THE NUTRITIVE VALUE OF SOY FLOUR AND SOY PROTEIN ISOLATE FOR HUMAN ADULTS

by

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Economic advantages associated with the direct utilization of vegetable protein have led to increased efforts to produce human foods from protein-rich vegetable sources. In the context of human nutrition, soybean protein represents an abundant vegetable protein resource. The importance of soy protein in nutrition lies in its high content of essential amino acids particularly lysine, leucine, and isoleucine, which have been shown to be limiting amino acids in the majority of cereal proteins. Total sulfur-containing amino acids have been found to be first limiting amino acids in soy protein. Food science and technology are continually increasing the potential of the soybean as a protein source for present and future populations. The use of processed soy in human foods has increased dramatically in the last decade.

Isolation of soy protein involves the exposure of the protein to heat and alkali (Horan, 1974). Isolated soy protein may then undergo further alkaline processing to produce the spun fibers used in the preparation of meat analogs (Horan, 1974; Rosenfield and Hartman, 1974). Soy protein, properly processed, could be an important source of amino acids in the human diet. Any processing procedure should not adversely alter the nutritive value of the product. However, the process of isolation which includes the elimination of certain protein fractions,

as well as the use of variable temperature and pH changes may reduce the nutritive value of the product.

Several investigators have reported lower protein efficiency ratio values for alkali-treated soy protein than for untreated protein (Bressani et al., 1967; Cogan et al. 1968; Badenhop and Hackler, 1970; 1973). Other studies have shown that alkaline processing reduces net protein utilization and digestibility of soy protein (De Groot and Slump, 1969).

Cogan et al. (1968), De Groot and Slump (1969), Badenhop and Hackler (1970), Robinson et al. (1971), and Woodard and Short (1973) all suggest that the decrease in nutritive value with alkali processing is primarily due to the destruction of certain amino acids. Supplementation with amino acids has not always restored the nutritive value of processed soy protein (Bressani et al., 1967; De Groot and Slump, 1969). Therefore, amino acid destruction alone is not a sufficient explanation for the observed decrease in nutritive value.

Changes in the availability of amino acids resulting from alkaline processing may contribute to the decrease in nutritional value. Decreased utilization of amino acids could be caused by alkaline racemization of the amino acids leading to the formation of less readily utilizable forms (De Groot and Slump, 1969). Availability of amino acids in a protein can also be influenced by changes in the overall amino acid pattern (Harper, 1969; Kofranyi, 1972).

The nutritive value of soy protein isolate has not been determined extensively in the human. This experiment was designed to compare the

nutritive value of soy flour, which has not undergone alkaline processing, and soy protein isolate when fed to human subjects. Nitrogen balance was used as a criterion for evaluation of the two protein sources. The protein nutritive values of soy flour and soy isolate were determined. The essential amino acid pattern of the soy isolate was then adjusted to that of the soy flour in order to determine if soy flour and soy isolate have comparable nutritive values when their essential amino acid patterns are equalized. The protein quality of the soy flour, soy isolate, and soy isolate adjusted to soy flour was further evaluated by determining protein efficiency ratios in growth studies with rats.

CHAPTER II

REVIEW OF LITERATURE

Alkaline processing used in the production of processed soy protein may result in a lowered nutritive value of the product. The decrease in nutritive value could be due to direct destruction of amino acid residues in the protein, or to a decrease in availability of these amino acids. Changes in availability could be the result of racemization of amino acid residues leading to the formation of less readily utilizable forms. Another factor which could affect the availability of amino acids is change in amino acid pattern of the protein as a result of processing. The activity of inhibitory factors in the raw product, the availability of trace minerals, and the formation of toxic derivatives may be affected by processing. These factors may contribute to loss of nutritive value in the final product.

Commercial Production of Soy Flour and Isolate

The soy product used in the largest volume by the food industry is defatted soy flour (Kellor, 1974). Defatted soy flour is processed from whole soybeans which have been defatted by solvent extraction techniques (Kellor, 1974). The defatted flakes are then treated with moist heat, cooled, and ground to a particle size finer than 100 mesh, U.S. Standard Sieve Size (Kellor, 1974). The proximate composition of defatted soy flour is shown in Table 1 (Horan, 1974).

TABLE 1

Typical composition of soy flour and soy isolate[1]

(Moisture-free basis)

Component	Percent	
	Soy flour	Soy Isolate
Protein	56.0	96.0
Fat	1.0	0.1
Fiber	3.5	0.1
Ash	6.0	3.5
Carbohydrates (soluble)	14.0	0.0
Carbohydrates (insoluble)	19.5	0.3

[1]Horan, 1974

Soy protein isolates are prepared from defatted soy meal, flakes, or flour by removing carbohydrate and fiber in order to concentrate the protein to a level of not less than 90 percent (Alden, 1975). The isolation process involves an initial stage in which the defatted material is treated with a mildly alkaline, aqueous medium. The protein and carbohydrates are solubilized and the insoluble fiber is removed by screening or centrifugation (Alden, 1975). The soluble protein is then precipitated with acid at pH 4.5. The precipitated protein is washed with water and resolubilized with sodium hydroxide. The resulting protein dispersion is spray dried to produce the final product (Alden, 1975). The proximate composition of isolated soy protein is summarized in Table 1 (Horan, 1974).

Isolated soy protein may undergo further alkaline processing to produce the spun fibers used in the preparation of meat analogs. In the spinning process, soy protein isolate is slurried with alkali to raise the pH to 10 or 11 (Cole, 1973; Alden, 1975). The protein dope so formed is forced through spinnerettes into an acid bath (Cole, 1973; Rosenfield and Hartman, 1974; Alden, 1975). The fibers are then bound and treated with colors and flavors to simulate different meat products (Cole, 1973; Horan, 1974; Alden, 1975).

The Nutritional Value of Processed Soy Protein

Bressani et al. (1967) evaluated the protein quality of isolated soy protein, spun fibrils, and a soy protein textured food made from the isolate. Studies were carried out with rats and dogs. Bressani

et al. (1967) reported that the PER (protein efficiency ratio) and NPU (net protein utilization) values for the spun fibers were lower than those for the protein isolate.

Cogan et al. (1968) looked at the effect of processing conditions on the protein nutritive value of isolated soy protein and of intermediate fractions obtained during the isolation process. They reported that the PER value (1.98) of the isolate was lower than that of the original meal (PER=2.48).

De Groot and Slump (1969) conducted feeding studies with rats to study the effect of alkali treatment on soy protein under varying conditions of pH (7 to 12.2), time (1 to 8 hours), and temperature (23° to 80°C). They reported that net protein utilization was considerably reduced in all samples treated at pH 12.2. Alakli treatment also reduced true digestibility. The effect was enhanced by increased time and temperature.

Badenhop and Hackler (1970) studied the effect of soaking soybeans in sodium hydroxide solution as a pretreatment in the preparation of soy milk. They reported a decrease in the PER value of soy milk from 2.41 to 1.70 as the pH of the sodium hydroxide solution increased from 6.55 to 9.18. Meyer (1971) reported that PER values for soy isolates were lower than those for soy protein concentrates which have not undergone alkaline treatment.

Information on the nutritive value of processed soy protein in human subjects is limited. Most of the studies have used textured foods processed from extruded, defatted products, spun concentrated

products, or isolated soy protein. The protein quality of these textured foods has generally been compared with the protein quality of foods which the textured soy protein might be expected to replace in the diet.

Bressani et al. (1967) evaluated the protein quality of a soy protein textured food when fed to children. The textured food was prepared from isolated soy protein fibrils (28.8%), with added egg albumin (12.3%), and wheat gluten (11.8%). Nitrogen equilibrium was obtained when the subjects received 138 mg/kg of body weight/day of nitrogen from the soy protein textured food, as compared with 97 mg/kg of body weight/day of nitrogen from milk. Bressani et al. concluded that the protein quality of the soy protein textured food was approximately 80 percent that of milk.

Kies and Fox (1971) compared the nutritive value of a textured soy protein product (prepared from an extruded product), methionine-enriched textured soy protein, and beef in adult subjects. When fed at a level of protein intake of 4.8 grams of nitrogen per day, nitrogen balances were -0.70 (textured soy protein), -0.45 (textured soy protein plus methionine), and -0.30 (beef) grams of nitrogen per day. At that level of intake, none of the test protein sources fully met the requirements of the subjects, but beef was superior to textured soy protein on the basis of the nitrogen balance data. Fortification of the textured soy protein with methionine at a level of one percent by weight was partially effective in improving the protein value of the product.

Korslund, Kies, and Fox (1973) compared the nutritive value of textured soy protein, methionine-enriched textured soy protein, and

beef in adolescent males. They found that at a level of intake of 4.96 grams of nitrogen per day, nitrogen balances were -0.08, +0.48, and +0.32 grams of nitrogen per day respectively. Nitrogen retention was significantly higher with methionine-enriched textured soy protein and beef as opposed to textured soy protein alone.

Doraiswamy (1972) fed two soy protein products, processed to resemble ground beef, to adult subjects. The two products used were an extruded, defatted product, and a spun concentrated product. Both soy protein products gave poorer nitrogen retention than did either ground beef or whole egg when all were fed at a level of intake of 4 grams of nitrogen per day.

Morse et al. (1972) compared the nutritive value of casein-lactalbumin and spun soy protein when the essential amino acid patterns of both were adjusted to equal the FAO provisional pattern. In the adjusted spun soy protein 35 percent of the essential amino acid nitrogen was supplied by crystalline amino acids. The ratio of essential amino acid nitrogen to total nitrogen in the diet was 0.35. Morse et al. (1972) concluded that, on the basis of nitrogen balance data, spun soy protein and casein-lactalbumin supplemented to a common essential amino acid pattern and fed at a level of 0.45 grams of protein/kilogram of body weight/day were utilized equally well.

Effects of Processing on Protein Quality

Destruction of amino acids

One possible change due to processing is direct destruction of amino acid residues in the protein. Hill (1965) reported the possible

destruction of serine, arginine, cystine, and cysteine on exposure of proteins to alkaline hydrolysis.

Cogan et al. (1968) found that isolated soy protein was nutritionally inferior to the toasted meal from which it was prepared. Available lysine and methionine were found to be lower in the isolate.

De Groot and Slump (1969) found a decreased cystine, lysine, and serine content in soy protein exposed for increasing periods of time to pH 12.2 at temperatures above 40°C. Badenhop and Hackler (1970) reported that alkaline treatment of soybeans prior to soy milk production caused a partial destruction of the amino acid cystine. Cystine content decreased from 1.74 g/16 gN to 1.30 g/16 gN as pH increased from 6.55 to 9.18. Robinson et al. (1971) reported that a reduction in PER of soy milk processed at pH values above 8.0 was related to the decrease in cystine content. Woodard and Short (1973) observed that alkaline treatment of soy protein (0.1N NaOH) for 8 hours at 60°C resulted in a decrease in lysine, serine, methionine, threonine, and arginine content.

Changes in amino acid availability

Racemization of amino acids.

Chemical and physical treatments used in the conversion of soybean protein to a more highly processed form could change amino acid availability. Unsuccessful attempts to restore the nutritive value of processed soy proteins to that of the untreated protein by amino acid supplementation have led to the conclusion that the problem is not one of amino acid destruction alone.

De Groot and Slump (1969) reported that a decreased net protein utilization value for alkali treated protein could not be completely alleviated by supplementation with methionine, lysine, or a combination of the two. They conducted *in vitro* digestion and absorption tests and showed a decreased rate of threonine absorption from the alkali treated protein. Although threonine content of both the treated and untreated proteins was similar, the decreased utilization of threonine could be responsible for the difference in nutritive value after methionine supplementation. Supplementation with threonine caused an increase in the net protein utilization value. De Groot and Slump suggest that the decreased utilization of threonine might be caused by alkaline racemization of the amino acid leading to the formation of D-threonine which is not utilized by the rat.

Several investigators have suggested that alkaline processing can result in racemization of amino acids. Levene and Bass (1928) reported that alkaline treatment of casein caused racemization of the amino acid residues in the intact protein. They found complete racemization of methionine after treatment with 1N NaOH at 125°C for two hours. Neuberger (1947) noted that slight racemization of amino acids occurred during alkaline hydrolysis and that acidic amino acids were more readily racemized. Raben (1955) reported racemization of amino acids in ACTH (adreno cortico tropic hormone) concentrate after treatment with 0.1N NaOH at 100°C for three minutes.

Pickering and Li (1964) observed extensive racemization of methionine, histidine, and arginine after ACTH concentrate had been

boiled in 0.1N NaOH for ten minutes. Serine and phenylalanine were racemized to a lesser extent. Geshwind and Li (1964) reported that alkali heat treatment of B-melanocyte stimulating hormone caused extensive racemization of arginine, histidine, methionine, and phenylalanine.

Pollock and Frommhagen (1968) studied the racemizing effect of 0.5N NaOH on gelatin, albumin, and fulvic and humic acids for 24 and 96 hours. The amino acids most highly racemized were alanine, aspartic acid, phenylalanine, glutamic acid, and lysine. Tannenbaum et. al (1970) reported on the processing of fish protein concentrate, a procedure which utilizes high pH (12.5) and high temperature (95^{o}C). They concluded that direct destruction of amino acids by the treatment was probably limited, but that extensive racemization of constituent amino acids did take place. Provansal et al. (1975) observed that treatment of sunflower protein with 0.2M NaOH at 80^{o}C for one hour caused racemization of lysine.

Utilization of D-amino acids.

D-amino acids are not naturally found in mammalian tissues and, therefore, in order to be utilized they must first be converted to the L-isomer (White et al., 1952). The rate at which a D-amino acid will be utilized by the organism depends on the rate at which it can be converted to the L-isomer. Krebs (1935) observed that the liver and kidney contain D-amino acids oxidases which catalyse the conversion of D-amino acids to the L-isomers. The process involves oxidative deamination of the D-amino acid to the corresponding α -keto acid, followed

by reamination of the α-keto acid to the corresponding L-isomer (Ratner et al., 1940). Berg (1959) categorized the essential amino acids as poorly, intermediately, or readily invertible according to the ability of the D-form to promote growth in rats when the single D-amino acid was fed mixed with L-amino acids. D-methionine is readily invertible and promoted growth as effectively as did the L-form (Wretland and Rose, 1950). D-lysine has not been shown to undergo inversion at all (Berg, 1936). D-tryptophan lies near the methionine end of the invertibility scale (Berg, 1934). D-arginine (Winitz et al., 1956), D-phenylalanine (Womack and Rose, 1946), and D-histidine (Cox and Berg, 1934) follow D-tryptophan on the scale. D-valine (Berg, 1959) and D-leucine (Rechicigl et al., 1958) are inverted at a rate which will support growth under certain conditions. D-isoleucine is inverted at a very slow rate and lies near the lysine end of the invertibility scale (Greenstein et al., 1957). D-threonine does not even support moderate growth in young rats (West and Carter, 1938).

Studies with animals have shown that there are several limitations affecting the utilization of D-amino acids. The D-isomers of the essential amino acids have been shown to be absorbed by passive diffusion whereas the L-isomers are preferentially absorbed by an active process when both isomers are injected into isolated intestinal loops (Matthews and Smyth, 1952; Nakamura et al., 1974). Crampton et al. (1951) reported that D-isomers were excreted more readily than the L-isomers when the DL-amino acids were injected into rats. Albanese (1945) suggested that a renal tubular threshold exists, preventing the

rapid absorption of D-amino acids and accounting for their greater urinary loss. Christenson et al. (1952) demonstrated decreased cellular uptake of the essential amino acids in their D-form.

In the early work to determine the utilization of essential amino acids (D-form), each amino acid was fed singly in a mixture of L-amino acids. Certain D-amino acids have been shown to compete with one another for sites on the D-amino acid oxidases. Gerulat and Berg (1958) showed that D-leucine inhibits the oxidation of D-valine by D-amino acid oxidase. D-lysine has been shown to inhibit the oxidative deamination of D-alanine, D-phenylalanine, and D-valine (Yoshimoto, 1958). The rate of inversion of D-amino acids and their rate of utilization can, therefore, be influenced by the presence of other D-amino acids in the diet. Wretland (1952) found that D-methionine promoted the same rate of growth in rats as did L-methionine when five of the eight other essential amino acids were fed in the L-form. However, it induced much slower growth when all of the other eight were fed in the racemic form. Philips and Berg (1954) noted that simultaneously replacing several of the essential L-amino acids with their D-forms (readily invertible when fed singly) produced an extremely low growth response in rats. Similarly, Wachter and Berg (1960) found that the presence of the D-forms of the poorly invertible amino acids in the diet interfered with the inversion of the more readily invertible amino acids and, therefore, caused a reduction in growth rate. Kamath and Berg (1964) also noted that the growth response in rats could be greatly reduced by the presence of poorly invertible D-amino acids and concluded that they were interfering with the process involved in the inversion

of readily invertible amino acids. Competition between the D-amino acids for the D-amino acid oxidase could be expected to retard the oxidative deamination of even readily invertible amino acids to such an extent that too little of the L-form could be produced to support even moderate growth. Kamath and Berg (1964) suggest that a limit is imposed on the overall capacity of the organism to invert these amino acids whose D-forms are readily invertible when fed singly.

Data on the utilization of D-amino acids by man is fragmentary. Albanese et al. (1944) conducted a series of experiments to determine the utilization of the D-forms of the essential amino acids in humans by means of nitrogen balance. They concluded that man was capable of using only D-methionine at rates which allowed for nitrogen balance and growth similar to that produced by its L-isomer. Rose (1957) conducted a series of experiments to determine amino acid requirements in man. To determine the utilization of the D- and L-forms of the amino acids, Rose (1957) measured nitrogen balance after feeding the D-, DL-, or L-form. Rose et al. (1955b) showed that man was able to utilize D- phenylalanine to some extent, but was only capable of inverting 0.5 grams per day of the amino acid. This amount was not enough to maintain nitrogen equilibrium. The minimum requirement for L-phenylalanine has been shown to be approximately 1.1 grams per day. D-phenylalanine in amounts up to 2.0 grams per day could not meet the requirement. DL-phenylalanine fed in amounts equal to or slightly in excess of the L-phenylalanine requirement was capable of maintaining the subjects in nitrogen equilibrium. The only other D-amino acid

reported to be as equally well utilized as the L-form was D-methionine (Rose et al., 1955a). Rose et al. (1955a) found that the D- and DL-forms of methionine had similar abilities to support nitrogen equilibrium when fed at levels of 0.8 to 1.1 grams of amino acid. They concluded that L-methionine showed no greater metabolic activity than did D-methionine. L-methionine was never tested.

Harper and Uryeyama (1948) measured plasma levels of D- and L-methionine after ingestion of DL-methionine. They observed that absorption rates of both isomers were similar but that rates of removal from the blood differed with L-methionine being removed at a faster rate than D-methionine. Camien et al. (1952) reported that on ingestion of DL-methionine by man, there was a slight increase in excretion of L-methionine in the urine and a marked increase in the urinary excretion of D-methionine. They concluded that D-methionine was not as well utilized as L-methionine. Efron et al. (1969) noted an increased level of methionine in the urine of infants fed DL-methionine. Kies et al. (1975) compared the effectiveness of supplementing oatmeal with L-, DL-, or D-methionine at levels of 0.58 and 1.16 grams per day. Supplementation with L-methionine at both levels significantly improved nitrogen retention over that of the unsupplemented diet. Some improvement in nitrogen retention was observed when DL-methionine was supplemented. No effect on nitrogen retention was noted as a result of D-methionine supplementation. Zezulka and Calloway (1975) fed soy protein isolate at levels of 4.5 grams of nitrogen in diets containing a total of 9.0 grams of nitrogen. The diets were supplemented with

either L-methionine, N-acetyl- L-methionine, or D-methionine to bring total sulfur-containing amino acids up to 900 mg. Supplementation with L-methionine and N-acetyl- L-methionine allowed subjects to maintain nitrogen equilibrium. D-methionine supplementation resulted in negative nitrogen balance.

Changes in amino acid pattern.

The nutritive value of a dietary protein depends upon the pattern and quantity of essential amino acids furnished to the body by that protein after absorption (Mauron, 1969). The overall pattern of available amino acids is more important in determining quality than are simply the absolute amounts of each of the essentials (FAO/WHO, 1965). The pattern or proportions of essential amino acids in a protein is known as the amino acid balance. Mauron (1969) defines a balanced protein as one in which the essential amino acids are present in proportions corresponding to the requirements for optimal performance. In diets with inadequate protein content, a surplus of essential amino acids may have an adverse effect (Harper, 1956). This situation is known as amino acid imbalance. An amino acid imbalance can result from a change in the proportions of amino acids in a protein. Mauron (1969) defines an imbalanced protein as one in which the pattern of essential amino acids is so disproportionate to requirements that adverse effects are created. An unbalanced protein contains all the essential amino acids in proportions different from the requirements, but close enough to them, so that no adverse effects are created (Mauron, 1969).

An amino acid imbalance can be created when an increase or a decrease in a single amino acid reduces the utilization of other amino acids in the protein (Harper, 1969). Studies with rats suggest that even a small increase in the concentration of certain amino acids can increase the amounts of others needed to maintain a given rate of growth, when the total protein intake is low (Benton et al., 1956; Jones, 1962).

The essential amino acid patterns of soy flour and soy isolate are shown in Table 2 (Wolf and Cowan, 1975) along with the amino acid pattern for egg protein. Most of the amino acid levels in soy protein equal or exceed the levels in egg with the exception of the sulfur-containing amino acids. Alkaline-processing of soy protein can result in changes in the amino acid pattern of the product. De Groot and Slump (1969) reported a decrease in cystine, lysine, and serine content in soy protein treated with alkali at pH 12.2. They also reported an apparent increase in isoleucine, leucine, tyrosine, phenylalanine, and valine. Woodard and Short (1973) reported that treatment of soy protein with 0.1N NaOH for eight hours resulted in losses of some amino acids and apparent increases in others. Lysine, cystine, serine, methionine, threonine, and arginine decreased in content. Valine, isoleucine, leucine, and phenylalanine appeared to increase. Badenhop and Hackler (1973) reported that as the pH of a sodium hydroxide solution used to treat soy protein increased from 6.5 to 8.97, cystine content decreased, while threonine content increased.

TABLE 2

Essential amino acid composition for soy flour, soy isolate, and egg protein[1]

Amino acid	mg/g total essential amino acids		
	Flour	Isolate	Egg
Isoleucine	119	121	129
Leucine	181	194	172
Lysine	161	152	125
Phenylalanine	117	134	114
Tyrosine	91	93	81
Cystine	37	34	46
Methionine	37	27	61
Threonine	101	93	99
Tryptophan	30	34	31
Valine	126	120	141
Protein score	68	56	100

[1]Wolf and Cowan, 1975

The proportion of non-essential amino acid nitrogen and the ratio of essential nitrogen to total nitrogen in the diet, can influence amino acid requirements (Campbell, 1963). When the non-essential amino acids are in short supply, some of the essentials furnish nitrogen more readily than others for the synthesis of non-essential amino acids (Snyderman et al., 1962; King, 1963). If the essential nitrogen to total nitrogen ratio is too high, essential amino acids will be used as a source of nitrogen for the non-essential amino acids (Campbell, 1963). This will have the effect of altering the essential amino acid pattern of the protein.

The question as to what extent amino acid imbalance has an impact on amino acid requirements is controversial. The extent to which the amino acid pattern of a diet may be unbalanced without affecting amino acid requirements has not been adequately studied. Harper (1969) points out that it is difficult to produce amino acid imbalances in human diets. In natural foods, most proteins are balanced or unbalanced. Mauron (1969) states that with the increasing use of protein isolates and supplemented proteins, imbalanced proteins will become more evident in the human diet.

Other factors affecting the nutritive value of soy protein

The effect of heat treatment.

Trypsin inhibitor activity in soy isolate is a possible source of lowered nutritive value (Longenecker et al., 1964; Bressani et al., 1967; Cogan et al., 1968). Proper heat treatment is required to

inactivate trypsin inhibitors and improve the nutritive value of soy protein (Rackis, 1974). The ideal temperature required to bring about an improvement in nutritive value depends on duration of treatment, moisture content, and particle size of the soy protein (Rackis, 1965; Albrecht et al., 1966; Wolf and Cowan, 1975). Maximum protein efficiency ratio values have been reached when only 79 percent of trypsin inhibitor activity has been destroyed (Rackis, 1974).

Although heat treatment is beneficial in situations where heat-labile inhibitors of the digestive enzymes are present, the availability of amino acids in the diet can be reduced by heat treatment. There are at least two types of chemical reactions involved in heat processing which affect the availability of amino acids without causing their destruction. In cross-linkage reactions, certain amino acids can react with other protein constituents forming enzyme-resistant bonds (Bigwood, 1972). Amino acids with reactive nitrogen groups may react with reducing sugars in the Maillard reaction (Donoso et al., 1962). Miller et al. (1965) showed that digestibility of proteins can be greatly reduced by prolonged heat treatment without causing any appreciable destruction of amino acids. They treated fish protein at 116°C for 27 hours. Except for cystine, there was no destruction of amino acids. However, there was a marked reduction in availability of lysine, methionine, and tryptophan.

There is some evidence to suggest that heating of proteins may lead to the racemization of amino acids. Hayase et al. (1975) treated casein and lysozyme at temperatures from 180° to 300°C for twenty

minutes. Racemization of amino acids could be detected in samples heated at 180°C. The extent of racemization increased as the temperature increased to 250°. The following amino acids were racemized: aspartic acid, glutamic acid, alanine, phenylalanine, leucine, isoleucine, valine, and proline.

Control of heat treatment is important in that the degree of change in nutritive value of the protein depends on the duration of heating, temperature, and moisture conditions (Rackis et al., 1975). Nitrogen solubility index and protein dispersibility index values in the range of 12 to 25 percent are indicative of adequate heat treatment for good protein nutritive value (De, 1971).

The effect of trace mineral availability.

A decrease in the availability of zinc with processing is a possible source of lowered nutritive value of soy protein (Rackis, 1974). Lease (1970) stated that although differences in protein efficiency ratio values of soy isolates could be indicative of differences in amino acid composition, the primary factor affecting the nutritive value of isolates is the need for supplemental zinc. Processing conditions used in the manufacture of isolates decrease the solubility of protein-phytic acid- zinc complexes formed in the isolate (Lease, 1970).

The effect of formation of toxic derivatives.

The formation of toxic derivatives such as lysino-alanine (LAL) during alkali processing may also cause a reduction in nutritive value

of soy isolates. Bohak (1964) isolated LAL from alkali treated ribonuclease. He postulated that LAL was formed by the addition of the ε-amino group of a lysine residue to the double bond of a dehydroalanyl residue formed by β-elimination from serine or cysteine. Woodard and Alvarez (1967) identified cytomegalic lesions in the kidneys of rats fed alkali treated "alpha protein". De Groot and Slump (1969) treated soybean meal with alkali at pH 12.2 for one to eight hours at 23° to 80°C. They found a decrease in lysine content and an increase in LAL content of the protein as severity of treatment increased. Digestibility and net protein utilization values in the alkali treated protein decreased as LAL content increased. Woodard and Short (1973) observed kidney lesions in rats similar to those reported by Woodard and Alvarez (1967). The occurrence of the lesions was related to alkaline processing (0.1N NaOH at 60°C for eight hours) and the appearance of LAL in the soy protein.

CHAPTER III

MATERIALS AND METHODS

Nitrogen Balance Study

Subjects

Eighteen women students from the Department of Human Nutrition and Foods served as subjects for the study. Subjects ranged in age from 20 to 25 years (Table 3). Weight of subjects ranged from 47.3 to 68.2 kilograms (Table 3). Hemoglobin and hematocrit concentrations were determined for each subject prior to the study. All values were within the normal range for women of this age group (Table 3).

All subjects maintained their normal schedules throughout the study. Subjects reported to Solitude House for all meals. Subjects consumed the same diet for the first two days of the study. On day three, subjects were assigned randomly on a weight basis, to one of the three treatment groups so that the average weight for each group was approximately the same initially (Table 4). Each group consisted of six subjects.

Diets

The 12-day experiment comprised a 2-day nitrogen depletion period, a 5-day nitrogen adjustment period, and a 5-day experimental period. Nitrogen intake during the nitrogen depletion period was maintained at 1.0 gram of nitrogen per subject per day. Initial feeding of a near protein-free diet reduces the time required for human subjects to adjust

TABLE 3

Age, weight, hemoglobin, and hematocrit of subjects on the initial day of study

Subject number	Age	Weight kg	Hemoglobin g/100 ml	Hematocrit vol %
1	23	68.2	12.5	37.0
2	20	63.6	15.5	40.0
3	21	47.3	14.3	40.0
4	23	63.2	15.7	45.0
5	25	56.8	15.0	41.0
6	23	58.0	14.3	40.0
7	23	67.7	14.0	37.5
8	23	59.1	15.7	44.5
9	22	55.5	15.0	39.5
10	21	54.5	15.0	45.5
11	21	51.8	14.0	42.0
12	22	58.2	13.7	41.5
13	25	52.3	14.3	40.0
14	23	53.6	13.7	36.0
15	22	65.9	14.0	38.5
16	24	60.5	16.0	45.5
17	21	49.1	14.2	38.5
18	24	63.6	14.3	40.0
mean	22.6	58.3	14.5	40.6

TABLE 4

Weight distribution of subjects

on three dietary treatments

Diet					
1 (flour		2 (isolate)		3 (isolate)	
Subject number	Weight kg	Subject number	Weight kg	Subject number	Weight kg
1	68.2	2	63.6	6	58.0
4	63.2	3	47.3	12	58.2
8	59.1	5	56.8	14	53.6
9	55.5	7	67.7	15	65.9
10	54.5	13	52.3	17	49.1
11	51.8	16	60.5	18	63.6
mean ± S.D.	58.8 ± 5.57		58.0 ± 6.83		58.1 ± 5.66

to low protein diets (Kies and Fox, 1970). The 1.0 gram of nitrogen was supplied by a basal diet consisting mainly of low nitrogen fruits and vegetables (Appendix 1). The principle source of energy in the diet used during this period was a wheat starch bread (Appendix 3).

During the adjustment and the experimental periods, nitrogen intake was maintained at 5.0 grams of nitrogen per subject per day. Dietary treatments included: 1) Basal diet plus 4.0 grams of nitrogen supplied by soy flour; 2) Basal diet plus 4.0 grams of nitrogen supplied by soy isolate; 3) Basal diet plus 3.09 grams of nitrogen supplied by soy isolate. Diet 3 was supplemented with amino acids to match the amino acid pattern of diet 1. Nonessential nitrogen was added to diet 3 to bring the total level of nitrogen (not including nitrogen supplied by the basal diet) in this diet to 4.0 grams. The soy protein used in the three diets was incorporated into a starch bread product. A total nitrogen intake of 5.0 grams was selected for use because previous studies have shown that this amount is slightly inadequate to meet protein needs (Kies et al., 1975). This level then is a sensitive one for evaluation of protein quality.

Wheat starch was used in the preparation of cookies used in the diet in order to add calories without the addition of excess protein to the diet. The basal diet was analyzed for nitrogen content prior to the study and provided approximately 1.0 gram of nitrogen per subject per day. The basal diet and calculated protein and food energy values for the basal diet (Watt and Merrill, 1963) are shown in Appendix 1. Recipes used in the preparation of foods in the basal diet

are given in Appendix 2. Individual portions of each food and beverage were weighed and subjects were required to consume all food provided.

During the 2-day nitrogen depletion period the principle source of energy in the diet was a low-protein yeast bread made with wheat starch. During adjustment and experimental periods, soy flour or soy isolate replaced some of the wheat starch in the bread. Recipes for the breads used during the study are given in Appendix 3.

Prior to the study 1.5 grams of instant tea and 3.0 grams of instant coffee were analyzed for their nitrogen content. Each subject was given a choice of drinking tea or coffee throughout the study, and was required to drink the total amount provided each day. Since nitrogen found in coffee and tea furnishes no amino nitrogen for cell metabolism (Hegsted et al., 1946), the amount of nitrogen taken in by each subject in these beverages was subtracted from the daily urine nitrogen.

Vitamin[1] and mineral[2] supplements were provided to ensure that all subjects received nutrients to meet or exceed the National Research Council's recommended dietary allowances (1974) with the exception of protein. Calcium requirements were not met by the supplements. The requirement for calcium is 800 mg/day. Subjects received approximately 760 mg of calcium/day. The difference from requirement was not expected

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[1]One vitamin tablet supplied: 5000 U.S.P. units of vitamin A, 400 U.S.P. units of vitamin D, 1.5 mg thiamin, 1.7 mg riboflavin, 20 mg niacinamide, 60 mg ascorbic acid, 2 mg pyridoxine, 0.1 mg folic acid, 10 mg pantothenic acid, 5 mcg cyanocobalamin, and 18 mg iron (as ferrous fumarate 55 mg).

[2]Two mineral tablets supplied: 464 mg calcium, 360 mg phosphorus, 10 mcg vitamin D.

to influence nitrogen retention during the 12-day study.

Initially the basal diet provided approximately 1390 kilocalories, with the yeast breads providing approximately 500 kilocalories in the diet. Subjects were weighed daily before breakfast and caloric intake was adjusted so that each subject maintained her starting weight throughout the study. When subjects showed a tendency to lose weight, calories were added to the diet in the form of applesauce cookies, hard candy, and jelly beans. The final caloric intake was approximately 2300 kilocalories.

The flour used in this study was Soyafluff 200W[1], a defatted soy flour prepared with minimum moist-heat treatment. A typical proximate analysis for Soyafluff 200W is shown in Table 5.

The isolate used throughout the study was Promine D[1]. A typical proximate analysis for Promine D is shown in Table 5. Rat feeding studies in which Promine D was fed as the sole source of protein gave protein efficiency ratios in the range of 1.1 to 1.2[2].

In diets 1 and 2, 4.0 grams of nitrogen were provided by soy flour and soy isolate respectively. In diet 3, 3.09 grams of nitrogen were provided by soy isolate. Prior to the study, the soy flour and soy isolate were analyzed for nitrogen content. Soy flour provided 0.0837 grams nitrogen per gram of flour. Soy isolate provided 0.1491 grams of nitrogen per gram of isolate. Based on the nitrogen analysis, the

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[1]Central Soya Company, Chicago

[2]Central Soya Technical Service Manual, 1975

TABLE 5

Typical proximate analyses[1] of

Soyafluff 200W and Promine D

	Soyafluff 200W	Promine D
Moisture	6.5%	4.8%
Protein (Nx6.25)	53.0%	92.0%
Fat	1.0%	-
Crude fiber	3.0%	.25%
Ash	6.0%	4.0%
Water soluble protein (% of total protein)	60.0-70.0%	75.0%

[1]Central Soya Technical Service Manual, 1975

amounts of flour and isolate required to supply the above amounts of nitrogen to the three diets were calculated. These amounts of soy flour or soy isolate were incorporated into a yeast bread prepared with wheat starch (Appendix 3).

The bread was baked the day before it was to be used. Bread was baked in individual portions equalling one-half of the daily allotment for each subject. Before baking, the total amount of batter given by one recipe was weighed and divided equally on a weight basis into eight individual baking pans. The first seven loaves weighed out from each complete recipe were used. Each subject received two loaves of bread per day.

Prior to the study, the soy flour and isolate were analyzed for amino acid content. Lysine, threonine, leucine, isoleucine, phenylalanine, tyrosine, and valine were determined following hydrolysis in 6N HCl (Appendix 4). Amino acid analysis was carried out by ion-exchange chromatography on a Model NC-1P Technicon Amino Acid Analyzer. Cystine and methionine were analyzed following conversion to cysteic acid and methionine sulfone using the method of Schram et al. (1954), followed by chromatography. Tryptophan was determined according to the method of Graham et al. (1947). The results from the amino acid analysis are give in Table 6. The amino acid pattern of the isolate was matched to that of the flour in the following manner. Since isoleucine was found to be in the largest percent excess above the quantity found in flour, this amino acid was used to determine the total amount of nitrogen to be supplied by the isolate in diet 3. The

TABLE 6

Essential amino acid composition of soy flour and soy isolate used in three diets

Amino acid	Diet		
	1 (flour) g/4gN	2 (isolate) g/4gN	3 (isolate) g/3.09gN
Lysine	1.50	1.58	1.22
Methionine	0.31	0.25	0.19
Cystine	0.30	0.30	0.23
Threonine	1.00	0.95	0.73
Leucine	1.93	2.00	1.55
Isoleucine	0.95	1.23	0.95
Phenylalanine	1.18	1.28	0.99
Valine	1.25	1.28	0.99
Tryptophan	0.35	0.28	0.22

amount of isolate supplying 100 percent of the isoleucine found in flour supplied 3.09 grams of nitrogen. The amounts of essential amino acids plus cystine provided by this amount of isolate were calculated (Table 6). In order to bring the level of the essential amino acids, other than isoleucine, to that of the flour pattern, additional amounts of these amino acids were added to the diet (Table 7). The nitrogen supplied by these amino acids was calculated (Table 7). Ammonium citrate was added to diet 3 in order to bring the total nitrogen level of the diet (not including nitrogen supplied by the basal diet) to 4.0 grams. All subjects on diet 3 received one-third of the daily amount of amino acid and ammonium citrate supplements in juice at each of the three meals.

Food Compositing

Food composites were prepared on the following days: days 1 and 2 of the nitrogen depletion period, days 1, 3, and 5 of the nitrogen adjustment period, and days 1, 3, and 5 of the experimental period. During the nitrogen depletion period, all food included in the basal diet as well as the wheat starch bread was composited. During the nitrogen adjustment and experimental periods only food included in the basal diet was composited. Margarine was not included in the food composite as it does not blend easily. Caloric supplements were not included as the nitrogen from these sources was negligible (Watt and Merrill, 1963). Mineral and vitamin supplements were not included in the general composite. Each day one 24-hour food portion was collected into a liter pyrex container and refrigerated until the end of the

TABLE 7

Amino acids added to diet 3

Amino acid	Amount added (g)	%N[1]	Amount nitrogen added (g)
Lysine	0.28	19.16	0.05
Methionine	0.12	9.39	0.01
Cystine	0.07	11.66	0.01
Threonine	0.27	11.76	0.03
Leucine	0.38	10.67	0.04
Isoleucine	0.00	10.68	0.00
Phenylalanine	0.19	8.48	0.02
Valine	0.26	11.96	0.03
Tryptophan	0.13	13.72	0.02

[1]Merck Index of Chemicals and Drugs. Merck and Co., Inc., 1960

24-hour period. The composite was then transferred to a five-quart Waring blender, diluted with water to a weight of 2000 grams, and homogenized thoroughly. Immediately after blending, a 200 gram aliquot from the composite was stored in the freezer for nitrogen anaylsis.

Since the starch bread containing the soy flour or isolate varied among the three dietary treatments, it was analyzed separately for nitrogen content. A day's supply of bread from each of the three dietary groups was homogenized in a five-quart Waring blender and a representative sample used for immediate nitrogen determination.

Nitrogen in the basal diet and the bread samples was determined using a modified Kjeldahl-Gunning-Arnold method (A.O.A.C., 1970).

Urine and Fecal Collections

Daily urine collections were made throughout the twelve-day study. Urine was collected directly into wide-mouth polyethylene containers, containing 5.0 ml of 4 N HCl, transferred to a larger polyethylene container, and refrigerated until a 24-hour collection was completed. Twenty-four hour urine collections began with the second voiding of one day and continued through the first voiding of the following day. The complete 24-hour collection for each subject was transferred to a graduated cylinder, measured, and brought to a volume of two liters with distilled water. Aliquots from each urine collection were used to determine creatinine and nitrogen levels daily. Creatinine and nitrogen were determined using a Technicon Autoanalyzer II[1],

[1]Technical Instruments Corp., Tarrytown, N.Y.

Fecal collections were made throughout the twelve-day study. A marked collection corresponding to food intake during the five-day experimental period was used for nitrogen determination. Three fecal markers were given to mark two continuous five-day periods. Fecal markers were prepared using brilliant blue[2] mixed with methyl cellulose[3], 25 mg: 175 mg per capsule. Fecal markers were given before breakfast on days 3, 8, and 13 of the study. The fecal composite for all subjects included all fecal collections beginning with that containing the first marker, up to, but not including the next marked collection.

Feces were collected into waxed freezer containers. Containers were held in a freezer until the end of the experimental period. Marked feces were separated, transferred to a five-quart Waring blender, brought to a convenient weight (1500 - 2200 grams) with distilled water, and homogenized thoroughly. Aliquots of 50 grams were transferred to plastic bottles and stored in the freezer until used for nitrogen determination. Nitrogen was determined using a modified Kjeldahl-Gunning-Arnold method (A.O.A.C., 1970).

Response Parameters

The following data were collected for each of the three dietary treatments: 1) Nitrogen intake per subject per day based on an analysis of actual food intake; 2) Daily urinary nitrogen and creatinine

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[2]Sigma Chemical Co., St. Louis

[3]Fisher Scientific Co.

excretion; 3) Fecal nitrogen was calculated from a marked collection corresponding to the last five days of intake.

Statistical Analyses

An analysis of variance was used to determine if there were significant differences in excretion of urine and fecal nitrogen or nitrogen retention between the three dietary treatments.

Protein Efficiency Ratio Study

Protein efficiency ratios were determined by growth studies with male weanling rats according to the A.O.A.C. method (1970). The composition of the basal diet is shown in Table 8. The protein source was fed at a level of 10 percent protein (Nx6.25) and replaced some of the corn starch in the basal diet in order to keep the level of total calories approximately constant. The compositions of the test diets are shown in Table 9. The three dietary diets consisted of: 1) the basal diet with soy flour fed at a level of 10 percent protein; 2) the basal diet with soy isolate fed at a level of 10 percent protein; and 3) the basal diet with soy isolate fed at a level of 7.75 percent protein. The amino acid patterns of the soy flour and isolate in the three experimental diets are shown in Table 10. The third diet was supplemented with amino acids in order to match the amino acid pattern of the isolate to that of the flour in diet 1. Diet 3 was further supplemented with ammonium citrate to bring the total protein level (Nx6.25) of the diets to 10 percent. A fourth group of rats was fed the basal diet with casein at a level of 10 percent protein. The experimental diets were compared to

TABLE 8

Composition of the basal diet

Component	g/100g ration
Vitamin mix[1]	2.2
Mineral mix[1]	5.0
Corn oil	5.0
Alphacel[1]	2.0
Corn starch	85.8

[1]ICN Pharmaceuticals, Cleveland

TABLE 9

Composition of test diets for PER study

Component	Diet			
	1 (flour)	2 (isolate)	3 (isolate)	4 (casein)
	g/100g ration			
Vitamin mix[1]	2.2	2.2	2.2	2.2
Mineral mix[1]	5.0	5.0	5.0	5.0
Corn oil	5.0	5.0	5.0	5.0
Alphacel[1]	2.0	2.0	2.0	2.0
Corn starch	66.8	75.2	74.7	74.9
Soy flour[2]	19.2			
Soy isolate[2]		10.8	8.3	
Casein[1]				11.1
Supplemental amino acids:				
Lysine[1]			0.11	
Methionine[1]			0.05	
Threonine[1]			0.11	
Leucine[3]			0.15	
Phenylalanine[3]			0.07	
Valine[1]			0.10	
Tryptophan[3]			0.05	
Cystine[3]			0.03	
Ammonium citrate[4]			2.23	

[1]ICN Pharmaceuticals, Cleveland
[2]Central Soya, Chicago
[3]Sigma Chemical Co., St. Louis
[4]Fisher Scientific Co., Fair Lawn

TABLE 10

Essential amino acid composition of soy flour and soy isolate in experimental diets

PER Study

Amino acid	Diet		
	1 (flour)	2 (isolate)	3 (isolate)
	g/100g ration		
Lysine	.60	.63	.49
Methionine	.12	.10	.08
Threonine	.40	.38	.30
Leucine	.77	.80	.62
Isoleucine	.38	.49	.38
Phenylalanine	.47	.51	.40
Valine	.50	.51	.40
Tryptophan	.14	.11	.09
Cystine	.12	.12	.09

the casein control diet. Sources of nitrogen in the test diets are shown in Table 11.

Each test group consisted of eleven animals distributed by weight so that the average initial weight was approximately the same for all four groups (Table 12). The animals were placed in individual cages. Feed and water were available at all times. Daily feed consumption records were kept for each animal throughout the twenty-eight day experimental period. The amount of food spilled each day was estimated. Growth was measured by weight gain. Rats were weighed on days 1, 8, 15, 22, and 28. At the end of the twenty-eight day period, protein efficiency ratios were calculated for each of the three groups and for the reference casein group using the formula:

$$\text{PER} = \frac{\text{weight gain of test animal (g)}}{\text{protein consumed (g)}}$$

The PERs for each of the experimental diets were expressed as a percentage of the PER of casein. An analysis of variance was used to determine if there were significant differences between the PER values for each of the three experimental groups.

TABLE 11

Sources of nitrogen in test diets

Component	Diet			
	1 (flour)	2 (isolate)	3 (isolate)	4 (casein)
	g/100g ration			
Soy flour	1.6			
Soy isolate		1.6	1.24	
Casein				1.6
Supplemental amino acids:				
Lysine			.021	
Methionine			.004	
Threonine			.012	
Leucine			.016	
Phenylalanine			.006	
Valine			.013	
Tryptophan			.007	
Cystine			.003	
Ammonium citrate			.28	
Total	1.6	1.6	1.6	1.6

TABLE 12

Starting weight of rats on four test diets

Animal number	Diet			
	1 (flour)	2 (isolate)	3 (isolate)	4 (casein)
	Weight (g)			
1	70.8	64.3	76.6	65.8
2	73.2	80.8	68.6	67.7
3	78.2	77.1	72.3	77.5
4	67.6	72.6	80.4	79.8
5	77.3	73.7	68.3	73.4
6	64.4	68.3	77.5	75.5
7	69.8	77.6	65.0	65.0
8	74.3	70.8	70.5	71.0
9	81.5	67.3	63.2	62.7
10	65.8	69.0	73.5	70.5
11	68.5	65.3	67.0	68.5
mean $\pm$ S.D.	71.9 $\pm$ 5.2	71.5 $\pm$ 5.10	71.2 $\pm$ 5.18	70.7 $\pm$ 5.17

CHAPTER IV

RESULTS

Human Nitrogen Balance Study

The technique most generally accepted for the evaluation of protein utilization in the human is the nitrogen balance technique. The interpretation of nitrogen balance is based on the premise that equilibrium is attained in the adult when the supplies of essential amino acids and total nitrogen are adequate for replacement of endogenous losses and for tissue synthesis (Pike and Brown, 1975). Nitrogen balance is expressed as B=I-(U+F) where B is nitrogen balance, I is nitrogen intake, U is urinary nitrogen, and F is fecal nitrogen, in g/day.

Eighteen women served as subjects in a study designed to compare the utilization of soy flour and soy protein isolate in the human as measured by the nitrogen balance technique. A two-day period on a near-zero protein diet and a five-day adjustment period on a low-protein diet were used to stabilize the subjects at a point near nitrogen equilibrium. The diet consumed during this seven-day period was analyzed for nitrogen content.

The basal diet (Appendix 1) plus two loaves of wheat starch bread (Appendix 3), consumed during the first two days of the study, supplied 1.16 g N per person per day. On day three of the study, subjects were randomly assigned to the three treatment groups on a weight basis

(Table 13). The average initial weight of subjects was 58.21 kg and the average final weight was 57.63 kg (Table 14). The average weight change was -0.58 kg (Table 14). As subjects showed a tendency to lose weight, additional calories were added to the diet in the form of applesauce cookies, jelly beans, and hard candy. All subjects were given one additional applesauce cookie during days 3 to 7 of the study, and three additional applesauce cookies during days 8 to 12 of the study. These caloric sources were composited with the basal diet and analyzed for nitrogen content. Individual subjects received jelly beans and hard candy as needed in order to stop weight loss. These caloric sources were not analyzed for nitrogen content. The basal diet consumed during days 3 to 7 of the study supplied 1.00 g N per person per day. The basal diet consumed during days 8 to 12 of the study supplied 1.04 g N per person per day.

During the experimental period (days 8 to 12), the six subjects on diet 1 consumed the basal diet providing 1.04 g N per person per day, plus two loaves of soy flour bread (Appendix 3) supplying an additional 4.23 g N per person per day. Their total nitrogen intake was 5.27 g N per person per day. The six subjects on diet 2 consumed 1.04 g N from the basal diet and 4.24 g N supplied by two loaves of soy isolate bread (Appendix 3). The total nitrogen intake on diet 2 was 5.28 g per person per day. The six subjects on diet 3 consumed 1.04 g N from the basal diet and 3.33 g N from two loaves of soy isolate bread (Appendix 3). The soy flour and soy isolate had been previously analyzed for nitrogen and essential amino acid content (Table 15). The

TABLE 13

Experimental Treatments

Treatment[1]	g nitrogen added	number of subjects	mean weight day 1 (kg)
1. Soy Flour[2]	4.0	6	58.49
2. Soy Isolate[3]	4.0	6	58.11
3. Soy Isolate[4]	3.09	6	58.03
Ammonium citrate[5]	0.7		
Amino acids[6]	0.21		

[1]The basal diet provided 1.04 g N daily

[2]47.79 g of soy flour provided 4.0 g N

[3]26.83 g of soy isolate provided 4.0 g N

[4]20.72 g of soy isolate provided 3.09 g N

[5]5.65 g of ammonium citrate provided 0.7 g N

[6]Amino acids in the following amounts were added to treatment 3 in order to match the essential amino acid pattern to that of treatment 1: Lysine HCl, 0.33g; Methionine, 0.12g; Threonine, 0.27g; Leucine, 0.38g; Phenylalanine, 0.19g; Valine, 0.26g; Tryptophan, 0.13g; Cystine, 0.07g.

TABLE 14

Weight change of subjects

during study

Subject number	Weight (kg) Day 1	Day 12	Difference
1	68.2	66.8	-1.4
2	63.6	64.5	+0.9
3	47.3	48.2	+0.9
4	63.2	60.0	-3.2
5	56.8	55.5	-1.3
6	57.7	57.5	-0.2
7	67.7	66.8	-0.9
8	57.7	59.3	+1.6
9	55.5	55.9	+0.4
10	54.6	54.6	0.0
11	51.8	50.7	-1.1
12	58.2	57.7	-0.5
13	52.7	51.8	-0.9
14	53.6	52.7	-0.9
15	65.9	64.6	-1.3
16	60.5	60.0	-0.5
17	49.1	48.0	-1.1
18	63.6	62.7	-0.9
mean	58.21	57.63	-0.58
SD	±6.21	±5.56	±0.96

TABLE 15

Essential amino acid composition[1]
and nitrogen content of soy flour
and soy isolate

Component	g Amino Acid/16gN	
	Soy Flour	Soy Isolate
Lysine	6.0	6.3
Methionine[2]	1.2	1.0
Cystine[2]	1.2	1.2
Threonine	4.0	3.8
Leucine	7.7	8.0
Isoleucine	3.8	4.9
Phenylalanine	4.7	5.1
Tyrosine	4.3	3.6
Valine	5.0	5.1
Tryptophan[3]	1.4	1.1
Nitrogen	0.0837[4]	0.1491[4]

[1]Determined by ion-exchange chromotography (Appendix 4).

[2]Schram et al., 1954

[3]Graham et al., 1947

[4]g/g sample

essential amino acid pattern of the soy isolate in diet 3 was matched to that of the soy flour in diet 1. The amino acids added to diet 3 are shown in Table 7 along with the amount of nitrogen which they supplied to the diet. In order to equalize the nitrogen content of diet 3 with that of diets 1 and 2, 0.7 g of nonessential nitrogen was added in the form of ammonium citrate (5.65 g per person per day). The total nitrogen intake on diet 3 was 5.28 g N per person per day.

Subjects were allowed to drink either 3.0 g of coffee or 1.5 g of tea in water per day. Coffee supplied 0.09 g N per person per day. Tea supplied 0.05 g N per person per day. The nitrogen supplied by these sources was subtracted from the daily urine nitrogen. Daily urinary nitrogen values for the experimental period are shown in Appendix 6. Daily urine volumes and creatinine excretions are shown in Appendix 5. The values for creatinine were relatively constant except on days when the 24-hour urine collection was not complete. The creatinine values were used as a guide in the interpretation of the daily urinary nitrogen data. Subject 6 had an incomplete urine collection on day 10. Subject 13 has an incomplete urine collection on day 9. Therefore, data for those two days were not used, and average daily urine nitrogen was calculated on the basis of a four-day collection period for these two subjects. Subject 8 showed low nitrogen excretion on days 11 and 12 in relation to the other values. Creatinine excretion for this subject was also low on these two days suggesting incomplete collections. Therefore, these two values were eliminated from the analysis. Average daily urinary nitrogen for subject 8 was

calculated on a three-day basis. Daily urinary nitrogen excretion was high for subject 4. As this subject showed a weight loss of 3.2 kg, data for subject 4 was not included in the calculations of nitrogen balance for diet 1.

Nitrogen balance was calculated on the basis of the last five days of the study. Average daily urinary and fecal nitrogen excretions, digestibility values, and nitrogen balance values for each individual are shown in Table 16 along with mean values for each treatment group. Subjects completed questionnaires pertaining to mental and physical well-being on each day of the study. Information obtained from these questionnaires indicated no problems which could be related to nitrogen balance. Although individual subjects lost weight during the study, weight had stabilized in the last five days (Table 17). The differences in weight were not expected to influence the mean nitrogen losses. Two subjects on diet 1 showed negative nitrogen balance. Three subjects on diet 1 maintained positive nitrogen balance. All subjects on diet 2 were in negative balance. The individual subject range for diet 3 was wide and followed no apparent trend. Three individuals were in positive balance, three were in negative balance. An analysis of variance[1] (Appendix 8) indicated no significant differences in urinary or fecal nitrogen excretion or digestibility between the three groups. Although the mean nitrogen balance for subjects receiving the flour diet was slightly higher than the mean balance for the

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[1]The .05 level of significance was used for all statistical analyses.

TABLE 16

Nitrogen Balance Data

Treatment	Subject number	Grams Nitrogen Intake				Grams Nitrogen Excreted		Balance	% digestibility
		Basal diet	Bread	Amino acid + ammonium citrate	Total	Urine	Feces		
1. Flour		1.04	4.23		5.27				
	1					4.06	1.52	-0.31	71
	8					4.35[1]	1.09	-0.17	79
	9					4.37	0.78	+0.12	85
	10					3.91	1.34	+0.02	75
	11					3.84	1.37	+0.06	74
	mean ± SD					4.11 ± 0.22	1.22 ± 0.26	-0.06 ± 0.16	77 ± 5

[1]Average of 3 days

TABLE 16 cont'd

Treatment	Subject number	Grams Nitrogen Intake				Grams Nitrogen Excreted		Balance	% digestibility
		Basal diet	Bread	Amino acid + ammonium citrate	Total	Urine	Feces		
2. Isolate		1.04	4.24		5.28				
	2					4.38	1.02	-0.12	81
	3					4.01	1.29	-0.02	76
	5					4.38	1.20	-0.30	77
	7					4.82	1.22	-0.76	77
	13					4.67[2]	1.15	-0.54	78
	16					4.71	0.62	-0.05	88
	mean ± SD					4.50 ± 0.27	1.08 ± 0.22	-.30 ± 0.27	80 ± 5
3. Isolate supplemented		1.04	3.33	0.91	5.28				
	6					4.12[2]	0.93	+0.23	82
	12					3.73	0.75	+0.80	86
	14					5.32	1.04	-1.08	80
	15					5.38	0.92	-1.02	83
	17					3.60	1.32	+0.36	75
	18					4.74	1.06	-0.52	80
	mean ± SD					4.48 ± 0.71	1.00 ± 0.17	-0.21 ± 0.71	81 ± 3

[2]Average of 4 days

TABLE 17

Weight change of subjects during last five days of study

Subject number	Weight (kg) Day 8	Day 12	Difference
1	67.27	66.80	-0.47
2	64.10	64.50	+0.40
3	47.30	48.20	+0.90
4	60.00	60.00	0.00
5	55.50	55.90	+0.40
6	57.30	57.50	+0.20
7	66.40	66.82	+0.42
8	58.18	59.32	+1.14
9	54.55	55.91	+1.36
10	54.09	54.55	+0.46
11	50.45	50.68	+0.23
12	57.73	57.73	0.00
13	51.82	51.82	0.00
14	52.27	52.73	+0.46
15	64.55	64.55	0.00
16	60.00	60.00	0.00
17	47.73	47.95	+0.22
18	62.73	62.73	0.00
mean $\pm$ SD	57.33 $\pm$ 5.97	57.65 $\pm$ 5.81	+0.32 $\pm$ .44

supplemented isolate diet, and both were higher than that for the unsupplemented isolate diet, the differences in response were not significant[1] (Appendix 8).

Protein Efficiency Ratio Study

The protein sources tested, group averages of protein consumption, weight gain data, and protein efficiency ratios from the 28 day feeding study with rats are summarized in Table 18. Data for individual animals are shown in Appendix 7. No significant differences in PER values were found between the three test groups[1] (Appendix 8).

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[1]The .05 level of significance was used for all statistical analyses.

TABLE 18

Average Weight Gain, Protein Consumption

and PER Values During 28-day Rat Study

Diet	Average Wt. gain (g)	Average Prot. Consumption(g)	PER	PER % Casein
1. Soy Flour	62.6 ± 9.5[1]	38.9 ± 3.1	1.6 ± 0.2	59.70
2. Soy Isolate	50.3 ±10.8	34.8 ± 4.7	1.4 ± 0.2	53.36
3. Soy Isolate supplemented	57.6 ± 9.8	38.7 ± 3.3	1.5 ± 0.2	55.60
4. Casein	119.1 ±19.8	44.5 ± 5.0	2.7 ± 0.3	

[1]MEAN ± SD

CHAPTER V

DISCUSSION

Alkaline processing, used in the production of soy isolate, caused some compositional changes in the amino acid pattern of the protein (Table 15). There were apparent increases in the amounts of lysine, leucine, isoleucine, and phenylalanine in the isolate. The content of methionine, threonine, tyrosine, and tryptophan decreased in the isolate.

Since alkaline processing did change the distribution of essential amino acids in the isolate compared to the flour, it was considered possible to measure any difference in utilization of the two proteins by adjusting the essential amino acid pattern of the isolate to that of the flour. Amino acids were added to diet 3 so that its essential amino acid pattern was equivalent to that of the flour in diet 1.

Nitrogen retention was not significantly different between the three dietary treatments. Although alkaline processing did cause some compositional changes in the amino acid content of the isolate, any changes in amino acid composition or availability which may have occurred, were not extensive enough to have a significant effect on nitrogen retention.

Because of the nature of the compositional changes in the amino acid pattern of the isolate on processing, the total essential amino

acid content and the amount of nitrogen supplied by the essential amino acids was approximately the same for all three diets (Table 19). The ratio of essential nitrogen to total nitrogen was constant for all three diets (Table 19), but the proportion of each supplied by the test protein or crystalline amino acids differed. The amount of nonessential nitrogen was approximately the same for all three diets (Table 19). The amounts of each essential amino acid supplied in each of the diets is shown in Table 20 in relation to the daily requirements for the essential amino acids. All of the diets satisfied the daily requirements for essential amino acids. The similarity in nitrogen retention of the three diets reflects the similarity in total essential amino acid and nitrogen patterns of the diets.

There were no significant differences in excretion of urine or fecal nitrogen between the three diets. Apparent digestibility values were similar for all three diets. Therefore, there were no apparent differences in nitrogen absorption between the three diets.

In the isolation of soy protein, soybean flakes are treated in an alkaline medium at pH 7 to 8 (Circle et al., 1959). Bressani et al. (1967) noted a change in amino acid availability and a decrease in nutritive value of soy protein treated in alkali at pH 12. Cogan et al. (1968) reported a decrease in nutritive value of soy protein treated in alkali at pH 9.5 to 9.8. De Groot and Slump (1969) reported that alkali treatment (pH 12.2) resulted in decreased utilization of amino acids and decreased nutritive value of soy protein. Badenhop and Hackler (1973) noted a decrease in the nutritive value of soy protein treated in alkali

TABLE 19

Total Essential Amino Acid Content, Essential Amino Acid Nitrogen Content, Total Nitrogen Intake, Nonessential Nitrogen Intake and Essential Nitrogen to Total Nitrogen Ratio of The Three Diets

	Diet I flour	Diet II isolate	Diet III isolate supplemented
(1) Total Essential Amino Acids (g)[1]			
Basal diet	2.74	2.74	2.74
Flour	9.85		
Isolate		10.05	7.77
Crystalline amino acids			1.70
Total	12.59	12.79	12.21
(2) Essential Amino Acid Nitrogen (g)[2]			
Basal diet	.34	.34	.34
Flour	1.16		
Isolate		1.17	.92
Crystalline amino acids			.21
Total	1.50	1.51	1.47
(3) Total Nitrogen Intake (g)[1]	5.27	5.28	5.28
(4) Nonessential Nitrogen Intake (g)	3.77	3.77	3.81
(5) Essential Nitrogen/ Total Nitrogen	.28	.29	.28

[1]Experimental

[2]Calculated from percentages

TABLE 20

Total Essential Amino Acid Intake on the Three Diets (g)

Essential amino acid	Diet I flour + basal	Diet II isolate + basal	Diet III isolate + basal supplemented	Daily amino acid requirements (g)[3]
Lysine	1.86	1.94	1.86	.72
Methionine & Cystine[1]	.73	.67	.73	.60
Threonine	1.35	1.30	1.35	.48
Leucine	2.49	2.56	2.49	.96
Isoleucine	1.27	1.55	1.27	.72
Phenylalanine & Tyrosine[2]	3.11	2.77	2.76	.96
Valine	1.69	1.72	1.69	.64
Tryptophan[1]	.35	.28	.35	.18

[1]Cystine and tryptophan values were not calculated for the basal diet. Values given are from soy flour or soy isolate.

[2]Tyrosine was not added to the isolate in diet III. Value given is from the basal diet and 3.09g of isolate.

[3]Recommended Dietary Allowances, 1974, mean body weight = 60 kg

at pH 8 to pH 9.18. Woodard and Short (1973) reported that a decrease in the nutritive value of soy protein occurred with alkali treatment at pH 12.

One possible explanation for the decrease in nutritive value at high pH is a decrease in the utilization of amino acids caused by racemization of the amino acid residues in the intact protein. De Groot and Slump (1969) suggested that an observed decrease in the utilization of threonine in soy protein was due to racemization of the amino acid during processing at pH 12.2. Wesson (1975) treated soy protein at pH 6.7, 9, and 12 for varying lengths of time. She concluded that at a pH of 9.0, some racemization of amino acids did occur. Minimizing the time of treatment at that pH could limit the extent of racemization. At a pH of 12.0, racemization may be extensive within a very short period of time (Wesson, 1975).

Common conditions for the processing of isolated protein are extraction with dilute alkali (pH 7 to 9) at 50 to 55°C (Circle et al., 1959); Wolf and Cowan, 1975). Racemization of amino acids reported in the literature occurred when soy protein was treated at pH values higher than those generally used in the production of commercial isolates. The soy protein isolate used in the present study was extracted in a mildly alkaline aqueous medium.[1] Exact conditions of pH, time, and temperature are not known. In this case, one would not

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[1]Central Soya Technical Service Manual, 1975.

expect a decrease in utilization of amino acids due to racemization to occur to as great an extent as could be expected at higher pH values.

The results obtained with the PER study with rats (Table 18) were similar to those of the human balance study. Although the flour diet was better utilized that the supplemented isolate diet and both were better utilized than the unsupplemented isolate diet, there were no significant differences in PER values between the three groups. Protein efficiency ratios of 1.5, 1.4, and 1.3 (casein, 2.5) were obtained for flour, supplemented isolate, and unsupplemented isolate respectively. The flour, Soyafluff 200W, used in this study was treated with minimum moist heat treatment.[1] Although no data on PER values for Soyafluff 200W are given in the literature, values of about 2.3 are given for optimally heat processed flours.[1]

To optimize the nutritional value of defatted soybean products, they must be subjected to a heat treatment process (Kellor, 1974). The heat treatment partially or wholly destroys trypsin inhibitor activity which interferes with the digestion and utilization of soy protein (Kellor, 1974; Longenecker et al., 1964). Kellor (1974) showed that protein efficiency ratios of soy flour increased from 1.31 to 2.19 when the flour was treated with steam. Rackis et al. (1975) treated soy flour with live steam for 0 to 30 minutes. They found that PER values increased from 1.13 to 2.19, with the maximum PER value being reached after 9 minutes. The need to toast flour for human use is not known. (Wolf and Cowan, 1975).

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[1]Central Soya Technical Service Manual, 1975

Soy isolates are prepared with a controlled amount of heat treatment in order to preserve functional properties. Isolates differ in the amount of heat treatment which they have received in the manufacturing process. Some isolates may be free of growth inhibitors while others contain heat labile antinutritional factors (Wolf and Cowan, 1975). Huge (1961) reported that the nutritive value of a soy isolate[1] was not significantly improved by heat treatment and suggested that in the manufacture of the isolate, most of the inhibitors in the soybean are removed. Rackis et al. (1963) found that heating decreased trypsin inhibitor content of a commercial isolate but did not improve its PER significantly. Longenecker et al. (1964) showed that heating isolates at 105°C for 30 minutes improved the PER values for some isolates, but had no effect on others.

In much of the work reported in the literature, PER values for alkali treated soy protein were compared with PER values of non-alkali treated material which were as high as 2.4 (Cogan et al., 1968; Badenhop and Hackler, 1970). The PER of the flour in this study was only 1.5. Protein efficiency ratios for the flour and isolate used here did not differ significantly. The fact that the flour used in this study probably had not been optimally heat processed could mean that other factors including trypsin inhibitor activity were acting to lower its nutritive value. The similarity in nutritive value of the soy flour and soy protein isolate used here reflect the similarity

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[1]Promine, Central Soya Company, Chicago.

in their PER values. It might be useful to compare the isolate used in this study to a flour which has undergone optimal heat treatment.

According to the results of this experiment, soy flour and soy protein isolate are equally well utilized by the human. Nitrogen balance can be correlated with the pattern of essential amino acids provided by dietary protein (Allison, 1959). Alteration of the pattern of essential amino acids in a protein can change nitrogen retention in the body (Allison, 1969). The extent to which the proportions of essential amino acids in a protein can be altered without affecting amino acid requirements has not been adequately studied. This study showed that the extent to which the amino acid pattern of the isolate used here was altered by processing did not affect the nutritive value of the isolate for the human when compared to soy flour.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Several investigators have reported that alkali treatment, used in the production of soy protein isolates, decreases the nutritive value of the product (Bressani et al., 1967; Cogan et al., 1968; De Groot and Slump, 1969; Badenhop and Hackler, 1970). The decrease in nutritive value with alkaline processing has been related to the destruction of certain amino acids (Cogan et al., 1968; Badenhop and Hackler, 1970; Robinson et al., 1971). However, amino acid destruction alone is not a sufficient explanation for the observed decrease in nutritive value (Bressani et al., 1967; De Groot and Slump, 1969). Decreased utilization of amino acids could be caused by alkaline racemization of the amino acids (De Groot and Slump, 1969) or by changes in the overall amino acid pattern of the protein (Kofranyi, 1972). This research was undertaken to compare the nutritive value of soy flour, which has not undergone alkaline processing, and soy protein isolate when fed to human subjects.

Eighteen adult women participated in a 12-day nitrogen balance study. After adjustment to the low protein intake, the subjects were assigned to one of three dietary treatments with six subjects in each group. The three dietary treatments consisted of: 1) Basal diet plus 4.0 grams of nitrogen supplied by soy flour; 2) Basal diet plus 4.0 grams of nitrogen supplied by soy isolate; 3) Basal diet plus 3.09

grams of nitrogen supplied by soy isolate. Diet 3 was supplemented with crystalline amino acids in order to match the essential amino acid pattern of diet 3 with that of the flour in diet 1. Diet 3 was further supplemented with nonessential nitrogen in the form of ammonium citrate in order to bring the total nitrogen level of diet 3 up to that of diets 1 and 2. The basal diet provided 1.04 grams of nitrogen and approximately 1390 kilocalories. The soy protein was baked into a yeast bread product providing approximately 500 kilocalories. Total nitrogen content of food, urine and feces was determined using a modified Kjeldahl-Gunning-Arnold method (A.O.A.C., 1970). Nitrogen balance data was calculated for each of the three treatment groups.

The average nitrogen retention for the subjects on the flour diet was -0.06 grams of nitrogen per day. Three subjects on this diet maintained positive balance. Two subjects were in negative balance. The average nitrogen retention on the isolate diet was -0.30 grams of nitrogen per day. All subjects on this diet were in negative nitrogen balance. The average nitrogen retention on the supplemented isolate diet was -0.21 grams of nitrogen per day. Three subjects on this diet were in positive balance. Three subjects were in negative balance. There was a great deal of individual variability within group 3. An analysis of variance showed that there were no significant differences in nitrogen retention between the three groups.

Amino acid analysis of the flour and isolate showed that some changes in amino acid composition did occur in the production of the isolate. Although the isolate tended to be a poorer protein source

than the flour, the changes in amino acid composition or availability of amino acid in the isolate were not large enough to have a significant effect on nitrogen retention. Trypsin inhibitor activity in the flour may have contributed to its low nutritive value.

Since alkaline processing may be responsible for the lowered nutritive value of soy protein isolates, and since the use of processed soy protein in the human diet is increasing, further assessment of the effect of processing on nutritive value of soy protein is important.

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Appendix 1

Basal Diet Menu

Food	Weight (g)	Kcals[1]	Protein[1] (g)
Breakfast			
Grapefruit juice	125	51	0.63
Margarine	10	72	0.06
Apple butter	20	37	0.08
Applesauce	100	41	0.20
Lunch			
Apple juice	250	118	0.25
Margarine	10	72	0.06
Apple jelly	15	41	0.02
Tomato soup	165	60	1.32
Lettuce	55	7	0.46
Tomato	40	9	0.44
Cucumber	25	4	0.15
Carrot	50	21	0.55
Green pepper	20	4	0.24
Italian dressing	28	149	0.06
Applesauce cookies	25	115	0.07

[1]Watt and Merrill, 1963.

Appendix 1 cont'd

Food	Weight (g)	Kcals[1]	Protein[1] (g)
Dinner			
Cranberry juice	100	65	0.10
Ginger ale	150	47	0.00
Margarine	10	72	0.06
Apple jelly	15	41	0.02
Cabbage	55	13	0.72
Apple	45	26	0.09
Peach	50	39	0.20
Pear	25	19	0.05
Poppy seed dressing	20	111	0.02
Applesauce	100	41	0.20
Applesauce cookies	25	115	0.07

[1]Watt and Merrill, 1963.

Appendix 2

Recipes used in basal diet

Applesauce Cookies

Ingredient	Weight (g)
Shortening	76
Margarine	85
Sugar	295
Applesauce	246
Wheat starch	650
Cinnamon	6
Nutmeg	1
Soda	4
Vanilla flavoring	1 Tbsp
Lemon juice	1 Tsp
Baking powder	2
Salt	6

Cream shortening, margarine, and sugar. Stir in applesauce, vanilla, and lemon juice. Blend in all dry ingredients. Weigh out 25 gram portions. Flatten with a spatula dipped in water. Bake at 350^{o} for 20-25 minutes.

Appendix 2 cont'd

Italian Dressing

Ingredient	Weight (g)
Salad oil	686
Sugar	480
Vinegar	309
Onion flakes	33
Dry mustard	18
Salt	15

Mix all ingredients in a bowl. Blend thoroughly.

Poppy Seed Dressing

Ingredient	Weight (g)
Sugar	152.0
Dry mustard	2.5
Salt	4.0
Vinegar	63.0
Onion flakes	5.0
Salad oil	204.0
Poppy seeds	2.5

Mix all dry ingredients except poppy seeds in bowl and blend in vinegar and onion flakes. Add oil slowly, beating after each addition. Stir in poppy seeds.

Appendix 3

Bread Recipes

Wheat Starch Bread

Ingredient	Weight (g)
Wheat starch	395.3
Dry yeast	9.0
Sugar	25.0
Salt	6.0
Glycerol monostearate[1]	1.0
Shortening	38.0
Water (105° - 115°)	296 ml

Measure wheat starch into bowl. Set aside. Spray individual loaf pans with PAM. In large mixing bowl, dissolve yeast, sugar, salt, and glycerol monostearate in warm water. Let stand 5 minutes. Place heatproof bowl of hot water to side in oven. Heat to "warm" or 150° for five minutes. Turn oven off. Add shortening and half the wheat starch to yeast mixture. Beat until smooth. Add remaining wheat starch. Beat until smooth. Weigh total batter. Divide into eight equal portions. Pour into individual pans. Place pans in oven beside bowl of hot water. Let rise 30 minutes. Set oven at 350°. Bake for 10 minutes. Cover with aluminum foil. Bake at 400° for 25 minutes.

[1]Fisher Scientific Co.

Appendix 3 cont'd

Soy Flour Bread
Diet 1

Ingredient	Weight (g)	
Wheat starch	204.0	
Soy flour	191.2	
Sugar	25.0	
Dry yeast	9.0	
Glycerol monostearate[1]	1.0	
Shortening	38.0	
Cinnamon	3.0	
Water		526 ml
Salt	6.0	

Weigh out wheat starch and soy flour. Add cinnamon. Dissolve sugar, salt, yeast, and glycerol monostearate in 296 ml of warm water. Let stand five minutes. Place bowl of hot water in oven. Turn over to "warm" or 150° for five minutes. Add shortening and half of the starch-flour to yeast mixture. Add 230 ml of warm water and rest of starch-flour mixture alternately. Beat until smooth. Weigh total batter. Divide into eight equal portions. Pour into eight individual loaf pans. Let rise in oven for 30 minutes. Turn oven to 350°and bake for 10 minutes. Cover with aluminum foil and bake at 400° for 20 minutes.

[1]Fisher Scientific Co.

Appendix 3 cont'd

Soy Isolate Bread
Diet 2

Ingredient	Weight (g)
Wheat starch	288.0
Soy isolate	107.3
Sugar	25.0
Dry yeast	9.0
Salt	6.0
Glycerol monostearate[1]	1.0
Shortening	38.0
Cinnamon	3.0
Warm water	481 ml

Weigh out wheat starch and isolate. Add cinnamon. Dissolve yeast, sugar, salt, and glycerol monostearate in 296 ml of warm water. Let stand five minutes. Place bowl of hot water in oven. Turn over to "warm" or 150° for five minutes. Add shortening and half of the starch-isolate mixture to yeast mixture. Add 185 ml of warm water and the rest of the starch-isolate mixture alternately. Beat until smooth. Weigh total batter. Divide into eight equal portions. Pour into eight individual pans. Let rise in oven for 30 minutes. Turn over to 350° and bake for 10 minutes. Cover with aluminum foil and bake for 20 minutes at 400°.

[1]Fisher Scientific Co.

Appendix 3 cont'd

Soy Isolate Bread
Diet 3

Ingredient	Weight (g)	
Wheat starch	312.5	
Soy isolate	82.8	
Sugar	25.0	
Salt	6.0	
Yeast	9.0	
Glycerol monostearate[1]	1.0	
Shortening	38.0	
Water		466 ml
Vanilla flavoring	1.0	
Cinnamon	2.0	

Weigh out wheat starch and soy isolate. Add cinnamon. Dissolve yeast, sugar, salt, and glycerol monostearate in 296 ml of warm water. Let stand five minutes. Place bowl of hot water in oven. Turn over to "warm" or 150° for five minutes. Add shortening and half of the starch-isolate mixture to the yeast mixture. Add 170 ml of water and rest of starch-isolate mixture alternately. Add vanilla. Beat until smooth. Weigh total batter. Divide into eight equal portions. Pour into eight individual loaf pans. Let rise in oven for 30 minutes. Turn over to 350° and bake for 10 minutes. Cover with aluminum foil and bake at 400° for 20 minutes.

[1]Fisher Scientific Co.

Appendix 4

Amino Acid Analysis

Samples of approximately 0.1g of soy flour, 0.05g of soy isolate and 1.0g of food were weighed into 20 ml capacity ampules. Ten ml of 6N HCl was added to each ampule. The ampules were sealed under a slight vacuum after flushing the ampules with nitrogen. The ampules were then heated at 110° for 12, 24, 36, and 48 hours. The hydrolysates were allowed to cool to room temperature. The ampules were opened and the contents filtered through glass wool. Aliquots of 1.0 ml were taken from each hydrolysate and placed in a vacuum desiccator over sodium hydroxide pellets. The hydrolysates were dried under reduced pressure for approximately 18 hours and resuspended in 10 ml (flour and isolate) or 5 ml (food) of sodium citrate buffer, pH 2.0.

Amino acid analysis was done by ion-exchange chromatography on a model HC-1P Technicon Amino Acid Analyzer. The total nmoles for each amino acid were calculated in the following manner: The peak height (absorbance units) was multiplied by the peak width (mm) taken at half height. This value was multiplied by a conversion factor determined from analysis of an amino acid standard.

In order to estimate the time of hydrolysis giving maximum release of each amino acid, the data were analyzed using a curvilinear regression technique provided by the Department of Animal Science, Virginia Polytechnic Institute and State University.

Appendix 5

Daily Urine Volumes and Creatinine Excretion

Subject number	Day 8		9		10		11		12	
	1	2	1	2	1	2	1	2	1	2
1	1.25	1570	1.26	1400	1.26	1730	1.31	1420	1.20	1030
2	1.01	1200	1.15	1020	1.22	1750	1.26	1350	1.28	1800
3	0.96	1380	1.04	1460	0.88	1420	1.01	1500	1.09	1720
4	1.30	1510	1.21	1440	1.26	840	1.40	1570	1.38	1020
5	1.25	1260	1.21	1580	1.27	1590	1.13	2100	1.32	2350
6	1.26	1380	1.18	1480	0.63[3]	1020	1.14	1000	1.12	890
7	1.45	1120	1.27	740	1.52	1050	1.47	1740	1.52	1620
8	1.16	880	1.06	750	1.17	860	0.58[3]	860	0.54[3]	930
9	1.34	1490	1.34	1720	1.34	1520	1.22	1280	1.21	2260
10	1.00	900	1.18	1480	1.04	2306	1.25	1540	1.16	2200
11	0.87	1860	0.91	2260	0.61	2880	1.06	2020	0.99	2540
12	1.13	1280	1.06	800	1.16	1090	1.31	1350	1.00	1700
13	1.34	900	1.07[3]	800	1.33	930	1.33	1160	1.36	1520
14	1.32	1100	1.34	1270	1.31	1320	1.46	1660	1.36	1310
15	1.18	1290	1.14	1160	1.16	1480	1.22	1330	1.22	2000
16	1.37	1240	1.37	1200	1.39	1110	1.28	1230	1.36	1520
17	0.98	1060	1.00	900	0.81	660	0.95	720	1.04	618
18	1.43	2240	1.06	1320	1.34	1680	1.15	1580	1.46	1900

[1]Creatinine excretion (grams per day)

[2]Urine volume (ml)

[3]Nitrogen values corresponding to these creatinine values were not used in the calculation of mean daily urine nitrogen excretion.

Appendix 6

Daily Urine Nitrogen Excretion

Subject number	Day				
	8	9	10	11	12
1	4.16	4.55	4.64	3.56	3.38
2	4.03	4.14	4.48	4.34	4.89
3	4.16	4.36	3.79	3.69	4.07
4	6.05	5.34	4.30	5.03	5.05
5	4.12	4.50	4.30	4.02	4.94
6	4.67	4.23	2.30[1]	4.12	3.46
7	5.45	4.36	4.80	4.30	5.19
8	4.25	4.18	4.61	2.09[1]	1.99[1]
9	4.81	4.18	4.52	3.95	4.41
10	3.20	4.41	4.13	3.91	3.91
11	3.79	4.27	3.67	3.78	3.72
12	4.20	3.16	3.61	4.16	3.50
13	5.32	3.86[1]	4.39	4.43	4.55
14	5.08	5.84	5.52	5.32	4.84
15	5.36	5.20	5.43	5.28	5.61
16	5.08	4.60	4.57	4.34	4.98
17	4.03	3.85	3.12	3.30	3.72
18	5.76	3.98	4.84	3.98	5.15

[1]Values not used in calculation of mean daily urine nitrogen excretion

Appendix 7

Weight Gain, Protein Consumption, and PER Values for Rats Over 28-day Period

	Flour Diet			Unsupplemented Isolate Diet		
Animal number	Weight gain (g)	Protein consumed (g)	PER	Weight gain (g)	Protein consumed (g)	PER
1	70.0	40.69	1.72	45.0	32.07	1.40
2	60.5	39.70	1.52	63.0	40.54	1.55
3	77.5	42.37	1.83	65.0	43.07	1.51
4	71.0	41.84	1.70	45.5	33.96	1.34
5	52.2	40.72	1.28	35.5	26.91	1.30
6	69.5	37.96	1.83	55.0	33.93	1.62
7	44.0	31.64	1.39	57.0	34.53	1.65
8	61.5	36.21	1.70	36.0	28.93	1.24
9	66.0	41.14	1.64	46.0	35.61	1.29
10	58.0	36.99	1.57	42.0	37.51	1.12
11	58.0	39.64	1.46	63.0	35.83	1.76

Appendix 7 cont'd

Animal number	Supplemented Isolate Diet			Casein Diet		
	Weight gain (g)	Protein consumed (g)	PER	Weight gain (g)	Protein consumed (g)	PER
1	57.0	39.31	1.45	128.0	41.68	3.07
2	61.0	37.34	1.63	109.0	38.85	2.81
3	42.0	35.41	1.19	137.0	45.50	3.01
4	53.0	44.64	1.19	112.0	47.62	2.35
5	70.0	40.56	1.73	167.0	55.06	3.03
6	48.0	38.46	1.25	104.5	48.32	2.16
7	74.0	42.60	1.74	99.0	38.09	2.60
8	48.5	33.77	1.44	99.0	39.45	2.51
9	67.0	36.53	1.83	117.0	43.31	2.70
10	55.5	40.70	1.36	124.0	45.63	2.72
11	57.3	35.80	1.60	114.0	45.75	2.49

Appendix 8

Analysis of Variance Tables

Urinary Nitrogen Excretion

Source of variation	degrees of freedom	mean square	F ratio
Between treatments	2	.2580	.9680
Within treatments	14	.2660	
Total	16		

Fecal Nitrogen Excretion

Source of variation	degrees of freedom	mean square	F ratio
Between treatments	2	.0647	1.1085
Within treatments	14	.0583	
Total	16		

Digestibility

Source of variation	degrees of freedom	mean square	F ratio
Between treatments	2	.0024	1.1921
Within treatments	14	.0020	
Total	16		

Nitrogen Retention

Source of variation	degrees of freedom	mean square	F ratio
Between treatments	2	.0800	.312
Within treatments	14	.2580	
Total	16		

Protein Efficiency Ratio

Source of variation	degrees of freedom	mean square	F ratio
Between treatments	2	.0814	1.9799
Within treatments	30	.0411	
Total	32		

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THE NUTRITIVE VALUE OF SOY FLOUR AND SOY PROTEIN ISOLATE FOR HUMAN ADULTS

by

Lillian Janette Taper

(ABSTRACT)

A 12-day nitrogen balance study was designed to determine the protein nutritive value of soy flour and soy protein isolate in human adults. By equalizing the essential amino acid patterns of the flour and the isolate, it was considered possible to measure any difference in nutritive value of the two protein sources.

The subjects were 18 healthy women ranging in age from 20 to 25 years. A basal diet provided approximately 1.0 gram of nitrogen and 1400 kilocalories per person per day. The soy protein was incorporated into a low-protein yeast bread providing approximately 500 kilocalories per person per day. The following dietary treatments were used:

1. Basal diet plus 4.0 grams of nitrogen supplied by soy flour.
2. Basal diet plus 4.0 grams of nitrogen supplied by soy isolate.
3. Basal diet plus 3.09 grams of nitrogen supplied by soy isolate.

Diet 3 was supplemented with amino acids to match the essential amino acid pattern of the isolate to that of the flour in diet 1. Diet 3 was further supplemented with nonessential nitrogen in the form of ammonium citrate in order to bring the total nitrogen level of the diet up to that of diets 1 and 2. The following data was collected

for each dietary treatment: 1) Urinary nitrogen excretion; 2) fecal nitrogen excretion; 3) nitrogen intake based on an analysis of actual food intake.

After a 7-day adjustment period to allow subjects to adjust to the low nitrogen intake, nitrogen retention was calculated from data collected during a 5-day experimental period. Nitrogen retentions for the three groups were: flour, -0.06; isolate, -0.30; and supplemented isolate, -0.21 grams of nitrogen per person per day. Analyses of variance showed no treatment effects on urinary or fecal nitrogen excretion or nitrogen retention. It was concluded that, under the conditions of this experiment, soy flour and soy protein isolate are equally well utilized by the human adult.