essentially the same bands as did isolate G. The γ- and δ-

bands, however, appear more intense and there appears to be an additional band with lower electrophoretic mobility than the δ- band.

b. With Urea (Fig. XIII)

Again, like those of the isolates of the white kidney bean, the electropherograms of the navy bean isolates, in the presence of urea, show bands which are grouped together in the region of the α- band. The α- band appears to have been fragmented. The electropherograms of isolates F, G and I are similiar except that the bands of isolate G are less intense. Isolate H show one less band than do the other three isolates. It is possible that urea caused fragmentation of the α- band and polymerisation of other protein molecules to give larger units which did not enter the gel.

(iii) Lima Bean Isolates

a. Without Urea (Fig. XIV)

Isolate J shows four bands which could correspond to the α-, β -, γ - and δ- bands of isolate A of the white kidney bean. Isolate K show only three bands corresponding to the α-, β - and δ- bands. The γ - band is not apparent. Isolate L show bands which are similiar to those obtained for isolate K.

b. With Urea (Fig. XV)

In the presence of urea, the electropherograms of the lima bean isolates show fragmentation of some of the bands. Isolate J gave six bands, five of which are faint. The major band could correspond to the α- band obtained with gels containing no urea. The other five bands are probably due to fragmentation of other bands. Isolate K gave five bands, one major band corresponding to the α- band and four other minor bands. Isolate L gave four bands, a major band corresponding to the α- band and three other minor bands. In all the isolates there is indication that there was some protein material which did not enter the gel.

From the results of the electrophoretic analysis of the isolates prepared the following conclusions can be drawn:

(1) The protein isolates of the white kidney bean, navy bean and lima bean are all electrophoretically heterogenous.

(2) The corresponding protein isolates of the different beans are similiar in some respects and different in others. A feature which is common to all of the isolates is the presence of a major band (α- band). The β- and δ- bands are also present in all of the isolates. The γ- and α_1- and α_2- bands are present in some isolates but absent in others. These observa-

tions are in accordance with what has been found by previous workers. Klotaz (1962) found that only some of the proteins of seeds of different Phaseolus species were similiar. Andriaanse and Robbers (1970) found that electrophoretic patterns of the corresponding globulin fractions were not the same for different cultivars of Phaseolus vulgaris.

(3) The protein isolates of the same bean are similiar in some respects but different in others. All isolates of the same bean gave the characteristic α- band. Generally the crystalline protein isolate gave more bands than non-crystalline isolates. Hammersten (1918) reported that the legumin isolated from pea by extraction with salt solution was different from that obtained by extraction with dilute alkali solution followed by acid precipitation.

(4) All of the isolates gave at least three of the four bands designated as α-, β -, γ - and δ - globulins by Ishino and Ortega (1975). The relative intensities of the bands were also similiar to those observed by these workers.

(5) The electrophoretic behaviour of the corresponding protein isolates of the different beansis different in the presence of urea. Protein isolates of the

kidney bean gave electropherograms which indicated that there was aggregation of protein molecules. The α- band of the navy bean seemed to be fragmented by urea to give at least two bands but there was also aggregation of some of the protein molecules. The α- band of the lima bean seemed to be unaffected by urea but the other bands were fragmented.

II. AMINO ACID ANALYSIS OF PROTEIN ISOLATES

1. Review of Literature

Protein isolates from seeds have been analysed for amino acid content since the early part of the twentieth century. Osborne and Heyl (1908) determined the amino acid composition of legumelin from pea seeds and found that the amino acid composition of this protein was different from other proteins isolated from the pea.

Danielsson (1956) considered that it was very improbable that different plants could synthesize proteins with exactly the same amino acid sequence.

Tandon et al (1957) indicated that the amino acid composition of different varieties of kidney bean (Phaseolus vulgaris) was influenced by several factors. They showed that the lysine, methionine and tryptophane content of the beans depended on environmental factors while the lysine and tryptophane contents were different for different varieties of beans.

Bressani et al (1961) found that beans of Phaseolus species were deficient in the sulphur containing amino acids, cystein and methionine. They also reported that the beans contained low quantities of tyrosine.

Jaffé and Hanning (1965) determined the amino acid composition of different protein fractions isolated from the

black kidney bean (Phaseolus vulgaris). They showed that the different protein fractions had different amino acid compositions.

Pant and Tulsiani (1969) found that the amino acid composition of different varieties of Phaseolus vulgaris beans were different. They suggested that maturity and age of seeds, soil, genetic and other environmental factors influence the chemical composition of the seeds.

de Moraes and Angelucci (1971) determined the amino acid composition of twelve different varieties of Phaseolus vulgaris beans. Their results demonstrated that amino acid composition of beans of different varieties may differ markedly. Aspartic acid, glutamic acid and tryptophane were shown to be relatively high in all varieties while the methionine contents were relatively low in all varieties.

Evans et al (1973) found that the subfractions of certain protein fraction isolated from the navy bean (Phaseolus vulgaris) were very different in amino acid content. The amino acid composition of the major subfraction of the protein which was isolated with sodium chloride solution (1 per cent) is given in Table XXIV.

Palmer et al (1973) analysed for amino acid content the ungerminated and germinated seeds of kidney beans (Phaseolus vulgaris). The amino acid composition of the globulin fraction from the ungerminated seed is given in Table XXIV.

Maneepun et al (1974) determined the amino acid composi-

tion of a protein concentrate obtained from the lima bean by extraction with water at pH 6.3. The protein was precipitated at pH 5.0. Table XXIV shows the results of the analyses.

TABLE XXIV

AMINO ACID COMPOSITION OF PROTEIN FROM PHASEOLUS BEANS

Amino Acid	Lima bean protein concentrate[a] g/16g N.	Kidney bean protein[b] g/16g N.	Navy bean protein fraction[c] g/100g protein
Aspartic acid	17.06	12.2	13.0
Threonine	9.84	4.6	3.5
Serine	7.15	6.1	6.5
Glutamic acid	14.15	16.0	13.3
Proline	4.22	5.4	3.5
Glycine	3.97	4.1	3.3
Alanine	4.74	4.4	3.3
Valine	4.15	6.0	5.2
Cystein	2.65	0.5	N.R.
Methionine	1.13	1.1	0.6
Isolencine	4.49	5.3	5.2
Leucine	7.81	9.4	9.2
Tyrosine	3.88	4.0	2.9
Phenylalanine	4.72	6.7	6.8
Lysine	6.61	7.6	6.4
Histidine	2.92	3.2	2.9
Arginine	5.01	5.9	5.0

[a] Maneepun et al (1974).
[b] Palmer et al (1973).
[c] Evans et al (1973).
N.R. - Not Reported.

2. Experimental, Results and Discussion

a. Materials and Method

Samples (8 mg) of the protein preparations were placed in clean dry test tubes (13 x 100 mm) along with concentrated hydrochloric acid (1 ml). The tubes were then placed in a beaker containing concentrated hydrochloric acid up to the same level as that in the tubes. The beaker was placed in a desiccator and concentrated hydrochloric acid was placed in the base of the desiccator. The desiccator was sealed and clamped by means of two wood flanges which were bolted together (Lauer, 1971). The desiccator was flushed with nitrogen and evacuated (with the aid of a vacuum pump) three times. After the final evacuation it was heated in an oven at 110°C. for twenty-four hours.

The samples were cooled then dried under vacuum in a desiccator containing sodium hydroxide flakes. The dried hydrolysates were dissolved in sodium citrate buffer solution (pH 2.2, 5 ml) immediately before the amino acid determination.

The protein hydrolysates were analysed by means of an Amino Acid Analyser (Beckman, Model 120C).

The necessary reagents were prepared as described in the Instructional Manual, Model 120C, Beckman Amino Acid Analyser (1965) and are listed below:

(i) Sodium Citrate Buffer Solution (0.20N, pH 2.2).

Sodium citrate (19.6 g), conc. hydrochloric acid (16.5 ml), thiodiglycol (5 ml), caprylic acid (0.1 ml), benzyl alcohol (5 ml), volume adjusted to 1 litre with distilled water.

(ii) Buffer A Sodium Citrate Buffer Solution (0.20N, pH 3.28).

Sodium citrate (78.4 g), conc. hydrochloric acid (50.3 ml), thiodiglycol (10 ml), caprylic acid (0.4 ml), benzyl alcohol (20 ml), volume adjusted to 4 litres with distilled water.

(iii) Buffer B Sodium Citrate Buffer Solution (0.20N, pH 4.25).

Sodium citrate (78.4 g), conc. hydrochloric acid (33.5 ml), thiodiglycol (10 ml), caprylic acid (0.4 ml), benzyl alcohol (20 ml), volume adjusted to 4 litres with distilled water.

(iv) Buffer D Sodium Citrate Buffer Solution (0.32N, pH 5.28).

Sodium citrate (137.3 g), conc. hydrochloric acid (20.2 ml), caprylic acid (0.4 ml), benzyl alcohol (20 ml), volume adjusted to 4 litres with distilled water.

(v) Ninhydrin Reagent.

Methyl Cellosolve (Ethylene Glycol Monomethyl Ether),

(3 litres), sodium acetate buffer solution (4N, 1 litre), ninhydrin, (80.0 g), stannous chloride (1.600 g).

For the determination of the basic amino acids a 23 x 0.9 cm tube containing Beckman Custom Spherical Ion-Exchange Resin PA 35 and elution buffer of pH 5.28 (Buffer D) were used.

For the determination of the acidic and neutral amino acids a 69 x 0.9 cm tube containing Beckman Custom Spherical Ion-Exchange Resin PA 28 and elution buffer of pH 3.28 (Buffer A) and pH 4.25 (Buffer B) were used.

The operating directions for a four hour protein hydrolysate analysis, as described in the Instruction Manual, Model 120C, Beckman Amino Acid Analyser (1965), were followed.

The volume of sample which was applied to the column was 0.4 ml. Column temperature was maintained at 55°C. and effluent flow rate of buffer solution and ninhydrin reagent was 102 ml per hour.

The Analyser was calibrated using a Beckman Standard Amino Acid Mixture containing 17 amino acids.

b. Results and Discussion

Tables XXV, XXVI and XXVII give the amino acid composition of the protein hydrolysates prepared from the isolates of the white kidney bean, the navy bean and the lima bean.

TABLE XXV

AMINO ACID COMPOSITION OF PROTEIN ISOLATES

FROM WHITE KIDNEY BEAN

Amino Acids	g Amino Acid per 100 g Protein				
	Isolate A[a]	Isolate B[b]	Isolate C[c]	Isolate D[d]	Isolate E[e]
Aspartic Acid	15.42	10.47	15.53	16.27	14.32
Threonine	3.13	2.98	3.38	4.47	2.77
Serine	6.12	6.82	5.59	6.08	5.96
Glutamic Acid	22.57	24.74	22.81	22.25	21.17
Proline	3.85	4.83	3.52	3.96	3.47
Glycine	3.22	2.49	4.09	3.93	2.96
Alanine	3.62	2.62	3.12	3.90	3.09
Cystein	0.35	0.29	0.21	0.30	0.23
Valine	4.67	4.13	5.63	5.70	4.48
Methionine	0.92	0.96	0.62	1.03	1.01
Isoleucine	4.54	4.23	4.93	5.25	4.20
Leucine	7.87	7.49	8.63	9.64	5.03
Tyrosine	2.62	2.51	4.63	4.06	0.87
Phenylalanine	4.48	4.18	5.95	5.04	1.25
Lysine	7.57	8.09	7.53	7.48	7.99
Histidine	2.29	2.71	2.62	2.31	2.58
Arginine	4.88	5.55	4.66	4.20	5.55

A[a] - crystalline isolate extracted with citric acid solution.

B[b] - spheroidal isolate extracted with citric acid solution.

C[c] - precipitate extracted with malic acid solution and precipitated by diluting with water.

D[d] - alkali extract, precipitated with acid.

E[e] - crystalline isolate extracted from isolate D with citric acid solution.

TABLE XXVI

AMINO ACID COMPOSITION OF PROTEIN ISOLATES

FROM NAVY BEAN

Amino Acid	g Amino Acid per 100 g Protein			
	Isolate F[a]	Isolate G[b]	Isolate H[c]	Isolate I[d]
Aspartic Acid	12.15	10.8	9.75	11.64
Threonine	3.23	2.78	3.19	3.22
Serine	6.13	5.01	5.19	6.02
Glutamic Acid	18.81	15.92	17.04	16.85
Proline	3.18	2.54	3.24	2.67
Glycine	3.87	3.29	3.89	3.01
Alanine	3.86	3.00	4.09	3.19
Cystein	0.25	0.17	0.38	0.28
Valine	5.61	4.16	4.53	3.22
Methionine	0.99	0.46	0.53	0.85
Isoleucine	4.67	3.69	4.17	4.36
Leucine	7.90	7.19	5.69	7.41
Tyrosine	2.97	2.78	2.40	2.92
Phenylalanine	5.58	4.46	3.58	5.23
Lysine	6.59	5.15	6.91	5.32
Histidine	2.74	1.57	2.33	1.65
Arginine	4.48	3.33	5.50	3.36

F[a] - crystalline isolate extracted with citric acid solution.
G[b] - spheroidal isolate extracted with citric acid solution.
H[c] - alkali extract, precipitated with acid.
I[d] - needle-like precipitate extracted with citric acid solution.

TABLE XXVII

AMINO ACID COMPOSITION OF PROTEIN ISOLATES FROM LIMA BEAN

Amino Acid	g Amino Acid per 100 g Protein		
	Isolate J[a]	Isolate K[b]	Isolate L[c]
Aspartic Acid	10.37	11.59	15.48
Threonine	2.98	3.22	3.12
Serine	4.96	4.88	6.50
Glutamic Acid	14.61	16.88	19.56
Proline	3.88	4.49	3.85
Glycine	3.51	4.67	4.30
Alanine	3.67	4.63	3.85
Cystein	0.21	0.35	0.39
Valine	4.90	6.65	5.35
Methionine	0.46	0.64	0.71
Isoleucine	3.92	4.24	5.78
Leucine	7.17	8.01	9.25
Tyrosine	3.11	3.47	3.45
Phenylalanine	2.93	4.76	5.23
Lysine	7.95	7.90	7.50
Histidine	2.10	2.84	3.02
Arginine	4.66	5.71	5.87

J[a] - crystalline isolate extracted with citric acid solution.
K[b] - spheroidal isolate extracted with citric acid solution.
L[c] - alkali extract precipitated with acid.

It would be noted from Table XXV that the amino acid compositions of the different isolates from the white kidney bean are quite similiar. The methionine contents of the various isolates from the kidney bean are quite low and in particular the isolate (isolate I) prepared by malic acid extraction and precipitated by dilution with water. The methionine contents of the other four isolates are about 1 per cent and agree favourably with that reported by Palmer et al (1973). The lysine contents of the various isolates are relatively high with values ranging from 7.48 to 8.09 g per 100 g of protein. Isolate E, shows relatively low phenylalanine and tyrosine contents. Cystein, like methionine, is present in very small quantities in all of the isolates. This agrees with the work of Bressani et al (1961) who found that Phaseolus beans were deficient in both the sulphur-containing amino acids, cystein and methionine.

The observations noted for the white kidney bean also apply to the navy bean. Certain amino acids, however, are present in lower concentrations in the navy bean than in the kidney bean eg. aspartic acid, glutamic acid and lysine. Pant and Tulsiani (1969) and de Moraes and Angelucci (1971) also noted that different varieties of Phaseolus vulgaris beans have different amino acid compositions.

The observations noted for the Phaseolus vulgaris beans (kidney beans and navy beans) also apply to the lima bean (Phaseolus lunatus). The lima bean isolates do not differ markedly

in amino acid composition from the isolates of the two Phaseolus vulgaris varieties.

The results show that the amino acid compositions of the crystalline protein isolates of the three beans, do not differ markedly from those of the other non-crystalline isolates. In fact, the different isolates of the same bean have amino acid compositions which are more similiar than are the corresponding isolates from the different beans.

SUMMARY

1. White kidney beans, navy beans and lima beans yield crystalline protein when extracted with citric acid solution and DL-malic acid solutions under certain conditions of pH and normality.

2. The yield of protein from lima beans (Phaseolus lunatus) using citric acid solutions and DL-malic acid solutions as extractants show no general relationship with pH. White kidney beans and navy beans (Phaseolus vulgaris) show a general increase in protein yield to a point of maximum yield as the pH of the extraction is increased.

3. The yield of protein from lima beans, white kidney beans and navy beans increased to a point of maximum yield as the normality of the extractants were increased. Protein was isolated from lima beans at much lower normalities than those protein was isolated from white kidney beans and navy beans.

4. The yield of protein from lima beans, white kidney beans and navy beans increased as the size of meal particles decreased to a diameter of 0.50 mm. Beyond this, the protein yield decreased as the particle size of the meal decreased.

5. The yield of protein from white kidney beans and navy beans increased as the period of extraction increased up to thirty minutes. The yield of protein from lima beans increased as the period of extraction was increased up to twenty minutes.

6. The yield of protein from white kidney beans and lima beans increased as the extraction temperature was increased from room temperature (27°C.) to 45°C. The yield of protein from navy beans increased as the temperature of extraction was increased up to 40°C.

7. The various isolates from the different varieties of beans as well as from the same variety of bean gave somewhat different electropherograms. In general the crystalline protein isolates had a greater number of electrophoretic components than did the non-crystalline isolates.

8. Five protein fractions from white kidney beans, four fractions from navy beans and three fractions from lima beans have been analysed for amino acid composition. The amino acid compositions of the crystalline proteins do not differ markedly from those of the non-crystalline isolates from the same beans.

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