ABSTRACT

BIOLOGICAL VALUE OF PEA PROTEINS
AS INFLUENCED BY GENETIC VARIATION

By

Satinder Bajaj

The biological value of 32 varieties of peas (Pisum sativum L.) grown under similar field conditions was determined using weanling rats by means of net protein utilization (NPU) and protein efficiency (PER). Different varieties of peas, when fed as the sole source of protein at a 10% level in an otherwise adequate diet, varied from 0.18-0.78, in comparison to casein, in their ability to support growth and nitrogen retention in 3-week-old Sprague Dawley rats. From the analysis of rat growth curves, the pea varieties were separated into those that produced fairly good growth and those that barely maintained the initial weight of rats. Carcass protein expressed as a percentage of body weight was higher in pea fed (20-22%) than in casein fed rats (18-19%). This was probably associated with a difference in the body fat content. PER and NPU were fairly constant when the experiments were repeated, indicating

the value of these biological techniques in evaluating protein quality. The total nitrogen content of different varieties of peas provides a poor indication of the biological value of their nitrogen containing compounds.

Nitrogen distribution in protein fractions was studied in varieties of peas selected to represent high, low, and mediocre biological values. Peas with a higher PER contained more albumin nitrogen than those of lower PER. For data from 21 varieties of peas, a quadratic function of the albumin content had a high coefficient of correlation (R = 0.949) with PER. The equation:

$$Y = 24.7X - 13.6X^2 - 8.8$$

provides a quick, simple and accurate method for evaluating protein quality of peas. The nitrogen in the other fractions did not correlate with the protein quality.

The protein quality of the peas was evaluated by a microbiological method using Streptococcus zymogenes. The microbiological values were expressed as a percentage of response produced by casein. These values of pea meals were similar to the PER and NPU when expressed as a percentage of casein PER and NPU. When the major extractable protein fractions were assayed by the microbiological method, albumin had a higher biological value (117-128) than either globulin (64-100) or the residual fraction (28-56). The biological value of the albumin fraction from different varieties of peas was not significantly different

($P<0.05$), whereas differences in the values of the globulin and the residual fraction were significant.

The extractable protein from all varieties of peas, when subjected to starch gel electrophoresis was represented by 3-4 major bands. The two slower moving bands were associated with the globulin and the others with the albumin fraction. In the pea extract the combined globulins moved as a single streak with two bands faintly discernible. The addition of reducing agents (7M urea and 2-Mercaptoethanol) dissociated the globulin (3-bands vicilin; 4-bands legumin) but not the major albumin band.

The study demonstrates that, total nitrogen is a poor index of protein quality of peas, both quantity and quality of peas vary in different varieties, and that the quantity of albumin correlates highly with the PER of the peas, an observation which can be utilized to predict the PER of peas. The albumin fraction which contains one major band is of better quality than the other protein fractions studied in the pea extract.

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INTRODUCTION

Plant geneticists have for a long time bred plants to alter specific characters such as color, size, shape, disease resistance and yield. Biologists studying plants and animals have shown that specific proteins are characteristic of certain families (Dalby and Lillevik, 1969; Boulter and Thruman, 1968). The nutritional implications of this type of work are obvious, namely that it is possible that quantitative as well as qualitative variation in protein occurs with genetic variation, and can be further enhanced by making suitable crosses.

With the increasing world population and the ensuing food shortage which threatens to affect even the economically developed countries, scientists have begun to look seriously at many hitherto unexplored areas for protein rich foods. Algae and leaves are being examined as possible sources of protein. Although such foods can be used in animal feeds or make interesting academic studies, acceptability is an important factor in human nutrition. Most of these unusual sources of food need either to be processed suitably or a tremendous effort

has to be put in toward educating people to accept the unusual foods where they may be required. These might be the countries that are not industrially developed and where communication is a problem.

In any search for sources of human protein foods several important properties need attention:

1. The foodstuff should have a high content of protein and if an increase in the protein is made, the contribution of the food-stuff to the protein in the diet should be significant.
2. It should not have any toxic factors, especially if they are difficult to remove or destroy.
3. It should be easy to grow and the yield must be sufficient to make its production economical.
4. If possible, it should be easy to store and transport. This becomes especially important in tropical countries where there is very little refrigeration.

Considering these requirements cereals and legumes appear ideally suited to provide an increasing portion of the world's protein needs. Although cereals are consumed in larger quantities as staple foods, legumes contain more protein (20-30% vs 10-20% in cereals).

According to an FAO estimate (Food balance sheet, FAO, 1957) the world production of legume seeds amounted to 28.3 million metric tons. India, the largest producer of

legumes had an estimated production of 10.617 million metric tons; on a per capita basis this amounted to 60.8g/caput/day. The United States had the second highest production, 0.914 million metric tons, which amounted to 10.6g/caput/day.

A great deal of effort is currently being focused upon increasing the lysine content of cereals. This interest stems from the work of Mertz and his collaborators (Mertz, 1966; Dimler, 1966; Mossé, 1966), who showed that the lysine content of corn was controlled primarily by a single gene (Opaque-2) which is the locus of synthesis of glutelin (alcohol insoluble protein). Most of the lysine and tryptophan is associated with this protein. By altering the quantity of glutelin the lysine content of corn could be altered. Furthermore the high lysine corn had a biological value similar to that of casein.

Mattern et al. (1968) from the University of Nebraska, under a project financed by the U.S. Agency for International Development have launched a world wide project for screening 9,500 varieties of wheat from the USDA collection, for protein and lysine. A similar project has been undertaken by Swaminathan and Austin (1968), at New Delhi, India.

In most countries where populations depend heavily upon vegetable sources of protein, the cereal diets are

usually supplemented with legumes whenever people can afford them. The legumes contain sufficient lysine to supplement cereals (Patwardhan, 1956). The improvement of the biological value of protein in legumes therefore deserves more attention than is currently being given to it.

The governments of some countries are seriously considering fortifying cereals with lysine, the validity of such supplementation is questionable in view of the fact that amino acids can produce toxicities and imbalances as demonstrated by experiments with rats (Harper, 1964). One solution to this problem would be to increase the production of legumes to make them available to most people, especially those varieties which have been selectively bred to increase their protein content and their biological quality.

Before an enthusiastic use of legume proteins is made, toxic factors which are associated with many legume seeds need attention. Very few toxic factors have been reported in peas (Pisum sativum L.), in fact, peas are considered such a good source of food that they are incorporated into baby foods. Peas are consumed in both the dry and green form; in the United States the green pea is used more frequently.

The protein fractions of peas are better characterized than most other legumes, yet more information

concerning the distribution of amino acids in the protein fractions, the quantity of each fraction present in different varieties as it relates to the biological quality, and simple assays for these proteins are required to aid the geneticist in making suitable crosses to improve the quality and quantity of protein.

REVIEW OF LITERATURE

PROTEINS IN PEAS AND OTHER LEGUMES

Several reviews have appeared on plant proteins, seed proteins and nitrogenous components of plant material (Steward and Street, 1947; McCalla, 1949; Danielsson, 1956; Stahman, 1963; Altschul et al., 1966). These frequently refer to pea proteins particularly in reference to comparison with other legume seeds.

Most of the original work on the classification of pea proteins is credited to Osborne and Campbell (1896, 1898). According to their definition which, due to its simplicity, is the most universally accepted, pea proteins are defined as follows:

Albumins: are soluble in water and coagulable by heat.

Globulins: are insoluble or sparingly soluble in water, but their solubility is greatly enhanced by the addition of neutral salts like sodium chloride. Many globulins are easily prepared from plant and animal tissue since they are readily extracted by salt solution and precipitated from saline by dilution with water.

The fundamental work on seed proteins was done by Osborne who started in 1891 and during the following 30 years published more than 100 papers on the subject (for

references see Osborne, 1924). He and his coworkers used mild methods of preparing their proteins and many of their results are still valid. According to Osborne, pea proteins contained a third unidentified protein, legumelin in addition to an albumin and a globulin fraction.

Osborne showed that the seed proteins from different members of the Leguminoseae family could be separated into a few simple fractions; each probably containing one protein. Osborne thought that the different proteins were homogenous and consisted of individual proteins. From the seeds of Pisum sativum, Ervum lens and Vicia faba he isolated two globulins, legumin and vicilin, and a third unidentified protein legumelin. The elementary composition (C,H,N.O,S) of the individual proteins from all the three plants was identical, but small distinct differences between proteins existed even when extracted from the same plant. For example the sulfur content of legumelin, legumin and vicilin was 1.06, 0.5 and 0.16% respectively. This finding is of importance since, in purified proteins, the sulfur might be expected to be present as sulfur containing amino acids, which are limiting in most legume proteins. It is interesting to note that other workers have not been able to isolate legumelin.

Since the methods used by Osborne were crude, particularly for determining homogeneity and purity of proteins, many recent experiments utilize Osborne's work as a

base for the study of proteins but check the homogeneity by more modern methods.

Location of Pea Globulins

The globulins of the pea are reported to be localized in distinct subcellular particles called protein bodies, which can be isolated and probably contain globulins only (Varner and Schidlovsky, 1963a). There is little or no globulin outside these bodies. The protein bodies are spherical in shape and distinctly delineated by a membrane similar in structure to the plasma membrane (Varner and Schidlovsky, 1963b). With the introduction of electron microscopy there has been renewed interest in protein bodies. Protein bodies or aleurone grains were discovered by Hartig (1856) and later studied in detail by Pfeffer (see Sachs, 1874). Pfeffer concluded that:

1. Protein bodies occur widely in both starch bearing and oil bearing seeds.
2. Some protein bodies contain crystalline inclusions of salts.
3. They are probably surrounded by membranes.
4. They contain most of the cellular protein but none of the oil.
5. Their formation commences only during the later stages of ripening of seeds (that is the time when the storage proteins or the globulins are synthesized).

6. They swell, coalsce and disappear early in germination having fulfilled their function in the nourishment of the embryo.

Extraction Procedures and Homogeneity of Pea Proteins

Agreement on the homogeneity of pea proteins is not universal due to various procedures employed for extraction and purification. Osborne's method (1896) consisted of extracting the seeds with naptha to free them of oils, and then extracting with 10% sodium chloride to remove the proteins; the globulins were precipitated by aqueous dilution of the salt solution. The precipitate was washed with alcohol and then dried at 110C over sulfuric acid.

Danielsson (1950) modified Osborne's method omitting the naptha extraction, and the alcohol washing which might denature the proteins. The globulins were precipitated from the extract with ammonium sulfate, and further resolved into their component proteins by the isoelectric precipitation of legumin at pH 4.7.

Wetter and McCalla (1949), working at about the same time as Danielsson, were unable to detect single proteins in their purified fractions when these were observed on free boundary electrophoresis. More recent experiments suggest two well defined globulin fractions which can be separated under carefully controlled conditions. Wetter

and McCalla used long extraction procedures for the removal of lipids which might have altered the chemical composition of the proteins. In addition they might not have carried the separation of the protein to its conclusion, where they would appear as single proteins on electrophoresis.

Workers studying pea proteins from a physiological rather than a chemical standpoint, need to quantitatively fractionate proteins, under such conditions that precise, repeatable results are secured. Accordingly Raacke (1957) modified Danielsson's method after a careful study of results obtained on altering the experimental conditions. In her study, the globulins were separated from the albumins by lowering the salt concentration by dialysis. For the quantitative estimation of legumin and vicilin, both Raacke and Danielsson used an ultracentrifuge.

Chromatographic methods have been used to separate pea proteins. Varner and Schidlovsky (1963a) using DEAE cellulose to separate pea proteins observed four peaks instead of the two due to globulins. Grant and Lawrence (1964) similarly separated pea globulins on DEAE cellulose, and they could observe only two peaks corresponding to legumin and vicilin. Grant and Lawrence (1964) studied the chromatographically separated proteins on polyacrylamide gels under the influence of various dissociating agents. They observed two distinct bands corresponding to the two globulins when no dissociating agents were added. Addition

of urea gave six bands, four of which were attributed to legumin and two to vicilin. Under the influence of sodium dodecylsulfate 12 bands were obtained, 6 of which were attributed to legumin, 4 to vicilin. Two bands were not identified. Grant and Lawrence (1964) concluded that they might be due to random reassociation of the component fractions.

Goffman and Vaintraub (1960) evolved a simple technique for monitoring the homogeneity of seed proteins. This method, based on paper electrophoresis, emphasizes the correct application of the sample to the paper so that the proteins to be separated migrate in the opposite directions.

From the observations on the extraction procedures it appears that the homogeneity of proteins, the quantity of protein extracted, and other properties depend to a large extent on the extraction procedure employed, for this reason it is necessary for workers to report the exact experimental details and employ the gentlest methods possible for the extraction of proteins.

Physical Properties of Pea Globulins

1. Danielsson (1950) investigated pea globulins on free boundary electrophoresis in the pH range 3.7-9.3, and found both vicilin and legumin to be homogenous giving single peaks. The iso-electric pH of legumin was found to be 4.8, a factor utilized in the separation of the two globulins.

2. Ultracentrifuge study of pea globulins (Danielsson, 1949b) showed the globulins to be homogenous in the ultracentrifuge. The sedimentation constants were determined to be 12.4S for legumin and 8.1S for vicilin.

3. The molecular weight was 186,000 for vicilin and 331,000 for legumin (Danielsson, 1949b).

Chemical Composition of Pea Proteins

The elemental composition of pea proteins was studied by Osborne and Campbell (1896). Nitrogen content of proteins extracted by Danielsson and Lis (1952) were similar to those obtained by Osborne. These results are reported in Table 1. From the values reported it appears that legumin contains 18% nitrogen and vicilin contains 17.4% nitrogen which would require the use of conversion factors of 5.55 and 5.75 respectively. The albumin fraction however does contain 16% nitrogen. The sulfur content of the albumin fraction is twice that of the globulins which is significant since sulfur containing amino acids are limiting in peas.

Danielsson and Lis (1952) studied the amino acid composition of pea proteins. Their results are reported in Table 2. From their report, the lysine and tryptophan content of the albumin fraction appears to be 2-3 times higher than that of the globulins.

Table 1.--Composition of legumin, vicilin, and legumelin according to Osborne and Campbell (1896).

Element	Pea	Lentil	Horse bean	Vetch	Average
		Legumin			
Carbon	51.74	51.73	51.72	51.69	51.72
Hydrogen	6.90	6.89	7.01	6.99	6.95
Nitrogen	18.04	18.06	18.06	18.02	18.04
Sulfur	0.42	0.40	0.39	0.43	0.41
Oxygen	22.90	22.92	22.82	22.82	22.88
		Vicilin			
Carbon	52.36	52.13	52.38	-	52.29
Hydrogen	7.03	7.02	7.04	-	7.03
Nitrogen	17.40	17.38	17.52	-	17.43
Sulfur	0.18	0.17	0.15	-	0.17
Oxygen	23.03	23.30	22.91	-	23.08
		Legumelin			
Carbon	53.6	-	-	53.55	
Hydrogen	6.93	-	-	6.99	
Nitrogen	16.14	-	-	16.46	
Sulfur	1.00	-	-	1.02	
Oxygen	22.55	-	-	22.27	

Table 2.--Amino acid content (% of weight) of some seed proteins. (From Danielsson and Lis, 1952.)

Amino Acid	Legumin	Vicilin	Albumin
Tryptophan	0.4	1.3	2.3
Tyrosine	4.2	4.2	5.5
Arginine	11.5	13.1	5.1
Histidine	2.4	3.0	3.2
Lysine	4.6	3.5	10.3
Glutamic	22.7	30.1	10.3
Aspartic	15.7	16.3	14.5

Grant and Lawrence (1964) studied the amino acid composition of legumin and vicilin on the Stein and Moore automatic amino acid analyzer. Their observation indicates that one of the vicilin fractions contains a large portion of the essential amino acids. The results obtained by Danielsson and those obtained by Grant and Lawrence (1964) are in poor agreement, possibly due to differences in the purity of the proteins, and the methods used for the estimation of amino acids.

Residue Proteins

Van Etten et al. (1961) determined the amino acid composition of seed meals obtained from 27 genera of 13 botanical families. Biological values as estimated by rat

and chick growth showed a majority of the seeds were deficient in methionine - cystine, lysine, or both. Three genera of legume families contained canavanine. The same workers found hydroxy proline in 63 out of 99 solvent extracted acid hydrolysed seed meals. Solubility studies indicated that the compound is a part of the seed coat and the pericarp. Seed meals derived from the kernel alone did not contain any hydroxy proline. Lamport (1965) has shown hydroxy proline to be a part of the primary cell wall protein.

Non-Protein Nitrogen of Pea Seed

Most seeds contain large amounts of amide nitrogen and are noted for a variety of non-protein amino acids. The latter may sometimes amount to as much as 2% of the dry weight of the seed (Fowden, 1964). Some examples of unusual amino acids in legume seeds are pipecolic acid in beans and homoserine in germinating pea seeds.

Phylogenetic Relationship of Globulins in Legume Seeds

Studies of the families leguminoseae and Malvaceae (Shadmanov, 1964) showed that phylogenetically old species

were characterized by low quantities of nitrogen (and hence protein) in comparison to higher forms. A relationship was suggested between the position of a plant on the evolution scale and the nature of the storage protein. In older forms of bean plants there was a predominance of the more difficultly soluble (legumin with higher molecular weight) protein over the more easily soluble form (vicilin with lower molecular weight). That is in the phylogenetically younger plants, vicilin predominates. Fox et al. (1964) electrophoresed proteins to establish such relationship within the family leguminoseae.

Physiological Function of Pea Proteins

The genetic selection of certain varieties would be greatly facilitated if the time of synthesis and the physiological role of the component proteins were known.

Albumins The albumin fraction is heterogenous in composition and the role ascribed to it is mainly enzymic. Among the enzymes isolated from it are: a proteolytic enzyme (Young and Varner, 1959), a peroxidase, a catalase, a phosphatase, an amylase and an aldolase (Hatz and Leuthardt, 1967).

Danielsson (1952) worked on protein synthesis in ripening pea seeds and showed that the total protein increased

on ripening. In unripe seeds all of the protein (100%) was present as albumin. On ripening of the seeds, although the absolute amount of albumin increased, there was a great drop in the percentage of protein present as albumin (17%).

Since the albumin fraction is heterogenous it is possible that more than one function might be allocated to it.

Function of the Globulins

While the albumins appear to be enzymic in function, globulins are considered storage proteins. During synthesis, the lower molecular weight vicilin appears first. The appearance of globulins coincides with the appearance of protein bodies.

Quantitative Variation in Protein as Influenced by Genetic Variation

It appears possible to make a precise science of the study of peas or other legumes based on the quantity of protein in these seeds. If no toxic factors are present in the seed, the increment in the quantity of protein in the absence of improvement in quality is desirable. Hegsted (1962) calculated that human diets based on wheat alone could provide sufficient amino acids for growth. Bolourchi (1967) showed the ability of wheat diets to maintain adult human subjects in nitrogen equilibrium. Campbell (1963), showed that with casein, maximum growth per unit protein intake was obtained when it was fed at a 7% level. With plant proteins, maximum growth was obtained when they were fed at a 15% level in the diet. These observations indicate the usefulness of increasing the quantity of protein in legumes or other seeds.

Pesola (1955) studied the variation in protein quantity of peas as influenced by genetic variation and climatic conditions. He concluded that the variation in protein content of peas is a varietal character, and showed that the protein content could be increased by the selection of high protein lines. Pesola suggested that the protein content may vary with the varying intensity of the symbiotic action of different pea varieties and root nodule bacteria. Climatic conditions were likewise shown to influence the protein content.

Evans et al. (1947) studied the effect of fertilizer treatment, environmental conditions and genetic variation, on protein, cystine and methionine content of peas. They showed that the protein content was determined by the location where the plants were grown and by breeding. The methionine content could not be altered by any of the conditions studied, however, the cystine content could be increased by the use of sulfur containing fertilizers. Since methionine is required partly to synthesize cystine in the mammalian system, an increase in cystine content by the use of sulfur containing fertilizers might be important.

Esh et al. (1959) studied the effect of environmental conditions and genetic strain on the protein content of legume seeds. They found variation in protein content (nitrogen X 6.25) when seeds of different strains were grown in the same locality. A variation of as much as 60% was sometimes noted. Similarly, location influenced the protein content, a variation of 13-34% was observed with changes in locality where the peas were raised.

The quantity of protein is influenced by genetic variation. This has been shown for several major seed crops; such as, wheat (Mattern et al., 1968) and sorghum (Swaminathan and Austin, 1968). These workers are using this information to cross high protein lines and obtain nutritionally superior varieties.

In addition to the effect of genes in changing protein quantity, herbicides such as simazine (2-chloro-4, 6-bis ethyl amino-s-trizaine) have been shown to increase the protein content of peas and oats (Ries et al., 1967). In addition to increasing the protein content, this herbicide was reported to increase the yield (Schweizer and Ries, 1969).

Biological Value

Although many studies have been done on the influence of genes on the quantity of protein, very little work has been done to show the effect of genes on protein quality. The quality of protein in foods depends on several factors, such as, the digestability of protein, the amino acid composition, the availability of the amino acids and the presence of toxic factors. Systematic studies to observe differences in the digestability of protein, the amino acid composition, the availability of amino acids and the toxic factors as influenced by genetic variation have only been initiated in the past few years.

Mertz et al. (1965) showed that the protein quality of high lysine corn was very similar to that of casein. This improvement in the biological value of corn resulted from an increase in the lysine content. Most of the lysine in the corn was reported to be associated with the alcohol insoluble protein fraction called glutelin (Dimler, 1966). Although chemical scores were obtained for the alcohol soluble proteins (prolamines) to show that they were of poor nutritional quality (Mossé, 1966) the same was not done for the glutelin fraction.

A limited number of studies on the nutritional quality of proteins in legume seeds (Esh, 1958; Niyogi et al., 1932) as influenced by genetic variation showed that the protein content of these seeds varied from 20-30%,

the digestability from 75-95%; and the biological value from 45-70%.

Evans and Bandemer (1967) studied the nutritional value of legume seeds. The two varieties of peas studied (Alaska and First and Best) by these authors had different biological value as estimated by rat growth. The results secured by these authors were not corrected for food intake, but expressed as the ratio of growth produced when the experimental diet was fed compared to that produced by the casein ration. Such results are reported to be highly variable (Campbell, 1963). According to the amino acid content, First and Best had a higher quantity of lysine, methionine and cystine; despite this the biological value of Alaska pea was higher (51 Vs 41 for First and Best). Supplementation of the pea ration with methionine at 0.5% level improved the biological value of Alaska pea from 51 to 124%. Methionine has been shown to be a limiting amino acid of peas and other legumes. Tryptophan is occasionally reported to be a limiting amino acid of legume seeds, however no studies with supplementation of tryptophan were conducted.

Similar studies on supplementation of methionine to pea diets at 0.1% level resulted in a dramatic improvement of the PER of peas from 0.5 to 2.35 (Russel et al., 1946).

Mitchell and Block (1946) postulated a relationship between a limiting amino acid in a dietary protein and its

biological value. It appears possible that PER of cereal proteins should be influenced by lysine and legume proteins by methionine and possibly tryptophan which are limiting amino acids in the respective foodstuffs. Suggestions are not wanting that nutritive value of cereal proteins should be improved by the addition of the amino acid which is the limiting factor. This suggestion is not capable of implementation on a large scale in the realm of human nutrition. It seems far more practicable to attempt to improve the nutritional value of cereal proteins by incorporation in the diet of other foodstuffs (such as legumes) which will supply the deficient amino acids. In fact Patwardhan (1956) observed that in Indian dietaries, a combination of legumes and cereals provide sufficient lysine for daily adult requirements.

Phansalkar and Patwardhan (1956) observed that the supplementation of cereal protein with legume protein improved the biological value and egg replacement value of the mixture in human subjects.

Phansalkar *et al.* (1957) studied the effect of supplementation of cereals with legumes by the rat growth method. The PER of wheat, rice and millets was improved when these were supplemented with legumes. Red gram (*Cajanus cajan*) was more effective than other legumes (*Cicer arietinum*, *Phaseolus mungo* and *Phaseolus radiatus*) as a supplementary source.

In an extensive review, Swaminathan (1967) tabulated the supplementary effect of plant proteins. The supplementation of peas with barley in the ratio of 1:1 gave a PER of 1.98 when either of these alone had PER values below 1.5.

Several attempts have been made to prepare nutritious milk substitutes based on vegetable protein. Soy bean and peanut milk was reported to be successful in feeding infants in China. Dean (1953) reported that highly nutritious spray dried food could be prepared from a blend of barley malt and soya bean, and that about half of the milk in the diet of infants up to 1 year of age could be replaced by soy bean foods without affecting growth and nutritional status. Several brands of proprietary foods based on soy bean are manufactured and used in the U.S.A. for feeding infants who are allergic to cow's milk (Meyer, 1960).

Chick pea (Cicer arietinum) has been used in protein foods based on oil seed meals (Parpia et al., 1964). This food was reported to be effective in treating protein malnutrition in children. Scrimshaw et al. (1960) found that a mixture of corn and black bean protein was not effective in curing protein malnutrition, but the addition of lysine and tryptophan to the diet significantly increased the nitrogen retention in malnourished children. Hansen et al. (1960) reported that a mixture of corn and pea meal did not initiate cure in three cases of Kwashiorkor.

Factors Affecting the Nutritive Value of Plant Proteins

1. Toxic Factors.--These substances are present in a large number of foods and exert a deletrious affect on the utilization of proteins and the growth of the animal. A number of toxic factors have been reported in legume seeds (Mickelsen and Yang, 1966), among those reported are: a growth depressant in raw soya bean meal (Saxena et al., 1962) and in kidney beans (Kakade and Evans, 1965), and trypsin inhibitors in raw soy bean meal (Ham et al., 1944), and anti thyroid compounds in legume seeds (Greer and Astwood, 1948). Although peas have not been reported to contain toxic factors, as evaluated by improvement of growth following heat treatment, it is significant that Greer and Astwood reported that unlike the anti thyroid compounds of other legume seeds the anti thyroid activity of peas was not destroyed by heating.

2. Effect of Heat Processing.--Heat produces both beneficial and deletrious effects on the nutritive value of proteins. The beneficial effects are due to inactivation by heat of the trypsin and growth inhibitors, hemagglutinins and other toxic factors present. The adverse effects are due to the decrease in the availability of certain essential amino acids, such as lysine and methionine, as a result of reaction with reducing sugars and carbonyl compounds present in foods. This mechanism (Maillard's reaction) has been

extensively studied (Patton, 1955; Ellis, 1959). The loss of amino acid depends on the severity and length of heat treatment and the moisture content of the food. In general, foods, such as legumes containing trypsin and growth inhibitors, show marked improvement in the nutritive value of their proteins on processing.

3. Amino Acid Imbalance, Deficiency, Toxicity, and Antagonism.--Partial deficiency of any one essential amino acid adversely affects the utilization of proteins for the maintenance of nitrogen equilibrium and growth. Certain amino acids when present in quantities that greatly exceed the requirements, adversely affect the growth of the animals. These effects have been ascribed to: (a) amino acid imbalance, (b) amino acid toxicity and (c) amino acid antagonism (Food and Nutrition Board, 1963). Amino acid imbalance may be defined as a condition in which increase in the concentration of certain amino acids brings about an increase in the amount of other amino acids needed to maintain a given growth rate when protein intake is low (Harper, 1959). In a two week study using gluten, the requirements for lysine as a percentage of the diet and the total amount of lysine required to support growth were both increased (Munaver and Harper, 1959).

The quantity of excess amino acid required to cause toxicity vary greatly. Methionine, cystine, tyrosine, tryptophan, and histidine present in excess quantities are

more toxic than arginine, lysine, threonine, isoleucine and valine (Sauberlich, 1961; Salmon, 1958). The signs and symptoms of toxicity include depression in growth, increased mortality, and histological changes in the liver, skin and pancreas. In amino acid antagonism, excess of the amino acid depresses the utilization of a structurally similar amino acid; e.g., excess of leucine depresses utilization of isoleucine, as in corn proteins (Harper et al., 1955) and excess of lysine depresses utilization of arginine (Jones, 1962).

4. Amino Acid Availability.--This depends on (a) digestibility coefficient of a protein and (b) rate of release of amino acids during digestion. There is evidence that some of the essential amino acids present in proteins may not be fully released after digestion, and the rate of release of different amino acids varies from protein to protein during digestion (Mauron et al., 1955). For example, the ten essential amino acids were all highly available from peanut flour and wheat (92-100%), whereas in cotton seed meal the availability ranged from 64.5-93.4%. The utilization of lysine from 19 food proteins ranged from 49-98%, and of methionine from 48-83% (Guthneck et al., 1953; Schweigert and Guthneck, 1954). Raw soy bean meal gave the lowest values for the availability of both lysine and methionine.

From the review of literature several indications towards the direction of research were obtained. The focal points of interest are:

1. In peas and legume seeds sulfur containing amino acids and tryptophan are limiting.
2. The quantity and quality of seed proteins have been shown to vary with varietal difference.
3. An alcohol insoluble protein fraction contained a high concentration of the limiting amino acid of maize seeds. The genes responsible for the synthesis of this fraction were located and used to obtain nutritionally superior variety of maize.
4. Work with pea proteins indicated that 2 or 3 distinct proteins could be obtained from it.
5. If sulfur in pea proteins could be utilized as an index of sulfur containing amino acids, the albumin fraction which contained twice the amount of sulfur might be expected to have a high biological value. In addition the albumin fraction contained two to three times the concentration of lysine and tryptophan present in either of the globulins.
6. The availability of the amino acids from the water soluble albumin fraction might be higher.

Most indications point to albumin fraction as one of good protein quality.

REFERENCES

Altschul, A. M., Yatsu, L. Y., Ory, R. L. and Engleman, E. M. (1966). Seed proteins. Ann. Rev. Plant Physiol. 17, 113-136.

Boulter, D. and Thruman, D. (1968). Acrylamide gel electrophoresis of proteins in plant systematics. Systematics Assoc. Special volume 2, 39-48 (ed.) Hawk, J. G.

Bolourchi, S. (1967). Wheat as a source of protein for human adults. Effect of wheat protein on nitrogen balance, urea, and amino acid metabolism. Ph.D. Thesis Michigan State University.

Campbell, J. A. (1963). Methodology of protein evaluation. A critical appraisal of methods for evaluation of protein in foods. Div. Food Tech. and Nutr. Faculty of Agric. Sci. American Univ. of Beirut, Lebanon.

Dalby, P. L. and Lillevik, H. A. (1969). Taxonomic analysis of electrophoretic blood serum patterns in cotton rat, Sigmoidon. Biological series 4 (3), Publications of the Museum, Michigan State University.

Danielsson, C. E. (1949a). Investigations of legumin and vicilin. Acta Chem. Scand. 3, 41-49.

Danielsson, C. E. (1949b). Seed globulins of the Gramineae and Leguminoseae. Biochem. J. 44, 387-400.

Danielsson, C. E. (1950). An electrophoretic investigation of vicilin and legumin from seeds of peas. Acta Chem. Scand. 4, 762-771.

Danielsson, C. E. (1952). A contribution to the study of the synthesis of the reserve proteins in ripening pea seeds. Acta Chem. Scand. 6, 149-159.

Danielsson, C. E. (1956). Plant proteins. Ann. Rev. Plant Physiol. 7, 215-236.

Danielsson, C. E. and Lis. H. (1952). Differences in the chemical composition of some pea proteins. Acta Chem. Scand. 6, 139-148.

Dean, R. F. A. (1953). Plant Proteins in Child feeding. Med. Res. Counc. (Brit.) Special Report 279.

Dimler, R. J. (1966). Alcohol insoluble proteins of cereal grains. Fed. Proc. 25, 1670-1675.

Ellis, G. P. (1959). The Maillard reaction. Advances in carbohydrate chemistry 14, 63.

Esh, G. C. (1958). J. Proc. Inst. Chemists (India) 30, 21.

Esh, G. C., De, T. S., and Basu, U. P. (1959). Influence of genetic strain and environment on the protein content of pulses. Science 129 (3342), 148-149.

Evans, R. J., St. John, J. L., Craven, P. M., Hadock, J. L., Wells, D. G., and Swenson, S. P. (1947). Some factors influencing the protein cystine and methionine content of dry peas. Cereal Chem. 24 (2), 150-156.

Evans, R. J. and Bandemer, S. L. (1967). Nutritive value of legume seed proteins. Agric. Food Chem. 15 (3), 439-443.

FAO, Food Balance Sheet, FAO, Rome, Italy (1959).

Food and Nutrition Board (1963). Evaluation of protein quality. Public. 1100 Natl. Acad. Sci. - Nat. Res. Counc. Washington, D. C.

Fowden, L. (1964). The chemistry and metabolism of recently isolated amino acids. Ann. Rev. Biochem. 33, 173-204.

Fox, D. J., Thruman, D. A., and Boulter, D. (1964). Studies on the proteins of seeds of the Leguminoseae. 1. Phytochemistry 3, 417-419.

Goffman, Y. Y. and Vaintraub, I. A. (1960). The use of paper electrophoresis in checking homogeneity of seed proteins. Biochemistry (U.S.S.R.) 25 (6), 1049-1054.

Grant, D. M. and Lawrence, J. M. (1964). Effects of sodium dodecyl sulfate and other dissociating reagents on globulins of peas. Arch. Biochem. Biophys. 108, 552-561.

Greer, M. A. and Astwood, E. B. (1948). The antithyroid effect of certain foods in man as determined with radioactive iodine. Endocrinology 43, 105-119.

Guthneck, B. T., Bennet, B. A., and Schweigert, B. S. (1953). Utilization of amino acids from foods by the rat. J. Nutr. 49, 289-294.

Ham, W. E. and Sandstedt, R. M., and Mussehl, F. E. (1944). A proteolytic inhibiting substance in the extract from unheated soyabean meal. J. Biol. Chem. 154, 505-506.

Hansen, J. D. L., Schendel, H. E., Wilkins, A. and Brock, J. K. (1960). Pediatrics. 25, 258.

Harper, A. E. (1959). Amino acid balance and imbalance. 1. Dietary level of protein and amino acid imbalance. J. Nutr. 68, 405-418.

Harper, A. E. (1964). Amino acid toxicities and imbalances. In Allison, J. B. and Monroe, H. N. (eds.) Mammalian protein metabolism. 2, 119.

Hartig, T. (1856). Botan. Z. 14, 257. In Bonner, J. and Varner, J. E. (eds.), p. 775. Plant Biochemistry. Acad. Press.

Hatz, C., Leuthardt, F. (1967). Isolation and characterization of fructose diphosphate aldolase from Pisum sativum. Biochim. Biophys. Acta 139, 460-468.

Hegsted, D. M. (1962). The role of wheat in the World's food supply. Report of conference. April 30th-May 2nd at Albany, California.

Jones, J. D. (1962). Observations on the toxicity of lysine. (Abstract) Fed. Proc. 21, 1.

Kakade, M. L. and Evans, R. J. (1965). Growth inhibition of rats fed navy bean fractions. Agric. Food Chem. 13, (5) 450-452.

Lamport, D. T. A. (1965). The protein components of primary cell walls. Advanc. Botan. Res. 2, 171-218.

Mattern, P. J., Johnson, V. A., Schimdt, J. W. (1968). Improvement of the nutritional quality of wheat through increased protein content and improved amino acid balance. Fifth semiannual progress report. Dept. Agronom. Univ. Nebraska, Lincoln.

Mauron, J., Mottu, F., Bujard, E. and Egli, R. H. (1955). The availability of lysine, methionine and tryptophan in condensed milk and milk powder. In vitro digestion studies. Arch. Biochem. Biophys. 59, 433-451.

McCalla, A. G. (1949). Nitrogenous constituents of plants. Ann. Rev. Biochem. 18, 615-638.

Mertz, E. T., Bates, L. S. and Nelson, O. E. (1964). Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science 145, 279-280.

Mertz, E. T., Vernon, O. A., Bates, L. S. and Nelson, O. E. (1965). Growth of rats fed opaque-2 maize. Science 148, 1741-1742.

Mertz, E. T. (1966). Improving the nutritional value of cereal grains by the use of gene mutations and selective breeding. Fed. Proc. 25, 1662.

Meyer, H. F. (1960). Infant foods and feeding practices. Thomas, Springfield, Illinois.

Mickelsen, O. and Yang, M. G. (1966). Naturally occurring toxicants in foods. Fed. Proc. 25 (1), 106-123.

Mitchell, H. H. and Block, R. J. (1946). Some relationships between amino acid content of proteins and their nutritive value for the rat. Biochem. J. 163, 599-620.

Mossé, J. (1966). Alcohol soluble proteins of cereal grains. Fed. Proc. 25, 1663-1669.

Munaver, S. M. and Harper, A. E. (1959). Amino acid balance and imbalance. 11. Dietary level of protein and lysine requirement. J. Nutr. 69, 58-64.

Niyogi, S. P., Narayana, N. and Desai, B. P. (1932). Ind. J. Med. Res. 19, 1041. In Influence of genetic strain and environment on the protein content of pulses. Esh, G. C., De, T. S. and Basu, U. P. (1959). Science 129, p. 148.

Osborne, T. B. (1924). The vegetable proteins. 2nd edition. (Longmans, Greens and Co., London, England) 168.

Osborne, T. B. and Campbell, G. F. (1896). Legumin and other proteids of pea and vetch. J. Am. Chem. Soc. 18, 583-608.

Osborne, T. B. and Campbell, G. F. (1898). The proteids of pea, lentil, horse bean and vetch. J. Am. Soc. 20, 410-427.

Parpia, H. A. B., Narayana Rao, M., Rajagopalan, R. and Swaminathan, M. (1964). J. Nutr. Diet. (1), 114.

Patwardhan, V. N. (1956). Ind. J. Med. Sci. 10, 1. In Nutritive value of vegetable proteins. Part 1. Phansalkar, S. V., Ramachandran, M. and Patwardhan, V. N. (1957). Ind. J. Med. Res. 45, 612.

Patton, S. (1955). J. Dairy Sci. 38, 457. In Albanese, A. A. (ed.) Newer methods of nutritional biochemistry. 3, 210 Acad. Press, N.Y.

Pesola, V. A. (1955). Protein content of field pea seeds as a varietal character. Acta Agral. Fenn. 83, 125-132.

Phansalkar, S. V. and Patwardhan, V. N. (1956). Utilization of animal and vegetable proteins. Nitrogen balance at marginal protein intakes and the determination of minimum protein requirements for maintenance in young Indian men. Ind. J. Med. Res. 44, 1-10.

Phansalkar, S. V., Ramachandran, M. and Patwardhan, V. N. (1957). Nutritive value of vegetable proteins. Part 1. Protein efficiency ratios of cereals and pulses and the supplementary effect of addition of leafy vegetables. Ind. J. Med. Res. 45 (4), 611-621.

Raacke, I. D. (1957a,b,c). Protein synthesis in ripening pea seeds. 1. Analysis of whole seeds. 2. Development of embryos and seed coats. 3. Study of the pods. Biochem. J. 66, 101-110, 110-113, 113-116.

Ries, S. K., Chmiel, H., Dilly, D. R. and Filner, P. (1967). Increase in nitrate reductase activity and protein content of plants treated with simazine. Proc. Natl. Acad. Sci. 58 (2), 526-532.

Russel, W. G., Taylor, M. W., Mohroff, T. G. and Hirch, R. R. (1946). The nutritive value of the protein of varieties of legumes and the effect of methionine supplementation. J. Nutr. 32, 313-325.

Saxena, H. C., Jensen, L. S. and McGinnis, J. (1962). Failure of amino acid supplementation to completely overcome growth depression effect of raw soybean meal in chicks. J. Nutr. 77, 259-263.

Sachs, J. (1874). Text book of Botany. 51. Leipzig.

Salmon, W. D. (1958). Am. J. Clin. Nutr. 6, 487-

Sauberlich, H. E. (1961). Studies on the toxicity and antagonism of amino acids for weanling rats. J. Nutr. 75, 61-72.

Schweigert, B. S. and Guthneck, B. T. (1954). Utilization of amino acids from foods by the rat. III. Methionine. J. Nutr. 54, 333-334.

Schweizer, C. J. and Ries, S. K. (1969). Protein content of seed: Increase improves growth and yield. Science 165, 73-75.

Scrimshaw, N. S., Behar, M., Wilson, D., Viteri, F., Arroyave, G. and Bressani, R. (1960). Am. J. Clin. Nutr. 9, 196. In Albanese, A. A. (ed.) Newer methods of nutritional biochemistry. 3, 229.

Shadmanov, R. K. (1964). Evolyutsionnya Biokhim. Rast. 128-137 (N.V. Tsistsin, ed. Moscow)

Smirnova, T. I., Poglazov, B. F. and Kretovitch, V. L. (1959). Biochemistry (U.S.S.R.). 24, 695-697. In Altchul, A. M., Yatsu, L. Y., Ory, R. L. and Engleman, E. M. (1966). Seed proteins. Ann. Rev. Plant Physiol. 17, 116.

Stahman, M. A. (1963). Plant proteins. Ann. Rev. Plant Physiol. 14, 137-158.

Steward, F. C. and Street, H. E. (1947). The nitrogenous constituents of plants. Ann. Rev. Biochem. 16, 471-502.

Swaminathan, M. (1967). Availability of plant proteins. In Albanese, A. A. (ed.) Newer methods of nutritional biochemistry 3, 197-237. Acad. Press.

Swaminathan, M. S. and Austin, A. (1968). Studies on protein quality of sorghum, millet, wheat and other cereal grains for the period 1967-1968. Ann. Progress Report Cummings Laboratory, Ind. Agric. Res. Inst. New Delhi, India.

Van Etten, C. H., Miller, R. W., Wolff, I. A. and Jones, Q. (1961). Agric. Food Chem. 9, 79-82, 433-435. In Stahman, M. A. (1963). Plant proteins. Ann. Rev. Plant Physiol. 14, 144.

Varner, J. E. and Schidlovsky, G. (1963a). Intracellular distribution of proteins in pea cotyledons. Plant Physiol. 38, 139-144.

Varner, J. E. and Schidlovsky, G. (1963b). Intern. Seed Protein Conf. U.S.D.A., New Orleans.

Wetter, L. R. and McCalla, A. G. (1949). Electrophoretic properties of pea proteins. Canad. J. Res. C 27, 96-102.

Young, J. L. and Varner, J. E. (1959). Enzyme synthesis in the cotyledons of germinating seeds. Arch. Biochem. Biophys. 84, 71-78.

PART I

BIOLOGICAL VALUE OF THE PROTEIN IN DIFFERENT VARIETIES OF PEAS

BIOLOGICAL VALUE OF THE PROTEIN IN DIFFERENT VARIETIES OF PEAS

ABSTRACT

The biological value of 32 varieties of peas (Pisum sativum L.) grown under similar field conditions was determined using weanling rats, by means of net protein utilization (NPU) and protein efficiency ratio (PER). Different varieties of peas when fed as the sole source of protein at a 10% level in an otherwise adequate diet varied, in comparison to casein, from 0.18-0.78 in their ability to support growth and nitrogen retention as estimated by NPU and PER. From analysis of rat growth curves, the pea varieties were separated into those that produced fairly good growth and those which barely maintained the initial weight of the rats. Carcass protein as a percentage of body weight was higher in pea fed (20-22%) than in casein fed rats (18-19%). This was probably associated with a difference in the body fat content. The total nitrogen content in different varieties of peas provides a poor indication of their biological value.

INTRODUCTION

Legumes are an important source of dietary protein in developing countries for several reasons. In a few countries a large number of people are vegetarians for one reason or another and so depend on seeds as a primary source of protein. Legumes, in their dry state, can be stored for long periods of time and can be transported for human consumption with a minimum amount of special treatment. The ease of storage, preparation and transport of dry leguminous seeds makes them ideal protein sources for use in disaster areas.

Although India is the biggest consumer of legumes (FAO, 1957) with a per caput intake of 60.8g per day; the inhabitants of the United States and United Kingdom consume a fairly large quantity (10.6 and 11.0g respectively) of legumes per day. In the United States peas are used in baby foods and if special foods for the aged are marketed they might find use in them.

Most of the work on peas has been directed towards improving their appearance, yield or freezing and canning qualities. The little work that has been done on the nutritional value of peas (Esh et al., 1959; Pesola, 1955) has been limited to increasing their nitrogen content. Almost no work has been reported on the biological qualities of legume proteins from different varieties of seeds, although it appears logical to assume that such differences exist.

New interest arose in improving the nutritional value of seed proteins when Mertz and his collaborators (Mertz et al., 1964, 1965, 1966) showed that the biological value of maize protein could be improved to such an extent that it approached or equalled that of casein. This resulted from a threefold increase in lysine and tryptophan content of the maize.

The present study was conducted to evaluate the biological value of the nitrogen components of 32 varieties of peas, hoping thereby to find some varieties with superior biological value to be used in future breeding experiments.

EXPERIMENTAL

The peas were grown at the Horticulture Research Center, East Lansing in 1966. Rainfall was adequate for growth. The peas were dried on the vine and later harvested by hand. The seeds were stored until used at 35-40°F and low humidity. Alaska peas, a commercial variety (lot number 54401) grown under standard conditions of commercial seed production were used for comparison.

The peas were ground in a Wiley mill until the powder passed through a 20 mesh screen. All samples were analyzed for nitrogen by the Kjeldahl method (AOAC, 1965). The nitrogen value was multiplied by a factor of 6.25 (Hegsted, 1964) to facilitate the comparison of the

nitrogenous fraction of the peas with casein, which was used as the reference standard in the bioassays.

For the bioassays, the pea meal was added to a purified ration[1] at the expense of sucrose. All the samples were incorporated into the ration to provide a total nitrogen equivalent to 10% protein level.

Weanling male Sprague Dawley rats were maintained on grain ration[2] for a week. Five rats were assigned to each group in such a way that the group weights did not vary by more than 3g. An extra group of 5 rats in each assay was used as controls for the evaluation of changes in body protein content. Since the 32 varieties of peas could not be assayed in the same experiment, four separate experiments were performed. Casein was repeated in every experiment, and a few varieties of peas were assayed 2-3 times.

One group of rats in each assay (body composition controls) was sacrificed at the start of the experiment. The carcass (minus the gastro intestinal contents) of each of those rats was analyzed for nitrogen thereby providing values for the initial quantity of protein in the carcasses.

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[1]Purified ration: Corn oil 5%, Salt mix 4%, Vitamin mix 2.2% (obtained from Nutritional Biochemicals), Alpha cel 2%, Peas or casein to provide 10% protein (based on nitrogen x 6.25), Sucrose to make up 100%.

[2]Grain ration: Ground corn 60.7%, Soy bean meal (50% protein) 28%, Alfalfa (17% protein) 2%, Fish meal (12.5 protein) 2.5%, Dried whey (67% lactose) 2.5%, Lime stone (38% calcium) 1.6%, Dicalcium phosphate (18.5% P, 22-25% Ca) 1.75%, Iodized salt 0.5%.

The remaining animals were fed the experimental diets for a period of 3 weeks. Food was provided ad libitum. The weight gains and the feed intake was measured every week. At the end of the 3 week period the animals were sacrificed, the contents of the gastro intestinal tract removed, the animals were autoclaved, homogenized (Mickelsen and Anderson, 1959) and a sample thereof analyzed for nitrogen. From this value, the total nitrogen in the carcass was calculated. Biological values of the proteins in the diets were calculated as:

$$\text{PER} = \text{Weight gain / g protein eaten}$$

$$\text{NPU} = \frac{\text{Nitrogen retained in carcass}}{\text{Nitrogen intake}} \times 100$$

RESULTS AND DISCUSSION

The protein content based on the nitrogen values, of vine dried peas varied from 21-28% (Table 3). The nitrogen content may not be an accurate estimate of the total protein. It is possible that different varieties of peas contain different quantities of non-protein-nitrogen (NPN). The NPN may contain peptides and amino acids which are nutritional equivalents of protein, and other compounds which cannot be utilized by monogastric animals. To reduce the non-utilizable NPN (other than the nucleic acids necessary for protein synthesis) and improve the biological

Table 3.--Percent Protein in Peas, NPU, PER, Percent protein in carcass and the weight gain on pea diets in comparison to casein diet.

Protein Source[4]	Percent Protein in the Supplement (Nx6.25)	NPU[5]	Protein Efficiency Ratio	Percent Protein in Carcass	Experimental Weight Gain of Rats
Casein	87.5	52.34	2.78	18.83	75.61
66-1	24.4	32.57	1.44[3]	22.62[3]	33.8
66-2	24.4	25.95	1.28[3]	20.28	31.6
66-3	25.9	16.11	0.76[3]	21.21[3]	23.4
67-4	25.5	39.53	2.11[3]	18.73	39.2
66-5	25.8	9.66	0.46[3]	21.02[3]	7.73
66-6	25.4	30.59	1.40[3]	21.85[3]	33.8
66-7	27.2	36.84	1.80[3]	20.47	42.61
66-8	27.2	26.36	1.16[3]	22.73[3]	33.0
66-9	27.2	22.26	1.00[3]	22.27[3]	21.6
66-10	28.1	32.63	1.61[3]	20.27	20.4
66-11	28.2	30.93	1.46[3]	21.19[3]	34.6
66-12	22.9	31.00	1.56[3]	21.60[3]	37.4
66-13	28.5	28.10	1.49[3]	18.86	28.8
66-14	26.9	33.66	1.70[3]	19.8	35.8
66-15	26.7	37.50	1.86[3]	20.20	34.8
66-16	28.3	27.18	1.34[3]	20.30	27.2
66-17	27.7	31.99	1.69[3]	20.40	32.0
66-19	25.2	31.55	1.57[3]	20.10	24.8
66-20	23.5	33.75	1.67[3]	20.21	27.8

Table 3.--Continued

Protein Source[4]	Percent Protein in the Supplement (Nx6.25)	NPU[5]	Protein Efficiency Ratio	Percent Protein in Carcass	Experimental Weight Gain of Rats
66-22	21.6	35.48	1.83[3]	19.39	60.85[6]
66-23	24.8	37.70	1.88[3]	20.06	35.8
66-24	26.3	41.28	2.05[3]	20.14	35.2
66-26	25.1	35.71	1.76[3]	20.29	35.2
66-27	23.6	26.67	1.28[3]	20.84	22.0
66-28	23.7	23.47	1.16[3]	20.24	24.6
67-28	21.2	32.30	1.65[3]	19.58	20.8
66-31	26.7	44.44	2.20[3]	20.20	37.1
66-33	26.9	33.62	1.80[3]	18.68	36.6
66-34	28.5	34.37	1.85[3]	18.58	38.2
66-38	28.2	25.58	1.28[3]	19.99	13.0
66-52	23.5	26.27	1.31[3]	20.06	43.29[6]
Alaska	22.9	13.65	0.635[3]	21.5[3]	3.00

[3]Significantly different from casein at ($P<0.05$).

[4]Casein was vitamin-free assay protein - secured from General Biochemicals. The numbers refer to the different varieties of peas with the first two figures indicating the year when the peas were grown.

[5]$$\text{NPU} = \frac{\text{Nitrogen retained in carcass}}{\text{Nitrogen intake}} \times 100$$

[6]The weight gains are higher in this experiment because the animals were fed experimental diets for a period of 5 weeks instead of the 3 weeks in all other experiments.

value of protein in peas should be an important consideration in any pea breeding study.

The protein efficiency ratio is a function of weight gain and food intake. On the basis of weight gain, the best pea varieties (67-4, 66-24, 66-31; Table 3) were approximately half as effective as casein. The PER of these varieties was closer to casein than anticipated, possibly due to the lower food intake of pea-fed rats. The reduced intake of food on pea diets may be due to depression of appetite brought about either by poor protein quality or the presence of toxic substance(s) in the uncooked peas. That there is a difference in the response of the animal to the two types of diets (casein vs. peas) is suggested by the observation that the size of the stomach and cecum of the rats fed pea diets were 2-3 times larger than those of the casein fed rats.

The PER of the same sample when assayed more than once remained fairly constant (Std. dev. for PER = 0.12, and NPU = 2.12, Table 4). Small deviations occurred when periods as long as 6 months intervened between 2 assays. Similar results have been observed by other workers (Chapman et al., 1959; Campbell, 1963; Jansen, 1962). The variation was much greater when the value of the PER was low, for the higher values the deviation in the results of the same sample between assays was surprisingly small. The greater deviation in the values for peas of low biological value

stems from the fact that small fluctuations in body weight gains of rats markedly influence the values.

Table 4.--Variation in PER and NPU Between Experiments.

Source of Protein	Experiment 1 PER	Experiment 1 NPU	Experiment 2 PER	Experiment 2 NPU	Experiment 3 PER	Experiment 3 NPU	Experiment 4 PER	Experiment 4 NPU
Casein	2.77	52.16	2.67	56.0	2.89	50.20	2.80	51.00
66-5	-	-	0.25	9.60	0.67	7.60	0.46	11.8
66-7	-	-	1.80	34.90	1.79	36.60	1.74	39.06
66-28	-	-	-	-	1.30	23.52	1.02	23.42
66-14	-	-	-	-	1.68	35.22	1.72	32.1
66-33	-	-	-	-	1.82	35.20	1.78	32.70
66-24	-	-	-	-	2.00	39.1	2.10	43.5
67-4	2.20	40.8	-	-	-	-	2.02	38.40

Standard deviation: A common standard deviation was calculated for each parameter since the variances ($P < 0.01$) were equal.

Standard deviation for PER = 0.12.

Standard deviation for NPU = 2.12.

The net protein utilization is based on the fraction of consumed nitrogen retained in the carcass. Obviously nitrogen retention in the carcass is determined by weight gain and carcass composition. Since the ratio of weight gain to food intake is already determined by PER, the factor of importance in differentiating NPU from PER is

the percentage of protein in the carcass. The percentage of protein in the carcass of rats fed some varieties of peas was significantly higher ($P<0.05$) than in casein fed rats (Table 3). The higher protein percentage in the carcass of pea fed animals suggests that they had a smaller amount of fat in their carcass.

The PER and NPU values for casein are within the range of reported values (Morrison, 1964).

From the rat growth curves (Figure 1), pea diets can be designated as supporting: (1) fairly good growth, (2) mediocre growth and (3) bare maintenance of initial weight. Rats fed poor quality diets usually showed an initial loss of weight which was regained in the latter part of the assay.

For the peas assayed, both PER and NPU varied 3-4 fold between the best and poorest quality peas. Furthermore, the best and the poorest varieties (67-4 vs. 66-5) had similar quantities of nitrogen showing that the superiority in protein quality is not related to total nitrogen content.

The observation that some varieties of peas (66-24, 66-15, 66-7, 66-34) contain large amounts of high quality protein suggests that it might be possible to improve these qualities by selective crossing. The peas should be bred to be appealing from a visual and nutritional point of view.

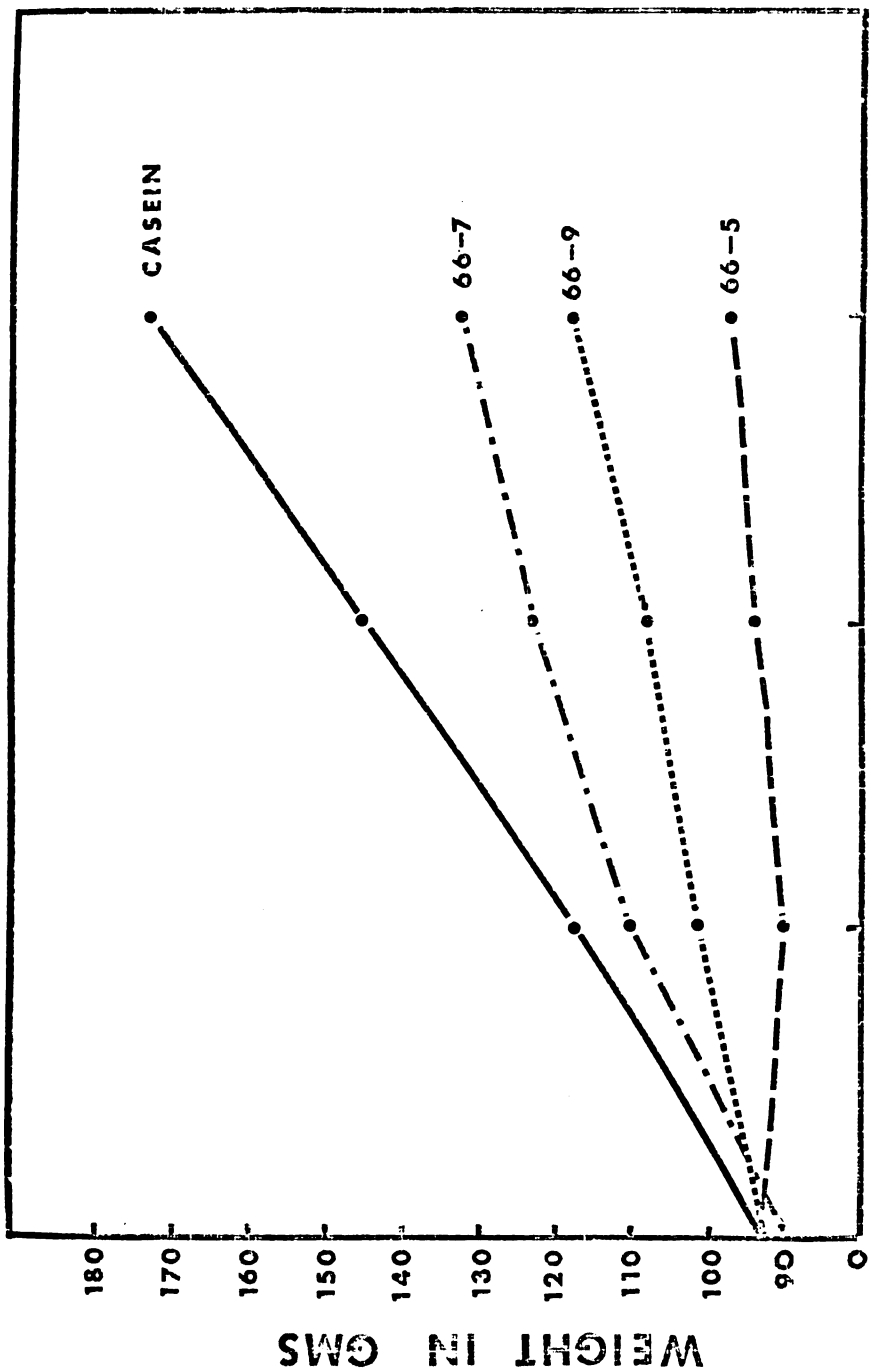


Figure 1. Growth curves of rats fed pea and casein diets.

REFERENCES

AOAC (1965). Official methods for analysis (Association of official agricultural chemists) Washington, D. C. 10th edit., 774-775.

Campbell, J. A. (1963). Methodology of protein evaluation. A critical appraisal of methods for evaluation of proteins in foods. Div. of Food Tech. and Nutr. Faculty of Agric. Sci. American Univ. of Beirut, Lebanon. Public. No. 21.

Chapman, D. G., Castillo, R., and Campbell, J. A. (1959). Canad. J. Biochem. Physiol. 37, 679.

Esh, G. C., De, T. S., and Basu, U. P. (1959). Science 129, 148-149.

FAO (1957). Food balance sheet. FAO, Rome, Italy.

Hegsted, D. M. (1964). Proteins. In Beaton, G. H. and McHenry, E. W. (eds.) Nutrition, a comprehensive treatise. Vol. 1, p. 116. Acad. Press.

Jansen, G. R. (1962). J. Nutr. 78, 231.

Mertz, E. T. and Bates, L. S., and Nelson, O. E. (1964). Science 145, 279-280.

Mertz, E. T., Vernon, O. A., Bates, L. S., and Nelson, O. E. (1965). Science 148, 1741-1742.

Mertz, E. T., Mossé, J., Dimler, R. J., and Nelson, O. E. (1966). Fed. Proc. 25, No. 6, Part 1, p. 1662.

Mickelsen, O., and Anderson, A. A. (1959). The J. Lab. and Clin. Med. 53, No. 2, p. 282.

Morrison, A. B. (1964). Some aspects of the nutritive value of proteins. In Schultz, H. S. and Anglemier, A. F. (eds.) Symposium on foods: Proteins and their reactions. Westport, Conn. Avi. p. 364.

Pesola, V. A. (1955). Acta Agral. Fenn. 83, 124-132.

PART II

NITROGEN DISTRIBUTION AND BIOLOGICAL VALUE OF PROTEIN FRACTIONS IN DIFFERENT VARIETIES OF PEAS

NITROGEN DISTRIBUTION AND BIOLOGICAL VALUE OF PROTEIN FRACTIONS IN DIFFERENT VARIETIES OF PEAS

ABSTRACT

The distribution of nitrogen in the protein fractions of 9 varieties of peas was studied. The percentage of albumin nitrogen in the pea meal related well ($R = 0.998$) with the protein efficiency ratio of pea meals. The percentage of nitrogen in the other fractions did not relate to the protein quality of pea meals ($R<0.3$).

The biological values of the intact pea meals were estimated by a microbiological method using Streptococcus zymogenes. These microbiological values were expressed as a percentage of the response produced by casein. The values so secured were similar to the protein efficiency ratios and net protein utilization values expressed as a percentage of the comparable value for casein. When the major extractable protein fractions were assayed by the microbiological method, the albumin fraction had a higher value (117-128) than either globulin (64-100) or the residual fraction (26-56). The biological value of the albumin fraction from different varieties of peas was not

significantly different ($P<0.05$), whereas the differences in the values of the globulin and the residual fraction were significant.

INTRODUCTION

The usually accepted procedure for determining the protein content of foods is based on their nitrogen content. The nitrogen values can be converted into figures for protein by means of factors which do not distinguish between amino acids, proteins and other nitrogen containing compounds.

The proteins in some types of foods (meat, dairy products and eggs) are recognized as being of high biological value. The biological values quoted in hand books and food composition tables are averages of values observed in different varieties of the same food. This fact is generally overlooked both by nutrition scientists and plant geneticists. The need to rectify the situation became apparent when it was shown that some varieties of maize contain more lysine and tryptophan than others (Mertz et al., 1964).

Quality of protein in a food depends on its constituent protein fractions. Two factors are of importance in this respect; the biological value of the different protein fractions and their concentration in the food. If one protein fraction has a higher biological value then it

must either (a) have a better combination of essential amino acids, especially those that are limiting in the food or (b) the amino acids present must be more readily available.

That certain proteins are better endowed with limiting amino acids than others has been demonstrated in the alcohol insoluble protein (glutelin) of maize (Dimler, 1966). Varieties of maize which contain more lysine and tryptophan have a high glutelin content and a biological value similar to that of casein (Mertz, et al., 1965; Nelson et al., 1965).

To establish a basis for similar studies with peas, it appeared advisable to utilize the experience gained with maize. This would require:

1. Screening a number of varieties to find those with high and low biological values.
2. The quantitative separation of protein fractions and the correlation of the concentration of the different protein fractions with the biological values of each variety.
3. The evaluation of the biological value of different protein fractions in different varieties of peas.

Information such as this should provide the plant geneticists with a start toward incorporating nutritional quality into their breeding programs.

Although the proteins of peas have been studied by a number of investigators (Osborne and Campbell, 1896, 1898;

Danielsson, 1949a,b, 1950, 1952; Danielsson and Lis, 1952; Raacke, 1957) the techniques for extraction are essentially refinements of the method originally proposed by Osborne and Campbell. The latter considered pea proteins to be comprised of three major fractions, an albumin and two globulins, which they named legumin and vicilin. A number of enzymes have been associated with the albumin fraction which is consequently regarded to be heterogenous (Raacke, 1957). The globulin fraction has been resolved into two well defined proteins, legumin and vicilin, which can be separated by isoelectric precipitation of legumin at pH 4.7 (Danielsson, 1950). Legumin and vicilin are homogenous in free boundary electrophoresis and the ultracentrifuge, under the conditions given by Danielsson (1949b). Elemental analysis for carbon, hydrogen, nitrogen, sulfur and oxygen of the proteins extracted from different species of legume seeds including peas indicated that each legumin and vicilin had a constant composition irrespective of their source of origin. Differences between the vicilin and legumin fraction existed even when they were obtained from the same species of seeds (Osborne and Campbell, 1898). Osborne and Campbell (1898) also detected the existence of a third well defined protein in the albumin fraction which they called legumelin.

Previous studies (Bajaj et al., 1969a) showed different varieties of peas to have a threefold variation in

their biological value as studied by rat growth. On the basis of this observation, peas of contrasting biological value were selected for a study of the distribution of nitrogen in their major proteins, and an estimation of the biological value of these fractions was undertaken.

MATERIALS AND METHODS

The peas were grown at the Michigan State University, Horticulture Research Center plots in East Lansing, Michigan, during the summer of 1966. The rainfall was adequate for good growth. The peas were dried on the vine, hand harvested and stored at 35-50°F under low humidity. Alaska peas, a commercial variety (lot number 54401) grown under standard conditions of commercial seed production were used for comparison. The pea varieties were numbered 66-1, 66-2, ---etc. The first two figures refer to the year of harvest and the others are the variety number. Pea meals refer to dried pea seeds ground in a Wiley mill till the powder passed through a 20 mesh screen.

Casein.--as vitamin free assay protein was used as a reference standard. (Obtained from General Biochemicals.)

Dialysis tubing.--(Number 27/100) was obtained from Union Carbide.

Test organism.--The test organism, Streptococcus zymogenes identified as NCDO 592 was kindly supplied through the courtesy of the National Institute for Research in Dairying, Shinfield, England.

Centrifugation.--unless otherwise specified was conducted at 2000 RPM at room temperature (25°C).

Buffers.--Buffers of the following composition were used. Each liter contained the listed amount of reagents:

Extractant buffer: pH 6.8-7.0; 0.2M NaCl (11.6g); 0.03M Na_2HPO_4 (8.0g); 0.02M NaH_2PO_4 (2.7g)

Borate salt buffer: pH 9.2; 0.02M NaCl (11.6g); 0.3M Borax (4.7g)

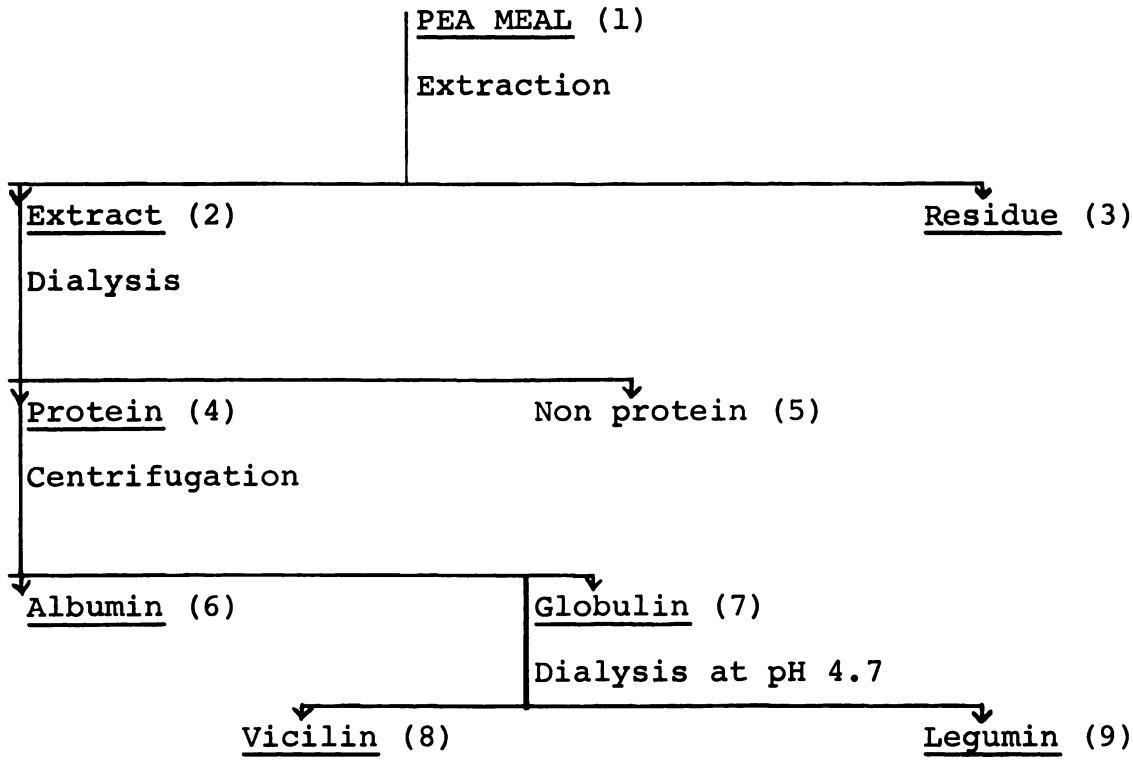
Acetate salt buffer: pH 4.7; 0.02M NaCl (11.6g); Sodium acetate (4g); Glacial acetic acid (2.94 ml)

EXTRACTION AND SEPARATION OF PROTEINS

The Osborne-Campbell procedure (1898) for extracting proteins from peas was elaborated by Danielsson (1950) and later modified by Raacke (1957). Since the quantitative distribution of nitrogen in the major protein fractions had to be considered, it was essential to obtain maximum possible extraction of nitrogen products from pea meals. The earlier procedures were slightly modified.

Pea meal extraction.--(Step 1 and 2 in Figure 2) The ground pea meal was extracted (1g : 10ml buffer) with extractant buffer in a Servall omnimixer operated at 16,000 RPM, for 4 minutes at room temperature (25°C). The extract together with a 5 ml portion of buffer which was used to wash and transfer it to a centrifuge tube, was centrifuged

Figure 2.--Scheme for fractionation of pea proteins*.



*Note the numbers used in the scheme are also used in the text where the details are provided.

for 4 minutes. The supernatant was decanted into a funnel lined with Whatman No. 1 filter paper and collected in a 50 ml volumetric flask. The residue in the centrifuge tube was re-extracted, centrifuged and filtered twice more. Finally 5 ml of additional buffer was used to wash and bring the total volume of the protein extract to 50 ml. The flask was set aside in the cold room (4°C) until the filteration was complete. Since some of the extract was absorbed on the filter paper, the volume of the filtered extract was made up to 50 ml with extractant buffer. This product was termed extract and aliquots of it were used for preparation of other proteins and analyzed for nitrogen.

Residue.--(Step 3 in Figure 2) The residue after extraction was washed to remove salts by dispersing in 20 ml portions of distilled water, centrifuging for 4 minutes, and discarding the supernatant (wash). After two such washings the residue was suspended in 10 ml of distilled water and lyophilized. The powder thus obtained was termed residue and used for microbiological assays.

Albumin.--(Step 6 in Figure 2) An aliquot of the extract was put into a dialysis bag and dialyzed against 100 times its volume of distilled water for 48 hours at 4°C. During dialysis the water was changed 4-5 times. At the end of the dialysis period a thick white precipitate of the globulins appeared. The contents of the dialysis tube were centrifuged for 1 hour and the supernatant (albumin) was

poured into a graduated cylinder and the volume recorded. Aliquots of albumin were analyzed for nitrogen, used in the microbiological assay, and the remainder lyophilized. The fluffy white powder obtained was termed albumin.

Globulins.--(Step 7 in Figure 2) The globulin precipitate, obtained after the preceding centrifugation was washed twice by dispersing in 20 ml of distilled water, and centrifuged to obtain the precipitated globulins. The supernatant wash containing salts was discarded. The washed globulins were finally suspended in 10 ml of distilled water and lyophilized.

Protein.--(Step 4 in Figure 2) A portion of the dialyzed extract was not further fractionated by centrifugation but lyophilized, for use in the microbiological assay and nitrogen analysis. This portion represented the total extractable protein since the non-protein nitrogen was assumed to have been removed by dialysis.

Vicilin.--(Step 8 in Figure 2) A portion of the washed globulin precipitate was dissolved in borate salt buffer (globulin from 1g pea meal in 20 ml buffer) and dialyzed against acetate salt buffer for 48 hours at 4°C. The contents of the dialysis tube were centrifuged for 1 hour. The supernatant vicilin was decanted into a measuring cylinder and its volume recorded. Aliquots of the vicilin were used for nitrogen and nitrogen analysis, and the remainder lyophilized.

Legumin.--(Step 9 in Figure 2) The precipitate from the preceding centrifugation was washed twice with 10 ml of distilled water to remove salts and then suspended in 10 ml distilled water and lyophilized.

The nitrogen in all the samples was estimated by the Kjeldahl method (AOAC, 1965).

MICROBIOLOGICAL METHOD

The microbiological method (Ford, 1960) was selected for evaluating the biological value of different protein fractions isolated from peas. This method has the advantage of requiring a small sample size and provides a better index of the amino acid availability than the amino acid analyzer. Streptococcus zymogenes was used as the test organism, this organism has the same requirement for exogenous amino acids as the growing rat and is able to utilize intact proteins.

The method of Ford (1960) with the following modifications was used:

1. The solution of salts used in the medium did not contain vanadous sulfate.
2. When sample size was insufficient, the dilution step was omitted.

The protein efficiency ratio (PER) and net protein utilization (NPU) values were obtained by rat assay, when the animals were fed pea diets at 10% protein level. The

details of this experiment are described in Bajaj et al. (1969a). The values of this assay were required to establish a common base for comparison of the microbiological values of pea meals to those obtained by rat assay so that the values for isolated proteins could be interpreted in the light of previous experiments.

RESULTS AND DISCUSSION

The nitrogen content of each protein fraction was expressed as a percentage of the pea meal. The latter were air dried and contained 6-7% moisture.

In the 9 varieties of peas studied in this experiment the nitrogen in the pea meal ranged from 3.71 (66-1) to 4.32 (66-9) (Table 5). The quantity of nitrogen which could be extracted from the pea meal ranged from 2.94-2.35 (66-28, 66-17) and was not related to the total nitrogen in the pea meal. The nitrogen in the extractable protein ranged from 1.93-2.51 (66-1, 66-24). The albumin nitrogen ranged from 0.53-1.06 (66-5, 66-24), a two-fold difference which was found to correlate highly with protein efficiency ratio estimated by rat growth (Bajaj et al., 1969a). The correlation coefficient of the albumin to the PER for these 9 varieties of peas was 0.998. On the basis of this observation the albumin content could be used to predict the PER of peas (Bajaj et al., 1969b). The globulin nitrogen

Table 5.--Nitrogen distribution in pea proteins percent pea meal.

Sample	Percent N in Meal	Percentage of the meal as							
		Extractable N	Residual N	Protein N	NPN	Albumin N	Globulin N	Vicilin N	Legumin N
66-1	3.71	2.35	1.36	1.93	0.42	0.64	1.29	0.58	0.71
66-5	4.03	2.93	1.10	2.23	0.70	0.53	1.70	0.93	1.16
66-7	4.24	2.93	1.31	2.34	0.59	0.70	1.63	0.90	0.84
66-9	4.32	2.82	1.50	2.18	0.63	0.63	1.55	0.56	0.99
66-23	3.82	2.64	1.18	2.29	0.35	0.82	1.47	0.58	0.89
66-24	3.97	2.93	1.04	2.51	0.42	1.06	1.45	-	-
66-26	3.79	2.48	1.31	2.25	0.23	0.68	1.57	0.60	0.97
66-28	3.74	2.94	0.80	2.48	0.46	0.62	1.85	-	-
Alaska	3.97	2.73	1.24	2.22	0.51	0.53	1.69	0.79	1.13

varied from 1.29-1.85 (66-1, 66-28) and neither this nor any other fraction studied correlated with ($R<0.3$) PER.

To evaluate the quality of protein in fractions, an initial comparison of the biological value of the intact pea meal estimated by the microbiological method was made with the PER values obtained by rat assay (Bajaj et al., 1969a). Since the values of the microbiological assay are expressed as a percentage of bacterial growth produced by the pea meal when compared with casein, a similar expression was obtained by dividing the PER or NPU values for these pea samples by that of casein. The NPU and PER ratios thus obtained were not significantly different ($P<0.05$) from the microbiological values of pea meals (Table 6).

The microbiological values of the albumin fraction ranged from 117-128% of the bacterial growth produced by casein (Table 7). These values were not significantly different ($P<0.05$) since the proteins are not absolutely pure and the assay involves a certain amount of variation. On the other hand the differences in the values of the globulin fraction, which ranged from 64-100% were significant ($P<0.05$). The microbiological values of the residue ranged from 28-56%. The peas of high biological value (66-24, 66-7, 66-23) had a residue of low biological value on the basis of the microbiological assay, while those that supported poor growth of rats (66-5 and Alaska) had a residue that supported good growth of the microorganisms.

Table 6.--A comparison of the biological values of pea meals as estimated by the microbiological assay and the values obtained by rat assay[1]

Sample	Microbiological	PER[2] Expt./ PER Casein	NPU[3] Expt./ NPU Casein
66-1	63±2	51	62
66-3	32±6	27	31
66-5	40±6	16	18
66-7	65±6	65	70
66-9	44±4	36	42
66-23	73±6	68	72
66-24	74±3	74	79
66-26	65±7	63	68
66-28	45±2	42	45
Alaska	30±8	23	26

[1]Values of PER and NPU were obtained from Bajaj et al. (1969a).

[2]PER = Weight gain of experimental rats / g protein eaten at 10% protein level.

[3]NPU = Gain in carcass nitrogen of experimental rats / g nitrogen consumed.

A graphical representation of the quantity of albumin nitrogen plotted against the microbiological values is a curve (Fig. 3), the shape of which is similar to the curve between PER and the albumin content of pea meals.

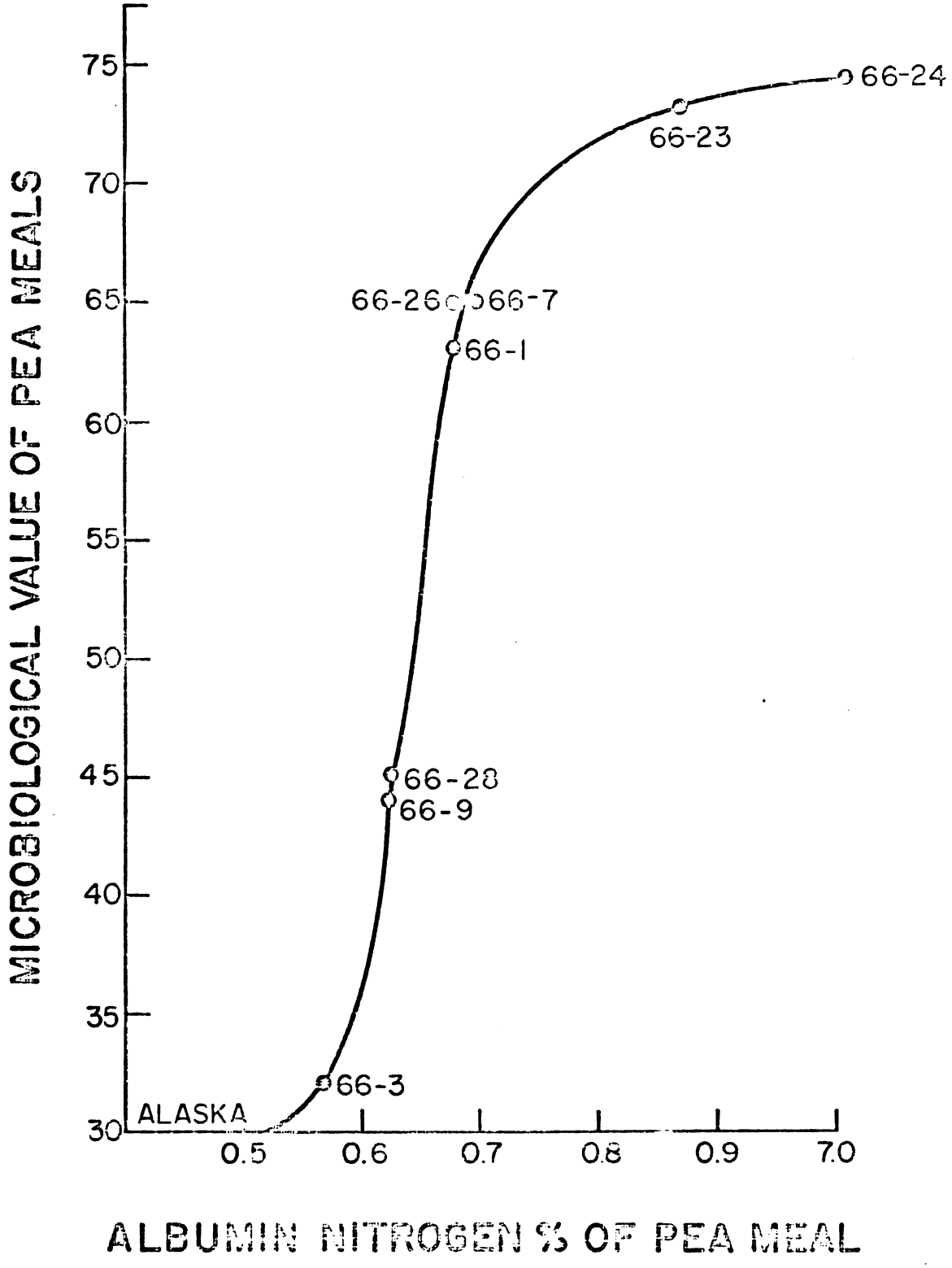


Figure 3 Relation of albumin to the microbiological values of pea meals.

Table 7.--Microbiological assay values* of protein fractions isolated from pea meals.

Sample	Protein fraction		
	Albumin	Globulin	Residue
66-3	118±10	85±9	48
66-5	119±8	64±2	56
66-7	117±8	71±6	29
66-9	121±7	90±5	28
66-23	126±9	94±6	29
66-24	128±9	76±3	28
66-26	120±3	93±6	32
66-28	120±5	100±6	48
Alaska	128±11	97±4	52

*All the values are expressed as the percentage of response produced by the sample when compared with the response secured with casein.

A possible explanation for the high biological value of the albumin fraction and its correlation to the protein quality of pea meals may reside in the sulfur content of the albumin fraction. Osborne and Campbell (1898) reported that the legumelin fraction (albumin) contained 1.06% sulfur whereas the globulins, legumin and vicilin, contained 0.5 and 0.16% respectively. The sulfur content in an isolated protein is present almost exclusively as amino acids, and sulfur containing amino acids limit the biological value of many legumes (Phansalkar et al., 1957).

The albumin fraction is reported to contain 3-4 times more lysine and 3 times more tryptophan than the globulin fraction (Danielsson and Lis, 1952), since both these are essential amino acids and tryptophan is the second limiting amino acid of legumes they may contribute to the superiority of the albumin fraction.

The possibility that the amino acids in the albumin fraction might be more easily available should also be considered. The albumins are water soluble proteins and may be composed of small proteins easily accessible to the proteolytic enzymes. The proportion and the availability of amino acids in the egg albumin has been shown to be perfect for mammalian nutrition (Delhumeau, 1962).

Genetic selection of pea varieties with high albumin content, and the biological value of these varieties should form basis of future experiments concerned with the incorporation of nutritional quality in peas.

REFERENCES

AOAC (1965). Official methods of analysis (Association of official Agricultural chemists, Washington, D. C. edit. 10), 744-745.

Bajaj, S., Mickelsen, O., Baker, L. R. and Markarian, D. (1969a). Biological value of the protein in different varieties of peas (in preparation).

Bajaj, S., Mickelsen, O., Lillevik, H. A., Baker, L. R. and Gill, J. L. (1969b). Prediction of protein quality of peas from albumin content (in preparation).

Danielsson, C. E. (1949a). Acta Chem. Scand. 3, 41-49.

Danielsson, C. E. (1949b). Biochem. J. 44, 387-400.

Danielsson, C. E. (1950). Acta Chem. Scand. 4, 762-771.

Danielsson, C. E. (1952). Acta Chem. Scand. 6, 149-159.

Danielsson, C. E., and Lis, H. (1952). Acta Chem. Scand. 6, 139-148.

Delhumeau, G., Velez Pratt, G., and Gitler, C. (1962). J. Nutr. 77, 52.

Dimler, R. J. (1966). Fed. Proc. 25, 1670-1675.

Ford, J. E. (1960). Brit. J. Nutr. 14, 485-497.

Mertz, E. T., Bates, L. S. and Nelson, O. E. (1964). Science 145, 279-280.

Mertz, E. T., Vernon, O. A., Bates, L. S. and Nelson, O. E. (1965). Science 148, 1741-1742.

Nelson, O. E., Mertz, E. T. and Bates, L. S. (1965). Science 150, 1469-1470.

Osborne, T. B. and Campbell, G. F. (1896). J. Am. Chem. Soc. 18, 583-608.

Osborne, T. B. and Campbell, G. F. (1898). J. Am. Chem. Soc. 20, 410-427.

Phansalkar, S. V., Ramachandran, M. and Patwardhan, V. N. (1957). Ind. J. Med. Res. 45 (4), 611-621.

Raacke, I. D. (1957). Bichem. J. 66, 101-110.

PART III

PREDICTION OF PROTEIN QUALITY OF PEAS FROM ALBUMIN CONTENT

PREDICTION OF PROTEIN QUALITY OF PEAS FROM ALBUMIN CONTENT

ABSTRACT

Varieties of peas (Pisum sativum L.) with higher protein efficiency ratios (PER) contained more albumin nitrogen than those of lower PER, as determined by rat bioassay. For data from 21 varieties of peas, a quadratic function of the albumin content had a high coefficient of correlation (R = 0.949) with PER. The equation provides a simple quick and accurate method for the evaluation of protein quality of peas. The genetic selection of varieties of peas containing more albumin may be important for future improvement of PER values of peas or other legumes.

INTRODUCTION

Until recently, the primary emphasis in plant breeding was on improving the appearance, yield or disease resistance of seed crops. Mertz and his collaborators (1964) showed the possibility of improving the nutritional quality of cereal protein by genetic selection. Since a large part of the world's population is dependent on seed proteins, a redirection of effort in plant breeding appears justified.

Previous work with maize bred for high lysine indicates that most of this amino acid is associated with the alcohol insoluble protein (glutelin) fraction (Dimler, 1966). However, a significant correlation between the concentration of glutelin or some other protein in maize and its biological value has not been reported.

EXPERIMENTAL

Albumins as defined by Osborne (1924) are the water soluble heat coagulable proteins. The original procedure for the extraction of pea proteins was elaborated by Danielsson and Lis (1952) and later modified by Raacke (1957). This procedure was further modified for the present study to obtain better extraction. The results secured depended on the extraction procedure employed (Table 8). Based on the results shown in columns 1 and 2 of Table 8, the procedure was devised whereby the data shown in column 3 were secured.

The field-dried peas used in this study were ground in a Wiley mill until the powder passed through a 20 mesh screen. The pea meal was homogenized for 4 minutes, with Standard buffer (pH = 6.8-7.0)[1] at room temperature (25°C). The homogenate was centrifuged to separate the residue from

---

[1]Standard buffer: pH 6.8-7.0, 0.2M NaCl, 0.3M Na_2HPO4, 0.02M NaH_2PO_4.

Table 8.--Effect of extraction time and procedure on the albumin nitrogen extracted.

	Albumin nitrogen percent of pea meal		
Sample[2]	Extracted[3] Overnight	Extracted[4] 2 minutes	Extracted[5] 4 minutes
66-16	0.461	0.528	0.630
66-19	0.431	0.469	0.650
66-4	0.514	0.560	0.614
66-34	0.525	0.331	0.702
66-24	0.514	0.603	1.060
66-14	0.513	0.580	0.626
66-12	0.606	0.485	0.660
66-31	0.513	0.651	0.779
Alaska	0.514	0.523	0.528
Average	0.510	0.525	0.694

[2]Sample: The first two figures represent the year of harvest, others the variety number.

[3]Extracted overnight with the magnetic stirrer, homogenizer was not used.

[4]Extracted 2 minutes, three times in a homogenizer.

[5]Extracted 4 minutes, three times in a homogenizer, as described in text.

the extract. To ensure maximum extraction, the residue was homogenized twice more with fresh buffer. When Alaska peas were thus extracted, 70.7% of the total nitrogen was obtained in the first extract, 7.6% in the second, and 4.4% in the third extract. The combined extract was filtered

through a Whatman No. 1 filter paper. The filtrate was dialyzed (dialysis tubing 27/100 - Union Carbide) against distilled water (1:100) for 48 hours at 4°C. During dialysis the water was changed 5-6 times. At the end of the dialysis period the globulins precipitated leaving the albumins in solution. The dialyzed solution was centrifuged for 1 hour at 5000 rpm. The albumin (supernate) was decanted into a graduated cylinder. The volume was recorded for use in subsequent calculations. The protein content of the albumin fraction was determined by the micro Kjeldahl method (Bradstreet, 1965).

The PER was determined according to the AOAC procedure (1960). For this, the pea meal was incorporated into a purified ration[2] to provide 10% protein. Weanling (3-week-old) male Sprague Dawley rats were fed the experimental diet for a period of 3 weeks. Casein[3] at 10% level was used as a standard of comparison. The low coefficient of variability of PER (Chapman et al., 1959; Campbell, 1963) was one reason why it was chosen for the evaluation of protein quality.

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[2]Purified ration: Corn oil 5%, Salt mix 4%, vitamin mix 2.2%, Alpha cel 2%, peas or casein to provide 10% protein, Sucrose to make up 100%.

[3]Casein: Vitamin free assay protein was obtained from General Biochemicals.

RESULTS AND DISCUSSION

The PER values of the varieties of peas studied ranged from 0.46-2.20 g weight gain / g of protein eaten, while the albumin content ranged from 0.52-1.06 g nitrogen / 100 g pea meal (Table 9). The direct use of nitrogen for the expression of protein eliminates the necessity of choosing a conversion factor. The moisture content was 6-7% in each of the pea varieties.[4]

In the first experiment only 9 varieties of peas were analyzed for albumin nitrogen. In these varieties of peas the PER was proportional to the albumin content. When a quadratic curve was fit to the initial data, 99% of the variation in PER could be explained by measured differences in albumin (R = 0.998). This high correlation suggested that the equation of the quadratic curve in albumin content as (X) could be used for the prediction of PER (Y).

$$Y = 23.2X - 12.8X^2 - 8.2 \text{ -------- } 1.$$

To check the validity of the equation, additional samples were selected, coded to conceal their identity, and analyzed for albumin. The predicted values of PER were calculated from equation 1. The differences of the predicted values from those obtained by rat assay ranged from 0.0 - 0.18, and the correlation was still high (R = 0.97).

The data from both the experiments were pooled and a new curve with the following equation was obtained:

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[4]Moisture determinations were made in a vacuum oven at 70°C.

Table 9.--Protein efficiency ratio and albumin content of pea varieties.

Sample	Albumin N Percent Pea Meal	PER
66-5	0.526	0.46
Alaska	0.528	0.63
66-3	0.573	0.74
66-38	0.607	1.30
66-28	0.624	1.16
66-14	0.626	1.45
66-9	0.628	1.00
66-16	0.630	1.34
66-1	0.640	1.44
66-19	0.650	1.57
66-12	0.660	1.56
67-28	0.666	1.65
66-4	0.674	1.82
66-26	0.679	1.76
66-7	0.705	1.80
66-34	0.707	1.86
66-17	0.721	1.70
66-15	0.724	1.90
66-31	0.779	2.20
66-23	0.819	1.88
66-24	1.060	2.05

$$Y = 24.7X - 13.6X^2 - 8.8 \text{ -------- } 2.$$

The correlation of the data from 21 varieties of peas to the final curve was R = 0.949.

At the lower levels of albumin, a unit change in albumin content produces a large change in the PER. The behavior of the curve outside the range of values studied cannot be predicted. The effect of increments in albumin above the values studied can be revealed only by further studies.

Examples of predictions across the range of albumin studied will illustrate the preciseness of the procedure. For albumin nitrogen content of 0.5, 0.7 and 1.0%, the predicted values (± standard errors) are 0.11 ± 0.37, 1.79 ± 0.06, and 2.27 ± 0.74, respectively. As in all regression analyses, predictions near the center of the range studied are considerably more precise than those made at the extreme values. The probable validity of predictions made from average albumin content may be expressed as a confidence interval about the predicted PER value. Given an albumin nitrogen of 0.7%, for example, one may have 95 percent confidence that the actual PER value for such cases will fall between 1.67 and 1.91. The length of this interval is well within the range of errors normally encountered in measuring PER.

Correlation such as those reported here have been observed in experiments relating biological values to the

availability of lysine and methionine (Ford, 1960; Campbell, 1963). It is possible that the albumin contains considerable lysine and methionine in a readily available form. Danielsson and Lis (1952) showed that the tryptophan and the lysine content of albumin fraction was 2-3 times higher than that of the globuline (tryptophan is one of the limiting amino acids of legume seeds).

Much effort has been spent searching for simple methods to evaluate protein quality. The method presented here, because of its simplicity and accuracy, merits use in routine assays. Increase in albumin content above present levels and its effect on the nutritional quality of protein should provide an interesting area for continued research, not only in peas but in other legumes as well.

REFERENCES

AOAC (1960). Association of official agricultural chemists. Official methods of analysis. 9th edit. Washington, D. C.

Bradstreet, R. B. (1965). The Kjeldahl method for organic nitrogen. Acad. Press.

Campbell, J. A. (1963). Methodology of protein evaluation. A critical appraisal of methods for evaluating proteins in foods. Div. of Food Tech. and Nutr. Faculty of Agric. Sci. American Univ. of Beirut, Lebanon, pp. 19 and 70.

Chapman, D. G., R. Castillo and J. A. Campbell (1959). Canad. J. Biochem. Physiol, 37, 679.

Danielsson, C. E. and H. Lis (1952). Acta Chem. Scand. 6, 139-148.

Dimler, R. J. (1966). Fed. Proc. 25, 1670.

Ford, J. E. (1960). Brit. J. Nutr. 14, 493.

Mertz, E. T., L. S. Bates and O. E. Nelson (1964). Science 145, 279-280.

Osborne, T. B. (1924). The vegetable proteins. 2nd edit. Longmans Green and Co., London, England.

Raacke, I. D. (1957). Biochem. J. 66, 101-110.

PART IV

A STARCH GEL ELECTROPHORETIC ANALYSIS OF THE NUTRITIONALLY SIGNIFICANT AND SALT-EXTRACTABLE PROTEINS OF PEAS

ABSTRACT

The salt-extractable proteins of peas (Pisum sativum L.) when analysed by starch gel electrophoresis showed 3 or 4 major components. The two slower moving bands corresponded to the globulin fraction, whereas the faster ones related to the albumin fraction. The unfractionated globulins of the pea protein extract migrated as a single streak with 2 bands faintly discernible. When an isolated preparation, of the two separated globulins, legumin and vicilin was subjected to electrophoresis, each migrated as a single band. The inclusion of dissociating and reducing agents (urea, 2-Mercaptoethanol) in the gel produced dissociation and/or scission of the globulins (3 new bands from vicilin, and 4 new bands from legumin) but had no effect on the major albumin component.

INTRODUCTION

According to the classical definition given by Osborne (1924) the proteins in peas are classified as albumins and globulins. The salt soluble proteins have been suggested to function as reserve proteins (Bonner and Varner, 1965). The two well defined proteins of the globulin fraction, legumin and vicilin, were isolated from peas by

Osborne and Campbell (1896, 1898). These researchers also isolated a water soluble protein fraction (albumin), and called it legumelin. The physico-chemical properties of globulins from peas have been studied extensively, and have been reported to be homogenous by both free boundary (Danielsson, 1950) and zonal electrophoresis (Grant and Lawrence, 1964) as well as by analytic ultracentrifuge studies (Danielsson, 1949). Tests on the albumin fraction indicated that several enzymes such as, phosphatase, amylase, and protease, were associated with it, and consequently it might be regarded as micro-heterogenous on polyacrylamide gels (Fox and Thruman, 1964).

The primary concern in most of the aforementioned electrophoretic characterizations of the pea proteins and their fractions has been homogeneity, number of protein components, and their distribution in plant seeds, to ascertain taxonomic relations.

Significant differences in the nutritional value of the total proteins from various varieties of peas have been reported from this laboratory (Bajaj et al., 1969a). Furthermore, the nutritional quality of meals from different varieties of peas was highly correlated to their percentage of albumin nitrogen (Bajaj et al., 1969b). The biological value of the albumin fraction was found to be higher than that of any other protein fraction of peas assayed by a

microbiological method using Streptococcus zymogenes (Bajaj et al., 1969c).

The aim of the present study was to make a preliminary survey of the distribution, homogeneity, association behavior and polypeptide chain structure of the proteins in peas as detected by starch gel electrophoresis. It is also desired to obtain any relation between the electrophoretically identifiable components of the pea protein fractions which were previously tested for biological values.

EXPERIMENTAL

Materials and Reagents

Starch.--Solubilized potato starch (partially hydrolysed) was obtained from Connaught Medical Research Laboratories, Toronto, Canada, and the same lot was used in all experiments.

Buffers.--The buffers contained in the starch gels or the buffer tanks were; 0.76M tris-citrate pH 8.6, 0.3M borate pH 8.6, 0.05M formate pH 3.1 (prepared according to Poulik, 1966).

Stain.--The dye used for staining proteins was amido Schwartz and was prepared and used as described by Poulik, (1966).

Proteins.--The sample proteins (total extractable protein and fractions thereof) were prepared from different pea varieties by procedures outlined in Part II.

Apparatus and Equipment.--The apparatus employed for horizontal starch gel electrophoresis was similar to that described by Sargent (1965). Other standard electrophoretic equipment such as a constant volt power supply, and electrolytically destaining unit were used are described by Bloemendal (1963).

Procedure

Electrophoresis was performed on 300 ml batch of starch gel under the following conditions:

1. A starch gel with no dissociating or reducing agent, under conditions of discontinuous buffers. Tris-citrate was used on the gel and borate in the tanks (method described by Scandalios, 1969).
2. A starch gel with no dissociating or reducing agent, in a continuous tris-citrate buffer (method described by Scandalios, 1969).
3. A starch gel with no dissociating or reducing agent in continuous formate buffer at acidic pH of 3.1 (method described by Scandalios, 1969).
4. A starch gel with 7M urea, in a discontinuous buffer tris-citrate on the gel and borate in the tanks (method described by Wake and Baldwin, 1961).

5. Starch gel with 7M urea and 2-Mercaptoethanol in a discontinuous buffer, tris-citrate on the gel and borate in the tanks (method described by Wake and Baldwin).

Samples.--The samples for electrophoresis were prepared by dissolving in a solution composed of 2% starch prepared in the same buffer that was used for making the gel (7M urea was included when used on the gel). 0.1-0.3ml of dissolved protein sample was directly micropipetted into the slots and covered.

Electrophoresis.--Electrophoresis was conducted at 10-20 milliamperes and 250 volts at 4°C.

After electrophoresis for 8-12 hours, the gel was sliced into 3 layers and the middle layer was retained for staining. The gel was submerged in the dye solution for 15 minutes after which it was electrolytically destained in 7% acetic acid.

RESULTS AND DISCUSSION

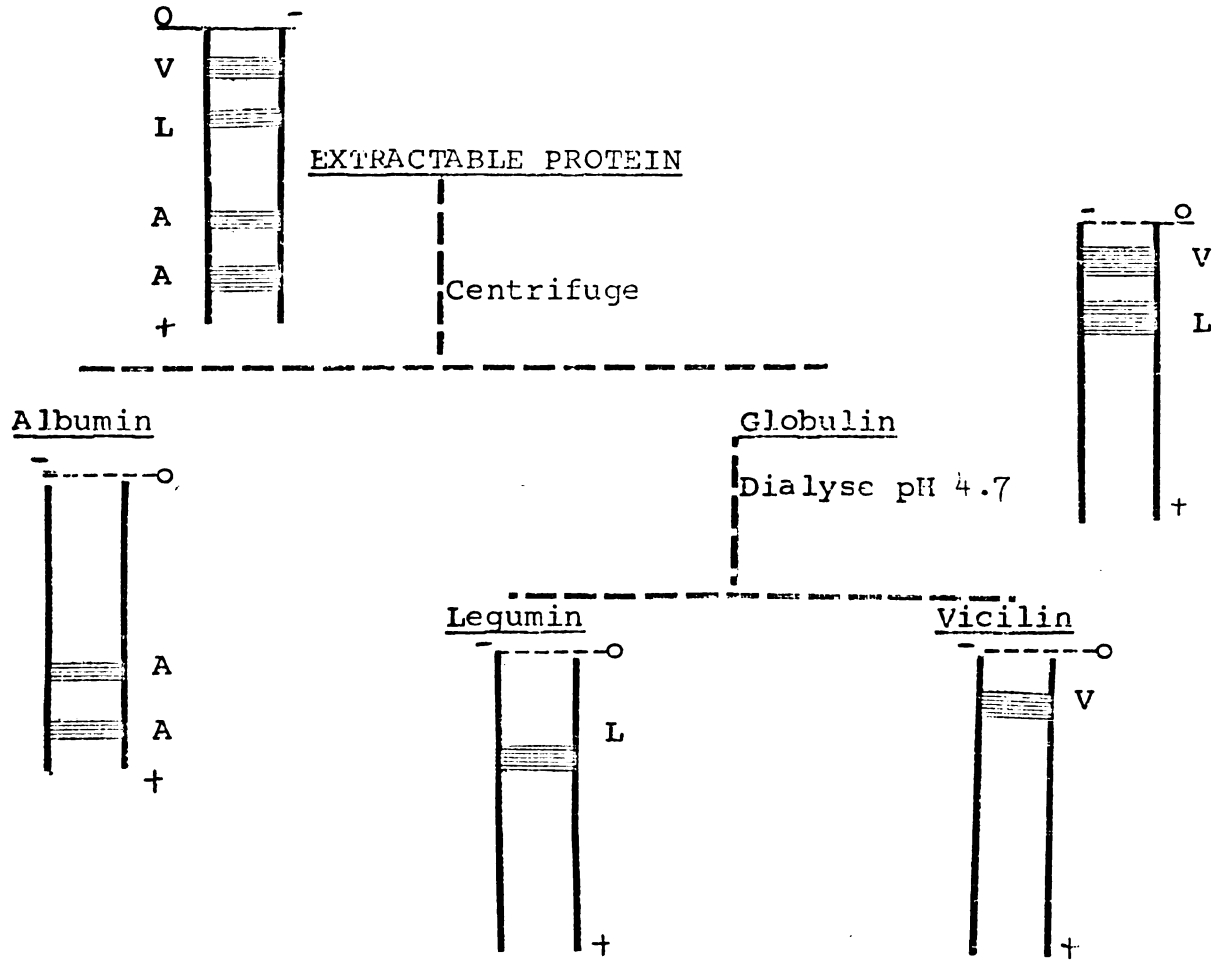
Condition I.--Electrophoretic analysis of the total salt-extractable proteins are seen in Figure 4. The two nearest the origin are heavily streaked (Plate I, slots 1-3) and are regarded as the pea globulins. The fastest migrating band suggests that it comes from the faster moving albumin component (Plate I, slots 4-6; Plate III, slots 1-6). The electrophoretograms of isolated preparations of legumin and vicilin show that legumin migrates ahead of vicilin.

Figure 4

Starch gel electrophoresis

of pea proteins

(No dissociating agents)



*Note the letters, O, V, L, A, refer to origin, vicilin, legumin and albumin respectively.

The presence of one or two major components in the albumin fraction has importance from a nutritional standpoint. If the genes controlling the synthesis of the albumin components could be identified, pea varieties could be selectively crossed to produce an increase in the nutritionally significant protein components. Although various enzymes have been identified in the water soluble extract of peas, these might not account to any great extent for the quantitative distribution shown by the albumin bands.

Condition II: The phenomenon of boundary self sharpening frequently occurs in systems with discontinuous buffers (Ornstein and Davis, 1962). Therefore to check the homogeneity of the albumin fraction, the electrophoresis was performed in a continuous tris-citrate buffer. Under such conditions at pH 8.6 the albumin fraction separated indiscretely, but for the varieties of peas studied two bands were faintly discernible in a heavy streak (Plate II, slots 1-3). The globulins did not resolve under these conditions as seen in Plate II, slots 4-6.

Condition III.--Starch gel without added reagents but run at acid pH of 3.1 in formate buffer, show that the albumin fraction appears mainly as one band (Figure 5) suggesting either (1) the albumin is a single complex at this pH, or (2) one of the two components observed at pH 8.6 has not acquired sufficient mobility.

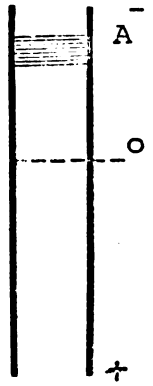
Figure 5

Starch gel electrophoresis

of pea albumins

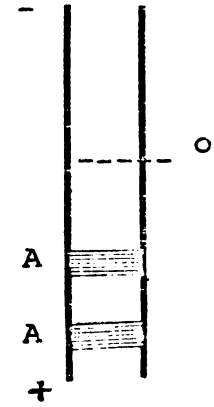
(Continuous buffer)

Formate buffer pH 3.1



Tris citrate buffer

pH 8.6



Condition IV.--The gels run with urea stained very poorly and did not yield much information and therefore they were discontinued.

Condition V.--Electrophoresis of the total salt-extractable protein under these conditions yielded 8 discernible bands (Figure 6; Plate VI, slots 1-3). The 3 closest to the origin can be identified as derived from vicilin (Plate V, slot 6). The major albumin band which migrated the furthests from the origin (Plate V, slot 5) still remained unaltered by the action of urea or 2-Mercaptoethanol. Among the remaining 4 bands which were obviously derived from legumin (by elimination), 2 represent proteins which have changed net charge and migrated towards the cathode in contrast to other proteins (Plate IV, slots 1-6).

From the data obtained it may be concluded that the albumin is composed of 1 or 2 major proteins depending upon the pea varieties studied. Since the albumin fraction was found to have nutritional importance, the identification of the component containing the more essential and available amino acids is important for selecting varieties containing proteins of good nutritional quality.

Figure 6

Starch gel electrophoresis

of pea proteins

(With 7M urea and 2-Mercaptoethanol)

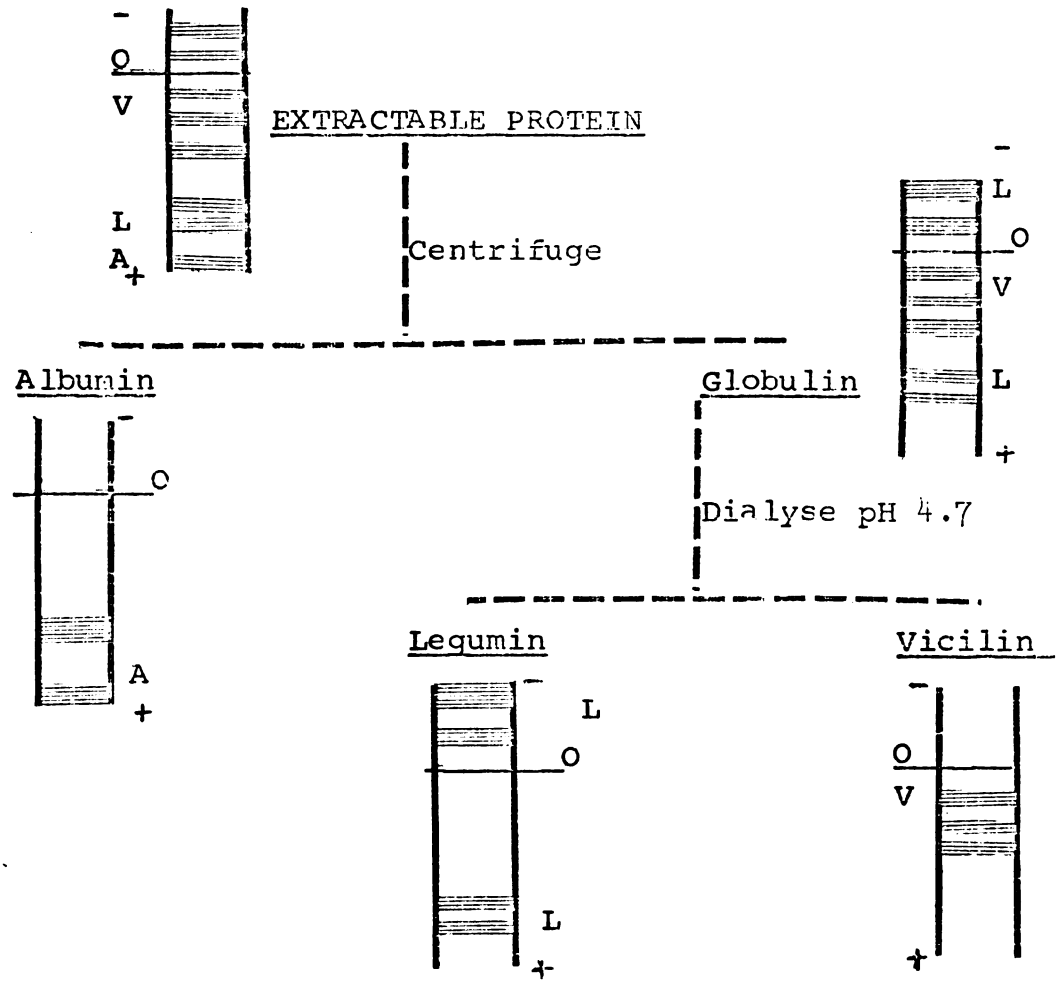
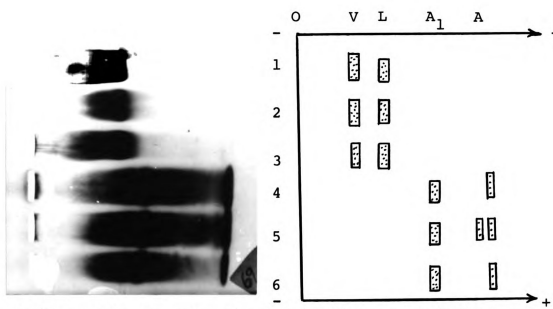
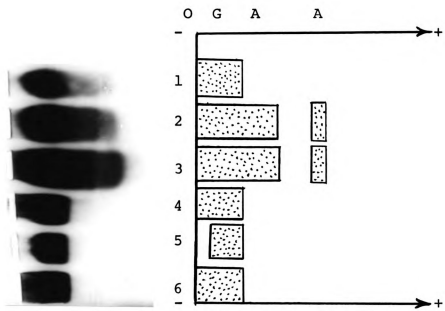


Plate I



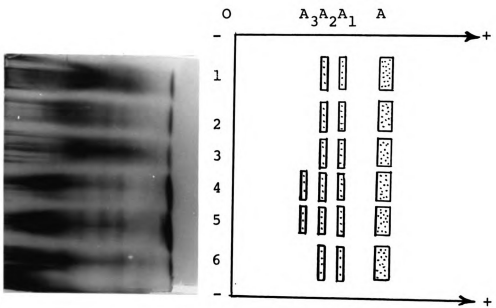
Starch gel electrophoretograms of the albumin and globulin fractions from 3 varieties of peas. Medium: Starch with no dissociating or reducing agents. Gel buffer: 0.76M tris-citrate at pH 8.6. Electrolyte buffer: 0.3M borate at pH 8.6. Running time: 8 hrs. at 4°C. Slots 1,2,3 contained 3% globulin from pea varieties Alaska, 66-24 and 66-15. Slots 4,5 and 6 contained 7% albumins from the corresponding pea varieties. The diagrammatic representation of the gel photographs show the location of the pea protein fractions from the origin (O) and are identified by the letter in the following plates as; vicilin (V), legumin (L) and albumin (A).

Plate II



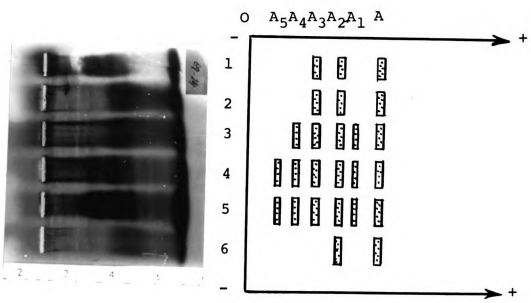
Starch gel electrophoretograms of the albumin and globulin from Alaska peas. Medium: Starch with no dissociating or reducing agents. Gel buffer: 0.76M tris-citrate at pH 8.6. Electrolyte buffer: 0.76M tris at pH 8.6. Running time: 8 hrs. Slots 1, 2, 3 contained albumin (A) at 1, 3, 5% concentration. Slots 4, 5, and 6 contained globulin (G) at 5, 3, and 5% concentration.

Plate III



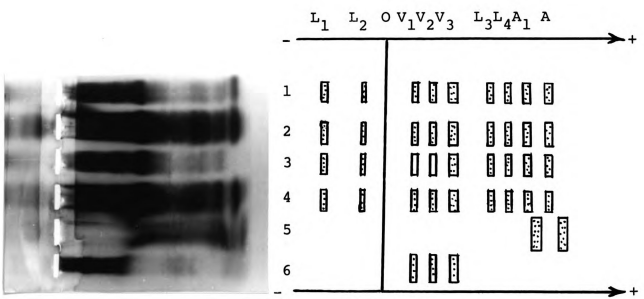
Starch gel electrophoretograms of albumin fraction from 3 varieties of peas. Medium: Starch with no dissociating or reducing agents. Gel buffer: 0.76M tris-citrate. Electrolyte buffer: 0.3M borate at pH 8.6. Running time: 8 hrs. at 4°C. Slots 1, 2, and 3 contained 2% albumin (A) from variety Alaska, 66-15, and 66-24. Slots 4, 5, and 6 contained 3% albumin from corresponding pea varieties.

Plate IV



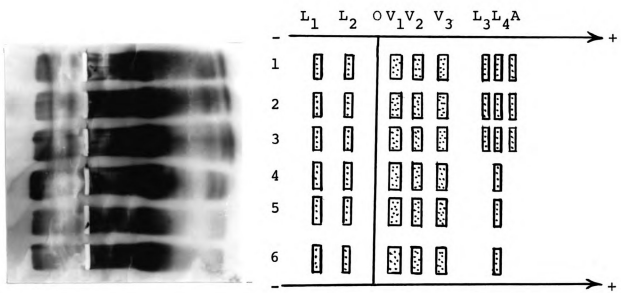
Starch gel electrophoretograms of the albumin fraction from 6 varieties of peas. Medium: Starch with 7M urea and 2-Mercapto ethanol. Gel buffer: 0.76M tris-citrate at pH 8.6. Electrolyte buffer: 0.3M borate at pH 8.6. Running time: 8 hrs. at 4°C. Slots 1-6 contained 4-5% albumin from Alaska, 66-24, 66-15, 67-4, 66-5, 66-7.

Plate V



Starch gel electrophoretograms of pea proteins. Medium: Starch with 7M urea and 2-Mercaptoethanol. Gel buffer: 0.76M tris-citrate at pH 8.6. Electrolyte buffer: 0.3M borate at pH 8.6. Running time: 8 hrs. at 4°C. Slot 6 contained 3% vicilin, slot 5 contained 2% albumin, slot 4 contained 5% globulin, slot 3 contained 2% globulin, slot 2 contained 6% globulin, and slot 1 contained 3% globulin from Alaska pea.

Plate VI



Starch gel electrophoretograms of pea proteins. Medium: starch with 7M urea and 2-Mercaptoethanol. Gel buffer: 0.76M tris-citrate at pH 8.6. Electrolyte buffer: 0.3M borate at pH 8.6. Running time: 8 hrs. at 4°C slots 1, 2, and 3 contained total salt extractable protein from varieties Alaska, 66-24 and 66-15 at 3% concentration. Slots 4, 5 and 6 contained corresponding globulins at 4% concentration.

REFERENCES

Bajaj, S., Baker, L. R. and Mickelsen, O. (1969a). Fed. Proc. (Abstr.) 28 (2), 811.

Bajaj, S., Mickelsen, O., Lillevik, H. A., Baker, L. R. and Gill, J. L. (1969b). Prediction of protein quality of peas from albumin content. (In preparation)

Bajaj, S., Mickelsen, O., Lillevik, H. A. and Baker, L. R. (1969c). Nitrogen distribution and biological value of protein fractions in different varieties of peas. (In preparation)

Bloemendal, H. (1963). Zone electrophoresis in blocks and columns. p 6.

Bonner, J. and Varner, J. E. (1965). Plant biochemistry. Acad. Press, p 774.

Danielsson, C. E. (1949). Biochem. J. 44, 387-400.

Danielsson, C. E. (1950). Acta Chem. Scand. 4, 762-771.

Fox, D. J., Thruman, D. A. and Boulter, D. (1964). Phytochemistry 3, 417-419.

Grant, D. R. and Lawrence, J. M. (1964). Arch. Biochem. Biophys. 108, 552-561.

Ornstein, L. and Davis, B. J. (1962). Disc electrophoresis. Distillation Products Industries, Div. Eastman Kodak Co.

Osborne, T. B. and Campbell, G. F. (1896). J. Am. Chem. Soc. 18, 583-608.

Osborne, T. B. and Campbell, G. F. (1898). J. Am. Chem. Soc. 20, 410-427.

Osborne, T. B. (1924). The vegetable proteins. 2nd edit. Longmans Green and Co., London, England, p. 168.

Poulik, M. D. (1966). Methods of Biochem. Anal. 14, 455-495.

Sargent, J. R. (1965). Starch gel electrophoresis In Methods in zone electrophoresis. p. 55.

Scandalios, J. G. (1969). Biochem. Genetics 3, 37-79.

Wake, R. G. and Baldwin, R. L. (1961). Biochim. Biophys. Acta 47, 225-239.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The 32 varieties of peas grown under similar field conditions were provided by the Department of Horticulture. The peas were vine ripened, harvested by hand, and stored (until used) at 35-40°F under conditions of low humidity.

The peas were ground into meal in the Wiley mill, and analyzed for nitrogen by the Kjeldahl method. The nitrogen was multiplied by a factor of 6.25 to obtain the quantity of crude protein in peas. The total protein thus calculated varied from 21-28% for different varieties of peas.

All 32 varieties of peas were assayed for protein quality using 3-week-old Sprague Dawley rats. Since the number of the pea varieties to be assayed was large, four separate experiments were performed. Based on the simplicity of the method and the reliability of results obtained in the first experiment, net protein utilization (NPU) and protein efficiency ratio (PER) were selected for use in subsequent experiments. Casein at a 10% level, was used in the standard diet for comparison in all experiments. Some of the pea varieties were assayed more than once to obtain an indication of the precision of methods. The PER of the

best and the poorest variety of peas varied fourfold (0.46-2.11), both these varieties (66-5, 66-4) had a similar quantity of protein (25.8, 25.5%). The PER values of some pea lines (66-4, 66-24) were close to that of casein, but the weight gain of the rats on pea diets was approximately half that secured on casein diets (42g vs 75g on casein). The reason for this difference was decreased food intake of pea-fed rats. Both PER and NPU yielded repeatable results, however PER had a lower standard deviation than NPU (0.12 vs. 2.12). The percentage of protein in the carcass of rats fed some varieties of peas (66-5, 66-7, 66-52 and Alaska) was significantly higher than casein fed rats ($P < 0.05$), this difference might be associated with a lower percentage of fat in the carcass of these animals.

To determine the distribution of nitrogen in the major extractable protein fractions, varieties of peas were selected to represent high, low and mediocre PER. Such a selection was expected to reveal contrasts in the distribution of nitrogen in different protein fractions as related to the PER of the pea meals.

The percentage of albumin nitrogen in the pea meal correlated highly with PER ($R = 0.949$). The equation of the quadratic curve which fit the data relating PER to albumin content could be used to predict PER values of pea meals. The percentage of nitrogen in the other protein fractions did not correlate with PER ($R<0.3$).

The biological values of the pea meals were estimated by comparing the growth response produced by adding pea meal to the media for Streptococcus zymogenes, with the growth response produced when casein was used. In the same way, NPU values for the pea meals secured by rat assays were calculated as a percentage of the values for casein. The resulting ratios and the microbiological values were not significantly different ($P<0.05$). When the protein quality of albumin, globulin and the residue was estimated by the microbiological method, albumin had a higher biological value (117-128) than either globulin (64-100) or the residual fraction (26-56). The reason for the high protein quality of the albumin fraction might be a superior amino acid composition in terms of essential amino acids, better availability or both. The biological value of albumin from different varieties of peas was not significantly different ($P<0.05$), but the differences in the globulin and residue were significant.

The extractable proteins of the pea were represented by 3-4 major bands when observed on starch-gel electrophoresis. The slow moving bands were enveloped in a heavy streak and represented the globulin fraction. An isolated preparation of vicilin (a globulin) attracted the globulins when they were in adjacent slots during electrophoresis. The preceding two observations might be interpreted to indicate that the globulin fraction is present as a complex,

consisting of two proteins which can be separated on the basis of solubility. The albumin fraction was represented by one major and several minor bands. The addition of reducing agents (7M urea and 2-Mercaptoethanol) dissociated the globulins (3 bands - vicilin, 4 bands - legumin) but not the major albumin band.

From the evidence presented it can be inferred that:

1. Nitrogen is a poor index of protein quality.
2. Variation in the quantity and quality of protein exists in different varieties of peas even if they contain the same percentage of nitrogen.
3. That 94% of the variation in the protein quality (PER) of the peas studied can be explained on the basis of differences in albumin content.
4. The albumin content of peas can be used to predict their PER.
5. The biological quality of the albumin is better than the other fractions.
6. The albumin fraction contains one or two major proteins.

APPENDIX

A. AMINO ACID CONTENTS OF SELECTED VARIETIES OF PEAS

Choice of Method

With the advent of the amino acid analyzer complete analysis of amino acids which required a long time became possible to obtain in 24 hours. The complete analysis of the essential amino acids can be used to give a fairly accurate estimate of the biological value (Oser, 1951) in most cases except where the proteins are undigestible. Mitchell and Block (1946) devised a system of chemical scores based on the amount of essential amino acid in greatest deficit in a protein compared to the level present in a reference protein selected for its nutritional excellence. Despite the emperical nature of this system the chemical scores were shown (Mitchell, 1954a) to have a high degree of correlation ($r = + 0.95$) with published biological values for a series of proteins. However as Mitchell pointed out in a review (Mitchell, 1954b) the chemical score is an index of the biological value of the protein for growth only, since it assumes that absence of an essential amino acid renders the protein completely unavailable for tissue maintenance, an observation not consistent with

reported observations (McCollum, 1911, Mason and Palmer, 1935). For scoring proteins by Mitchell's method the limiting amino acids of legume seeds need to be determined by methods other than the use of the amino acid analyzer, since some methionine, cystine, and tryptophan are lost by acid hydrolysis.

Oser's method of the integrated amino acid index for predicting biological value of proteins accounts for several factors not considered in Mitchell's methods. Oser's method (Oser, 1959) is based on the observation that for optimum utilization of food proteins all essential amino acids must be available and liberated for absorption at rates permitting mutual supplementation. The probability of such events occurring simultaneously is considered a function of the product of the probability of each essential amino acid being present at the site of protein synthesis. Since the essential amino acid index is a product function, the absence of an amino acid would yield a biological value of zero, which is not consistent with observations with most deficient proteins such as gelatin and gluten. In short term experiments the absent amino acids may be secured from the products of tissue catabolism. This method therefore requires that one assume the presence of small quantities of missing amino acids, to be able to correlate the estimates with reported biological values.

Experiments

The amino acid content of four selected varieties of peas was estimated on the Spinco 120-C Amino acid analyzer. The main purpose of this experiment was to determine if differences in amino acids could account for the observed differences in the biological values. The values reported in Table 10 indicate that methionine values alone cannot explain the differences in the biological value since the variety 66-5 contains the most methionine and has the lowest BV, similarly the essential amino acid index cannot explain the low biological value of this variety. The low BV of 66-5 appears to be due to some other reason than poor amino acid content. The BV of 67-4 as observed by the balance method was 70.7 which is quite like the value obtained by the essential amino acid index (70.9).

The observed values of the amino acids are within the range of reported values for Alaska pea (Biological data handbook) although they are frequently at the upper range.

Table 10.--Amino acid composition of pea meals g/100g nitrogen.

Amino Acid	67-4	66-5	66-7	Alaska pea	Alaska pea[1]	Alaska pea[2]
Tryptophan	12.1	9.3	12.3	10.2	11.9	-
Lysine	49.5	43.8	43.1	50.2	43.1	29.5, 8.9-43.6
Leucine	51.7	49.9	47.0	47.3	40.6	39.0, 29.3-54.6
Tyrosine	25.4	23.1	23.6	24.0	11.25	15.2, 10.0-25.8
Phenyl Alanine	34.8	31.2	31.2	32.5		24.0, 15.1-34.8
Valine	38.2	34.8	31.9	32.2	25.6	25.6, 19.4-32.8
Iso Leucine	30.8	35.1	29.0	28.1	23.1	28.7, 23.0-36.7
Methionine	2.6	3.9	2.1	2.4	4.4	5.0, 1.7- 9.7
1/2 Cystine	-	-	-	-	-	6.8, 3.9- 9.6
Threonine	29.9	25.6	27.3	25.6	25.6	22.9, 17.3-29.8
Arginine	48.7	56.4	63.1	66.9	59.4	55.5
Histidine	15.9	14.8	15.1	13.1	13.7	10.2, 6.1-19.8
Aspartic Acid	82.5	83.0	77.9	75.1	-	-
Serine	34.5	33.1	37.9	30.8	-	-
Glutamic Acid	86.0	131.1	103.0	110.9	-	-
Proline	31.1	22.0	26.0	26.5	-	-
Glycine	29.6	25.9	26.0	30.8		
Alanine	31.1	27.1	30.5	26.5	-	-
*EAA Index	70.9	71.8	64.5	64.1	-	-

[1]Alaska peas--Values from Evans and Bandemer (1967) converted to g/100g nitrogen.

[2]Alaska peas--Values from Biological data handbook.

*Egg protein was used as a standard for calculating essential amino acid index.

REFERENCES

Evans, R. J. and S. L. Bandemer (1967). Agric. Food Chem. 15, (3) 439-443.

Mason, I. D. and L. S. Palmer (1935). J. Nutr. 9, 489.

McCollum, E. V. (1911). Am. J. Physiol. 29, 215.

Mitchell, H. H. (1954a). Wiss. Abhandl. Deut. Akad. Land Wirtsch. 2, 279.

Mitchell, H. H. (1954b). Biological value of proteins and amino acid interrelationships. Quartermaster Food and Container Inst. Surveys. Natl. Res. Counc. Washington, D. C.

Mitchell, H. H. and R. J. Block (1946). J. Biol. Chem. 163, 599.

Oser, B. L. (1951). J. Am. Diet. Assoc. 27 (5), 396-402.

Oser, B. L. (1959). An integrated essential amino acid index. In Albanese, A. A. (ed.) Protein and amino acid nutrition., p. 281, Acad. Press.

B. MOISTURE CONTENT OF PEAS[1]

Table 11

Sample	Percentage Moisture	Sample	Percentage Moisture
66-1	6.81	66-17	6.95
66-2	6.90	66-19	6.82
66-3	6.93	66-20	7.00
66-4	7.01	66-22	6.93
66-5	7.00	66-23	6.86
66-6	6.61	66-24	6.90
66-7	6.89	66-26	6.87
66-8	6.99	66-27	7.11
66-9	6.78	66-28	6.67
66-10	7.12	67-28	6.70
66-11	6.88	66-33	6.80
66-12	7.00	66-34	6.81
66-13	6.69	66-38	7.15
66-14	6.87	66-52	6.77
66-15	7.10	Alaska	6.99
66-16	6.86		

[1]Moisture was estimated in a vacuum oven at 70°C for 48 hours.