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POTATO PROTEIN: NUTRITIONAL EVALUATION
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POTATO PROTEIN: NUTRITIONAL EVALUATION AND UTILIZATION

By

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ABSTRACT

POTATO PROTEIN: NUTRITIONAL EVALUATION AND UTILIZATION

By

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The purpose of this research was to a) study the nutritional quality of potato protein and b) prepare a high protein potato product and use it in breadmaking.

When the N distribution in the raw tuber was studied, it was found that the N content of potatoes is highest in the skin. The Protein Efficiency Ratio (PER) of the skin, however, is practically zero (Meister and Thompson, 1976a). The pith region of the tuber presented the next highest N content and the outer layer the lowest N content. It was also observed that the area near the apical end of the potato had a slightly higher N content than the stem end.

The free amino acids and amides extracted with distilled water showed variations in the levels among the raw potato cultivars (cv) analyzed. In general, the most abundant free amino acids were aspartic and glutamic, and the amides asparagine and glutamine. Free cystine was present as traces in cv Atlantic and Superior potatoes and was not detected in the other varieties. The range in content of free methionine was 0.8 to 2.5 g/100 g free amino acids plus amides.

The whole tuber boiled with intact skin lost the lowest amount of protein, 0.8 g/100 g of original protein content, in comparison to tubers boiled after cutting into halves and quarters either peeled or unpeeled. The highest protein loss, 10.4%, was observed when whole potatoes were peeled, cut into halves, and boiled. After boiling the cv Russet Burbank and Atlantic potatoes with intact skins either cut or uncut, the predominant amino acids in the boiled water were aspartic and glutamic. It was found that the whole tuber boiled with intact skin suffered a minimum loss of cystine and methionine; the largest loss of cystine and methionine was observed in the halved and quartered potatoes.

The protein quality of the potato as determined by the PER showed that the difference in the nutritional value of the unsupplemented or supplemented potato protein was reflected in their varying PERs. Russet Burbank potatoes chemically and organically fertilized presented close PER values 1.62 and 1.54, respectively, statistically not different. Also, the limiting amino acids, cystine and methionine were present in similar concentrations in the potatoes organically or chemically fertilized.

Dried whole eggs, and whey powder were used for supplementing the Russet Burbank potatoes. Commercially dried potato flour prepared from the cv Russet Burbank and mixed

with dried whole eggs in the ratio 65:35 potato protein to egg protein, had a PER which was 8% higher than the PER of whole egg powder. This ratio of potato protein to egg protein yielded a 54% increase in PER when compared to the potato flour alone. When the same commercially dried potato flour was mixed with dried whey in the ratio 90:10 potato protein to whey protein, the PER value of the mixture was similar to that of casein (2.48 versus 2.50) but 13% higher than the PER of the potato flour alone.

A potato flour fraction rich in protein (36.3%; Nx 6.25) was prepared from potato flour by air classification in a Walter Lab. Separator. Bread was made after substituting 5% and 10% of the wheat flour with this fraction named potato protein flour (PPF). A 32 and 51% decrease in loaf volume was observed as a result of this substitution, respectively. There was also a decrease in tenderness (Allo-Kramer Press) in the bread containing PPF. Taste and texture of the substituted bread were lower than those of the control.

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INTRODUCTION

Origin of potatoes and their potential to feed people

The potato (*Solanum tuberosum*) is a plant of Andean origin. Potatoes were first cultivated on the border of Peru and Bolivia. The potato is a widely adaptable crop.

For a long time the potato has been a reliable source of food for man. In its fresh state the potato has, on the average, 2.1% total protein. However, on a dry weight basis its total protein content, 10.4%, is not different from that of wheat grain (10-14%). Per cultivated unit area the amount of potato protein produced is higher than that of wheat, corn or rice. A hectare (2.47 acres) produces 226 Kg of potato protein as a world average; this is a yield greater than that in wheat grain protein (200 Kg/ha) or rice grain protein (168 Kg/ha) (FAO, 1972a).

The potato has a good potential to feed people in relation to other crops. Borgstrom (1969) stated that one hectare of land under potato cultivation can supply the protein requirement for 10 people while the protein of wheat can satisfy only 6 people.

In some developing areas the cost of protein of animal origin limits its consumption; many people get their

protein from plants. One example is South America where in some regions potatoes constitute a valuable protein and energy source for the human diet. Kies and Fox (1972) stated that the potato is a very important source of dietary protein for some groups of high consumption of it.

The objective of this study was a) to evaluate the nutritional quality of potato protein and b) prepare a high protein potato product and use it in breadmaking.

LITERATURE REVIEW

A. Botany of the potato tuber-physical structure

The potato is an enlarged underground tip of a rhizome (Fuller, 1963). The tuber is composed of periderm (peel), cortex, parenchyma and pith (Schwimmer and Burr, 1959) (Figure 1). A similar structure was presented by Schuphan (1959, 1960) for Solanum stenotomum Juz et Buk.

Schwimmer and Burr (1959) described the cortex as a narrow layer of parenchyma tissue, underlying the periderm. Parenchyma cells, high in starch content, lie on both sides of the vascular ring. The pith, forming a small central core with radiating branches to reach the eyes, contains less starch.

B. Nitrogen distribution within the tuber

According to Neuberger and Sanger (1942) the nitrogen content is highest in the periderm, and then decreases sharply in the cortex and rises again towards the pith. A similar distribution was reported by Monday and Rieley (1964), who also showed that throughout the tuber the percentages of total and soluble nitrogen, on a dry weight basis, are inversely related to specific gravity. Schuphan

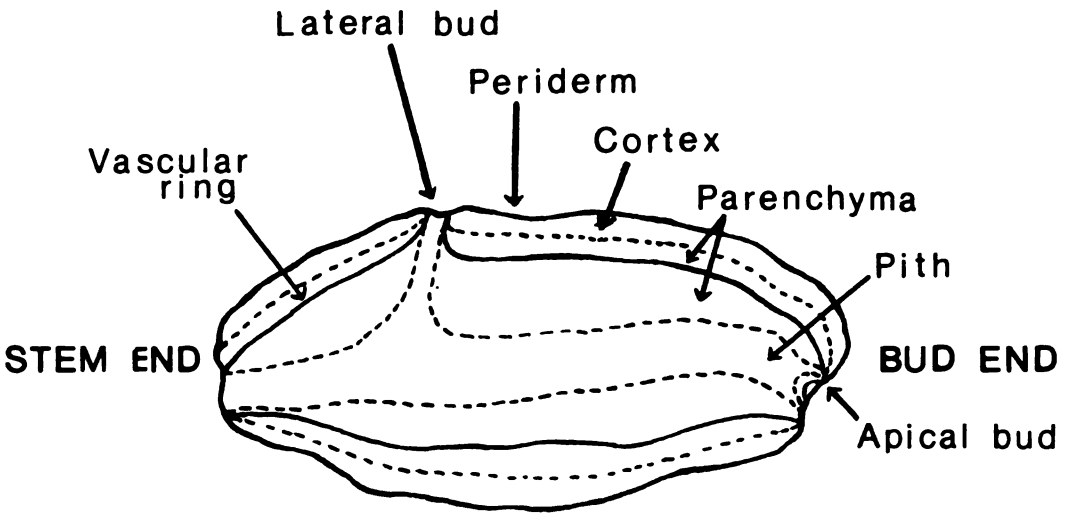

Figure 1. Structural features of potato tuber section.

(1970) found that the cortex and the area near the apical and lateral buds have a much higher concentration of essential amino acids than the inner layers. The better balance of essential amino acids appears to be associated with cell layers which are capable of cell division. An interesting observation is that protein crystals have been found in the outer layers.

During the growth of potato tubers, the nitrogen content decreases gradually on a dry matter basis and the portion of protein is higher in the immature tuber (Schuphan, 1970). The same author (1959) stated that small tubers have a nutritionally better pattern of essential amino acids. Of the essential amino acids, lysine and threonine increase with tuber weight, while most of the other amino acids show little change or drop slightly (Schuphan, 1970).

The potato protein has been studied by electrophoresis and by combined electrofocusing and electrophoresis. Macko and Stegemann (1969) and Luescher (1972) showed that the tuber proteins of different potato varieties are present in different proportions. Results of this kind may help the potato breeder.

C. Protein quality of potato

1. Nitrogen containing compounds of potatoes

The total nitrogen content can be broken down into a) true protein fractions soluble in various extracting

solutions; b) in an insoluble protein residue and c) non protein nitrogen (NPN) which includes inorganic nitrogen, amide nitrogen, free amino acid nitrogen, and numerous other constituents such as alkaloids, purines, pyrimidines, choline, enzymes, certain vitamins, quaternary ammonium compounds, etc.

The potato tuber contains on the average 2.1% total protein (%N x 6.25) on a fresh weight, but it can range from 1.5 to 4.0%. The total protein (2.1%) represents 10.4% of the total solids of the potato, and it can be as high as 17%. It is important to point out that a new protein conversion factor (7.5) has been recently proposed by Desborough and Weiser (1974). Should this factor be proven correct, old values must be multiplied by 1.2 to obtain the total protein content of potatoes.

The results presented in Table 1 are based on many analysis by various authors (Schreiber, 1961). In relation to Table 1, Markakis (1975) stated that it is doubtful that there is so much free NH_3 (3%) in potatoes; ammonia is formed from glutamine during hydrolysis.

Schuphan (1958) reported that the total protein is composed of 30 to 50% true protein. Meister (1977) found for NPN (g/100 g N) values from 38.8 to 54.8, and Luescher (1972) reported values from 50 to 61 (g/100 g N) NPN for three potato cultivars.

Table 1. Nitrogen containing compounds (crude protein) of the potato (Schreiber, 1961)

N Fraction	% of Total N
True protein N	50
Non protein N	50
Inorganic N	
Nitrate N	1
Nitrite N	trace
Ammonia N	3
Amide N	
Asparagine N	13
Glutamine N	10
Remaining N	
Free amino acid N	15
Basic N	8

2. Potato proteins and amino acids

Lindner et al. (1960) measured the relative amounts of different proteins in the potato and found that of the total protein, globulin I (tuberin) accounted for 76.4%; globulin II, 1.4%; albumin (tuberinin), 4%; prolamin, 1.8%; glutelin, 5.5%; and insoluble residue, 10.9%. These protein fractions have been classified according to the concept of solubility in various extracting solutions. Tuberinin is soluble in water; tuberin is soluble in neutral salts (i.e. NaCl); prolamin is soluble in 70% ethanol (by volume); glutelin is soluble in a 0.2% NaCl solution made up in 60 volume percent ethanol; the insoluble protein fraction in the solutions described above, is found mainly in the skin and outer cortex.

Protein crystals have been observed occasionally in the protoplasm of the outer layers of potato cells. Depending upon varieties, these crystals can also be synthesized in other tissues of the potato tuber (Hoelzl and Bancher, 1959).

Tuberinin is considered a heterogeneous albumin. Tuberin is a globulin ; it is slightly soluble in water, but its solubility is greatly enhanced by the addition of neutral salts, such as 2% sodium chloride; it is heterogeneous in composition.

Tuberin and tuberinin are present in a dissolved form in the cell sap of the potato. As soon as the cell wall is

ruptured these two proteins can be easily extracted (Hoelzl and Bancher, 1961). At a pH 6.8 both proteins have a negative isoelectric point (Groot et al., 1947). Together, they account for 30 to 60% of the total protein. Luescher (1972) isolated the various potato protein fractions from three cultivars, by the procedure outlined by Lindner et al. (1957). He found that of the total nitrogen, tuberin (globulin I) accounted for 29 to 35%, globulin II for 0.3 to 0.6%, tuberinin (albumin) for 1.0 to 1.2%, prolamin for 0.6 to 0.8%, glutelin for 0.1%, and the residue for 5 to 10%. Similar results were reported by Meister and Thompson (1976b) in a study of the Russet Burbank potato, except for the percentage of tuberin which was a little higher (39.2%).

Luescher (1972) also determined the sulfur amino acids (methionine and cystine) of tuberin and tuberinin by his own standardized procedure using the microorganism Streptococcus zymogenes. Tuberin and tuberinin from two of the cultivars tested contained more than twice as much methionine as cystine: on the average 2.8 g methionine versus 1.2 g cystine per 16 g N. However, this was not true for the other cultivarswhere methionine was considerably lower. Prolamin is an excellent source of cystine and the values ranged from 3.1 to 3.9 g/16 g N in the three cultivars. Breeding potatoes high in sulfur amino acids, particularly in methionine, is a likelihood for the future. The possibilities of genetic improvement of potato protein have been

discussed by Schwartze and Sengebush (1937); Siegle (1951); Reissig (1958); Schuphan (1970); Desborough and Weiser (1972); and by a planning commission of the International Potato Center (CIP, 1973). It is known that heavy nitrogen fertilization increases the percentage of total protein mainly due to an increase in aspartic and glutamic acids and the amides asparagine and glutamine. The concentration of lysine increases proportionally with fertilization, but the methionine content drops from 2.2 to 1.6% (Hoff et al., 1971). Mulder and Bakema (1956) reported that free methionine dropped from 1.9% when fertilized with 33 Kg N/ha, to 1.0% when 150 Kg N/ha of fertilizer was used. However, Luescher (1972) observed that a high free methionine content (2.07 g/16 g NPN) was obtained with 185 Kg N/ha. The extent of response in amino acid composition as influenced by fertilizer may be dependent upon genotype. Schuphan (1970) reported that when the level of nitrogen fertilizer per hectare was 120 Kg, the variety Bona had an essential amino acid Index (EAA-Index) of 64 and the variety Olympia 86. The EAA-Index among cultivars is influenced primarily by the ratio of protein N to non protein N (Reissig, 1958). The results reported by Talley et al. (1970) and Augustin (1975) confirm varietal differences for total protein, the ratio of free to total N and methionine, but all three are subject to modifications by year, location, fertilization, and very highly by genotype x environment conditions.

Woodward and Talley (1953) and Luescher (1972) found that no free tryptophan or free cysteine could be detected in the NPN fraction. Essential amino acids in the NPN fraction are present at a much lower level than in the protein fractions.

The methionine content of potatoes has been studied recently by Kaldy and Markakis (1972); Luescher (1971, 1972); Peare (1973); Desborough and Weiser (1974). According to Luescher (1972) the methionine content of potato families varies and free methionine is responsible for 93% of the variation in available methionine. Free methionine ranged from 0.34 to 2.07 g/16 g non-protein N. Free methionine contributed from 12 to 62% of all methionine present in the total protein.

Low temperature storage (40°F) does not appreciably affect the free amino acid composition of potatoes, but reconditioning to 75°F results in a disappearance or marked decrease in the content of free amino acids. A complete loss of the free basic amino acids, arginine, histidine and lysine, was observed during reconditioning (Habib and Brown, 1957). Fitzpatrick and Porter (1966), however, showed that low temperature storage (36°F) followed by reconditioning at room temperature resulted in an increase of the free amino acids, but no change in the amino acids of the true protein. However, Desborough and Weiser (1974) stored 12 genotypes at 38-40°F and found an average loss of 3% in total protein.

The free amino acids that are included in the NPN fraction are subject to variations as a result of storage, nutrition of the plant, year, location and treatment with chemicals such as ethylene chlorohydrin (Mulder and Bakema, 1956; Schwimmer and Burr, 1959; Talley et al., 1970). The free amino acid composition of potatoes has been studied by several investigators (Chick and Slack, 1949; Thompson and Steward, 1952; Mulder and Bakema, 1956; Kaldy, 1971). The free amino acid pool has been studied recently by Desborough and Weiser (1974), using dialyzed and non dialyzed half tuber samples of six genotypes. Free amino acids accounted for about 10% of the total amino acids and non essential amino acids constituted over 90% of the free pool. The authors concluded that the essential amino acids occur mainly as components of the potato protein. Free amino acids also have been studied in cooked potatoes, chips, canned, drum dried and french fried potato samples (Hughes, 1958; Jaswal, 1973). It is interesting to point out that, in general, the most abundant free amino acids are valine, arginine, aspartic acid, glutamic acid, and the amides asparagine and glutamine.

Hughes (1958) analyzed the amino acids of the heat coagulable fraction of potato nitrogen and reported the following composition in g amino acid per 16 g total N: arginine 5.5, histidine 2.4, isoleucine 6.8, leucine 11.1, lysine 8.3, phenylalanine 6.2, methionine 2.8, cystine 1.6,

threonine 5.7, tryptophan 1.8, valine 8.0, alanine 4.7, aspartic acid 13.0, glutamic acid 11.3, glycine 4.9, proline 5.1, serine 5.8, tyrosine 6.1, r-aminobutyric acid 0, and ammonia 1.7.

The amino acid composition of whole potatoes is shown in Table 2, which was presented by Markakis (1975) on the basis of chemical and microbiological analysis by several researchers.

The FAO report (1970) cites ranges for the methionine and cystine content of potatoes, ranging from 54 to 125 and 4 to 81 mg/g total N, respectively. These results may explain the differences in the nutritional value of potato protein as reported by various investigators (Schuphan and Postel, 1957; Luescher, 1972; Peare, 1973; Meister, 1977). Therefore, improvements in the quality and quantity of tuber protein are possible.

3. Nutritional characteristics of potato proteins

The potato makes important contributions to human nutrition. As an energy source it is considered good. However, today it is also being recognized for the nutritional quality of its protein.

The evaluation of the protein quality of potatoes has been tested chemically by amino acid analysis and biologically by animal feeding experiments, human feeding

Table 2. Amino acid composition of potatoes (mg/g total N)[1]

Amino acid (AA)	Average	% CV
Essential AA		
Isoleucine	257	27.6
Leucine	362	30.2
Lysine	342	43.5
Methionine	92	27.9
Cystine	55	77.2
Phenylalanine	280	24.8
Tyrosine	172	38.6
Threonine	233	24.3
Tryptophan	85	33.4
Valine	323	22.0
Non essential AA		
Arginine	305	14.9
Histidine	103	29.1
Alanine	297	20.5
Aspartic acid	1138	29.4
Glutamic acid	729	28.7
Glycine	214	18.6
Proline	213	16.1
Serine	236	14.8
Protein Score	70	
MEAA Index	72	

[1]Notations MEAA: modified essential amino acid Index
% CV: percent coefficient of variation = $\frac{\text{Standard deviation}}{\text{mean}} \times 100$, on data by FAO (1970).

experiments, and microbial growth.

4. Evaluation of protein quality in potato

The protein quality of potato will be considered in relation to:

a. Amino acid analysis

b. Streptococcus zymogenes assay and animal feeding experiments

c. Human feeding experiments

a. Amino acid analysis of the potato tuber

Allison (1959), Pike and Brown (1975) relate the quality of a dietary protein to the amount and kind of its amino acids. A diet must provide the essential amino acids required by the human body, but also the nitrogen of non-essential amino acids is nutritionally important. Snyderman et al. (1962) indicated that a food, for example milk, can satisfy the requirements of the essential amino acids, but not the requirements in total utilizable nitrogen for the human body. Also, a diet adequate for maintenance and repair may be inadequate for growth (Mitchell, 1947). Not only the quantity of protein, but also the amino acid balance of the protein is important. According to several investigators the potato protein has a good amino acid composition and balance for maintenance and growth promotion in humans. Block (1951) stated that tuberin, the main protein fraction of the potato, contained sulfur amino acids

similar to milk, but only about 75% of methionine and 50% of the cystine present in the protein of the whole egg.

Markakis (1975) using the protein score procedure developed by FAO/WHO (1965), calculated a protein score of 70 for the protein of the whole potato, based on the average of the amino acids present in potatoes (Table 2). This value compares favourably with other foods which have the following protein scores: beef 80, fish 75, soyflour 70, milk 60, wheat flour 50, maize 54, rice 65 (FAO/WHO, 1965; Payne, 1976). A protein score of 70 and a modified essential amino acid index (MEAA-Index) of 98 were calculated for the "pure" potato protein by Markakis (1975), based on the amino acid analysis reported by Hughes (1958).

The high MEAA index of this protein indicates that the potato protein is a better source than whole egg protein of every essential amino acid, except for the sulfur containing amino acids that are low compared to egg protein. But if the sulfur amino acids of the "pure" potato protein are compared to the FAO/WHO pattern (1973), the total 4.4 (methionine + cystine) is higher than 3.5 of the pattern (Table 3). However, the whole tuber has a lower value (2.9).

Knorr et al. (1977) found that the amino acid composition of a potato protein concentrate (PPC) was equal to or greater than that reported by FAO (1972b). Also, in the PPC a high methionine content (2.70 g/16 g N) was obtained.

Table 3. Essential amino acid composition of potato tuber, a heat coagulable fraction of it (HCF), whole egg protein and the reference pattern (FAO/WHO, 1973) (g/16 g N).

Amino acids	Potato protein		Whole egg	FAO/WHO reference pattern
	Whole tuber[1]	HCF[2]		
Lysine	6.2	8.3	7.8	5.4
Methionine + cystine	2.9	4.4	5.3	3.5
Phenylalanine + Tyrosine	8.2	12.3	9.3	6.1
Leucine	6.0	11.1	8.8	7.0
Isoleucine	4.2	6.8	5.9	4.0
Valine	5.7	8.0	7.1	4.9
Threonine	3.8	5.7	4.9	4.0
Tryptophan	1.5	1.8	1.4	1.0
Histidine	2.3	2.4	2.6	-
Total	40.8	60.8	53.1	35.9

[1]Kaldy and Markakis (1972).

[2]Hughes (1958).

Finally, the potatoes have a high percent of the amides asparagine and glutamine, 13% and 10% of the total nitrogen content, respectively (Schreiber, 1961). It has been suggested that some compounds of the potato, such as glutamine, have a substantial influence upon the efficiency of the utilization of the amino nitrogen. It is known that certain amino acids act as antagonists to each other. If these amides in the potato prevent this antagonism and afford efficient use of protein, they may have a good role in human nutrition (McCay, 1959).

b. Streptococcus zymogenes assay and animal feeding experiments

Ford (1960) developed a microbiological assay to test the quality of food proteins and demonstrated a good correlation between microbiological and biological experiments with the growing rat. Comparable results were obtained when the protein quality of potato was determined using the S. zymogenes assay, the meadow vole, and rat feeding (Peare, 1973; Meister and Thompson, 1976a).

The proteolytic bacterium S. zymogenes is strongly proteolytic and has an absolute requirement for exogenous methionine, tryptophan, arginine, histidine, leucine, isoleucine, valine and glutamic acid. Of the essential amino acids lysine, threonine and phenylalanine are not indispensable for the organism although the omission of any one of the culture causes a marked failure in growth rate.

The total growth of the organism on a given amount of sample protein has been expressed in percent of the growth on the same amount of casein protein.

Luescher (1971, 1972) used this organism to measure the biological value (BV) and available methione of potato protein. On the basis of many samples analyzed with this organism, the average for the BV of total potato protein was 84 and 78 for non-protein nitrogen (NPN). These values are in the range 61 to 89 reported by Schuphan (1959). Peare (1973) used the BV with S. zymogenes and the total percent protein to calculate the net protein content (BV x% total protein) of potatoes. He concluded that potato varieties having a high BV will not necessarily contain high amounts of net protein. Meister (1977) stated that the BV of potatoes depends on genetic features; he also studied the BV of potatoes using S. zymogenes and found values from 77 to 82. He observed that raising the protein content of the tuber may bring about a drop in the BV of the protein. Most of this drop would be due to an increase in the NPN which has a lower BV than the true protein.

Mitchell (1924) reported a BV for the potato protein of 68.5% when a diet at 5% protein level was fed to young rats and 66.7% when the diet contained 10% protein. Drum dried potato flakes of 5.28% protein content in the diet gave better growth than drum dried beans when the meadow vole (Microtus pennsylvanicus) was used as an experimental

animal (Rios et al., 1972). Also, the addition of methionine increased the PER values of the potato protein and improved the digestibility of the protein in the meadow vole (Rios, 1969).

When the heat coagulable fraction, tuberin, was tested a BV of 71 was obtained with rats compared to 68 for casein (Kon, 1928).

It has been demonstrated that the nitrogen of the intact potato supports growth at least as well as tuberin alone. But the NPN alone can not promote growth of rats (Slack, 1948; Chick and Cutting, 1943). Chick and Slack (1949) stated that the apparent digestibility of the potato protein was increased from 74 to 79% when the skin and outer cortex was removed. The peel obtained by the abrasive method did not support growth of the meadow vole, but weight loss was not observed (Meister and Thompson, 1976a). Peare (1973) determined with rats the nitrogen incorporation efficiency (NIE) of cooked whole potato flour and a high-protein potato flour according to the procedure of Stucki and Harper (1962). The NIE values as a percent of casein were for whole potato flour 74.3% and for the high protein flour 73.9%. No differences in protein quality or food consumption were noted.

Protein recovered from waste effluent of potato chip processing gave equal to or slightly higher protein efficiency index with meadow voles than total protein of whole

tubers (Meister and Thompson, 1976a).

Baciqalupo (1969) reported in boiled and dried potato a PER value with rats of 2.5 after adjustment with respect to casein. Joseph et al. (1963), testing different potato varieties, reported PER values from 0.95 to 1.99. Brune (1968, 1969), working with pigs, reported the BV for potatoes grown in the presence of a fertilizer; he observed that with an increase in the level of N fertilizer the BV decreased. Different levels of N fertilization resulted in different biological values for the potato protein. The biological values were 76, 82, 74 and 71% for zero, 50, 100 and 200 Kg of N fertilizer/ha, respectively. The results indicate that an increase in protein content in potatoes through N fertilization is possible only within certain limits. Similar information has been obtained with mice, rats and S. zymogenes.

Peare (1973) observed in a preliminary study that weanling rats, fed the raw concentrate of potato flour high in protein content at a level of 9% protein during 5 days, barely maintained their initial weight, but when the concentrate was cooked, weight gain was normal. The explanation to this could be due to adverse effects of feeding larger sized starch grains (Morrison, 1936) and also the presence of protease inhibitors such as trypsin and chymotrypsin inhibitors which have been found in the raw potato by Sohonie and Ambe (1955) and Rayan and Balls (1962).

Rayan (1966) pointed out that the potent chymotrypsin inhibitor of the potato is quickly destroyed by heating. Raw potato starch caused serious impairment of the net protein utilization (NPU) and the food efficiency values when fed to growing albino rats. A gross caecal enlargement was observed (El-Harith et al., 1976).

Whittemore et al. (1975) compared the nutritive value of dried cooked potato versus raw potato for pigs. In relation to the values obtained for cooked potato, raw potato had lower apparent digestibility coefficients for nitrogen, 0.76 versus 0.86. Similar results were reported earlier when the nutritive value of cooked potato flour, dried potato dice and raw potato mash were evaluated for pigs. The apparent digestibility coefficients for nitrogen were respectively, 0.89, 0.80, and 0.69 (Whittemore et al., 1973).

Felix and Whittemore (1975) working with young chicks found that growth rates and efficiency of food conversion were lower with a diet containing potato flakes than a diet containing maize. However, in the discussion of the results the authors stated that the protein quality of potato flakes is comparable with that of ground maize, for the young chick. Other unknown factors than the quality of the potato protein affected the results.

c. Human feeding experiments

In the case of protein, growth is used as criterion of adequacy for infants and children, and maintenance of body N content for adults (Payne, 1976). Hindhede (1913) maintained nitrogen equilibrium in three men on a potato diet. The same author claimed that a man was kept in N equilibrium for more than three months on a potato diet although the protein intake was 11-15 g daily. Similar results were obtained by Rose and Cooper (1917) with a young woman who consumed 1500 g potato per day. The experiment was performed for 7 days and the N balance was kept by an intake of 0.096 g N from potato/Kg body weight. A man and a woman were kept in N balance for 167 days on a diet in which the potato was the only source of nitrogen (Kon and Klein, 1928). According to this experiment the daily need for potato protein was 35.6 g for the man and 23.8 g for the woman on a 70 Kg body weight basis.

In nitrogen balance studies with college students, potato protein proved to have the best nutritive value in relation to other plant proteins tested, such as wheat flour, rice, kidney beans, soybeans, algae (Kofranyi and Jekat, 1965, 1967). In relation to animal proteins the nutritional value was almost equal to whole egg protein, but better than beef, tuna fish and whole milk. These authors used single foods and pairs of foods mixed at definite protein ratios and determined the minimum amount

of protein from each diet that was necessary for nitrogen balance maintenance, in three subjects. The average of the three subjects was 0.545 g N/Kg body weight for the potato protein and 0.505 g N/Kg body weight for the egg protein. When the 65:35 potato:egg mixture of proteins was used, the total protein requirement for N-balance was less than the requirement in egg or potato protein alone.

Other pairs of proteins were similarly tested, but none resulted in as low a minimum of protein requirement as the 65:35 potato:egg protein mixture. Thompson (1977) stated that he gave to one graduate student a diet in which the potato was the only source of nutrients. The student consumed during one month four pounds per day of a potato high in methionine. The diet was short in 230 calories that were supplemented with oil in the form of french fries. The subject gained four ounces and felt good after one month on the diet.

Kies and Fox (1972) showed that the protein quality is improved by adding purified L-methionine to the diet of human adults who consumed dehydrated potato flakes. The same authors demonstrated that neither leucine nor phenylalanine resulted in improvement of N-balance as methionine did. The digestibility of potato protein tested was 78%.

McCay (1959) stated that potato gruel made from specially prepared potato meal or the pulp of baked potatoes has been found by Germans to be of great service in the

feeding of infants or invalids. Hegsted (1957), in a theoretical comparative study of vegetable proteins, demonstrated that the protein of potato with a BV of 71 can provide the required protein for a child 6 months old with 800 calories intake, or a child one year old with an intake of 975 calories. Corn grits with a BV of 54 and white bread with a BV of 47 can not be used as the only source of protein, although theoretically with these proteins, the protein and amino acid requirement can be met.

5. Influence of processing on quality of potato protein

As a result of processing by different methods, i.e. baking, boiling (cooking), chipping, french frying, canning and drum drying, the quality of the protein of potatoes could be altered. Also, if the product has been prepared from fresh or stored tubers the protein quality can be different. Burton (1965) reported that during storage of some British potato varieties considerable sweetening occurs after more than 10-15 weeks of storage at 50^{o}F. However, 50^{o}F is an ideal storage temperature, because of the sprout suppression. Protein N decreases in sprouting tubers (Pol and Labib, 1968), thus making it advisable to avoid sprouting if the potato is going to be stored.

Exposure of tubers to low temperatures results in a rapid conversion of starch to hexoses due to a rapid increase in invertase activity (Pressey and Shaw, 1966). However, it is also known that there is a subsequent

disappearance of the sugars when the tubers are returned to higher temperatures.

Carbonyl amine reactions, including the reaction of reducing sugars and other aldehydes and ketones with amino acids, peptides, and proteins, may occur during heating, resulting in browning of the product (Maillard reaction). The effect of chipping on the nitrogeneous compounds of potatoes and the dark color in fried potato products has been studied by Shallenberger (1956), Chawan (1965), Fitzpatrick and Porter (1966, 1968). Fitzpatrick and Porter (1966) found that the greater the accumulation of reducing sugars in stored potatoes, the greater the loss of free amino acids after chipping. In 1968 the same authors studied the effect of fat frying versus microwave finishing on the extractable potato protein. In microwave finished chips the amino acids were more extractable. The reason for this is that in microwave finishing the chain on non-enzymatic browning reactions are interrupted, because the temperature of the chips never exceeds the boiling point of water (Fitzpatrick and Porter, 1968). Jaswal (1973) found the losses of bound and free amino acids in low specific gravity potatoes after chipping to be 36.9 and 44.8%, respectively. The corresponding losses in high specific gravity potatoes were 20.2% for bound amino acids and 33.2% for free amino acids. The same author reported losses of amino acids occurring during canning, drum drying

and french frying of potatoes. He found that canning caused almost as much destruction to amino acids as chipping, drum drying resulted in less damage and french frying caused insignificant loss in amino acids.

Hughes (1958) analyzed the amino acids in pre-peeled boiled potatoes and found that the amino acid composition is not very different from that of raw potato. However, Desborough and Weiser (1974) reported a 50% loss of total protein for 12 genotypes after boiling for 30 minutes.

6. The potato protein as a supplement of other proteins

Nutritional protein deficiency can be present in two ways: deficiency of total protein in the diet and deficiency of amino acids irrespective of total protein intake.

According to Jansen and Howe (1964), in diets consisting of rice or wheat only a deficiency in lysine and threonine is likely. In corn diets, lysine and tryptophan may be in short supply. In these foods the protein deficiencies, at least in relation to the amino acid balance can be overcome by addition of potatoes, because the complementarity between potatoes and cereals has been proven. Flodin (1953) and Markakis et al. (1962) stated that a combination of potatoes and cereals, especially corn and wheat, makes a desirable food mixture, because of the high lysine and tryptophan content in potatoes. Markakis et al. (1962) developed a fried chip based on cereal and potato products.

If the whole potato is dried, a very nutritious dry product is obtained with more than 10% protein in most cases.

Bacigalupo (1969) stated that a rat diet containing 6% protein from cotton seed flour (CSF) and 4% protein from potatoes had a PER value equal to 2.54 and a diet containing 5% protein from CSF and 5% protein from potatoes had a PER of 2.69 versus 2.70 for casein. The same author found that enrichment of wheat flour with potatoes plus CSF, increased the nutritive value of the bread about 100%.

If potato protein concentrates from waste effluent of potato chip processing or a high-protein air classified fraction of potato flour are available (Shaw and Shevy, 1972; Peare, 1973; Meister and Thompson, 1976b; Knorr et al., 1977), it is possible to use them to enrich the total protein content of different food mixtures.

Numerous investigations have been conducted on the incorporation of potato flour in bread (Harris, 1932; Trogonitz, 1939; Harris et al., 1952; Treadway, 1952; Braden, 1962; Reynoso and Bacigalupo, 1968; Bacigalupo, 1969; Reynoso et al., 1972; Jain and Sherman, 1974; Knorr, 1977).

Jain and Sherman (1974) used potato flakes at the level of 20-30% using the mechanical dough development method and the bread had good quality in color, texture and taste. Bacigalupo (1969) reported the use of 25 and 30%

potato flour with wheat flour for bread without involving great change of bread quality. Similar results were obtained by Reynoso et al. (1972), using up to 30% cooked mashed potatoes.

Harris et al. (1952) found that replacing 1 to 4% of wheat flour with potato flour increased the loaf volume and caused a slight change in the bread texture.

Substitution of the wheat flour up to 10% with starch-free potato protein recovered from potato starch waste effluents produced an acceptable bread with a longer shelf life than that of 100% wheat bread (Knorr, 1977). Some decrease in loaf volume and deformation was observed with increasing potato protein from 15 to 20%.

MATERIALS AND METHODS

Preparation of the Samples

For this study the following potato varieties were chosen:

Atlantic (high solid content)

Denali (high solid content)

Russet Burbank (medium solid content)

Superior (medium-low solid content)

Monona (low solid content)

The potatoes were grown on the Montcalm Experimental Farm of Michigan State University in 1977. They were fertilized with 217 lbs/A of N, 200 lbs/A of P_2O_5 and 72 lbs/A of K_2O. A separate plot of Russet Burbank was fertilized with cattle manure, only. Thereafter these treatments are referred to as chemical and organic samples, respectively.

After harvest the potatoes were stored at $40^{o}F$, and a relative humidity of 85%.

A. Nitrogen distribution

Six tubers were randomly chosen from each variety, washed and sliced according to Figure 2. Two raw tubers were sliced as shown over "Tuber 1", two raw tubers were sliced as shown over "Tuber 2" and two boiled tubers were cut as shown over "Tuber 3". The slices and the inner

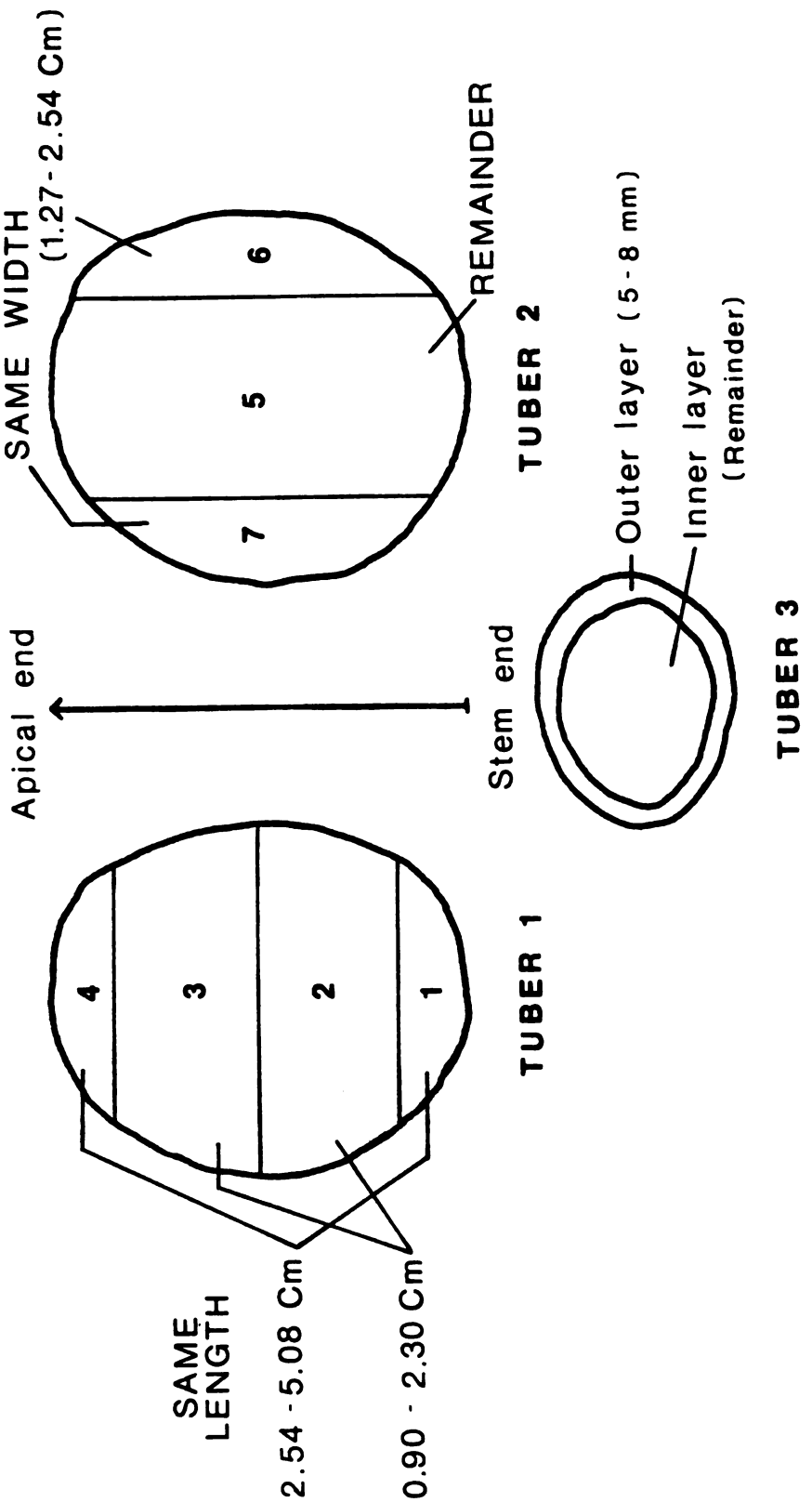

Figure 2. Sectioning of potato tuber for analysis.

and outer layers were frozen immediately and freeze-dried.

After freeze-drying the samples were ground in an electric grinder (Chemical Rubber Co.) and stored in plastic bags at a temperature lower than 14°F until they were analyzed.

B. Amino acid distribution

Amino acid analysis on the following samples were carried out: outer and inner layers of the potato varieties Atlantic, Russet Burbank, and Monona, and apical and stem ends of the variety Russet Burbank.

C. Protein and amino acid losses during boiling

From each variety eight tubers were chosen randomly, washed, dried and weighed before boiling with distilled water. Two groups of four tubers for each variety were prepared for boiling. The tubers were cooled to room temperature and weighed again.

The total solids (TS) were determined on boiled and fresh samples, according to AOAC (1975).

For each variety the following scheme was carried out for boiling, using the same volume of water (6 cups):

a) Whole tubers with skin
b) Whole tubers with skin cut longitudinally into halves.
c) Whole tubers with skin cut into quarters
d) Whole tubers peeled with a home peeler

e) Whole tubers peeled and cut into halves

f) Whole tubers peeled and cut into quarters

A narrow stainless steel spatula was inserted into the potatoes to see when they were cooked. After boiling, the water for all the samples was filtered hot using cheese cloth. The volumes were measured accurately after cooling at room temperature. The water of the whole tubers boiled with skins was concentrated by evaporation.

The boiling water was examined for the presence of protein and amino acids. The amino acids were analyzed in the boiled water from the cooked potatoes with intact skins, halved potatoes with skins and quartered potatoes with skins. Four ml of water after boiling the potato with intact skins were evaporated to dryness on a rotatory evaporator. The same procedure was followed for the halved and quartered potatoes but 2 ml of water were evaporated. The dried samples were used for amino acid analysis.

D. Amino acid analysis of potato tubers

1. Amino acid distribution

The following scheme was carried out for the amino acid distribution in whole potatoes.

a) The sulfur amino acid content in the whole raw Atlantic and Russet Burbank potatoes.

b) Amino acid distribution in the Russet Burbank potatoes, boiled with intact skins, chemically or organically fertilized. The samples for analysis were prepared from a group of four potatoes for each variety.

2. Free amino acid profile

Free amino acids were analyzed in the whole raw potato, varieties Atlantic, Denali, Russet Burbank, Superior and Monona. The samples for analysis were prepared from a group of four potatoes for each variety.

3. Available lysine in boiled potatoes

Available lysine was analyzed in the boiled potato, varieties Atlantic, Russet Burbank, and Monona. The samples for analysis were prepared from a group of four potatoes for each variety.

E. Amide analysis of potato tubers

The amides asparagine and glutamine were analyzed in the whole raw potato, varieties Atlantic, Denali, Russet Burbank, Superior and Monona. The samples for analysis were prepared from a group of four potatoes for each variety.

F. Protein efficiency ratio (PER)

1. Fertilization

The variety Russet Burbank, grown with chemical and organic fertilizer, previously stored for 6 months at 40^{o}F, was selected for this portion of the research. From each sample, approximately 30 kg of large and small washed tubers

were boiled with distilled water for 30 minutes. The tubers with skins were sliced, freeze-dried, and stored in plastic bags at 14°F.

2. Supplementation

Dried whole eggs, type W-1 (Henningsen Foods, Inc.) and whey powder (from cheddar cheese whey) were used for supplementing the commercially dried potato Russet Burbank. The whey powder was prepared as follows: the sweet-rennet cheese whey was collected at the time of the cheese-making operation and maintained at 50°F for 12 hours. The whey was pre-heated in a tank-jacket with steam at 115°F, fed to a vacuum evaporator, and concentrated to 23.5% total solids (TS), at 106°F.

The condensed whey (23.5% TS) was spray-dried using the following conditions in the spray-drier:

Inlet air temperature	160°F
Outlet air temperature	150°F
Feed tank pressure	40 psi
Atomizing pressure	30 psi

Total solids in the whey were determined refractometrically.

3. Preparation of diets

Before incorporation of the freeze-dried potato sample into diets, the material and the commercially dried potato buds were ground in a Wiley mill to pass through a 0.50 mm screen.

Eight diets were prepared: In six of them the sole source of protein was a) Russet Burbank potatoes, chemically fertilized; b) Russet Burbank potatoes, organically fertilized; c) Russet Burbank, commercially dried potato buds; d) Whey powder; e) Dried whole eggs; f) Casein. The other two diets were prepared using composite flours in which the ground commercially dried potatoes were mixed a) with whey powder in the ratio 90:10 potato protein to whey protein; b) with dried whole eggs in the ratio 65:35 potato protein to egg protein.

The composition of the basal diet is shown in Table 4. Diet c) and the two diets prepared using composite flours contained 8% protein. Each diet was thoroughly mixed in a Hobart blender. The diets were adjusted according to the proximate analysis of test materials so that all diets, including the reference (casein) diet, had the same composition, except for the nature of the protein. Male rats of Sprague-Dawley strain, 21 to 28 days old, were used, ten for each assay. After a 4-day acclimatization period, during which the rats were fed a standard rat diet the first two days and the experimental diet the last two days, they were randomly divided into groups. Rats were housed individually in metal cages with raised wire mesh floors. Water and diets were fed ad libitum.

The body weight of each rat was recorded on the first day of the assay period and again weekly up to the 28th day.

Table 4. Composition of basal diet for the PER

Ingredients	Amount %
Protein	10
Corn oil	8
Salt mixture[1]	5
Vitamin mixture[2]	1
Non-nutritive fiber	1
Corn starch and sugar	to complete 100

[1]USPXIX Salt mixture (Teklad Test Diets)

[2]Vitamin mix AOAC (Teklad Test Diets).

The food intake was determined weekly.

PER (weight gain/protein intake) was calculated from the weight gain and protein consumption of each rat and the values were adjusted to PER = 2.5 for the reference casein.

The prepared diets were refrigerated during the duration of the experiments.

G. Taste of the Russet Burbank potatoes chemically or organically fertilized

The triangle test was used to determine the preference (flavor differences) of the potato variety Russet Burbank, grown with chemical versus organic fertilizer.

Twenty-three untrained panelists received three samples, two of which were identical and they were asked to identify the odd sample.

The potato samples for tasting were chosen randomly from the batch of boiled potatoes used for the PER experiment. Each individual sample was prepared by cutting a slice from three different potatoes.

H. Preparation of a high protein potato flour

The potato protein flour was prepared as follows: whole tubers of low to medium solid content were freeze-dried, ground in a Wiley mill (.32 mm sieve) and subsequently air-classified in a Walter Lab. separator at the rate of 2.5-3.0 g flour per min. Air classification yielded three fractions: a) a small quantity of very fine particles; b) a

medium weight fraction; and c) a heavy fraction.

The second fraction was used in this part of the study. This fraction was named according to the new FDA regulation (1979) "potato protein flour" (PPF). It was used at levels of 5 and 10% substitution in wheat bread.

I. Bread

The basic formula for bread making with and without PPF are given in Table 5. Three loaves of bread were made with each flour formula. The straight dough method was used (HNF (Human Nutrition and Foods)403-404 Laboratory manual, Michigan State University).

Preparation of bread

The bread was prepared according to the method outlined in HNF (Human Nutrition and Foods) 403-404 laboratory manual (Michigan State University). Commercial bakery flour, bleached and bromated, was obtained from the General Food Stores, Michigan State University. The following steps were followed:

1. The yeast was allowed to hydrate in water at 113^{o}F for 5 minutes in the stainless steel bowl of the mixer.
2. The sugar, salt, fat and approximately half of the flour were added. The above ingredients were mixed for one minute at speed 1.

Table 5. Ingredients used to produce bread by the straight dough method

Ingredients	Control[1]	5% PPF[2]	10% PPF[2]
Bread flour	200 g	190 g	180 g
Potato protein flour	0 g	10 g	20 g
Water, 65%	130 g	130 g	130 g
Shortening, 10%	20 g	20 g	20 g
Salt, 1%	2 g	2 g	2 g
Sugar, 6%	12 g	12 g	12 g
Yeast (dry granular), 3%	6 g	6 g	6 g

[1]Kneading time 6 min.; Fermentation time 30 min; proof 40 min.; bake 400°F(HNF 403-404 p. 66).

[2]Kneading time 7 min.; Fermentation time 85 min; proof 45 min.; bake 400°F (Knorr, 1977).

3. Half of the remaining flour was added and mixed for another minute at the same speed.
4. The additional flour was added gradually with continuous mixing and the dough was kneaded at speed 1 during the time registered in Table 5.
5. The dough was shaped into a ball by folding in half about five times, put into a greased bowl and left to ferment during the time registered in Table 5, in a proofing cabinet at about 100°F.
6. The dough was punched down, portions weighing 225 g were removed, shaped into loaves using the sheeter, put into greased loaf pans of approximately 7.6x 15.2 cm. and left to ferment during the time registered in Table 5, to double in volume.
7. The loaves were baked in a pre-heated oven at 400°F until the crust was golden brown.
8. The bread was removed from the pan and allowed to cool. Then, it was wrapped with plastic film, and frozen for later examination.

The bread was thawed approximately four hours before analysis. Evaluations were carried out on the physical and sensory characteristics of the finished bread according to HNF 403-404 Lab manual. Physical measurements were color, tenderness, volume, pH, moisture. Sensory characteristics were carried out with a four member untrained panel. The panelists rated crust color, characteristics of the crust,

grain, crumb color, taste and texture.

The protein and ash content of the PPF were determined according to the AOAC method (1975).

Nitrogen Determination - Micro Kjeldahl

Samples containing approximately 8.0 mg dried protein were digested in duplicate for 1 hour, according to AOAC (1975).

Sulfuric acid of 1.84 specific gravity was used for digestion and potassium sulfate and mercuric oxide were added to raise the temperature. After cooling, the insides of the flasks were rinsed with deionized water and digestion was continued for an additional hour.

The digests were transferred into the distillation apparatus by using approximately 10 ml deionized water. The digestion mixture was neutralized with 10 ml of a 50% NaOH solution, containing 5% of sodium thiosulfate. Released ammonia was steam-distilled into 5 ml of 5% boric acid solution, containing 4 drops of methyl red-methylene blue indicator. The distillation was continued until the volume in the receiving flask reached 25 ml. The ammonium borate complex was titrated with 0.02 N HCl which had been accurately standardized against tris-hydroxy-amino methane as primary standard. Nitrogen was calculated from the following formula:

$$\% \text{ N} = \frac{(\text{ml HCl-ml blank})(\text{normality of HCl})(14.007)}{\text{mg of sample}} \times 100$$

Amino Acid Analysis

Acid hydrolysis. Amino acid analysis was performed on HCl hydrolysates of protein using the "New Amino Acid Analyzer Technikon", according to the procedure of Moore et al. (1958). Samples consisting of approximately 10 mg of protein were placed into hydrolysis tubes with screw caps. Two ml of 12 N HCl were added to the tubes, followed by the addition of 2 ml of norleucine standard solution (1 mM). Then, ten ml of 6 N HCl were added to the tubes. A stream of nitrogen was gently run into each tube for 1 minute. The tubes were capped quickly and screwed tightly. The tubes were autoclaved for 16 hours at 250°F for hydrolysis. After hydrolysis the tubes were cooled to room temperature and opened. The hydrolysate was then quantitatively filtered through Whatman No. 2 filter paper and the hydrolysate collected into a 100 ml flask. The filter paper was washed well with five times (5x) deionized water. Three washings were done but always keeping the volumes small. The hydrolysate was evaporated to dryness on a rotatory evaporator using a 131°F water bath. The dried sample was washed and dissolved with 10 ml of 5x deionized water and again taken to dryness. In all samples, three washings were performed to remove residual HCl.

The washed and dried hydrolysate was dissolved in 4 ml 0.01 N HCl and transferred carefully to a small tube and frozen. Later, the frozen hydrolysate was thawed and 1 ml of it diluted to a volume of 4 ml with lithium citrate buffer (0.2 N Li^+, pH 2.20 ± 0.01) (Pierce Chemical Company, Rockford, IL.). The solution was then filtered with a 0.20 µm Metricel filter using a syringe with a Gelman hypodermic adapter (Gelman Instrument Company); 60 µl aliquots were used for analysis.

The chromatograms were quantitated by the peak area method. Standard amino acid mixtures were analyzed using the same ninhydrin solution.

Methionine and Cystine Analysis

Since methionine and cystine undergo a variable amount of oxidation during acid hydrolysis, they must be analyzed separately. The methods of Schram et al. (1954) and Lewis (1966) were used. These methods involve performic acid oxidation of methionine and cystine to methionine sulfone and cysteic acid, respectively.

The performic acid solution was prepared by mixing one volume of 30% (w/w) hydrogen peroxide with nine volumes of 88% (w/w) formic acid. This mixture was allowed to stand for one hour at room temperature.

Samples representing 5 - 9 mg of protein were weighed into hydrolysis tubes with screw caps. The protein

was oxidized for 24 hours with 10 ml of performic acid at 39°F. After oxidation 2 ml of norleucine (1 mM) and 0.3 ml of hydrobromic acid were added. The performic acid was removed in a rotatory evaporator at 100°F.

The dried sample was hydrolyzed as previously discussed. The sulfur amino acid analysis was performed with a Beckman Amino Acid Analyzer, model 121, using a temperature of 134.6°F and a citrate buffer, pH 2.2. Standard methionine sulfone and cysteic acid mixtures were analyzed. The chromatograms were quantitated by the height-width method.

Free Amino Acid Profile

Free amino acid analysis was performed on distilled water extracts of raw, freeze-dried potatoes, using the "New Amino Acid Analyzer Technikon".

The procedure described by Booth (1971) was adapted to extract the free amino acids of raw freeze-dried potatoes. The free amino acids were extracted with distilled water. To 1 g of freeze-dried potato flour 20 ml of distilled water were added. The mixture was shaken at room temperature for four hours in a Junior Orbit Shaker (Lab-Line Instruments, Inc.) at 200 rpm. Then 20 ml of 5% trichloroacetic acid were added. The mixture was kept in the refrigerator overnight at 39°F, centrifuged at 5,100 g

for 10 minutes and filtered using Whatman No. 2 filter paper. Since acid hydrolysis was not employed, the free sulfur amino acids were not destroyed and could be quantitated directly.

The chromatograms were quantitated by the peak area method. Standard amino acid mixtures were analyzed using the same ninhydrin solution.

Available Lysine

Carpenter's direct 1-fluoro 2,4-dinitrobenzene (FDNB) method as modified by Booth (1971) was used.

The samples were fine enough to pass through a .125 mm sieve. A 0.8 g quantity of sample was placed in a 100 ml round bottom flask, with four antibump glass beads. Ten ml of $NaHCO_3$ solution (8% w/v) was added, the flask was gently shaken by hand and sonicated for 7-10 minutes until the sample was wetted. Care was taken so that the sample would not be widely scattered in the flask.

Fifteen ml of ethanol into which 0.5 ml of FDNB was dissolved (Eastman Kodak Co.) was added to each flask. The flask was shaken, gently at first, for 4 hours at room temperature in a Junior Orbit Shaker (of the Lab-Line Instruments, Inc.) at 200 rpm. Midway through the shaking period, the flask was twirled and the rpm increased to 210 to disperse the sample and to make sure that all particles

were wetted by the FDNB solution. After the shaking period, the ethanol was evaporated in a boiling water bath (it was checked by making sure the flasks lost 12.5 g of their weight).

The mixture was cooled, mixed with 30 ml of 8.1 N HCl and refluxed for 16 hours.

The heat was turned off, the condenser was washed with a little water and the flask disconnected. The contents were filtered, while still hot, through a Whatman No. 2 filter paper and rinsed repeatedly with hot distilled water until the total filtrate was almost 250 ml. When the filtrate was cooled it was made to volume and mixed. The hydrolysates were stored at $39^{o}F$, in the dark, until used for further analysis.

Two ml of the filtrate were pipetted into each of two stoppered test tubes, A and B. The contents of tube B were extracted three times with about 5 ml of peroxide-free diethyl ether. Most of the ether was removed with a water aspirator. The dissolved ether was removed by placing the tube in a steam bath until effervescence from the residual ether ceased. A drop of phenolphthalein solution was added followed by addition of NaOH solution (120 g/liter) until the first pink appeared. Two ml of carbonate buffer pH 8.5 (19.5 g $NaHCO_3$, and 1 g Na_2CO_3 dissolved in 250 ml of water and pH adjusted to 8.5) were added. Under the fume hood 0.06 ml of methyl chloroformate (Eastman Kodak Co.) were

added. The tube was firmly stoppered and shaken vigorously. Internal pressure was released cautiously. After about 8 minutes, 0.75 ml concentrated HCl were added dropwise with agitation to prevent frothing. The tube was shaken occasionally. The solution was extracted with ether 4 times as described above and the residual ether was removed by warming the tube in a steam bath. The tube was cooled and the contents made up to 10 ml with water. In the meantime, tube A was extracted three times with peroxide-free diethyl ether. Residual ether was removed and the contents made up to 10 ml with 1 N HCl.

The absorbance of both A and B were read at 435 nm against deionized water. Reading A minus reading B (the blank) is the net absorbance attributable to ε-dinitrophenyllysine (DNP-lysine).

With each set of samples analyzed, the absorbance of the standard ε-DNP-lysine solution, carried through the same procedure as the samples, was determined.

The standard solution was prepared by dissolving 254 mg of mono-ε-N-dinitrophenyllysine hydrochloride (ε-DNP-lysine, mol. wt. 348.8, lysine content 41.9%)(Sigma Chemical Company), in 200 ml of 8.1 N HCl. Two tenths of 1 ml of this solution were used in each run for tubes A and B. This amount contained 0.106 mg lysine when it was diluted to 10 ml and gave a net absorbance of .400 at 435 nm.

The available lysine content was calculated from the following formula:

$$c = \frac{WsxAuxVx100x100}{WuxAsxax(CP)}$$

c = content as g lysine/16 g N

Ws = weight of standard, expressed as mg lysine in standard solution used in each run (usually 0.2 ml of standard solution used containing 0.106 mg lysine)

Wu = weight of sample hydrolyzed in mg

As = net absorbance (absorbance tube A - Absorbance tube B) of standard solution

Au = net absorbance of unknown

V = total volume of filtrate (usually 250 ml)

a = aliquot of filtrate (usually 2 ml)

(CP) = crude protein of sample, 6.25xgN/100 g potato

A recovery factor was calculated by using ε-DNP-lysine as internal standard. Duplicate samples of each material were treated as described above as far as the addition of 8.1 N HCl, but approximately only half of the volume of the HCl was added. Standard ε-DNP-lysine solution was then added containing 7.95 mg lysine. More 8.1 N HCl was added to a total volume of 30 ml, and the procedure was continued as previously described. From the difference in available lysine between the two samples and the known amount of

lysine added into the second sample, the percent recovery factor was calculated. A second digestion was performed with the residue of the variety Russet Burbank. The residue was left in the tip of the filter cones after the last washing. Using 6 N HCl, the residue was washed, together with the antibump balls, back into the same round flask. Replicated residues were pooled. Twenty ml of 6 N HCl were used for the second digestion. The solution was refluxed 16 hours, filtered hot, washed, and made up to 100 ml and the DNP-lysine determined as described above.

The values of samples for available lysine were corrected only for half of the loss estimated by the internal standard, since it has been shown by Booth (1971) that the loss of protein-bound dinitrophenyl lysine (as the lysine of the sample) is only half of the loss of free ε-DNP-lysine (as that of the internal standard).

Amide Analysis

The amides asparagine and glutamine were analyzed on distilled water extracts, free of protein, using the "New Amino Acid Analyzer Technikon". The procedure used to extract the free amino acids was adapted to extract the amides asparagine and glutamine in raw potatoes. Since acid hydrolysis was not employed, the amides were not destroyed and could be quantitated directly.

RESULTS AND DISCUSSION

The discussion of the results will be presented in two parts. Part I discusses the nutritional quality of potato protein and Part II covers the preparation of an air-classified high protein potato flour and its use in bread-making.

Part I

Nutritional Quality of Potato Protein

A. Nitrogen distribution within the tuber

In Table 6 the results of an analysis of the nitrogen content distributed within the raw and boiled potato tuber of five freeze-dried potato varieties are presented. The results of Table 6 were obtained with the potato flour free of skin. The skin of the raw freeze-dried sections was removed with the tip of a stainless steel spatula. The skin of the boiled potatoes was removed by hand.

For the statistical study, the data of Table 6 for potato sections 1, 2, 3 and 4 (see Figure 2) were rearranged by averaging the N content of sections 2 and 3. The average of sections 2 and 3 was named 2' as it is shown in Table 7. These data were analyzed using Dunnett's test (Gill, 1978).

Table 6. Nitrogen distribution within the potato tuber in five freeze-dried varieties[1]

Section No.	% N (g/100 g sample)				
	Atlantic	Denali	Russet Burbank	Superior	Monona
1	1.33	1.47	1.41	1.91	2.09
2	1.39	1.59	1.55	1.97	2.21
3	1.65	1.64	1.58	2.10	2.26
4	1.49	1.75	1.53	2.10	2.11
5	1.91	1.85	1.90	2.09	2.35
6	1.65	1.55	1.69	1.72	2.06
7	1.67	1.65	1.76	2.01	2.05
Outer layer[2]	1.62	1.32	1.45	1.88	1.55
Inner layer[3]	1.74	1.47	1.54	2.00	1.57
periderm[4] (skin)					2.74

[1]Mean based on two different tubers.

[2,3]The whole potato was boiled with distilled water.

[4]Periderm removed after boiling and drying in an oven.

Table 7. Nitrogen distribution within the raw potato tuber in five freeze-dried varieties

Section No.	% N (g/100 g sample)				
	Atlantic	Denali	Russet Burbank	Superior	Monona
1	1.33	1.47	1.41	1.91	2.09
2, 3 $\bar{X}(2')$	1.52	1.62	1.57	2.04	2.24
4	1.49	1.75	1.53	2.10	2.11

[2'] Average of the N content of sections 2 and 3.

The results showed that there was no significant difference ($P<0.01$) between sections 1, 2' and 4, but a slight significant difference ($P<0.05$) was found in section 1 versus 4 (Table 7). Apparently the section near the apical end of the potato had slightly higher N content than the section near the stem end.

It was found also that the N content of the potato is higher in the dried peel flour compared to the N content of the potato flour of the outer and inner layers. The same results were observed when it was compared to the N content for potato sections 1, 2, 3, 4, 5, 6, and 7 (Table 6). The PER of the skin, however, is practically zero, according to previous work (Meister and Thompson, 1976a).

The varieties Russet Burbank and Monona displayed higher N contents in sections 2 and 3 than what were found in sections 1 and 4. In the varieties Atlantic, Denali and Superior, sections 3 and 4 had a higher N content than did sections 1 and 2. For the variety Monona, section 3 was found to have the highest N content. In all the varieties studied, the sections 2, 3 and 4 had higher N contents than section 1 (Table 6, Figure 3).

In relation to sections 5, 6, and 7, it was observed that section 5 had the highest N content in all five potato varieties (Table 6, Figure 3). However, the difference was not statistically significant ($P<0.05$). Section 5 corresponds roughly to the pith (Figure 1). When the skin is not

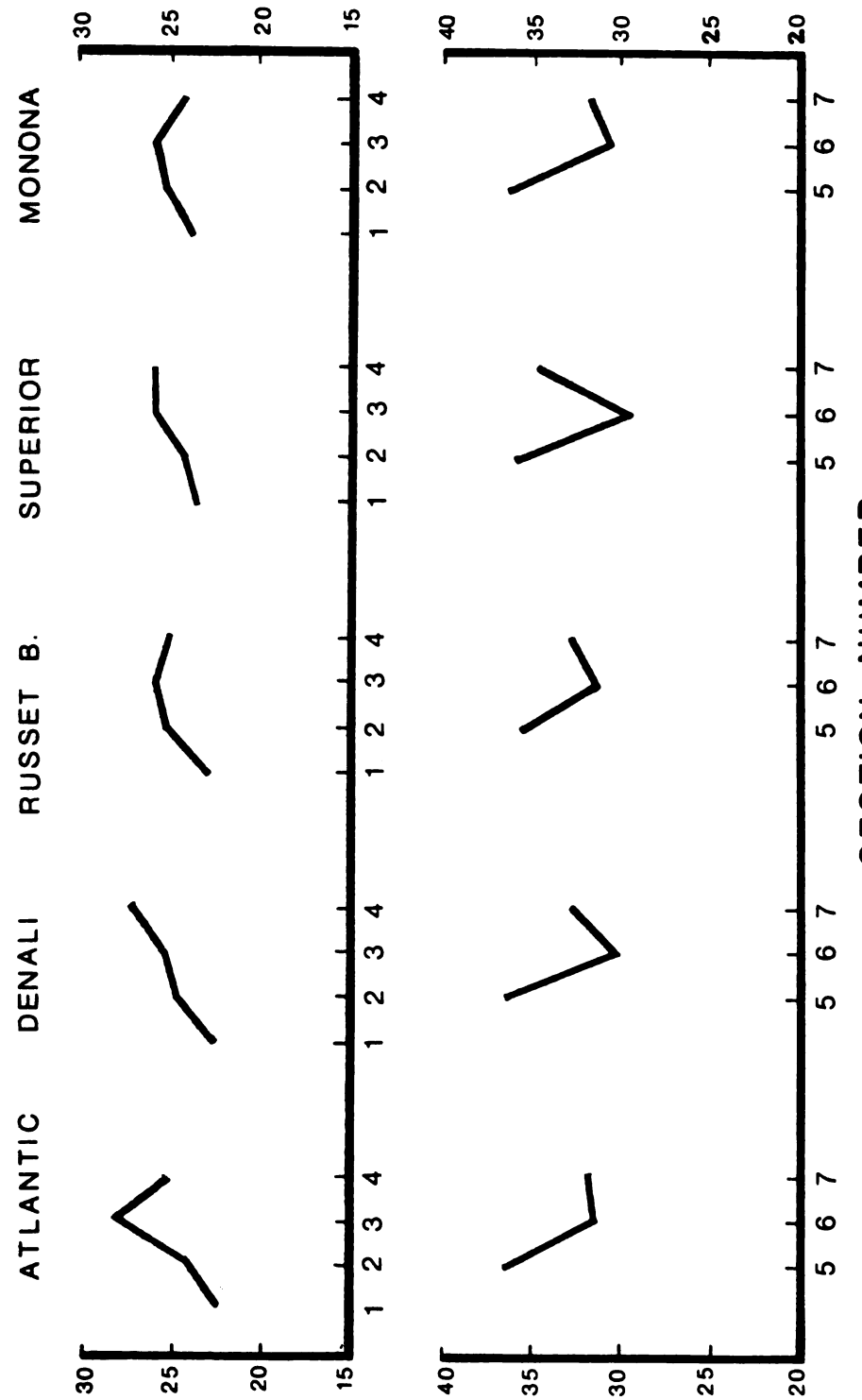

Figure 3. Percentage distribution of N in seven potato sections (see Fig. 2) for five varieties.

considered, the pith of the potato has the highest N content. If the outer layer of the potato is cut after boiling, the inner layer is found to have a higher N content than outer one (Table 6). This confirms the results presented above, that the N content tends to rise toward the pith of the potato. The outer layer was cut after observing visually two defined areas following the boiling of the potato.

B. Amino acid distribution within the tuber

The amino acids in the acid hydrolysate were calculated in micrograms and expressed as g residue per 100 g amino acid hydrolysate.

In Table 8 is presented the amino acid distribution in acid hydrolysates of the outer and inner layers of the boiled freeze-dried potatoes. The results showed higher amounts of aspartic and glutamic acids in the inner layer compared to the outer layer in the varieties Atlantic, Russet Burbank and Monona. The amino acid proline tended to be higher in the outer layer than in the inner layer (Table 8). Of the essential amino acids, valine, leucine, and lysine tended to be higher in the outer layer compared to the inner layer. Of the total content of amino acids in the acid hydrolysate, the essential amino acids threonine, valine, isoleucine, leucine, phenylalanine and lysine comprised 27.5% in the outer layer and 24.0% in the inner

Table 8. Amino acid distribution in hydrolysates of the outer and inner layers (see Figure 2) of boiled freeze-dried potatoes (g residue/100 g amino acid hydrolysate).[1]

Amino acid	Atlantic		Russet Burbank		Monona	
	outer layer	inner layer	outer layer	inner layer	outer layer	inner layer
Aspartic acid	29.9	36.5	36.9	37.9	36.1	41.0
Threonine	2.7	2.2	2.2	2.1	2.5	2.5
Serine	2.1	1.7	1.7	1.5	2.3	1.9
Glutamic acid	19.3	21.2	17.2	21.5	14.0	21.4
Proline	6.1	5.5	3.1	0.9	3.2	2.7
Glycine	3.6	1.8	2.2	2.1	4.5	1.7
Alanine	2.6	2.2	2.8	1.7	0.9	2.3
Valine	4.4	3.9	5.0	2.9	5.8	5.0
Isoleucine	3.3	2.9	3.1	2.7	2.6	3.1
Leucine	5.9	4.9	5.9	4.4	5.5	4.0
Tyrosine	3.6	2.4	4.7	6.7	5.0	2.2
Phenylalanine	3.7	4.6	4.0	4.8	4.3	2.9
Lysine	7.5	5.5	6.4	5.4	6.1	5.3
Histidine	1.9	1.1	1.8	2.2	1.5	1.4
Arginine	3.4	3.6	3.0	3.2	5.7	2.6
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1]Mean of duplicates. The values for cystine and methionine (g/16 g N) in the variety Russet Burbank, showed for cystine 1.30 and 1.20 for the outer and inner layer, respectively, and for methionine 1.54 and 1.37, for the outer and inner layer, respectively.

layer for the variety Atlantic; 26.6% in the outer layer and 22.3% in the inner layer for the variety Russet Burbank; and 26.8% in the outer layer and 22.8% in the inner layer for the variety Monona. These data indicate that there is also variation among varieties in relation to the distribution of the essential amino acids in the outer versus inner layers. A higher concentration of essential amino acids is observed in the outer layer in all the potato varieties studied. In order to protect methionine and cystine from destruction during acid hydrolysis, these amino acids were oxidized before hydrolysis to methionine sulfone and cysteic acid respectively, by performic acid. Analysis of the sulfur amino acids, methionine and cystine in the variety Russet Burbank, showed for cystine 1.30 and 1.20 g/16 g N for the outer and inner layer, respectively. For methionine it showed 1.54 and 1.37 g/16 g N, respectively, for the outer and inner layers.

Table 9 represents the amino acid distribution in acid hydrolysates of the apical and stem sections of the raw freeze-dried Russet Burbank potatoes. It was observed that the predominant amino acids in the apical section were aspartic and glutamic acids. In the stem section aspartic acid was the predominant amino acid. The apical section compared to the stem section had higher amounts of

Table 9. Amino acid distribution in hydrolysates of the apical and stem sections (see Figure 2) in the raw Russet Burbank potatoes (g residue/100 g amino acid hydrolysate)[1]

Amino acid	Russet Burbank	
	Apical section	Stem section
Aspartic acid	18.5	44.0
Threonine	2.9	5.9
Serine	2.6	6.4
Glutamic acid	20.5	3.7
Proline	7.6	5.2
Glycine	4.1	2.0
Alanine	4.4	2.6
Valine	8.8	5.0
Cystine	0.0	0.0
Methionine	1.2	0.8
Isoleucine	4.0	2.4
Leucine	6.1	6.2
Tyrosine	4.5	3.5
Phenylalanine	4.6	2.8
Lysine	6.5	6.4
Histidine	1.4	1.1
Arginine	2.3	2.0
Total	100.0	100.0
Cystine[2] (g/16 g N)	1.10	1.12
Methionine[3] (g/16 g N)	1.50	1.34

[1]Mean of duplicates

[2,3]These amino acids were oxidized before hydrolysis by performic acid, to protect them during acid hydrolysis.

glutamic acid, glycine, alanine, valine, Isoleucine, tyrosine, and phenylalanine. Lower contents of aspartic acid, threonine and serine were observed in the apical end in relation to the stem end (Table 9). The content of glutamic acid of the stem section was 3.7% compared to 20.5% of the apical end section. The amino acids leucine, lysine, histidine, and arginine seems to be similarly distributed in the apical and stem sections (Table 9). It was observed also that the total distribution of the essential amino acids threonine, valine, isoleucine, leucine, phenylaline, and lysine was high in the apical section, 32.9% compared to the stem section, which has 28.7%.

The sulfur amino acids were oxidized before hydrolysis to methionine sulfone and cysteic acid, as described previously.

The sulfur amino acids, methionine and cystine were analyzed in the stem and apical sections of the Russet Burbank potatoes. The values for cystine were 1.12 and 1.10 g/16 g N for the stem and apical sections, respectively. For methionine, 1.34 and 1.50 g/16 g N were obtained, respectively, for the stem and apical sections.

The N and amino acid distribution within the potato tuber could be a help to the potato breeder because the

biological value of the potato protein can be improved through plant breeding.

C. Protein and amino acid losses during boiling

Table 10 represents the percent protein loss from boiled potatoes compared to the original protein content, and raw sample. The results expressed as percentages of the raw sample seem insignificant. Therefore, the discussion of the percent protein loss during boiling will be related to the percent change from the original protein content, based at 100%. There are some differences among varieties in the protein losses during boiling under the types of cutting indicated in Table 10.

If the data for all varieties and for each type of cutting is averaged, it is found that the whole tuber boiled with skin suffered the lowest loss of protein, 0.8%, and the whole tuber peeled and cut into halves had the highest protein loss, 10.4%. Comparison of the peeled versus non-peeled types of cuttings of the potato, showed that the protein losses are higher in the peeled potatoes: 6.5% protein loss for the whole tuber when peeled versus 0.8% for the whole tuber with skin, 10.4% for the whole tuber when peeled and cut into halves versus 4.3% for the whole tuber with skin cut into halves, and 9.3% for the whole tuber when peeled and cut into quarters versus 4.8% for the whole tuber with skin cut into quarters.

Table 10. Percent protein loss (Nx6.25) in potatoes boiled in distilled water[1]

Variety	a		b		c		d		e		f	
Time (min)			time		time		time		time		time	
Atlantic	30	0.6 (0.015)	25	3.3 (0.086)	10	2.9 (0.075)	17	6.5 (0.170)	15	9.4 (0.245)	10	7.6 (0.198)
Denali	35	0.4 (0.011)	25	3.3 (0.085)	9	3.1 (0.081)	17	4.1 (0.106)	15	6.4 (0.167)	11	6.1 (0.158)
Russet Burbank	30	0.5 (0.013)	25	4.3 (0.112)	9	5.3 (0.138)	17	5.6 (0.146)	15	10.6 (0.275)	10	10.2 (0.264)
Superior	28	1.0 (0.023)	25	4.8 (0.111)	9	6.3 (0.144)	17	6.8 (0.156)	15	13.4 (0.308)	10	11.5 (0.265)
Monona	25	1.3 (0.028)	25	6.0 (0.132)	8	6.3 (0.138)	15	9.5 (0.210)	15	12.0 (0.265)	10	11.3 (0.249)
$\bar{X}$	29.6	0.8 (0.018)	25	4.3 (0.105)	9	4.8 (0.115)	16.6	6.5 (0.158)	15	10.4 (0.252)	10.2	9.3 (0.227)

[1]Expressed as percent change from the original protein content of raw potatoes. Percent protein of raw potatoes: Atlantic, 2.6%, Denali, 2.6%, Russet Burbank, 2.6%, Superior, 2.3%, and Monona, 2.2%. The data in parenthesis belong to protein loss, expressed as percent based on raw sample. Mean of duplicates from independent samples.

a = whole tuber with skin

b = whole tuber with skin cut longitudinally into halves

c = whole tuber with skin cut into quarters

d = whole tuber peeled with a home peeler

e = whole tuber peeled and cut into halves

f = whole tuber peeled and cut into quarters

Next, when comparing the types of cuttings only in potatoes with skin, the largest loss of protein was observed for the quartered tuber. The loss was 4.8% for quarters, 4.3% for halves and 0.8% for the whole tuber. Examining the area of the potato directly exposed to the water could explain this difference. The results of this study are lower compared to 50% loss of total protein reported by Desborough and Weiser (1974) after boiling halved potatoes with skin for 30 minutes.

When the peeled potato was boiled, the largest loss, 10.4%, of protein was observed with the whole tuber peeled and cut into halves; the loss in the whole peeled tuber was 6.5%.

The difference in protein loss between the peeled halved and the peeled quartered tuber might be due to the extent of time for boiling. The peeled quartered potato required less time for cooking, 10.2 min. versus 15 min for the peeled halved tuber (Table 10).

After boiling the potatoes, the water was examined for the presence of amino acids. Table 11 represents the amino acid distribution in the acid hydrolysates of the boiled water from the cooked potatoes. The predominant amino acids in the boiled water of Russet Burbank and Atlantic potatoes boiled with intact skins were aspartic and glutamic acids. These non-essential amino acids comprised 68.8% and 70.2% of the total acid hydrolysate for the Russet Burbank

Table 11. Amino acid distribution in hydrolysates of the boiled water from the cooked potatoes (g residue/ 100 g amino acid hydrolysate)[1]

Amino acid	Russet Burbank			Atlantic		
	a	b	c	a	b	c
Aspartic acid	43.3	36.1	39.2	48.1	37.5	42.3
Threonine	1.2	1.1	0.8	2.3	1.2	0.9
Serine	2.8	1.2	1.2	1.9	1.4	1.1
Glutamic acid	25.5	38.3	35.4	22.1	30.3	33.9
Proline	2.6	10.4	1.5	8.4	10.2	2.2
Glycine	3.7	1.8	0.7	2.7	1.3	1.4
Alanine	1.9	1.1	1.3	1.3	1.2	2.0
Valine	5.3	3.7	3.8	4.6	4.0	4.0
Isoleucine	2.8	1.2	3.4	1.2	1.3	1.6
Leucine	2.4	1.3	1.2	1.7	1.3	1.3
Tyrosine	3.8	1.0	2.5	1.7	1.8	2.1
Phenylalanine	3.4	1.4	2.4	1.4	2.1	2.4
Lysine	1.3	1.4	2.7	1.3	1.7	3.0
Histidine	traces	traces	1.4	traces	traces	0.7
Arginine	traces	traces	2.5	1.3	4.7	1.1
Total	100.0	100.0	100.0	100.0	100.0	100.0

[1]Notations:

Means of duplicates
a = whole tuber with skin

b = whole tuber with skin cut longitudinally into halves

c = whole tuber with skin cut into quarters

and Atlantic potatoes, respectively. Of the essential amino acids, valine comprised 5.3% for the Russet Burbank variety and 4.6% for the Atlantic variety.

The same predominant amino acids were found in the boiled water from the boiled halved potatoes with skins. Aspartic and glutamic acids comprised 74.4 and 67.8% of the total pool in the acid hydrolysate for the Russet Burbank and Atlantic potatoes, respectively.

Potatoes cut into quarters were observed also to yield predominantly aspartic and glutamic acids. In the water from the Russet Burbank variety 74.6% of the total pool consisted of these acids and 76.2% in the Atlantic variety. A decrease in threonine and proline was also observed whereas isoleucine, lysine and histidine increased when compared to other types of cuttings with the Russet Burbank and Atlantic potatoes (Table 11).

The sulfur amino acids, cystine and methionine, were analyzed in the boiled water from the Russet Burbank and Atlantic potatoes boiled under three cutting conditions. These conditions were intact skins, halved potatoes with skins and quartered potatoes with skins. The time for boiling with intact skins for the Russet Burbank and Atlantic potatoes is registered in Table 10.

In order to protect methionine and cystine from destruction during acid hydrolysis, these amino acids were oxidized

as indicated before.

Table 12 represents the loss of cystine and methionine from boiled potatoes, expressed as a percent (mg/100 g) from the original content of cystine and methionine, based at 100%. In the same table appears the losses of each sulfur amino acid per each 100 g of raw potatoes and protein. The losses expressed per each 100 g of raw potatoes and protein seem insignificant. Therefore, the discussion about the losses of each sulfur amino acid during boiling will be related to the percent from the original content of cystine and methionine, based at 100%.

Of the three cuttings, it was found that the whole tuber boiled with intact skins suffered a lower loss of cystine and methionine than the halved or quartered tubers. This result was observed in both Russet Burbank and Atlantic potatoes boiled with intact skins. In the boiled Russet Burbank potatoes, 0.24 g and 1.33 g were lost for each 100 g of cystine and methionine present in the raw potatoes. For the boiled Atlantic variety, losses per 100 g of cystine and methionine present in the raw potatoes were 0.13 g for the former amino acid and 0.79 g for the latter one (Table 12).

The largest loss of sulfur amino acids was observed for the halved and quartered potatoes boiled with intact skins. For the halved Russet Burbank potatoes the losses

Table 12. Cystine and methionine lost during the boiling of potatoes

Variety	cyst	met	cyst	met	cyst	met[4]
	mg		mg		mg	
	100 g raw potato		100 g protein		100 g original cyst, met	
Russet Burbank[1]	.085	.55	3.3	21.2	236	1,325
Russet Burbank[2]	.143	.80	5.5	31.0	393	1,938
Russet Burbank[3]	.190	1.20	7.3	46.2	521	2,888
Atlantic[1]	.044	.35	1.7	13.5	131	794
Atlantic[2]	.214	1.30	8.2	50.0	631	2,941
Atlantic[3]	.260	1.20	10.0	46.2	769	2,718

[1]Whole tuber with skin

[2]Whole tuber with skin cut longitudinally into halves

[3]Whole tuber with skin cut into quarters

[4]Mean of duplicates. The content of the sulfur amino acids of the raw potatoes was: cystine 1.4 and methionine 1.6 (g/16 g N) for Russet Burbank, and cystine 1.3 and methionine 1.7 (g/16 g N) for Atlantic. The protein content of raw Russet Burbank and Atlantic potatoes was 2.6 %.

were 0.39 g of cystine and 1.94 g of methionine per each 100 g of each sulfur amino acid present in the raw potatoes. For the quartered Russet Burbank potatoes the losses were 0.52 g of cystine and 2.89 g of methionine per each 100 g of each sulfur amino acid present in the raw potatoes. For the halved Atlantic potatoes the losses were 0.63 g of cystine and 2.94 g of methionine per each 100 g of each sulfur amino acid. For the quartered Atlantic potatoes the losses were 0.77 g of cystine and 2.72 g of methionine per 100 g of each sulfur amino acid (Table 12).

The loss for methionine is higher than for cystine for each type of cutting. One explanation of this might be that the potatoes have higher free methionine content than free cystine. It was found in this study that free cystine appears as traces in some varieties and in others it is not detected (Table 15).

The fact that the loss of sulfur amino acids during boiling of potatoes increases by cutting the potatoes, indicates that it is advisable to boil the potatoes with intact skins. Nutritionally, this is very important. Methionine and cystine are the limiting amino acids in potatoes; therefore, avoiding the loss of methionine and cystine is significant for the biological quality of the potato protein.

D. Amino acids of potato tubers

1. Amino acid distribution

Table 13 represents the sulfur amino acid content in the raw freeze-dried Russet Burbank and Atlantic potatoes.

Methionine and cystine were oxidized before hydrolysis, as described previously. The sulfur amino acid content of the Russet Burbank and Atlantic potatoes was: cystine 1.4 and methionine 1.6 g/16 g N for Russet Burbank, and cystine 1.3 and methionine 1.7 g/16 g N for Atlantic. These values of sulfur amino acids were used to evaluate the cystine and methionine losses during boiling of Russet Burbank and Atlantic potatoes (Table 12).

Table 14 represents the amino acid distribution in acid hydrolysates of the boiled Russet Burbank potatoes chemically or organically fertilized. The Russet Burbank potatoes chemically or organically fertilized showed similar patterns in the amino acid distribution. Apparently, slight variations were observed in serine, glutamic acid, glycine, isoleucine, and histidine.

In relation to the sulfur amino acids, similar values were observed in the boiled Russet Burbank potatoes chemically or organically fertilized (Table 14).

Table 13. Cystine and methionine content in raw freeze-dried potatoes (g/16 g N)[1]

Amino acid	Atlantic	Russet Burbank
Cystine	1.3	1.4
Methionine	1.7	1.6

[1]Mean of duplicates.

Table 14. Amino acid distribution in hydrolysates of the boiled Russet Burbank potatoes chemically or organically fertilized (g residue/100 g amino acid hydrolysate)[1]

Amino acid	Russet Burbank	
	Chemically Fertilized	Organically Fertilized
Aspartic acid	33.7	34.3
Threonine	2.1	2.1
Serine	2.3	1.5
Glutamic acid	18.0	21.1
Proline	2.9	3.0
Glycine	3.0	2.0
Alanine	2.8	2.8
Valine	5.0	4.4
Cystine	traces	traces
Methionine	0.9	0.5
Isoleucine	3.6	4.2
Leucine	4.8	5.1
Tyrosine	5.5	4.8
Phenylalanine	4.3	3.6
Lysine	5.0	5.4
Histidine	3.0	2.2
Arginine	3.1	3.0
Total	100.0	100.0
Cystine[2] (g/16 g N)	1.37	1.39
Methionine[3] (g/16 g N)	1.58	1.60

[1] Mean of duplicates

[2,3] These amino acids were oxidized before hydrolysis by performic acid, to protect them during acid hydrolysis.

2. Free amino acids

The determination of free amino acids in potatoes was warranted in this study because a) free amino acids are well utilized by the human body, and b) free amino acids can be leaked or rinsed during cooking or processing of potatoes.

Table 15 represents the free amino acid distribution and amide distribution in distilled water extracts from raw freeze-dried potatoes. The discussion about amide distribution in potatoes will be presented later. The results for free amino acids are expressed as g residue per 100 g of non-protein extract (free amino acids plus amides). In the distilled water extract the free essential amino acids threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine comprised summarily the following percentages of the total pool: 16.0% for Atlantic potatoes; 7.5% for Denali potatoes; 23.3% for Russet Burbank potatoes; 20.0% for Superior potatoes and 21.1% for Monona potatoes.

In the potato varieties Atlantic and Denali the predominant free amino acids in distilled water extracts were aspartic acid, glutamic acid, proline, valine and arginine. These same predominant amino acids were observed in the varieties Russet Burbank, Superior and Monona with the exception of proline that was present in lower percentages than observed in Atlantic and Denali varieties (Table 15).

In relation to the sulfur free amino acids it was found in this study that cystine was present as traces in the distilled protein free

Table 15. Free amino acid[1] and amide[2] distribution in water extracts from raw freeze-dried potatoes

	Atlantic	Denali	Russet Burbank	Superior	Monona
Amino acids					
Aspartic acid	8.4	15.3	9.9	14.8	8.9
Threonine	2.1	1.0	2.0	1.8	1.7
Serine	1.5	1.6	2.6	2.2	1.8
Glutamic acid	15.1	21.5	6.6	8.7	13.6
Proline	12.1	8.8	1.1	traces	0.5
Glycine	1.1	1.0	0.3	0.5	0.3
Alalanine	1.2	0.5	1.0	0.9	1.4
Valine	5.4	2.8	7.9	3.9	6.1
Cystine	traces	0.0	0.0	traces	0.0
Methionine	1.7	0.7	1.1	2.2	0.8
Isoleucine	1.2	0.8	2.7	2.5	1.9
Leucine	0.8	0.4	1.4	1.1	1.2
Tyrosine	1.6	0.4	4.0	12.0	4.8
Phenylalanine	2.0	0.8	3.5	5.5	4.9
Lysine	2.8	1.0	4.7	3.0	4.5
Histidine	1.8	1.1	2.0	4.3	2.3
Arginine	7.2	5.9	4.7	10.6	6.4
Amides					
Asparagine	17.9	18.1	20.1	14.4	16.1
Glutamine	16.1	18.3	24.4	11.6	22.8
Total	100.0	100.0	100.0	100.0	100.0

[1]Mean of duplicates (Grams residue/100 g free amino acids + amides).

[2]Mean of duplicates (Grams/100 g free amino acids + amides).

water extracts from Atlantic and Superior potatoes. These potato varieties had higher amounts in free methionine compared to Denali, Russet Burbank, and Monona potatoes. The free methionine content, expressed as g residue per 100 g of non-protein extract (free amino acids plus amides), were: 1.7, 0.7, 1.1, 2.2 and 0.8 for Atlantic, Denali, Russet Burbank, Superior and Monona potatoes, respectively (Table 15). The varieties Atlantic and Superior could be chosen to improve the sulfur containing amino acids in potatoes through plant breeding. Also, the wide range in content of free methionine, 0.7 to 2.2 g per 100 g of the non-protein extract (free amino acids plus amides), indicates that selection of potatoes high in methionine could be possible. Luescher (1972) suggested that with intensive N fertilization the ideal potato to select should be high in free methionine. Also, the possible presence of free cysteine should be kept in mind.

The variation of free amino acids among potato varieties is pronounced. Mulder and Bakema (1956) stated that the composition of the soluble non-protein fraction is much less constant than that of the true protein fraction. Some factors such as type of variety, mineral nutrition of the plant, environmental conditions, etc. might affect the composition of the nonprotein fractions.

E. Amides, asparagine and glutamine of potato tubers

Table 15 shows the free amide distribution in distilled water extracts from raw freeze-dried potatoes. The results

are expressed as g amide per 100 g of the non-protein extract (free amino acids plus amides).

In relation to amides it was found that the distribution of asparagine ranged from 14.4 to 20.1%, g per 100 g non protein extract (free amino acids plus amides), and glutamine ranged from 11.6 to 24.4% (Table 15). The amides comprised 34.0, 36.4, 44.5, 26.0 and 38.9% of the total pool (free amino acids plus amides), for the potato varieties Atlantic, Denali, Russet Burbank, Superior, and Monona, respectively. The results obtained in this study suggest a variation in the content of amides among potato varieties and also that the concentration of amides is high in potatoes. A high content of amides in potatoes could be useful from the nutritional point of view, since these amides (asparagine and glutamine) may prevent antagonism among amino acids (McCay, 1959).

F. Available lysine by the FDNB method

Table 16 shows the values of available bound lysine obtained by the FDNB method, in whole boiled potatoes with intact skins.

The results were obtained from the study of potato flour free of the skin which was removed by hand after boiling the potatoes.

Comparison of the amounts of available bound lysine in boiled potatoes showed that there were slight differences in the amounts of available lysine among the varieties analyzed.

Table 16. Available bound lysine in boiled potatoes by the FDNB method[1]

	Atlantic	Russet Burbank	Monona
Corrected values g available lysine/100 g protein	4.77	4.54 (5.08)[2]	4.94

[1]Mean based on two determinations made on one group of four freeze-dried boiled potatoes.

[2]The value in parenthesis belong to raw Russet Burbank potatoes.

The corrected values of available bound lysine (g/100 g protein) were: Atlantic, 4.77, Russet Burbank, 4.54, and Monona, 4.94.

Raw potato flour and cooked potato flour of Russet Burbank potatoes were tested. Available lysine in the raw freeze-dried material was 5.08 g/100 g protein versus 4.54 g in the boiled freeze-dried potato. These results demonstrated that there is a decrease of 10.6% in the availability of the lysine after boiling the Russet Burbank potatoes. When the residue left after the Hcl hydrolysis was subjected to a second Hcl hydrolysis, Russet Burbank potatoes were found to contain 0.17 g more lysine per 100 g protein.

Table 17 shows the percent weight loss during boiling of potatoes in distilled water. The results showed a decrease in weight of potatoes after boiling compared to raw weight. However, an increase in weight was observed in relation to raw potatoes, peeled or unpeeled, when they were overcooked. Presumably the starch bound more water in the overcooked, cracked potatoes (Van Den Berg et al., 1975). Therefore, to avoid overcooking, the cooked potatoes were carefully tested with a thin stainless steel spatula by inserting it into the tuber to determine when the potatoes were cooked.

Table 18 represents the total solids (TS) of raw and boiled potatoes. The results show that the TS of the boiled potatoes with intact skins is approximately that of the raw material. However, if potatoes are peeled or cut prior to boiling, the TS content increases. One

Table 17. Percent weight loss during boiling of potatoes in distilled water[1]

Variety	a	b	c	d	e	f
Atlantic	1.3	1.6	2.2	4.6	4.4	1.5
Denali	1.4	4.7	4.9	6.0	4.3	1.9
Russet Burbank	1.3	3.7	4.0	5.0	4.8	4.0
Superior	1.5	5.1	4.3	7.4	5.7	4.0
Monona	2.1	5.9	4.9	4.2	3.6	4.0

[1] Notations:

a = whole tuber with skin

b = whole tuber with skin cut longitudinally into halves

c = whole tuber with skin cut into quarters

d = whole tuber peeled with home peeler

e = whole tuber peeled and cut into halves

f = whole tuber peeled and cut into quarters

Mean of duplicates

Table 18. Percent total solids of raw and boiled potatoes[1]

Variety	Raw	a	b	c	d	e	f
Atlantic	21.6	22.7	23.6	22.5	24.1	22.9	25.7
Denali	23.0	22.7	23.2	24.7	23.7	23.5	25.4
Russet Burbank	21.0	19.3	21.3	20.3	22.2	21.7	23.4
Superior	17.5	19.5	18.0	17.1	17.4	19.6	19.8
Monona	16.3	14.9	-	15.7	16.5	17.9	20.0

[1]Notations:

a = whole tuber with skin

b = whole tuber with skin cut longitudinally into halves

c = whole tuber with skin cut into quarters

d = whole tuber peeled with a home peeler

e = whole tuber peeled and cut into halves

f = whole tuber peeled and cut into quarters

Mean of duplicates

explanation to this could be that there is a loss of the water present in the potato tissue. This fact could explain the weight loss during boiling of potatoes, too.

Table 19 shows the percent of the raw portion of potato removed by a home peeler; also, the total solids and N contents of this portion. An average of 10.1% of the raw potato was removed by the home peeler. This removed portion contained 14.9% TS, on the average, and 2.8% N on dry basis.

G. Protein efficiency ratio (PER)

1. Fertilization
2. Supplementation

The growth curves for the 8 groups of the rats fed the experimental diets are shown in Figure 4. Rats fed the supplemented diets, potato + dried whole eggs and potato + whey powder, grew at higher rates 5.07 and 2.71 g/rat/day, respectively, when compared to rats fed the commercial dried potato diet that grew at a rate of 2.00 g/rat/day. Those fed diets containing Russet Burbank potatoes chemically or organically fertilized (CF, OF) grew at rates of 1.79 and 1.63 g/rat/day, respectively; these rates were not statistically different.

In general the results indicated that rats fed the potato diets grew at slower rates than those fed whole dried eggs, casein and the supplemented diets.

The average values of food intake, protein intake, weight gain, food conversion ratio, and PER are shown in

Table 19. Percent of the raw portion of potato removed by a home peeler; percent total solids content of the removed portion; and percent N content of the removed portion on a dry basis.

Variety[1]	% portion	% TS[2]	% N
Atlantic	8.6	15.0	2.9
Denali	10.5	15.4	2.7
R. Burbank	10.6	16.4	3.0
Superior	10.5	14.2	2.8
Monona	10.2	13.5	2.8
$\bar{X}$	10.1	14.9	2.8

[1]Mean of duplicates

[2]Total solids.

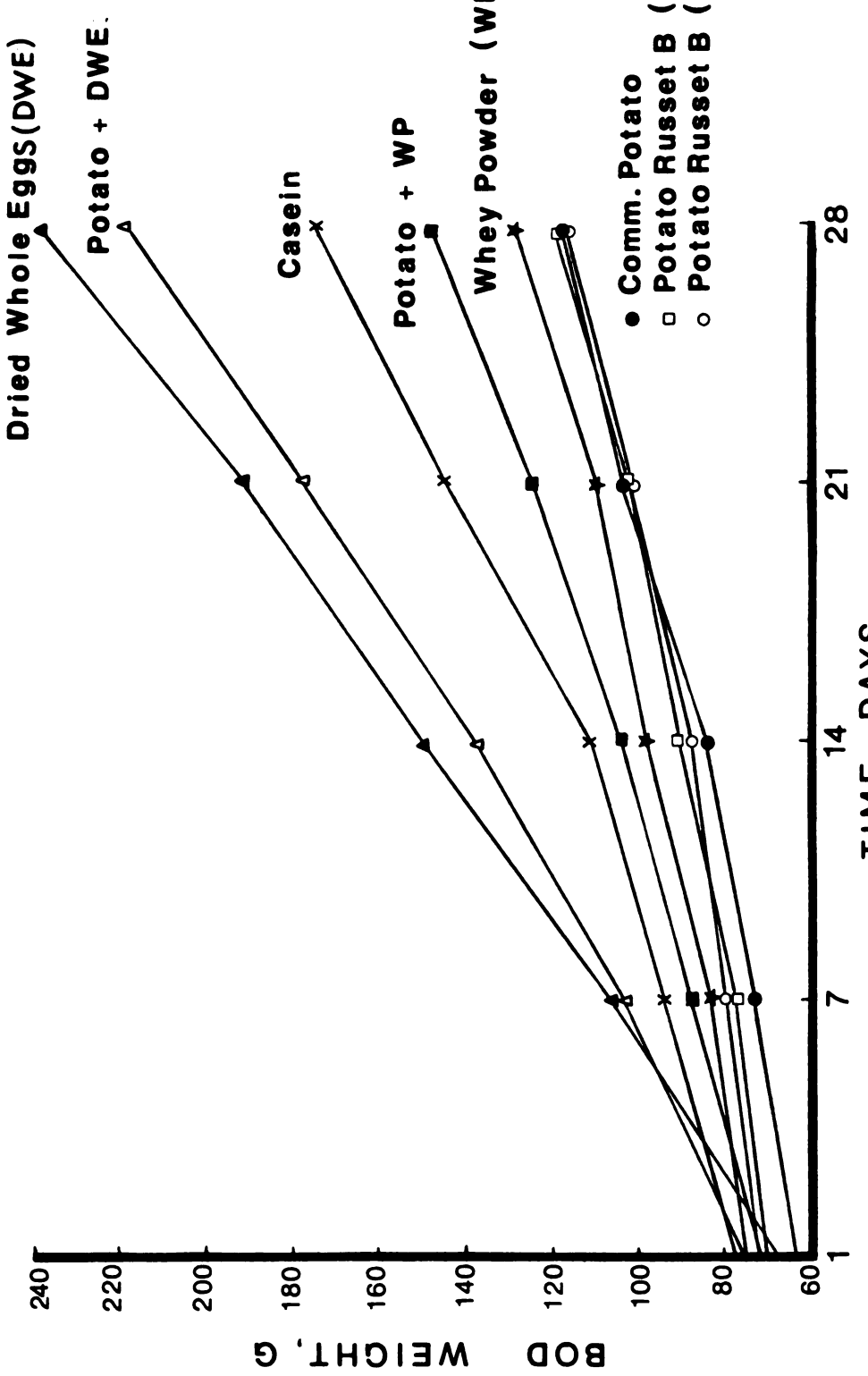

Figure 4. Average weight gain of weanling rats fed diets containing as a sole source of protein: a) chemically fertilized potatoes (CF); b) organically fertilized potatoes (OF); c) whey powder; d) dried whole eggs; e) commercial potato powder; f) commercial potato powder + whey powder; g) commercial potato powder + dried whole eggs; h) casein.

Table 20. The food conversion ratio (feed intake, g/weight gain, g) was 5.6 for the Russet Burbank potatoes, CF; 5.9 for the Russet Burbank potatoes, OF; 2.7 for dried whole eggs, 4.5 for potato + whey powder, 5.2 for the Russet Burbank potatoes, commercially dried; 3.2 for the Russet Burbank potatoes commercially dried + dried whole eggs; and 3.6 for casein.

For the whey powder, the PER and food conversion ratio could not be calculated, since the rats had diarrhea during the entire experiment, with the exception of two that apparently tolerated the lactose present in the whey powder. After one week of the experiment, the enzyme lactase (Maxilact (R) 20,000-Lab and Plant: Keyport, N.J.) was added to the water at 0.05 g/150 ml of water. No improvement in lactose tolerance was observed after the rats drank the enzyme-fortified water.

PER values were calculated from the formula weight gain, g/protein consumed, g. One group of rats was fed a diet containing casein, and was used as the control group to calculate the corrected (adjusted) PER values.

The adjusted PER values are presented in Table 20. The Russet Burbank potatoes chemically or organically fertilized produced close values of 1.62 and 1.54, respectively. This difference was not statistically significant. The similarity in the PER values observed with Russet Burbank potatoes, CF or OF, are in agreement with the sulfur

Table 20. Feed intake, protein intake, weight gain, feed conversion ratio and PER of cooked potatoes fed to rats for 28 days

Diet	Feed Intake	Protein Intake	Weight Gain	Feed Conversion Ratio	PER	Adjusted PER[1]
Potato Russet Burbank (CF)[2]	279.7 ± 25.5	28.0 ± 2.5	50.0 ± 8.7	5.6	1.79 ± .24	1.62[4c]
Potato Russet Burbank (OF)[3]	267.6 ± 20.3	26.8 ± 2.0	45.7 ± 2.3	5.9	1.71 ± .09	1.54[4c]
Whey powder	-	-	54.8 ± 12.0	-	-	-
Dried whole eggs	469.4 ± 62.8	46.9 ± 6.3	171.0 ± 16.8	2.7	3.65 ± .27	3.29[a]
Potato Russet Burbank[5] (commercially dried) + whey powder	344.6 ± 69.1	27.6 ± 5.5	75.9 ± 20.5	4.5	2.75 ± .37	2.48[b]
Potato Russet Burbank (commercially dried)	294.8 ± 52.7	23.6 ± 4.2	56.2 ± 14.2	5.2	2.38 ± .26	2.15[b]
Potato Russet Burbank[6] (commercially dried) + dried whole eggs	461.2 ± 43.5	36.7 ± 3.5	142.0 ± 15.7	3.2	3.87 ± .13	3.49[a]
Casein	351.7 ± 26.8	35.2 ± 2.7	97.5 ± 10.1	3.6	2.77 ± .17	2.50[b]

[a,b]Means with different superscripts are significantly different ($p<0.05$) (Dunnett's test)

Table 20 (cont'd.).

[1]Adjusted for casein PER = 2.5

[2,3]Chemically fertilized and organically fertilized, respectively.

[4]Statistical analysis done with these values for separate interpretation (student's t test to compare PER of potatoes CF versus OF)

[5]Commercially dried potato buds (Russet Burbank) mixed in the ratio 90:10 potato protein to whey protein.

[6]Commercially dried potato buds (Russet Burbank) mixed in the ratio 65:35 potato protein to egg protein.

amino acid composition. It was found that the sulfur amino acid content was similar in Russet Burbank potatoes, CF or OF (Table 14). Earlier reports indicate that methionine plus cystine are the amino acids which limit the nutritional value of potato protein.

The cooked Russet Burbank potatoes, CF, showed a lower PER, 1.62, than the 2.15 value observed for the same variety which was commercially dried. This difference may be due to these factors: a) some non-protein N is removed in the manufacture of potato powder, and b) the skin that was fed with the cooked potatoes is known to reduce the digestibility of protein.

The PER of the commercially dried potato flour prepared with the variety Russet Burbank and mixed with dried whole eggs in the ratio 65:35 potato protein to egg protein was 8.0% greater than the PER of the dried whole eggs. This ratio of potato protein to egg protein resulted in a PER which was 54% higher than that of the commercially dried potato, and 40% higher than that of casein (Figure 5). These results are in agreement with those of Kofranyi and Jekat (1965, 1967), who experimented with human volunteers.

When the same commercially dried potato flour was mixed with whey powder in the ratio 90:10, potato protein to whey protein, a PER value was obtained which was similar to that of casein (2.48 versus 2.50), but a 13% higher than

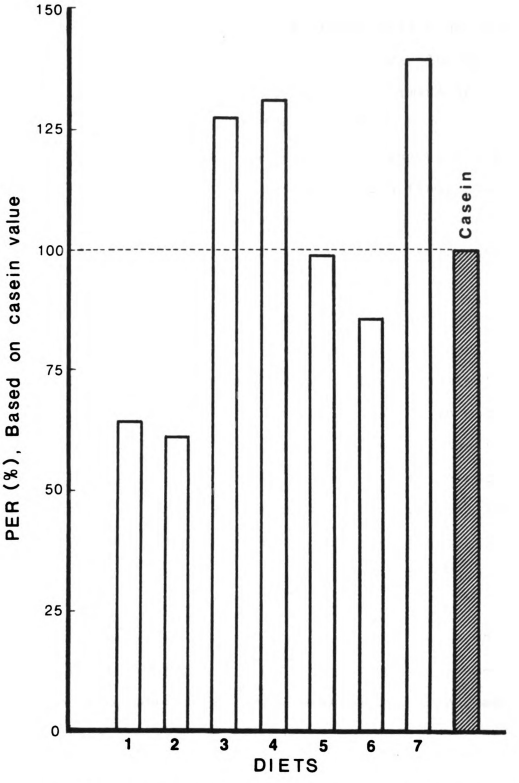

Figure 5. PER of diets calculated as percent of casein PER. 1 and 2=chemically and organically fertilized potatoes, respectively, 3=whey powder (from Cottom, 1974), 4=dried whole eggs, 5=commercial potato + whey powders, 6=commercial potato powder, 7=commercial potato + dried whole egg powders.

that of the commercially dried potato flour alone (Figure 5). This may be attributed to the relatively high content of whey in methionine plus cystine (4.1 g per 100 g protein according to Glass and Hedrick, 1976), the amino acids which limit the nutritional value of potato protein.

Lactose tolerance was observed after the rats ate the mix prepared with commercially dried potato flour plus whey powder mixed in the ratio 90:10, potato protein to whey protein. The rats did not have diarrhea. The whey powder can be a good supplement for potato protein.

Finally, it can be said that potato is an economical food and these research results suggest a high nutritional value. However, the low methionine content can limit it. But solutions to this limitation are on hand. To obtain a potato diet higher in methionine the following aspects can be considered (Kies and Fox, 1972): a) genetic selection of potato tubers having a high methionine level; b) addition of purified methionine in the industrial processing; c) education of consumers in usage of desirable food combinations.

In less developed areas such as South America which produces large quantities of potatoes, genetic selection of potato tubers high in methionine and food combinations should be kept in mind in order to have a potato diet higher in methionine. At present in South America it is economically unfeasible to consider the fortification

of potatoes with purified methionine during the industrial processing. Also, in some areas of South America potatoes can be an excellent supplement for lysine poor vegetative proteins such as wheat or other cereals.

H. Taste of Russet Burbank potatoes chemically or organically fertilized

There have been claims that "organically" grown foods taste better than those grown with chemicals.

In this research, no significant difference ($P<0.05$) in taste (flavor) was found for the Russet Burbank potatoes grown with organic or chemical fertilizer, when boiled potatoes were tested by 23 untrained panelists.

Part II

Preparation of a Potato Protein Flour and Its Uses in Bread Making

A. Preparation of a potato protein flour and bread

Three fractions were obtained from the prepared potato flour by air classification: a very fine fraction representing 1.9% of the flour, a medium weight fraction representing 11.2% of the flour, and a heavy fraction (mostly starch) representing 80.0% of the flour. Approximately 6.9% was lost during the process. This loss is excessive but it would not occur in a large commercial operation.

In this study the medium weight fraction known as potato protein flour (PPF) was used to prepare bread. This fraction contained 36.3% protein (Nx6.25) and 10.1% ash, and it was used as a replacement of 5% and 10% wheat flour, in preparing two types of "potato bread". No studies on breads with potato protein obtained by air classification were reported in the literature reviewed.

B. Bread analysis data

In Table 21, the physical characteristics of the control and PPF substituted bread are presented. The control had the best volume as expected. The loaf volume of the bread with 5% PPF was 31.5% lower and that with 10% PPF was 51.3% lower than the control. In similar experiments Knorr (1977) found that the loaf volume was influenced by the potato protein obtained by heat coagulation. Also, Jain and Sherman (1974) found that addition of potato flakes to bread decreased dough development time, dough stability, and dough strength. Poor volume is also found with excessive salts (Paul and Palmer, 1972). The PPF has a high ash content (10.1%) that could affect the volume. Kim and D'Appolonia, 1977, stated that the ratio of starch to protein is important in the dough because the effect of the protein in reducing the staling rate was due to dilution of the starch by the protein. The pH of the bread with PPF was lower (5.53) as compared to the control (5.85). This

Table 21. Physical characteristics of breads containing potato protein flour.

Physical test		Variables		
		Control	5% Potato Protein Flour	10% Potato Protein Flour
Volume, cc		825 ± 13.2[3]	565 ± 4.5	402 ± 5.0
Tenderness[1] (lb/g)		9.6 ± 1.2	11.3 ± 0.5	12.1 ± 0.7
Color[2]	L	75.6 ± 1.2	67.6 ± 0.7	61.5 ± 2.0
	a_L	-0.5 ± 0.2	0.7 ± 0.2	1.7 ± 0.5
	b_L	14.9 ± 0.2	16.8 ± 0.5	16.5 ± 0.8
pH		5.83 ± 0.03	5.53 ± 0.03	5.53 ± 0.03
moisture, %		30.5 ± 0.5	35.6 ± 0.8	38.4 ± 1.2

[1]Allo Kramer shear press (to measure textural characteristics of foods).

[2]Hunter colorimeter. The L values on this table indicate lightness. The a_L values (negative readings) indicate greenness. The a_L values (positive readings) indicate redness. The b_L values (positive readings) indicate yellowness.

[3]Mean of 3 replicates ± standard deviation.

could be due to the long fermentation time of the dough in the bread with potato. The percent of water retained in the bread increased with PPF. The values were 30.5 ± 0.5% for the control versus 35.6 ± 0.8% and 38.4 ± 1.2% for the bread with 5 and 10% PPF, respectively. One explanation could be the presence of potato starch in the potato protein. Van Den Berg et al. (1975) stated that potato starch has the highest water binding capacity of any starch because it has the lowest degree of association between the starch molecules. In the tenderness test, the control bread had the lowest value 9.6 ± 1.2 lb/g compared to 11.3 ± 0.5 and 12.1 ± 0.7 for 5 and 10% PPF bread. This indicates that bread with PPF was firmer in relation to the control, because the potato protein was less extensible than gluten.

The control had lighter color in the Hunter L range in relation to the bread with PPF (Table 21). In the a_L range the bread with PPF showed that redness increased as the PPF increased. In the b_L range the bread with PPF had higher yellowness in relation to the control. The sensory characteristics of breads containing PPF are presented in Table 22. These data were analyzed using f-test for orthogonal contrasts to compare the control bread versus 5%, 10% PPF bread. The panelists found a small difference in crust color between control and 5% PPF bread and a much larger difference between control and 10% PPF bread. The difference was not statistically significant ($P<0.05$).

Table 22. Sensory[1] characteristics of breads containing potato protein flour (PPF)

Variable	Sensory Characteristics					
	Crust Color	Crust Character.	Grain	Crumb Color	Taste	Texture
Control	4.3±0.3[2]	4.3±0.7[a]	4.4±0.1	4.9±0.1[a]	4.9±0.2[a]	4.8±0.2[a]
5% PPF + 95% wheat flour	4.2±0.2	3.6±0.2	3.3±0.6	3.5±0.2	3.9±0.6	3.5±0.5
10% PPF + 90% wheat flour	3.5±0.4	3.1±0.2	3.0±0.9	2.6±0.3	3.2±0.4	2.7±0.7

[1] Based on a scale of 1 to 5; 5 excellent.

[2] Mean of 3 replicates ± standard deviation.

[a] Control means with superscript are significantly different from average of experimental means ($P<0.05$). Control means without superscript "a" are not significantly different. (f-test for orthogonal contrasts).

For texture, bread with potato protein received lower scores in relation to the control. The results showed significant difference ($P<0.05$) between the control bread and 5% and 10% PPF bread. Grain was also rated lower for bread with PPF. The difference was not statistically different ($P<0.05$).

For taste, the bread with 5% PPF was closer to the control than the bread with 10% PPF that had the lowest score. The results showed significant difference ($P<0.05$) between the control bread and 5% and 10% PPF bread.

In general, the 5% PPF substitution in bread showed physical and sensory characteristics closer to the control than the 10% PPF substitution (Table 21, Figure 6). However, the loaf volume was markedly decreased with 5 and 10% PPF, 31.5% and 51.3%, respectively.

The potato protein flour or the potato flour itself could be promising substitutes in breadmaking in less developed areas where there is an abundance of potatoes.

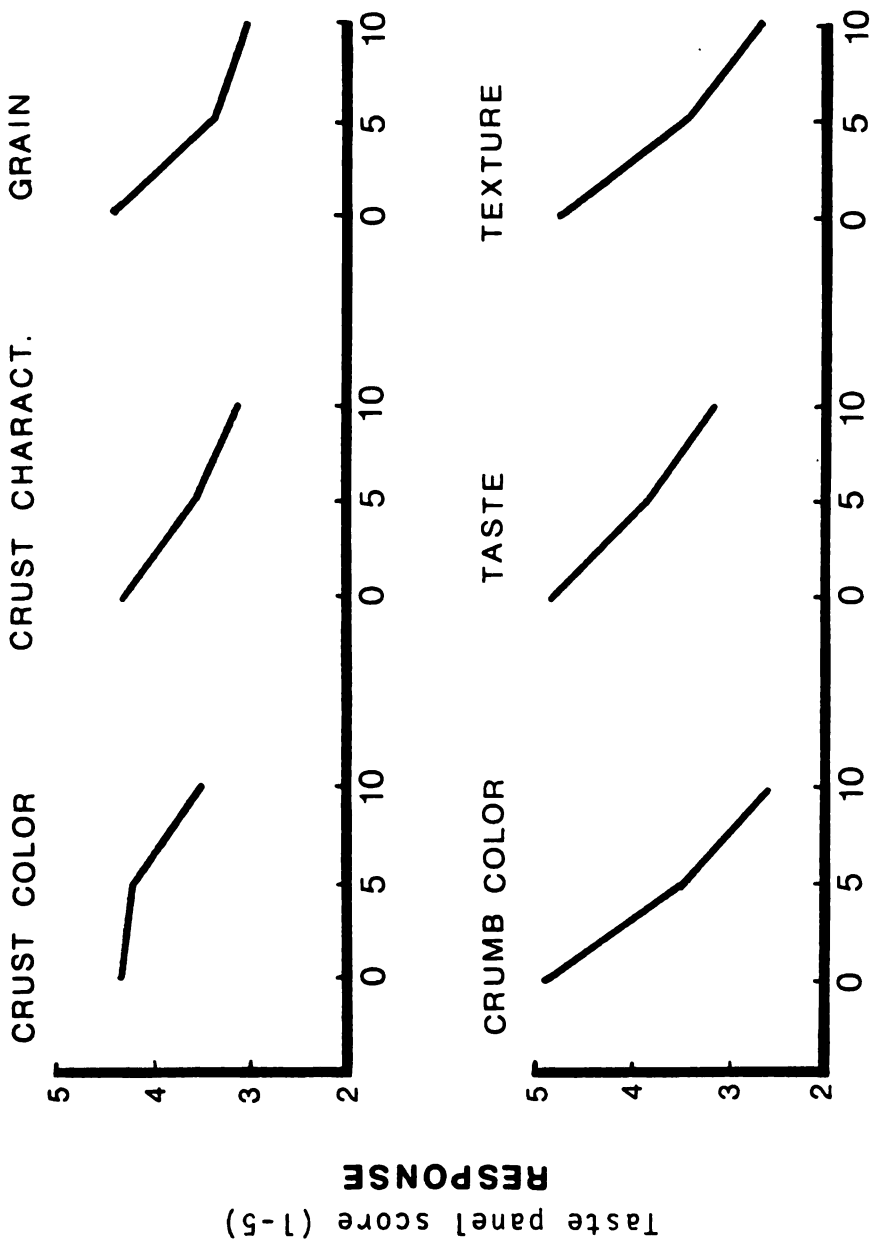

Figure 6. Taste panel score of breads containing potato protein flour (PPF).

SUMMARY

POTATO PROTEIN: NUTRITIONAL EVALUATION AND UTILIZATION

The purpose of this research was to a) study the nutritional quality of potato protein and b) prepare a high protein potato product and use it in breadmaking.

Nutritional Quality of Potato Protein

A. When the N distribution in the raw tuber was studied, it was found that the N content of potatoes is highest in the skin. The Protein Efficiency Ratio (PER) of the skin, however, is practically zero, according to previous work (Meister and Thompson, 1976a). The pith region of the tuber presented the next highest N content and the outer layer the lowest N content. It was also observed that the area near the apical end of the potato had a slightly higher N content than the stem end. The results showed higher amounts of aspartic and glutamic acids in the inner layer compared to the outer layer after boiling. Of the essential amino acids, valine, leucine, lysine and methionine, tended to be higher in the outer layer compared to the inner layer.

B. The free amino acids and amides extracted with distilled water showed variations in the levels among the raw cv potato varieties analyzed. In general, the most abundant free amino acids were aspartic and glutamic, and the amides asparagine and glutamine.

The content of the free essential amino acids threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine, in g residue/100 g of free amino acids plus amides, was: 16.0% in cv Atlantic, 7.5% in cv Denali, 23.3% in cv Russet Burbank, 20.0% in cv Superior and 21.1% in cv Monona. Free cystine was present as traces in Atlantic and Superior potatoes and was not detected in the other varieties. The range in content of free methionine was 0.7 to 2.2 g residue (0.8 to 2.5 g), indicating that selection of potatoes high in methionine could be possible.

C. The whole tuber boiled with intact skin suffered the lowest protein loss, 0.8 g/100 g of original protein content, in comparison to tubers boiled after cutting into halves and quarters either peeled or unpeeled. The highest protein loss, 10.4%, was observed when whole potatoes were peeled, cut into halves and boiled. After boiling the cv Russet Burbank and Atlantic potatoes with intact skins either cut or uncut, the predominant amino acids in the boiled water were aspartic and glutamic. It was found that the whole tuber boiled with intact skin suffered a minimum loss of cystine and methionine; the largest loss of cystine and methionine was observed in the halved and quartered potatoes. The average values for the halved potatoes were 0.5 g cystine and 2.4 g methionine per 100 g of each of these amino acids present in the raw potatoes. Russet Burbank potatoes showed a decrease of 10.6% in the availability of the lysine after boiling.

D. The protein quality of the potato as determined by the Protein Efficiency Ratio (PER) showed that the difference in the nutritional value of the unsupplemented or supplemented potato protein was reflected in their varying PERs. Russet Burbank potatoes chemically and organically fertilized presented close PER values 1.62 and 1.54, respectively, statistically not different. Also, the limiting amino acids, cystine and methionine were present in similar concentrations in the potatoes organically or chemically fertilized.

Dried whole eggs, and whey powder were used for supplementing the Russet Burbank potatoes. Commercially dried potato flour prepared from the cv Russet Burbank and mixed with dried whole eggs in the ratio 65:35 potato protein to egg protein, had a PER which was 8% higher than the PER of whole egg powder. This ratio of potato protein to egg protein yielded a 54% increase in PER when compared to the potato flour alone. When the same commercially dried potato flour was mixed with dried whey in the ratio 90:10 potato protein to whey protein, the PER value of the mixture was similar to that of casein (2.48 versus 2.50) but 13% higher than the PER of the potato flour alone.

Preparation of a Potato Protein Flour and Bread Supplemented With It

A potato flour fraction rich in protein (36.3%; Nx6.25) was prepared from potato flour by air classification in a

Walter Laboratory Separator. Bread was made after substituting 5% and 10% of the wheat flour with this fraction named potato protein flour (PPF). A 32 and 51% decrease in loaf volume was observed as a result of this substitution, respectively. There was also a decrease in tenderness (A11O-Kramer Press) in the bread containing PPF. The panelists found a small difference in crust color between control and 5% PPF bread and a much larger difference between control and 10% PPF bread. Both differences were not statistically significant. According to an untrained taste panel the score for taste and texture of the substituted bread were lower than those of the control, statistically different.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allison, J.B. 1959. The efficiency of utilization of dietary proteins. In Protein And Amino Acid in Nutrition. Ed. by A.A. Albanese, Acad. Press, New York.

Association of official analytical chemists. 1975. Methods of Analysis, AOAC, Washington, D.C.

Augustin, J. 1975. Variations in the nutritional composition of fresh potatoes. J. Food Sci. 40:1295.

Bacigalupo, A. 1969. Protein-rich cereal foods in Peru. In Protein-Enriched Cereal Foods for World Needs. Ed. by Max Milner. The American Association of Cereal Chemists, Inc.

Block, R.J. 1951. The Amino Acid Composition of Proteins and Foods. Charles C. Thomas, Publisher, Springfield, Illinois, U.S.A.

Borgstrom, G. 1969. Too Many: A Study of Earth's Biological Limitations. The Macmillan Company, New York.

Booth, H.V. 1971. Problems in the determination of FDNB-available lysine. J. Sci. Fd. Agric. 22:657.

Braden, B. 1962. A study of potato flakes in bakery foods. The Baker's Digest 36:46.

Brune, H. 1968, 1969. Die ernaehrung sphysiologische Bedeutungder N-Verbin dungen in pflansen im Hinblich auf die stickstoffduenhung. Qual. Plant Mater. Veg. XVIII:134.

Burton, W.G. 1965. The sugar balance in some British Potato varieties during storage. I. Preliminary observations. Eur. Potato J. 8:80.

Chawan, T.B. 1965. Effect of storage temperature and atmosphere on the chipping quality of potatoes. M.S. thesis, Michigan State University, East Lansing, Michigan.

Chick, H., and E.B. Slack. 1949. Distribution and nutritional value of the nitrogenous substances in the potato. Biochem. J. 45:211.

Chick, H. and M.E. Cutting. 1943. Nutritive value of the nitrogenous substances in the potato. Lancet 245: 667.

CIP. 1973. Potato protein quality. Report of the International potato center's planning conference on protein quality. Held at CIP. Lima, Peru. Nov. 26-30, 1973.

Cotton, R.H. 1974. Soy products in bakery goods. Utilization of soy protein in foods. Session A.J. of the Amer. Oil Chemists' Society. 51:116A.

Desborough, S.L. and C.J. Weiser. 1972. Protein comparisons in selected phureja-haploid tuberosum families. Amer. Potato J. 49:227.

Desborough, S.L. and C.J. Weiser. 1974. Improving potato protein. I. Evaluation of selection techniques. Amer. Potato J. 51:185.

El-Harith, E.A., J.W.T. Dickerson and R. Walker. 1976. On the nutritive value of various starches for the albino rat. J. Sci. Fd. Agr. 27:521.

FAO. 1970. Amino acid content of foods and biological data on proteins. Rome, Italy.

FAO. 1972a. Production Yearbook. Rome, Italy.

FAO. 1972b. Food composition table for use in East Asia. FAO, Rome.

FAO/WHO. Expert group. 1965. Protein requirements. Geneva, Switzerland.

FAO/WHO. 1973. Energy and protein requirements WHO, Geneva: Technical reports series No. 522.

FDA regulation of vegetable protein products. 1979. Nutrition policy issues No. 3. General Mills. Minneapolis, MN.

Felix, J.P. and C.T. Whittemore. 1975. Nutritive value of cooked potato flakes for the young chick. J. Sci. Fd. Agr. 26:261.

Fitzpatrick, T.J. and W.L. Porter. 1966. Changes in the sugars and amino acids in chips made from fresh, stored and reconditioned potatoes. Amer. Potato J. 43:238.

Fitzpatrick, T.J. and W.L. Porter. 1968. Microwave finishing of potato chips: effect on the amino acids and sugars. Amer. Potato J. 45:103.

Flodin, N.W. 1953. Amino acid and proteins, their place in human nutrition problems. J. Agr. Food Chem. 1: 222.

Ford, J.E. 1960. A microbiological method for assessing the nutritional value of proteins. Br. J. Nutr. 114: 485.

Fuller, H.J. 1963. General Botany, Barnes and Noble Inc., New York, N.Y.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. The Iowa State University Press. Ames, Iowa. Vol. I.

Glass, L. and T.I. Hedrick. 1976. Nutritional composition of sweet and acid type dry wheys. J. of Dairy Sci. 60:185.

Groot, E.H., L.W. Jansen, A. Kentie, H.K. Oosterhuis, and H.J. Trap. 1947. A new protein in potatoes. Biochem. Biophys. Acta 1:410.

Habib, A.T., and H.E. Brown. 1957. Role of reducing sugars and amino acids in the browning of potato chips. Food Technol. 11:85.

Harris, R.H. 1932. The effects of cooked potato in baking bread and its relation to crude protein and baking strength.

Harris, R.H., L.D. Sibbitt, and O.J. Banasik. 1952. Effect of potato flour on bread quality changes with age. Cereal Chemistry 29:123.

Hegsted, D.M. 1957. Theoretical estimates of the protein requirements of children. J. Am. Diet. Assoc. 33:225.

Hindhede, M. 1913. Studies on protein minimum. Skand. Arch. Physiol. 30:97 (in German).

HNF (Human Nutrition and Foods), 403-404. Laboratory manual. The role of fats, carbohydrates and proteins in food systems. Department of Food Science and Human Nutrition. Michigan State University, East Lansing, Michigan.

Hoelzl, J. and E. Bancher. 1959. Ueber Vorkommen und sorten-abhaengigkeit der Eiweisskristale in der kartoffel-knolle. Bodenkultur 10:110.

Hoelzl, J., and E. Bancher. 1961. Ueber geloestes und Kristallisiertes eiweiss in der kartoffelzelle (solanum Tuberosum). Qual. Plant. Mater. Veg. 8:1.

Hoff, J.E., C.M. Jones, G.E. Wilcox, and M.D. Castro. 1971. The effect of nitrogen fertilization on the composition on the free amino acid pool of potato tubers. Amer. Potato J. 48:390.

Hughes, B.P. 1958. The amino acid composition of potato protein and of cooked potato. Brit. J. Nutr. 12:188.

Jain, S. and P. Sherman. 1974. The influence of partial replacement of wheat by potato flakes on the texture of bread prepared by the chorleywood process. Proc. IV Int. Congress Food Sci. and Technol. 2:248.

Jansen, G.R. and E.E. Howe. 1964. World problems in nutrition. Amer.J. Clin. Nutr. 15:262.

Jaswal, A.F. 1973. Effect of various processing methods on free and bound amino acid content of potatoes. Amer. Potato J. 50:186.

Joseph, A.A., R.N.R. Choudhuri, K. Indiramma, M.N. Rao, M. Swaminathan, A. Sreenivasan, and V. Subrahmanyan. 1963. Food Sci. (Mysore) 12:255.

Kaldy, M.S. 1971. Evaluation of potato protein by amino acid analysis and dye-binding. Ph.D. Thesis, Michigan State University, East Lansing, Michigan.

Kaldy, M.S. and P. Markakis. 1972. Amino acid composition of selected potato varieties. J. Food Sci. 37:375.

Kies, C. and H.M. Fox. 1972. Effect of amino acid supplementation of dehydrated potato flakes on protein nutritive value for human adults. J. Food Sci. 37: 378.

Kim, S.K. and B.L. D'Appolonia. 1977. Bread staling studies. I. Effect of protein on staling rate and bread crumb pasting properties. Cereal Chem. 54:207.

Kofranyi, E. and F. Jekat. 1965. The biological value of potato proteins. Porschber. Landes Nrhein-Westf. No. 1582 (in German).

Kofranyi, E. and F. Jekat. 1967. The biological value of food proteins. Hoppe-Seyler's Z. physiol. Chem. 34884 (in German).

Knorr, D. 1977. Potato protein as partial replacement of wheat flour in bread. J. Food Sci. 42:1425.

Knorr, D., G.O. Kohler, and A.A. Betschart. 1977. Potato protein concentrates: the influence of various methods of recovery upon yield, compositional and functional characteristics. J. of Food Processing and Preservation 1:235.

Kon, S.K. 1928. The nutritional value of tuberin, the globulin of potato. Biochem. J. 22:261.

Kon, S.K. and A. Klein. 1928. The value of whole potato in human nutrition. Biochem. J. 22:258.

Lewis, O.A.M. 1966. Short ion-exchange column method for the estimation of cystine and methionine. Nature 209: 1239.

Lindner, K., S. Jaschik, I. Korpaczy, R. Polner and P. Vardi. 1957. Der ernaehrungswert der ungarischen kartoffeln. Acta chimica Acad. Sci. Hungarical 11: 151.

Lindner, K., S. Jaschick and I. Korpaczy. 1960. Amino acid composition and biological value of potato protein fractions. Qual. Plant. Mater. Veg. 7:289 (in German).

Luescher, R. 1971. Evaluation of methods to determine the sulfur containing amino acids in potatoes. M.S. thesis, Michigan State University, East Lansing, Michigan.

Luescher, R. 1972. Genetic variability of "available" methionine, total protein, specific gravity and other traits in tetraploid potatoes. Ph.D. thesis, Michigan State University, East Lansing, Michigan.

Macko, V. and H. Stegemann. 1969. Mapping of potato proteins by combined electrofocusing and electrophoresis. Hoppe-Zeyler's Zeit schr. F. physiol. chem Bd. 350:917.

Markakis, P. 1975. The nutritive quality of potato protein. In Protein Nutritional Quality of Foods and Feed. Part 2, (M. Friedman, ed.) Marcel Dekker, New York.

Markakis, P., T.M. Freeman and W.H. Harte. 1962. Method of producing a chip-type product. U.S. patent 3,027,258.

McCay, C.M. 1959. The nutritive value of potatoes. In potato processing, ed. by W.F. Talburt and O. Smith. The AVI Publishing Co., Westport, Conn.

Meister, E. 1977. Genetic improvement of yield and nutritive value of tetraploid potatoes. Ph.D. thesis, Michigan State University, East Lansing, Michigan.

Meister, E. and N.R. Thompson. 1976a. Protein quality of precipitate from waste effluent of potato chip processing measured by biological methods. J. Agr. Food Chem. 24:924.

Meister, E. and N.R. Thompson. 1976b. Physical-chemical methods for the recovery of protein from waste effluent of potato chip processing. J. Agr. Food Chem. 24:919.

Mitchell, H.H. 1924. Biological values of proteins at different levels of intake. J. Biol. Chem. 58:905.

Mitchell, H.H. 1947. Protein utilization by the adult rat. The lysine requirement. Arch. Biochem. 12:293.

Monday, M.I. and P.B. Rieley. 1964. Relationship of specific gravity to the nitrogen and ascorbic acid content of potatoes. Amer. Potato J. 41:417.

Moore, S., D.H. Spackman, and W.H. Stein. 1958. Chromatography of amino acids on sulfonated polystyrene resin. Anal. Chem. 30:1185.

Morrison, F.B. 1936. Feeds and Feeding. 20th ed. The Morrison Publishing Co., Ithaca, New York.

Mulder, E.G. and K. Bakema. 1956. Effect of N, P, K, and Mg nutrition of potato plants on the content of free amino acids and on the amino acid composition of the protein of the tubers. Plant and Soil Sci. 7:135.

Neuberger, A., and J. Sanger. 1942. The nitrogen of the potato. Biochem. J. 36:662.

Paul, P.C. and H.H. Palmer. 1972. Food Theory and Applications. John Wiley and Sons, Inc., New York.

Payne, P.R. 1976. Nutritional criteria for breeding and selection of crops: with special reference to protein quality. Plant Foods for Man. 2:95.

Peare, R.M. 1973. Potato protein as affected by: A. Variety and environment and B. Air separation of the dried flour. M.S. thesis, Michigan State University, East Lansing, Michigan.

Pike, R.L. and M.L. Brown. 1975. Nutrition: An Integrated Approach. John Wiley and Sons, Inc., New York.

Pol, G. and A.I. Labib. 1968. Effects of fertilization and storage conditions on the nutritive value of potatoes of different varieties. In Potatoes: Production, Storing, Processing. ed. by O. Smith. The AVI Publishing Co., Westport, Conn.

Pressey, R. and R. Shaw. 1966. Effect of temperature on invertase, invertase inhibitor, and sugars in potato tubers. Plant Phys. 41:1657.

Rayan, C.A. 1966. Chymotrypsin inhibitor I from potatoes: reactivity with mammalian, plant, bacteria, and fungal proteinases. Biochemistry 5:1592.

Rayan, C.A. and A.K. Balls. 1962. An inhibitor of chymotrypsin from Solanum tuberosum and its behavior towards trypsin. Proc. Natl. Acad. Sci. U.S. 48: 1839.

Reissig, H. 1958. Veber die moeglichkeiten einer zuechterischen verbesserung der biologischen wertigkeit von Kartoffelwiweiss-Zuechter 28:51-60.

Reynoso, Z. and A. Bacigalupo. 1968. Ensayo de panificación con mezcla de trigo-papa y su evaluación biológica. I. Congreso de la sociedad Latinoamericana de nutrición, Caracas.

Reynoso, Z., A. Bacigalupo, and A. Reggiardo. 1972. Investigaciones tecnológicas y nutricionales sobre el uso de la Papa en la producción de pan. Universidad Agraria. Lima, Perú.

Rios, B.J. 1969. Protein value of potato and navy bean powders: nutritional evaluation using the meadow vole (Microtus pennsylvanicus). M.S. thesis, Michigan State University, East Lansing, Michigan.

Rios, B.J., N.R. Thompson and C.L. Bedford. 1972. Protein in potato flakes: evaluation by the meadow vole (Microtus pennsylvanicus).Amer. Potato J. 49:255.

Rose, M.S. and L.F. Cooper. 1917. The biological efficiency of potato nitrogen. J. Biol. Chem. 30:201.

Schram, E., S. Moore, and E.J. Bigwood. 1954. Chromatographic determination of cystine as cysteic acid. Biochem. J. 57:33.

Schreiber, K. 1961. Chemistry and biochemistry. In R. Schick and M. Klinkowski (eds.) the Potato. VEB Deutscher Landwirtshaftsverlag, Berlin (in German).

Schuphan, W. 1958. Proteins et amino acids. Teneurs en amino acids indispensables des vegetaux alimentaires et de leur diverses organes. Qual. Plant. Mater. Veg. 3-4:19.

Schuphan, W. 1959, 1960. Die Indianerkartoffel Solanum stenotomum Juz. et Buk. Studien uber essentialle aminosauren in kartofeln. Qual. Plant. Mater. Veg. 26:1.

Schuphan, W. 1959. The influence of increasing nitrogen fertilizers on the content of essential amino acids and the biological albumine evaluation of potatoes. Z. Pflanzerer nach. Dveng, Bodenk. 86:1.

Schuphan, W. 1970. Control of plant proteins: the influence of genetics and ecology on food plants. In R.A. Lawrie (ed.) Proteins as Human Foods. AVI Publishing Co., Westport, Conn.

Schuphan, W. and W. Postel. 1957. The biological value of potato protein. Naturwiss enschaften 44:40 (in German).

Schwartze, P. and V. Sengebush. 1937. Eine methode zur bestimmung des Rohproteingehaltes in Zuchmaterial. ber Zuechter: p. 256.

Schwimmer, S. and H.K. Burr. 1959. Structure and chemical composition of the potato tuber. In W.F. Talburt and O. Smith (eds.). Potato Processing. AVI Publishing Co., Westport, Conn.

Shallenberger, R.S. 1956. Browning reaction in potato chips. Ph.D. thesis. Cornell University, Ithaca, New York.

Shaw, R. and W.C. Shevy. 1972. Production of potato starch with low waste. Amer. Potato J. 49:12.

Siegle, K. 1951. Das Kartoffeleiseiss, seine steigerung und verwertung. Z.F. Ackerbau-und Pflanzenbau 93:208.

Slack, E.B. 1948. Nitrogen constituents of the potato. Nature 161:211.

Sohonie, K. and K.S. Ambe. 1955. Crystalline inhibitors of trypsin from potato. Nature 176:972.

Snyderman, S.E., L.E. Holt, J. Danois, E. Roitman, A. Boyer, and M.E. Balis. 1962. "Unessential" nitrogen: a limiting factor for human growth. J. Nutr. 78:57.

Stucki, W.P. and A.E. Harper. 1962. Effects of altering the ratio of indispensable to dispensable amino acids in diets for rats. J. Nutr. 78:278.

Talley, E.A., T.J. Fitzpatrick, W.L. Porter. 1970. Chemical composition of potatoes. VIII. Effect of variety, location and year of growth on the content of nitrogen compounds. Amer. Potato J. 47:231.

Thompson, N.R. 1977. Plant protein production course. Botany, lecture. Michigan State University, East Lansing, Michigan.

Thompson, J.F. and F.C. Steward. 1952. The analysis of the alcohol-insoluble nitrogen of plants by quantitative procedures based on paper chromatography. II. The composition of the alcohol-soluble and insoluble fractions of the potato tuber. J. Exp. Bot. 3:170.

Treadway, R.H. 1952. Uses of potato starch flour in the United States. Amer. Potato J. 29:83.

Trogonitz, K. 1939. The influence of additions of potato products on the baking quality of wheat flour, Zts chr, f.d. ges. getscidewesen, 26. 14.

Van Den Berg, C., F.S. Kaper, J.A.G. Weldring and I. Walters. 1975. Water binding by potato starch. J. Food Technol. 10:589.

Whittemore, C.T., A.G. Taylor, F.W.H. Elsey. 1973. The influence of processing upon the nutritive value of potato. J. Sci. Fd. Agri. 24:539.

Whittemore, C.T., A.G. Taylor, I.W. Moffat, and A. Scott. 1975. Nutritive value of raw potato for pigs. J. Sci. Agri. 26:255.

Woodward, C.F. and E.A. Talley. 1953. Review of the nitrogenous constituents of the potato. Amer. Potato J. 30:205.