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EFFECTS OF PROCESSING AND MATURITY ON CERTAIN ANTINUTRITIONAL FACTORS IN SOYBEANS

by

Keshun Liu

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ABSTRACT

EFFECT OF PROCESSING AND MATURITY ON CERTAIN ANTINUTRITIONAL FACTORS IN SOYBEANS

By

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Seeds of two soybean cultivars [Glycine max. (L.) Merr.], Beeson 80 and Pella, were harvested at four maturity stages and subjected to soaking (24 hr), cooking (20 min), steaming (20 min), and soaking-cooking. Samples were freeze-dried and analyzed for protein, oil, ash, trypsin inhibitory activity (TIA), oligosaccharides and phytic acid. As seeds matured, oil increased slightly, protein was constant, ash remained constant after an initial high value and phytic acid increased. TIA increased in Beeson 80 and decreased in Pella. Oligosaccharides were present in very low amounts early in seed development. In mature soybeans, soaking slightly reduced oligosaccharides, but had no effect on phytic acid and TIA. Cooking or steaming reduced oligosaccharides and TIA, but had no significant effect on phytic acid. Soaking-cooking increased phytic acid, decreased oligosaccharides greatly and inactivated TI completely. In immature soybeans, changes in oligosaccharides and phytic acid were similar to those with mature soybeans except that steaming slightly increased sucrose content. TIA decreased more readily upon processing in immature soybeans than in mature ones.

DEDICATION

To my sisters

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INTRODUCTION

Direct consumption of fresh immature soybeans [*Glycine max.* (L.) Merr.] is very popular in China and in some other oriental countries. In Chinese, the immature soybean is called "Mao Dou" or "Qing Dou", meaning green bean. In Japanese, it is called "edamame". Steamed or boiled in water before or after shelling, traditionally for not less than 20 min, these fresh green beans can be served either as a delicious and nutritious green vegetable with the main meal or as snacks with wine and beer.

In the West, due to increasing production of soybeans and popularization of vegetarian life-styles, development of acceptable food products from green immature soybeans has been suggested to further increase the variety of soybean products. In fact, frozen and canned immature soybeans have appeared in Western markets.

In general, immature soybeans have some advantages over mature ones. These include higher content of ascorbic acid and β-carotene, lower amounts of antinutritional factors and higher protein availability. Furthermore, their green color and soft texture enhance their appeal as a vegetable.

One of the major factors that limit use of soybeans in human diet is the presence of certain naturally occurring

substances, termed antinutritional factors. These substances, including trypsin inhibitor, oligosaccharides (mainly, raffinose and stachyose) and phytic acid, can cause adverse physiological responses or diminish availability of certain nutrients to animals and humans. For example, trypsin inhibitors reduce the digestibility of soybean proteins; oligosaccharides cause flatulence associated with soybean consumption, while phytic acid decreases the availability of certain minerals by chelating them. There are numerous reports in the literature concerning this subject, including the presence of these antinutrients in soybeans, the analytical methods to measure them and the proper processing treatments to reduce them. However, most of these reports have been limited to mature soybeans. There are no data available on the oligosaccharides in developing soybeans, and little information on the effect of processing treatments (soaking, cooking, steaming and soaking-cooking) on trypsin inhibitors, oligosaccharides and phytic acid in immature soybeans. The purpose of this investigation was to provide information on these subjects.

LITERATURE REVIEW

I. Soybeans as a Food Source

With a steady increase of the world population, demand for both protein and edible oil is ever increasing. Because soybeans contain about 40% protein and 20% oil, they have become one of the most promising crops to fulfil this need. Indeed, the world production of soybeans has steadily been increasing. For example, in 1970, the world total soybean production was 32 million metric tons, while in 1985, it reached 90 million metric tons (USDA, OCS-8 1985).

Throughout history, the Chinese and other oriental people have developed various forms of soybean foods. Traditionally, oriental soybean products can be classified into two groups, non-fermented and fermented soyfoods. For both kinds, the name, the brief description and the use for each variety are presented in Table 1.

For people in western countries, due to the relative short history of soybean use, the flavor and texture of soybean products are comparatively strange. Therefore, in the West, the use of soybeans as a food is not as extensive

Table 1. Classification of oriental soyfoods *

Oriental Non Fermented Soyfoods

SOYFOODS	LOCAL NAMES	DESCRIPTION	USES
Fresh green soybeans	Mao-tou (Chinese) Edamame (Japanese)	Picked before mature, firm, bright green	Boiled or steamed in the pods and shelled. Served as hors d'oeuvre (often with wine or beer), or shelled before cooking served as fresh green vegetable.
Soybean sprouts	Huang-tou-ya (Chinese) Daizu-no-moyashi (Japanese)	Bright yellow beans with 3-5cm sprouts	Served as vegetable in dishes or in salad (parboiled).
Soynuts	Huoh-huang-tau (Chinese) Iri-mame (Japanese)	Roasted whole soybeans or cotyledons by deep-frying or dry heat. Seasoned by salt, garlic or coated with chocolate	Served as snack.
Soymilk	Tou-chiang or tounai (Chinese) To-nyu (Japanese) Kongkuk (Korean)	Water extract of soybeans, resembling cow's milk, with plain, sweetened and flavoured	Served hot as breakfast drink or served cold as soft drink.
Soy flour	Huang-tou-fen (Chinese) Kinako (Japanese)	Ground roasted dry soybeans with nutty flavour	Used as filling or coating for pastries.
Soy protein-lipid film	Tou-fu-pi (Chinese) Yuba (Japanese)	Creamy-yellow film formed over the surface of simmering soymilk, and moist sheets, flakes or sticks	Cooked and used as meat with chewy texture.
Soybean curd	Tou-fu or Tao-fu (Chinese) Tofu (Japanese) Tubu (Korean) Taboo (SE Asia)	White curd cubes coagulated from soymilk with a calcium or magnesium salt soft to firm (by pressed curds)	Served as dishes with or without further cooking.

Oriental Fermented Soyfoods

SOYFOODS	LOCAL NAMES	ORGANISMS USED	DESCRIPTION	USES
Soy Sauce	Chiang-yu (Chinese) Shoyu (Japanese) Ketjap (Indonesian) Kanjang (Korean) Toyo, see-iau (S.E. Asia)	Aspergillus, Pediococcus, Torulopsis and Saccharomyces	Whole soybeans or defatted soy flake and wheat	Dark brown liquid, salty taste suggesting the quality of meat extract, a flavouring agent.
Miso	Tou-chian, wei-chong (Chinese) Miso (Japanese) Doenjang (Korean) Tau-cho (Indonesian) Soybean Paste	Aspergillus, barley Pediococcus Saccharomyces, Torulopsis and Streptococcus	Whole soybean, rice or reddish brown paste,	Light yellow to dark smooth or chunky, salty and flavoured. A flavouring agent.
Tempeh	Tempeh (Indonesian) Tempei (Japanese)	Rhizopus	Whole soybeans	Cooked soft beans bound together by mycelia as cake, a clean fresh and yeasty odour. Cooked and served as main dish.
Natto	Natto (Japanese) Tau-nou or Thua-no (Thailand) Kenima (Nepal)	Bacillus Natto	Whole soybeans	Cooked beans bound together and covered with viscous, sticky substance, produced by the bacteria, ammonium odour, musty flavour. Served with cooked rice as a dish.
Fermented tofu	Tou-fu-ru or Tao-ru (Chinese) Fu-nyu (Japanese) Sufu (Indonesian) Tohuri (Philippinse) Chinese cheese	Actinomucor, Mucor	Soybean curd (firm tofu)	Creamy cheese-type cubes salty. A condiment, served with or without further cooking
Soy Nuggets	Tou-shih (Chinese) Tao-si (Philippinese) Tau-chee (Malaysian) Tauco (Indonesian) Hamanatto (Japanese)	Aspergillus, Streptococcus, and Pediococcus	Whole soybeans Wheat flour	Nearly black soft bean, salty flavour resembling soysauce. A condiment.

* From Byrne, 1985.

as in the Orient. However, this situation is now beginning to change. The move is towards products made from tofu, tempeh and miso, and the emphasis is on more imaginative uses of soybeans in modern prosessing systems such as snackfood products. With advancing technology, it is not surprising that more and more edible products can be seen in western markets, which contain soybean derivatives.

II. Chemical Composition of Soybeans

Soybeans are composed of approximately 8% hull, 90% cotyledon, and 2% germ (hypocotyl and plumule). The chemical composition for soybeans depends on variety, soil and other factors. Table 2 gives the proximate composition for whole beans and their three fractions. Carbohydrates in the table include polysaccharides, and oligosaccharides such as sucrose, raffinose and stachyose.

Table 2. Composition of soybeans and their fractions *

Fractions	Protein (Nx6.25) (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Whole bean	40	21	34	4.9
Cotyledon	43	23	29	5.0
Hull	8.8	1	86	4.3
Hypocotyl	41	11	43	4.4

* Moisture free basis (from Kawamura, 1967).

III. Factors Affecting Nutritional Quality of Soybean Products

The nutritional quality of soybean products is affected by several factors:

1) Contents of protein, oil and other nutrients.
2) Quantity and availability of the amino acids which make up the protein of such products.
3) Contents in certain chemical substances, which cause adverse physiological responses in man. Commonly, they are termed antinutritional factors.
4) Degree of elimination of these antinutrients by certain processing treatments such as heating, germination etc.

IV. Antinutritional Factors in Soybeans

Liener (1981) classified antinutritional factors into two groups according to their reponses to heat: heat-labile and heat-stable (see Table 3).

Among these antinutrients, trypsin inhibitors, flatulence factors and phytates may be considered as key determinants to the nutritional quality of soybeans for humans.

An attempt will be made to evaluate their nutritional significance and biologically detrimental effects, particularly in human diet. Later on, in the following sections, discussion will be made on how these effects may be eliminated to some extent by appropriate processing methods.

Table 3. Antinutritional factors in soybeans

Heat-labile	Heat-stable
Trypsin inhibitors	Saponins
Hemagglutinins	Estrogens
Goitrogens	Flatulence factors
Antivitamins	Lysinoalanine
Phytates	Allergens

1. Trypsin inhibitors

Trypsin inhibitors (TI) are proteinase inhibitors. They belong to a family of proteins which inhibit a wide variety of proteinases, mainly trypsin.

Osborne and Mendel (1917) made the first significant observation that soybeans had to be heated in order to support the growth of rats. TI proved to be responsible for reducing digestibility of proteins by inhibiting trypsin activities. Later, a second observation was made by Desikachar and De (1947), and Liener (1949) that preparations of TI were capable of inhibiting growth even when incorporated into diets containing predigested protein or free amino acids. This indicated that inhibition of proteolysis by TI was not the sole factor responsible for growth depression. Subsequently, another significant finding was made by Chernick et al. (1948). They found that raw soybeans and the TI itself could cause hypertrophy of the pancreas, which is accompanied by an increase in the secretory activity of the pancreas. This led to the suggestion that the growth depression caused by the TI might be the consequence of an endogenous loss of essential amino acids being secreted by hyperactive pancreas.

More recent experiments suggested that protein from raw soybeans is in itself refractory to enzymatic attack unless denatured by heat (Green et al., 1973). Therefore, it would

appear that the TI and the refractory nature of the soybean protein act through a common mechanism to inhibit the growth of rats.

2. Flatulence factors

One of the important factors limiting the uses of soybeans in human diet is the flatulence associated with their consumption. Steggerda et al. (1966) conducted an experiment in which four male adults consumed various toasted soybean products commercially manufactured. They revealed that flatulence resulted mainly from the low-molecular-weight carbohydrate fractions of soybeans containing α-galactosidic and β-fructosidic linkages, namely raffinose and stachoyse (Figure 1). In other kinds of legumes, verbascose may be included. Hymowitz et al. (1972) analyzed soybean seeds of as many as 60 selected lines for sucrose, raffinose and stachyose contents. They found that ranges in values (expressed as g/100g seed) were 2.5-8.2 for sucrose, 0.1-0.9 for raffinose and 1.4-4.1 for stachyose.

Flatulence is generally attributed to the fact that man is not endowed with the enzyme called α-galactosidase necessary for hydrolyzing the α-galactosidic linkages of these oligosaccharides to yield readily absorbable sugars (Gitzelman and Aurricchio, 1965). Consequently, the intact oligosaccharides enter the lower intestine where they are metabolized by microorganisms, resulting in the production of such gases as carbon dioxide, hydrogen, nitrogen, methane

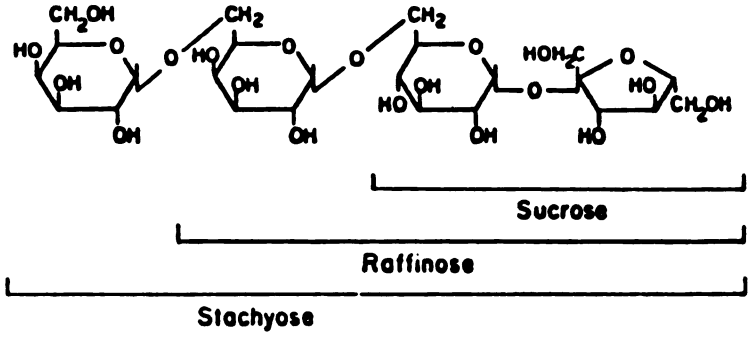


Figure 1. Structures for oligosaccharides

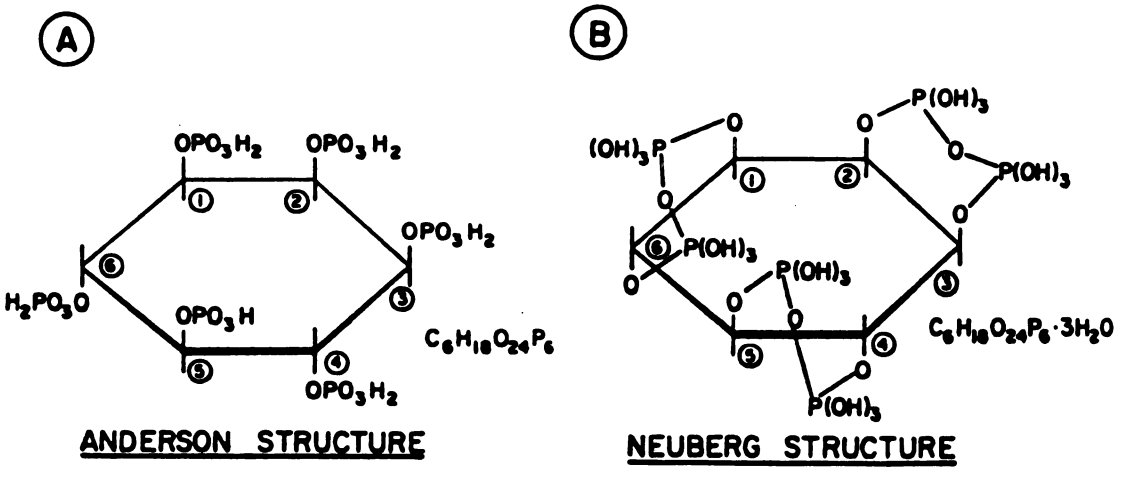


Figure 2. Proposed structure for phytic acid

etc., depending on the individual diet and microflora spectrum.

3. Phytic acid

Phytic acid, the hexaphosphate of myoinositol (Figure 2), is found mainly in plant seeds, where it functions as a reserve material for phosphorus. In soybean seeds the amount of phytic acid present varies with variety, ranging fron 1.01-1.47% (Lolas et al., 1976). To plants themselves, the presence of phytic acid is desirable, but when these plants are used as food, it is undesirable, because it readily chelates with such di- and tri-valent metal ions as Ca, Mg, Zn, Cu and Fe, to form insoluble salts. Such complexes are poorly absorbed from the intestine, resulting in reduced availability of these minerals from soybean products (Jaffe, 1981).

In addition to binding with minerals, phytic acid was found to bind strongly with proteins at certain pH values. Therefore, it can affect the properties of proteins (Okubo et al., 1976).

V. Elimination of Antinutritional Factors in Soybeans

1. Trypsin inhibitors

Since TI are proteins, they are readily inactivated by various heat treatments. These include live steam (a process called toasting), heating in boiling water, dry roasting,

dielectric heating, microwave radiation, micronization and extrusion cooking (Liener, 1981). Figure 3 shows the effect of general heat treatment on trypsin inhibitory activity (TIA) and protein efficiency ratio (PER) of soybean proteins.

Other methods such as soaking and germination were also reported to eliminate TIA in soybeans to some extent although they were not as effective as heating (Ologhobo and Fetuga, 1983)

Recently, Orf and Hymowitz (1979) discovered a soybean line from Korea which lacked TI. This might provide an approach for eliminating TI through genetic breeding.

2. Flatulence factors

Flatulence factors are heat-stable, and heat treatments cannot reduce their contents. There are certain techniques that have been reported to reduce the content of oligosaccharides. These include soaking, enzymatic hydrolysis and germination (Liener, 1981).

The fact that there is a considerable variation in oligosaccharide content of different soybean varieties may give the hint that genetic changes by plant breeding is, at best, only a long-term solution to the problem.

3. Phytic acid

Phytase can hydrolyze phytic acid, but unfortunately, monogastric animals like man have little or no intestinal

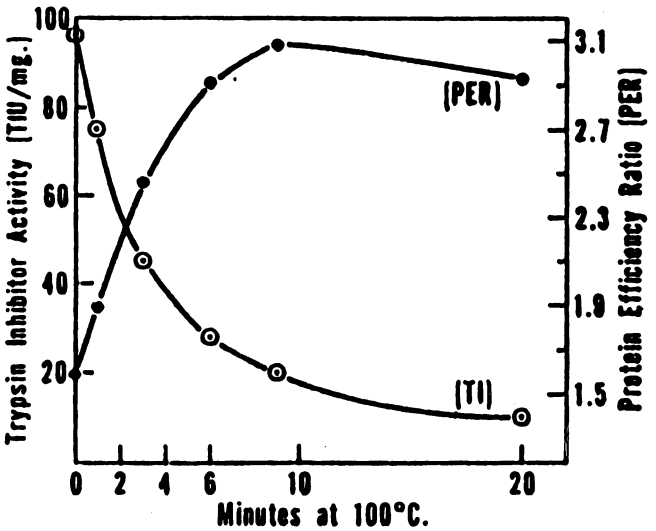


Figure 3. Effect of heat treatment on trypsin inhibitory activity and protein efficiency ratio of soybean proteins

(From Rackis, 1972).

phytase activity. Therefore, it would be advantageous to develop methods to eliminate it. However, since phytates are tightly bound to soya proteins, no commercial methods have been developed to eliminate them completely. At a laboratory scale, several procedures have been used such as precipitating phytates as the barium salt, dialyzing them against a sodium chloride solution, treating them with a strong anion exchange resin, and controlling the pH during preparation of soybean isolates (Jaffe, 1981).

Certain microorganisms containing phytase could cleave phosphates from phytic acid during soybean fermentation (Erdman, 1979).

VI. Measurements of Antinutritional Factors

1. Measurement of trypsin inhibitors

Although various methods of column chromatography, affinity chromatography and electrophoresis have proved valuable for the isolation and characterization of diverse TI, these methods are not suitable for quantitation. At present, methods for TIA analysis are mainly colorimetric, based on interaction among TI, trypsin and a substrate. There are two kinds of substrates used, natural substrates like casein and synthetic substrates like benzoyl-DL-arginine-p-nitroanilide (BAPNA).

The common method employing casein is the one originally

described by Kunitz (1947); it involves the spectrophotometric determination of the breakdown products produced by a given concentration of trypsin in the presence and absence of the inhibitor. However, the rate of hydrolysis of casein by trypsin was later reported not to follow zero order kinetics under the condition defined by Kunitz (Jacobbson, 1955). This poses a question of reliability.

Erlanger et al. (1961) first introduced a synthetic substance, BAPNA. They found that hydrolysis of BAPNA by trypsin not only followed a zero order, but also can be directly traced colorimetrically. Later on, Kakade et al. (1969) reported that the use of BAPNA is preferable over casein in tern of simplicity and accurancy, provided that the competitive nature of the inhibition was taken into consideration. Subsequently, a collaborative study by a Committee on Soybean Trypsin Inhibitor Analysis resulted in several modifications of the original procedure (Rackis et al., 1974 and Kakade et al., 1974).

However, Hamerstrand et al. (1981) found that the extrapolation procedure for data interpretation suggested by Kakade et al. (1969) could lead to an erroneously high value for TIA in soybean products, since this extrapolation used data that are not in the region in which zero order kinetics are followed. In order to obviate this problem, they developed a further modified procedure in which TIA was determined from a single dilution of a sample extract that inhibited trypsin in the range of 40-60%.

2. Measurement of oligosaccharides

Reports on the determination of oligosaccharides in legumes involved a number of techniques. These include chemical and enzymatic methods, the colorimetric method, the gravimetric method, thin layer chromatography (TLC), paper chromatography (PC), gas-liquid chromatography (GLC), and liquid chromatography (LC) (Cegla and Bell, 1977). However, most of these methods have their limitations. The enzymatic procedure is highly specific, but the reagents required for large numbers of analysis are expensive. The colorimetric method is applied to pure solutions or mixtures of sugars. The gravimetric method does not distinguish among different carbohydrates. TLC and PC are useful only for qualitative determination. GLC requires derivatization of the sugars, although it is one of the most accurate methods. For LC, it takes a long time to finish a single separation.

Conrad and Palmer (1979) reported a procedure for analysis of carbohydrates in legumes by high pressure liquid chromatography (HPLC). This method has then been considered as an adequate means for rapid analysis of soluble sugars. However, because soybeans contain many substances other than sugars, there is a strong interference with separation and quantitation of sugars. Also there is a high risk of column contamination.

In order to separate sugars from other components, several methods have been reported including precipitation of proteins with 15% trichloroacetic acid (TCA) or with lead

acetate solution and purification of sugars on TLC or on preparative column of hydroxylapatite. Cegla and Bell (1977) concluded that TLC was the best method to separate unwanted compounds from sugars although it was very tedious. Therefore, development of an accurate and simplified extracting procedure with least risk of column contamination for HPLC analysis of sugars still remains a challenge.

3. Measurement of phytic acid

Figure 4 summarizes the various methods developed for analysis of phytic acid in soybeans as well as in other materials.

Most assays for phytic acid employ ferric chloride to precipitate ferric phytate. The determination may be based on the analysis of either phosphorous or iron in isolated ferric phytate (Nahapetian and Bassir, 1975), or indirectly on the determination of the residual iron (Young, 1936) or the residual phosphorus (Tangkongchitr et al., 1981) in the supernatant after precipitation. Alternatively, sodium hydroxide may be used to convert the precipitate to sodium phytate and ferric hydroxide. It is convenient to analyze for iron after taking up the ferric hydroxide in acid (Wheeler and Ferrel, 1971).

Most of these methods are time-consuming. Furthermore, interpretation of the iron content either in the precipitate or in the supernatant into phytate content is based on assuming certain molar ratio of iron to phytate phosphorus

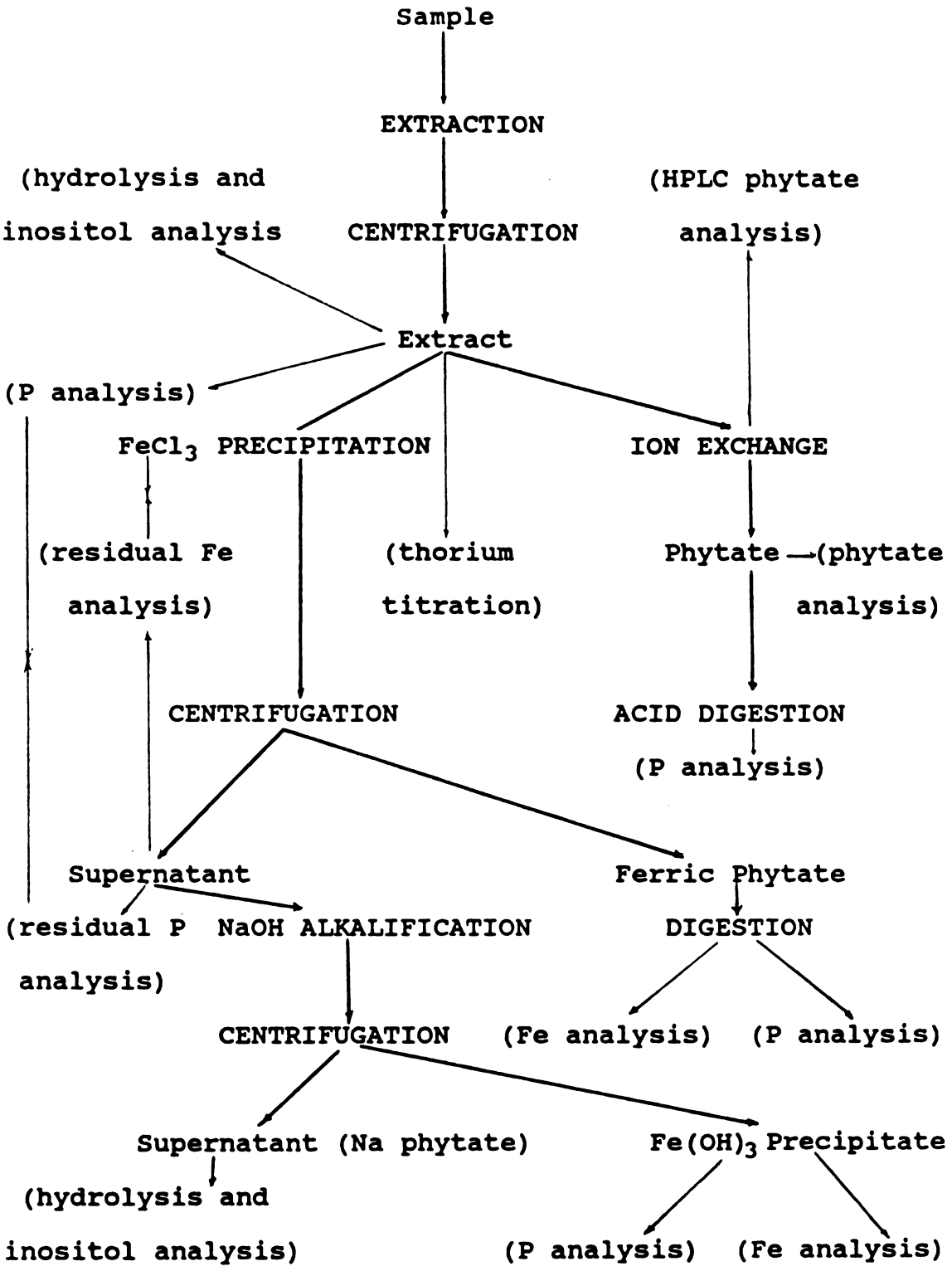


Figure 4. Scheme for approaches to phytic acid analysis

(ranged from 3:6 to 4:6 as reported previously), resulting in poor reliability and repeatability.

An Anion exchange separation of phytate from the extract was reported accurate (Harland and Oberleas, 1977).

Recently, a HPLC system has been developed to quantitate phytic acid (Knuckles et al., 1982). It has proved to be a rapid method with more reproducible results as compared with traditional methods.

For extracting phytate from soybeans, different acid solutions were used, including 3% trichloroacetic acid (TCA) (Wheeler and Ferrel, 1971), 0.5N HCI (Makower, 1970), and 1.2% HCl + 10% Na_2SO_4 or 3% TCA + 10% Na_2SO_4 (Thompson and Erdman, 1982).

VII. Comparison of the Food Value for Immature and Mature Soybeans

The high consumption of immature soybeans in China and other parts of the world can not be explained by tradition alone. It is likely that nutritional considerations justify the popularity of this foodstuff.

1. Chemical composition

1) Fresh Weight and Total Solids. According to Rackis et al. (1972), the maximum fresh weight of soybeans was reached 44 days after flowering, when these seeds were still green but the pods began to yellow. The dry matter

accumulated with maturation, more slowly at early stages and faster at later ones.

2) Protein. On a dry basis, protein content was constant regardless of maturity, while on fresh weight basis, it accumulated with maturation (Rubel et al., 1972).

3) Oil and oil Composition. On a dry basis, crude oil increased with maturation, faster at early stages and more slowly at later ones. Regarding oil composition, it appeared that with maturation, palmitic, stearic and linolenic acids decreased while oleic and linoleic acids increased. However, the ratio of saturated vs. unsaturated fatty acids remained the same (Rubel et al., 1972).

4) Minerals. Limited data are available concerning the effect of maturity on ash content in soybeans. Rasmussen (1978) reported that lower content in boron and phosphorous and higher content in zinc were noticed in meal prepared from immature soybeans compared with that prepared from mature soybeans.

5) Vitamins. Perhaps, one of the major nutritive advantages of immature soybeans over mature ones is the higher contents in ascorbic acid and β-carotene in immature soybeans (Bates and Matthews, 1975).

6) Carbohydrates. It has been found that during maturation, large amounts of starch accumulated and reached a maximum at the green mature stages. During subsequent development to full maturity, starch practically disappeared and the sugar content increased (Bils and

Howell, 1963).

2. The Essential amino acid pattern of proteins

In general, the essential amino acid pattern of soybean proteins is considered rich in lysine and poor in sulfur-containing amino acids, that is, methionine and cysteine. Table 4 shows the essential amino acid composition of proteins from both immature and mature soybeans for comparison.

3. Antinutritional factors

1) Trypsin Inhibitors. Reports on the change of TI in developing soybeans are somehow contradictory. Collins and Sanders (1976) found that as soybeans matured the amount of TI in the extracts of beans increased, while Yao et al. (1983) reported that TI remained constant during the development of soybeans. The divergent results may be due to the different varieties of soybeans used by the two groups.

2) Oligosaccharides. No data have been reported on the changes of flatulence factors in developing soybeans. However, a study made on peas by Gautier (1979) showed that total galactoside content was correlated with increase of total solids during maturation of peas; raffinose appeared first, followed by stachyose and verbascose. Furthermore, in fresh peas the amount of these oligosaccharides was too low to cause digestive disorder.

Table 4. Essential amino acid pattern of proteins from immature and mature soybeans

Amino Acid	(Gram amino acid per 16 grams N)	
	Immature [a]	Mature [b]
Lysine	8.5	6.5
Methionine	0.8	1.0
Cysteine	/	1.2
Tryptophan	1.0	1.8
Threonine	1.9	3.7
Isoleucine	6.6	4.2
Leucine	7.1	8.0
Phenylalanine	5.0	4.9
Valine	5.6	4.9

a. From Standal, 1967.

b. From Evans and Banserner, 1963.

3) Phytic Acid. Phytic acid content was reported increasing with maturity of soybeans from 0.81 to 1.26%, on a dry basis (Yao et al., 1983).

4. In vivo studies of protein digestibility

Perhaps, the most effective and reliable method to evaluate the nutritional quality of soybeans is in vivo studies. Table 5 shows that immature soybeans appeared to supply protein of higher nutritive value than that of mature soybeans both before and after autoclaving (Everson et al., 1944). Standal (1963) found that net protein utilization (NPU) and protein efficiency ratio (PER) of immature soybeans, as measured with rats, was comparable to that of casein and lean beef.

Table 5. In vivo (rats) studies on [a,b] the nutritive value of soybean proteins

Soybeans	Raw	Autoclaved
Mature	0.484	1.700
Immature	1.101	2.013

a. From Everson et al., (1944).

b. Values represent protein efficiency ratio (PER)

5. Organoleptic properties

One of the major constraints in the use of soybeans is their characteristic flavor, described as beany and bitter. Fresh immature soybeans may have an advantage over mature ones because of their lower flavor intensity and softer texture (Rackis et al., 1972). Since the lipoxygenase activity was found to be related with beany flavor (Mattick and Hand, 1969), the lower lipoxygenase activity in immature soybeans might partly explain the lower flavor score of immature beans as compared with that of mature beans.

6. Difference in variety---Garden Type soybeans

Usually, soybeans for direct consumption are the varieties called "Garden types". Garden type soybeans are not basically different from field varieties but generally larger in size, higher in protein and lower in oil and yield. They also have a better flavor and texture than regular field beans (Smith and Circle, 1978).

MATERIALS AND METHODS

I. Soybean Cultivation

Two soybean cultivars, "Beeson 80" and "Pella" were obtained from the Department of Crop and Soil Sciences, Michigan State University. Seeds were sown in the open field on June 6, 1985. No fertilizers were used.

II. Seed Harvesting

The seeds were harvested at four different maturity stages on the basis of both the solid content and the organoleptic properties of the beans. The beans were respectively defined as Immature I, Immature II, Immature III and mature beans.

Tables 6 and 7 show the dates of harvest, the total solids contents and the color characteristics of the beans at each stage of maturity for two cultivars. The total solid content was determined by drying the seeds in a vacuum oven at 60C for 24 hr. The mature seeds were harvested when thoroughly field-dried.

Only the seeds from the middle of the plants were picked

Table 6. Harvest date, total solids and color characteristics of maturing Beeson 80 soybeans

Stage of Maturity	Date of Harvest	Total Solids (%)	Color description Pods	Seeds
Immat. I	9/9	30.0	Green	Green
Immat. II	9/17	35.3	Green	Green
Immat. II	9/26	38.4	Yellow	Light-green
Mature	10/18	92.2	Brown	Buff-brown

Table 7. Harvest date, total solids and color characteristics of maturing Pella soybeans

Stage of Maturity	Date of Harvest	Total Solids (%)	Color description Pods	Seeds
Immat. I	9/9	28.6	Green	Green
Immat. II	9/22	35.1	Green	Green
Immat. III	9/30	38.6	Yellow	Lightgreen,dark spots
Mature	10/23	91.6	Brown	buff-brown,dark spots

up and transported to the laboratory for immediate hand shelling. Within 6 hours the fresh seeds were subjected to various processing treatments.

III. Fresh Seed Processing

Immature II, III and Mature seeds were subjected to the following processing treatments. Immature I seeds were too small for processing.

1) Soaking for 24 hr;
2) Steaming over boiling water for 20 min;
3) Cooking in boiling water for 20 min;
4) Soaking for 24 hr and then cooking in boiling water for 20 min.

Each treatment was duplicated. The size for each independent sample was adjusted to about 35 g on a dry basis. Each weighed sample was wrapped in cheese cloth and marked before processing. The processed samples were drained and cooled before freezing.

IV. Sample Preparation

The frozen raw and processed samples were first freeze-dried in a freeze dryer for about 3 days and then ground in a blender to pass through No. 20 mesh. Finally, the ground sample was stored in a plastic bag until analyzed.

V. Analysis of Raw Samples

Nitrogen content was determined by micro-Kjeldahl (AOAC, 1980). The factor 6.25 was used for converting N-content to protein content. Oil and ash contents were measured according to AOAC (1980).

VI. Analysis for Trypsin Inhibitory Activity (TIA)

1) Tris-buffer (0.05 M, pH 8.2) containing 0.02M $CaCl_2$. 6.05g tris (hydroxymethylamino methane) and 2.94g $CaCl_2 \cdot 2H_2O$ were dissolved in 900 ml of water. The pH was adjusted to 8.2, and the volume was brought to 1 liter with water.

2) Substrate Solution. 40 mg of benzoyl-DL- arginine-p-nitroanilide (BAPNA) hydrochloride were dissolved in 1 ml of dimethyl sulfoxide and diluted to 100 ml with tris-buffer prewarmed to 37C. The solution was prepared before each run of measurements and kept at 37C immediately after preparation.

3) Trypsin Solution. In 200 ml 0.001M HCl solution, 4 mg of accurately weighed trypsin were dissolved. The solution could be stored in a refrigerator for about two weeks without considerable loss of activity.

4) Preparation of Soybean Samples for Assay. 500.0 mg of soybean sample (50 mesh) were extracted with 50 ml of 0.01N NaOH for 2 hr on a mechanical shaker. The pH of the

suspension was in the range of 8.4-10.0. After the extract was allowed to stand for 10 minutes, a 10 ml aliquot was pipetted into a 100 ml volumetric flask and diluted to the mark. For raw soybean samples, 0.8 ml of the diluted suspension could inhibit trypsin in the ranges of 40-60%.

5) Color Measurement. Portions (0.0, 0.4, 0.8, 1.2 and 1.4 ml) of each soybean suspension of three independent samples were pipeted into 17 test tubes and adjusted to 2.0 ml with water. The 16th tube was another trypsin standard corresponding to 0.0 ml sample suspension. The 17th tube was a reagent blank. After 2 ml of trypsin solution was added to each tube, the tubes were placed in a waterbath at 37C. Two min later, 5 ml of BAPNA solution previously warmed to 37C was added to each tube at exact intervals of 10 sec. The tubes were then shaken for mixing. Exactly 10 min later, the reaction was terminated by adding 1 ml of 30% acetic acid solution to each tube at the same time intervals (10 sec). After thoroughly mixing, the content of each tube was filtered (Whatman No.5) and the absorbance of the filtrate was measured at 410 nm against a reagent blank. The reagent blank was prepared by adding 1 ml of 30% acetic acid to test tube containing trypsin and water (2ml each) before 5 ml of BAPNA solution was added.

6) Expression of Activity. One trypsin unit (TU) is arbitrarily defined as an increase of 0.01 absorbance at 410 nm per 10 ml of the reaction mixture under the conditions used herein. TIA is expressed in terms of trypsin units

inhibited (TUI). The TIA in soybeans is expressed as TUI/mg dry sample by averaging the results of 4 portions of diluted inhibitor solution taken for assay.

VII. Analysis for Oligosaccharides

As pointed out earlier, HPLC for the measurement of oligosaccharides is convenient and accurate, but contamination of the column remains a problem. The HPLC system originally reported by Conrad and Palmer (1976) was therefore modified as follows:

1) Extracting the Soybean Samples. 2.2 g dried soyflour in a 40-50 ml round bottom polyethylene centrifuge tube were first defatted by treating it with 40 ml hexane, followed by centrifuging. This was repeated twice with 20 ml hexane. Then the defatted flour was dried in a vacuum oven at 35C overnight before it was refluxed with 25 ml of 75% alcohol solution in a waterbath at 80C for 4 hr with occasional shaking. The mixture was allowed to cool before centrifuging. The supernatant was saved and the precipitate was washed twice with 10 ml alcohol solution. Then, 1 ml 10% lead acetate was added to the conbination of supernatants before holding them in the waterbath for 8 min. The tube was then centrifuged and the precipitate was washed once. This was repeated once to precipitate the remaining proteins. After this step, about 0.4 ml 10% oxalic acid was added to the mixture to precipitate the excess lead (this

was optional). The supernatant was now concentrated at temperature below 50C to a volume of approximate 15 ml, using a vacuum evaporator. The concentrated solution was then transfered to a 25 ml flask and diluted to mark with water.

2) HPLC System. The HPLC system was assembled from a Waters Model M-45 pump, a Waters RI-401 differential refractometer detector, a Guard-Pak procolumn module with silica cartridge in it (Waters) and a Waters μ-Bondapak/ carbohydrate column (3.9mm x 30cm). A Kontes model 100 recorder was used for detector response reading.

Samples were loaded onto the column through a Waters 7010 injector with a 20 ul loading loop. The bi-solvent system, acetonitrile/water of 80/20 resulted in good resolution. The flow rate was adjusted at 3ml/min. Detector attenuator was held constant at 4X and chart speed of recorder was kept at 0.5 cm/min.

3) Final Sample Clean-up Procedure. Immediately before injection, the sample extract was passed through a 0.22 um membrane filter (Millipore Corp.)with a prefilter, utilizing a syringe and then passed through a Waters Sep-Pak C18 Cartridge for final sample clean-up.

4) Standard Sugar Solutions. For identifying the soybean sugars, a standard mixture of sucrose (0.3%), raffinose (0.05%) and stachyose (0.1%) was used. For quantifying, the same standard sugar mixtures were prepared with four concentrations of each standard sugar in the range

of 0.1-0.6% for sucrose, 0.02-0.10% for raffinose and 0.03-0.21% for stachyose.

VIII. Analysis for Phytic Acid

A modified procedure for analysis of phytic acid was introduced here according to the method by Wheeler and Ferrel (1971) for extraction and precipitation and to the method by Makower (1970) for color measurement.

1. Extracting and precipitating

1) Weigh 1.2 g finely ground (20 mesh) soybean sample into a 125 ml Erlemeyer flask
2) Extract sample with 40 ml 3% TCA + 10% sodium sulfate solution on a mechanical shaker.
3) Pour into a 40-50 ml round bottom polyethylene centrifuge tube, centrifuge the suspension at 20,000xg for 15 min.
4) Transfer 10 ml aliquot of suspension into another tube to which 3 ml ferric chloride solution (made to contain 6 mg ferric chloride/ml in 3% TCA solution) has been added (note the order is important).
5) Heat in boiling water for 50 min.
6) Cool in cold water for 30 min after heating.
7) Centrifuge for 15 min at 20,000xg.
8) Wash the precipitate twice by dispersing it in 20-25ml 3% TCA + 10% sodium sulfate solution and then

heating for 7 min, cooling for 10 min.

9) Repeat washing once with water.

10) Coagulate ferric hydroxide by adding 4 ml of 1N NaOH to the precipitate and heating for 40 min.

11) Centrifuge for 15 min.

12) Wash precipitate with 15ml deionized water.

13) Disolve precipitate in 3 ml of 1N HCl with heating for 15 min.

14) Transfer to 100ml volumetric flask and make to volume with 0.1N HCl solution.

2. Making iron standard solution

1) Make iron stock solution by dissolving 0.0484 g $Fe_2Cl_3 \cdot 6H_2O$ (analytical reagent grade) in 100 ml volumetric flask. Make to volumn with 0.1N HCl solution. This stock solution contained 100 µg Fe/ml.

2) Dilute to obtain 0, 5, 10, 15, 20, 25 and 30 ug Fe/ml with 0.1 N HCl solution.

3. Color measurement

1) Transfer 1ml aliquot of standard solution or unknown sample solution to a 25ml Erlemeyer flask.

2) Add 9 ml 0.1 N HCl solution.

3) Add 1 ml 10% hydroxylamine HCl solution.

4) Add 10 ml 2M sodium acetate solution.

5) Add 1 ml 0.1% orthophenanthroline solution.

6) Mix and let stand for 5 min.

7) Pour into two 10 ml uniform and transparent glass test tubes (Pyrex) for duplicate color reading at 510nm in a spectrophotometer (Spectronic 70, Bausch and Lomb Co.)

IX. Statistical Analysis of Data

Data were analyzed using analysis of variance at 5% level. A completely randomized design and a factorial design were used (Bhattacharyya and Johnson, 1977).

If the F-test proved significant, the Least-Significant-Difference (LSD) procedure was applied to determine whether a significant difference among means of different levels existed (Li, 1964).

RESULTS AND DISCUSSION

I. Compositional Change During Maturation

Changes in total solids, protein, oil and ash contents during seed development of two soybean cultivars are given in Table 8.

1) Total Solids. As seeds matured the total solids increased significantly. This result is expected because nutrients will accumulate as seeds grow. It was also noticed that the rate of accumulation varied with stages of maturity, slower at early stages and faster at later ones. These results agree with findings of Rackis et al. (1972) and Urbanski et al. (1980).

2) Crude Oil. On a dry basis, the crude oil content in two cultivars slightly increased with maturation. This result agrees with the data of Yao et al. (1983).

3) Protein. In Beeson 80, the protein content was constant, but in Pella, it decreased at the initial stage and increased at the final stage. It was also found that Pella showed a slightly lower protein content (mean of four maturity levels: 37.9%) than that of Beeson 80 (mean of 41.7%).

Table 8. Change of chemical composition in developing Beeson 80 and Pella soybeans

Stages of Maturity	Chemical Composition (% dry basis)							
	total solids		protein		crude oil		ash	
	Beeson 80	Pella	Beeson 80	Pella	Beeson 80	Pella	Beeson 80	Pella
Immature I	30.5 [a]	28.6 [a]	42.1 [a]	39.1 [a]	18.7 [a]	19.8 [a]	5.4 [a]	5.7 [a]
Immature II	35.3 [b]	35.1 [b]	40.4 [a]	36.3 [b]	19.0 [ab]	19.3 [a]	4.9 [b]	4.9 [b]
Immature III	38.4 [c]	38.6 [c]	41.3 [a]	36.3 [b]	19.7 [ab]	20.8 [ab]	4.8 [b]	4.9 [b]
Mature	92.2 [d]	91.6 [d]	42.9 [a]	39.6 [a]	20.2 [b]	21.2 [b]	5.0 [b]	5.0 [b]

[a] Means in the same vertical column bearing different superscripts differ significantly at 5% level.

[b] Means with a common underline in the same horizontal row do not differ significantly at 5% level.

4) Ash. The ash content was approximately 5% at the final three stages of maturation for both soybean cultivars but Immature I beans had a higher ash content than in the following stages. Limited data are available in the literature concerning this subject.

II. Trypsin Inhibitory Activity

1. Discussion on the modified procedure for TIA assay

If the modified procedure for the measure of TIA in soybeans was compared with the method described by Kakade et al. (1974) and with the method modified by Hamerstrand et al. (1981), the following four main points were made.

1) Although previous methods called for preparation of the substrate BAPNA solution daily, the degradation of the reaction activities between the substrate and trypsin in the solution could not be neglected even in a short time. The results showed that on the average, the activity of BAPNA solution decreased at a rate of 5.5% reduction of TU/hr. (Table 9). Therefore, it is suggested that preparation of the substrate solution be made for each run of measurements.

2) Since TIA values (expressed as TUI/ml extract) for all portions of diluted inhibitor solution taken for assay are interpreted against the trypsin standard, it is necessary to run multiple trials of the trypsin standard in order to obtain a reliable result. In the method modified by

Table 9. Degradation of BAPNA solution activity with time in terms of trypsin units

Time after Preparation	TU reduction (%)	TU reduction per hr. (%/hr)
1 hr 30 min	9.2	6.1
2 hr 50 min	14.6	5.1
4 hr	21.7	5.4
Average		5.5

Hamerstrand et al. (1981), this point seemed neglected. In this modified procedure, quadruplicate trypsin standard tests were made for each run.

3) For comparison of effects of the particle size of the soybean sample and extraction time on the final value of TIA, the samples with mesh size of 20, 50 and 100 were used and extracted for 1 and 2 hr. Results showed that there was no significant difference between samples with mesh sizes of 50 and 100 as long as the extraction time was increased from the original 1 hr to 2 hr. For immature and heat-treated soya samples, there were even no signficant differences among samples with mesh sizes of 20, 50 and 100. Thus the use of the samples with 50 mesh size minimized the labor for

preparing the samples with 100 mesh size.

4) As noticed earlier by Rackis et al. (1974), when TI activity (TUI/ml extract) was plotted as a function of the volume of inhibitor solution, three different curves resulted (Figure 5). Since curve A (negative curve) was limited only to extracts of raw soybean samples, the extrapolation procedure for data interpretation of the raw sample was suggested. However, according to the observations in this experiment with as many as 23 two-sample replicate sets of raw or soaked soybean samples, only a few negative curves were obtained (curve D in Table 10). Most curves were of A or B types.

Furthermore, if the distribution histogram was plotted for the percentage of trypsin inhibition at the lowest value of TUI/ml extract among four portions (0.4, 0.8, 1.2 and 1.4 ml) of diluted inhibitor solution (Figure 6), it was found that the highest frequency distribution was in the range of 40-60% trypsin inhibition, that is, at this range, the TIA value tended to be the lowest. Therefore, although the extrapolation procedure of data interpretation in the original method tended to give a high value of TIA, the determination of TIA from a single dilution of a sample extract that inhibited trypsin in the range of 40-60% as suggested by Hamerstrand et al. (1981) could go to the other extreme---to give the lowest value of TIA. As a result, we suggested that averaging the results from four levels of inhibitor provide the most reliable estimated value of TIA.

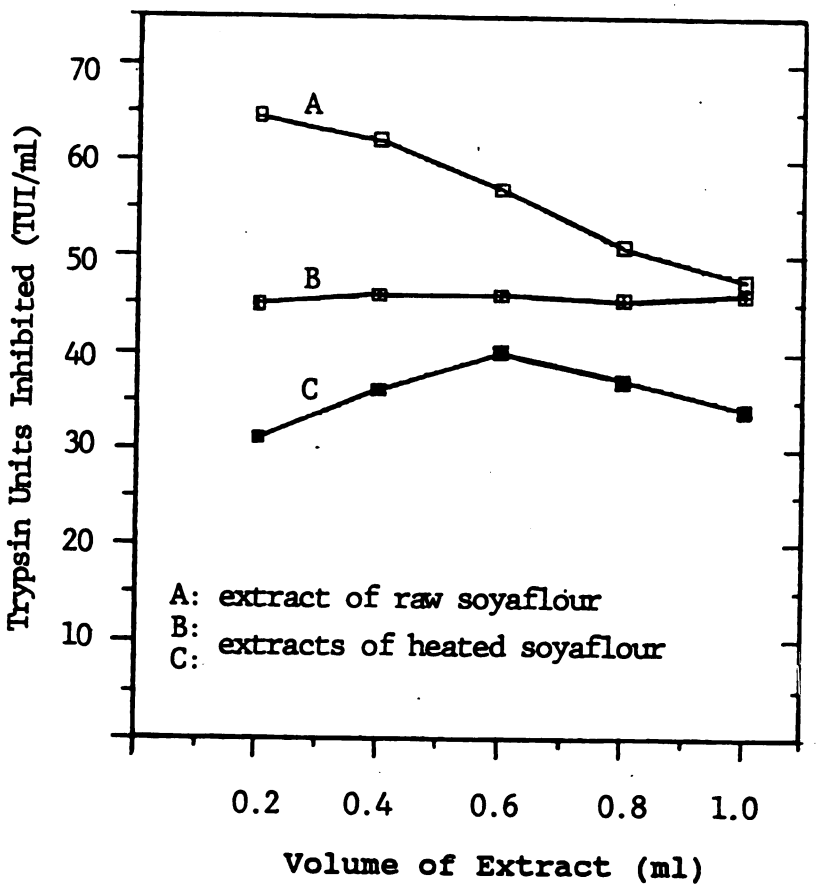
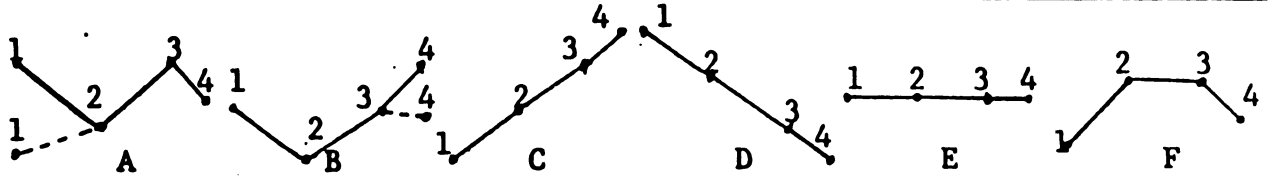


Figure 5. Trypsin inhibitory activity (TUI/ml extract) in relation to inhibitor solution

(From Rackis, 1974.)

Table 10. Values of trypsin inhibitory activity (TUI/ml extract) in relation to levels of inhibitor solution

Shapes of Curves						
	A	B	C	D	E	F
No. of Samples	16	25	1	2	1	1
Relative Frequency (%)	35	54	2	4	2	2

* Four portions (0.4, 0.8, 1.2 and 1.4 ml) of inhibitor solution were taken for the final color analysis.

No. 1, 2, 3 and 4 in the curve represent these 4 levels to the X-axis respectively.

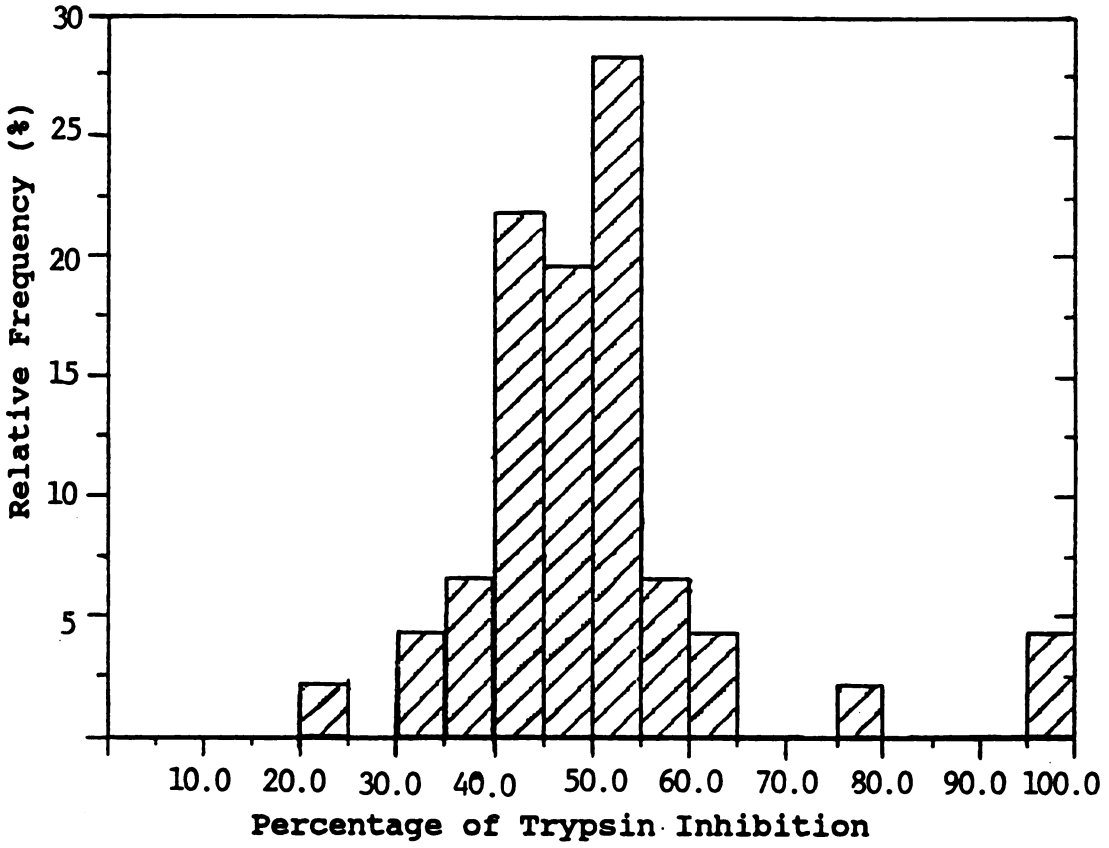


Figure 6. Distribution histogram for percentage of trypsin inhibition at the lowest TUI/ml value of four portions of the extract

(For the details refer to Appendix, Table A1).

2. Changes in TIA during maturation

Tables 11 and 12 show the TIA in raw and processed soybean samples of two cultivars harvested at different maturity stages. Beeson 80 showed an increase in TIA from 59.5 to 64.2 TUI/mg with a significant difference between Immature II and Immature III, while Pella showed a decrease from 59.4 to 56.0% TUI/mg with a significant difference between Immature I and Immature II. If the TIA for four maturity stages was averaged, the two cultivars appeared to be slightly different: 61.1 TUI/mg for Beeson 80 and 55.5 TUI/mg for Pella.

Divergent values and change patterns in TIA during maturation among soybean varieties were also reported by other workers (Collins and Sander, 1976; Yao et al. 1983). All these differences might be attributed to the different varieties used in different study groups.

It is interesting that the change in TIA is parallel to the change in protein content during maturation for the two cultivars studied here. In fact if TIA is plotted against protein content (Figure 7), a high correlation is obtained, with $r = 0.9069$. This relation may be explained by the fact that trypsin inhibitors are proteins.

Table 11. Effect of maturity and processing on trypsin inhibitory activity in Beeson 80 soybeans [a,b]

Date of Harvest	Stage of Maturity	Trypsin inhibitory activity (TUI/mg)				
		Raw	Soaked	Steamed	Cooked	S-cooked
9/9	Immt. I	59.5 b				
9/17	Immt. II	58.7 b	57.1 b	0.0	0.0	0.0
9/26	Immt. III	62.0 a	63.3 a	0.0	0.0	0.0
10/18	Mature	64.3 a	64.0 a	13.1	9.2	0.0

a. Means in the same vertical column bearing different subscripts differ significantly ($P < 0.05$).

b. Means with a common underline in the same horizontal row do not differ significantly ($P > 0.05$).

Table 12. Effect of maturity and processing on trypsin inhibitory activity in Pella soybeans [a,b]

Date of Harvest	Stage of Maturity	Trypsin inhibitory activity (TUI/mg)				
		Raw	Soaked	Steamed	Cooked	S-cooked
9/9	Imm. I	59.9 b				
9/22	Imm. II	52.6 a	53.8 a	0.0	0.0	0.0
9/30	Imm. III	53.4 a	55.7 a	0.0	0.0	0.0
10/23	Mature	56.0 a	56.6 a	6.3	6.6	0.0

a. Means in the same vertical column bearing different subscripts differ significantly ($P < 0.05$).

b. Means with a common underline in the same horizontal row do not differ significantly ($P > 0.05$).

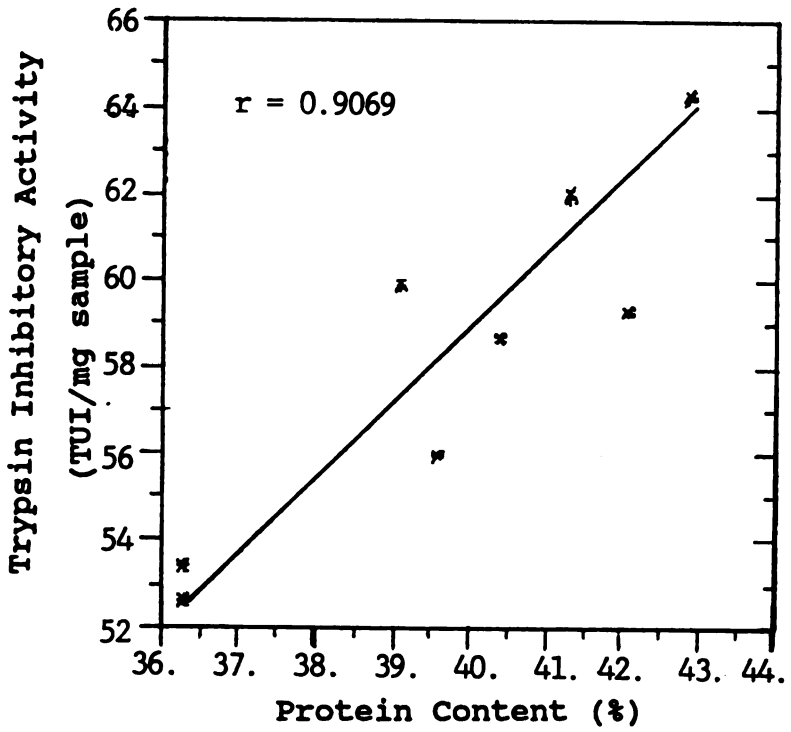


Figure 7. Correlation of trypsin inhibitory activity with protein content in two cultivars of soybeans harvested at four different maturity stages

3. Effect of processing on the TIA in different maturity soybeans

As shown in Tables 11 and 12, in both cultivars, steaming or cooking for 20 min completely inactivated the TI in immature soybeans. For mature beans these heat treatments reduced their TIA to a very low level. Soaking for 24 hr did not reduce TIA significantly, but it was necessary prior to cooking in order to bring a complete elimination of TIA in mature soybean samples.

Heat treatments are commonly used to inactivate the protease inhibitors of soybeans and other legumes (Liener, 1981). As for the effect of soaking on the reduction of TIA, striking differences were noticed among various legumes (Al-Bakir et al., 1982). These differences may be attributed to differences in chemical composition and seed coat texture.

In order to effect a complete inactivation of TI in mature soybeans, soaking was necessary prior to cooking, while for immature soybeans soaking was not necessary. This indicates that higher moisture content renders the TI more sensitive to heat. Results here seem to agree with data of Collins and Beaty (1980), that heating fresh green soybeans in boiling water destroyed their TIA rapidly.

III. OLIGOSACCHARIDES

1. HPLC chromatography

Figure 8(A) shows the HPLC separation of three standard sugars: sucrose, raffinose and stachyose. Figure 8(B) shows the HPLC chromatogram of the sugars present in a 75% ethanol extract of soybeans. Comparison of the two chromatograms indicates that soybeans contained sucrose, raffinose and stachyose in large amounts. Cegla and Bell (1977) obtained the similar results.

2. Changes of oligosaccharides in maturig soybeans

Quantitative results showed that during the maturation of two soybean cultivars, all three sugars increased (Figure 9). Sucrose appeared early in the seed development, followed by raffinose and stachyose. In young seeds, both cultivars contained a considerably low amount of oligosaccharides, while in full mature seeds, Beeson 80 contained 7.00% sucrose, 3.18% stachyose and 0.52% raffinose and Pella contained 7.20% sucrose, 2.71% stachyose and 0.64% raffinose. No data on the oligosaccharide contents in immature soybeans were found in the literature, but for mature soybeans our results agree with findings of Hymowitz et al. (1972).

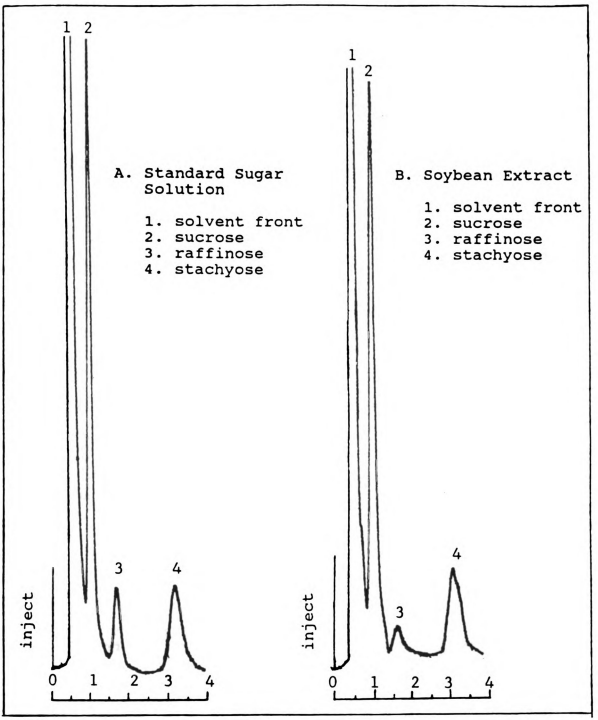


Figure 8. **HPLC chromatograms for sugar analysis:** Waters carbohydrate column; Solvent, acetonitrile/water (80/20); Flow rate, 3.0/min; Attenuation, 4X; Chart speed, 0.5/ cm/min.

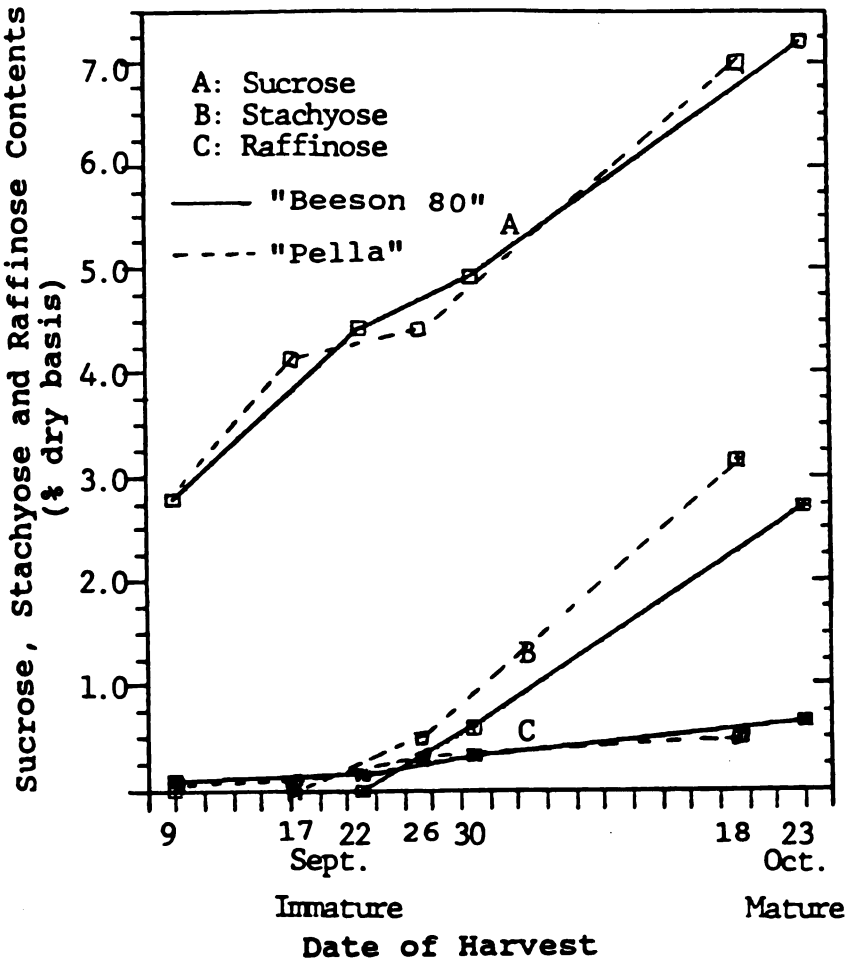


Figure 9. Changes of sucrose, stachyose and raffinose contents in maturing soybeans

3. Effect of processing on sucrose in different maturity soybeans

Figures 10 and 11 show the sucrose contents in raw and treated soybeans of two cultivars harvested at different maturity. Generally, upon all kinds of processing, sucrose content decreased by 7.9-46.6% in both cultivars except for steaming, which appeared to increase the sucrose content by 4.5-31.8% in young seeds. The combination of soaking and cooking caused the highest decrease in sucrose content.

The increase of sucrose content in young seeds as a result of steaming may be due to enhancement of certain enzymatic activities or due to some other factors unknown.

4. Effect of processing on stachyose in different maturity soybeans

Among the three sugars, stachyose may play the key role in causing flatulence because of its relatively high content. Figures 12 and 13 show the stachyose content in raw and processed soybeans of two cultivars harvested at the final two maturity stages: Immature III and Mature. Since the Immature II seeds contained considerably low amounts of stachyose no processing effect on stachyose could be derived.

In general, under various processing treatments, stachyose content in both cultivars harvested at the final two maturity stages was reduced by 12.0 - 49.4%. Soaking

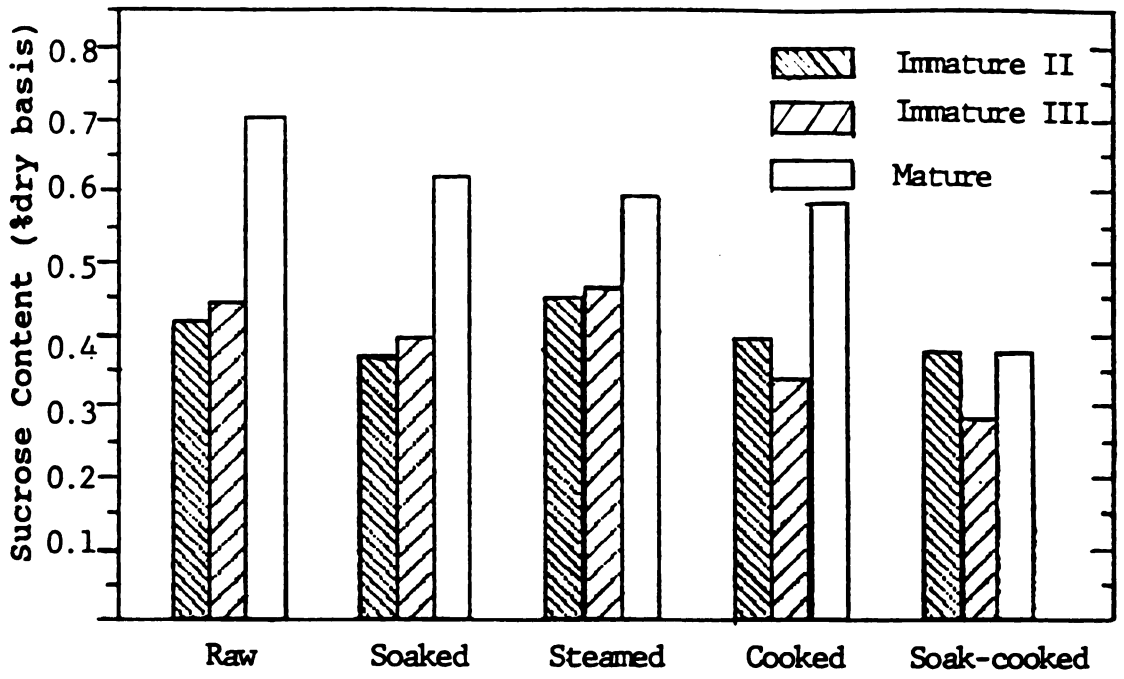


Figure 10. Effect of processing on sucrose in Beeson 80 soybeans of different maturity

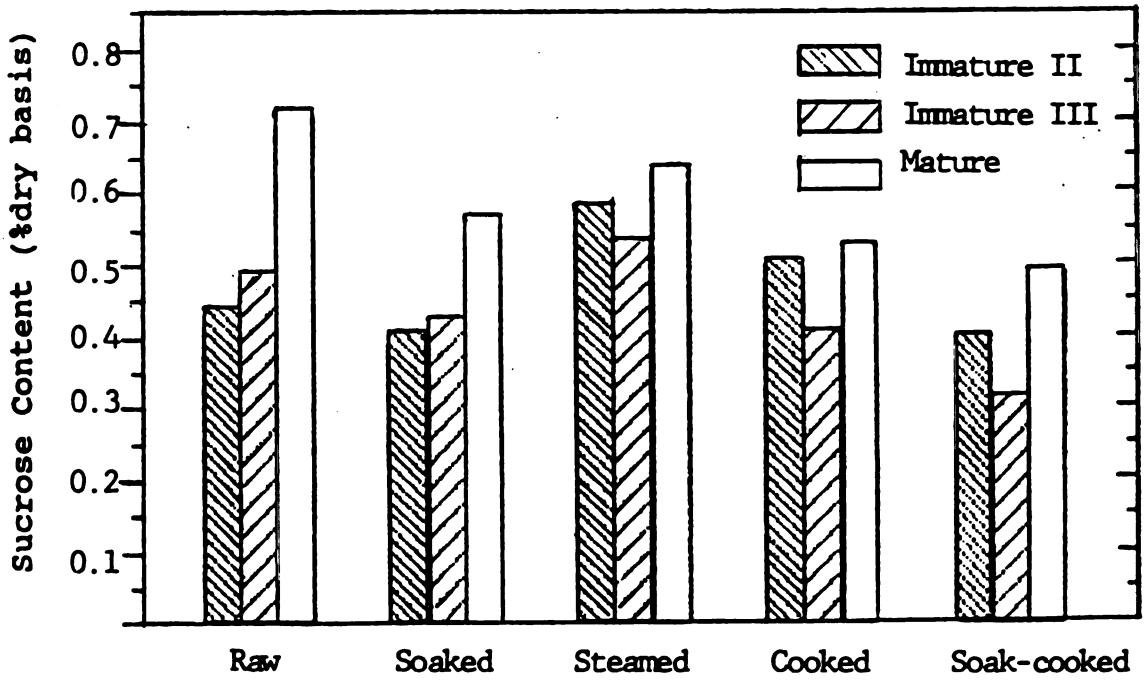


Figure 11. Effect of processing on sucrose in Pella soybeans of different maturity

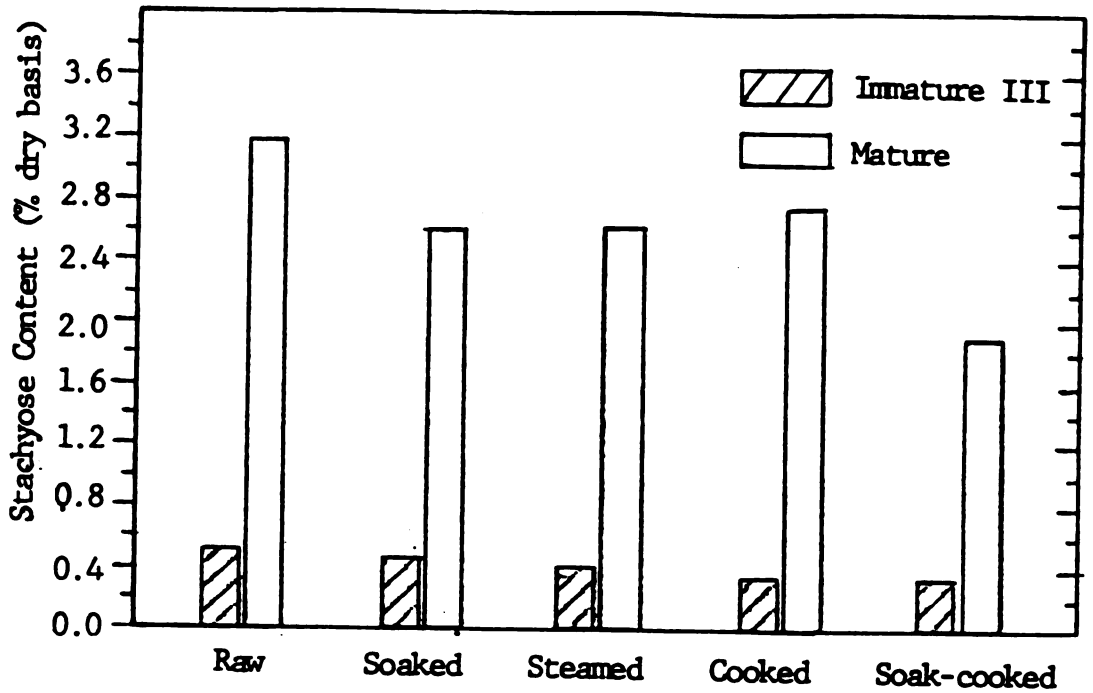


Figure 12. Effect of processing on stachyose in Beeson 80 soybeans of different maturity

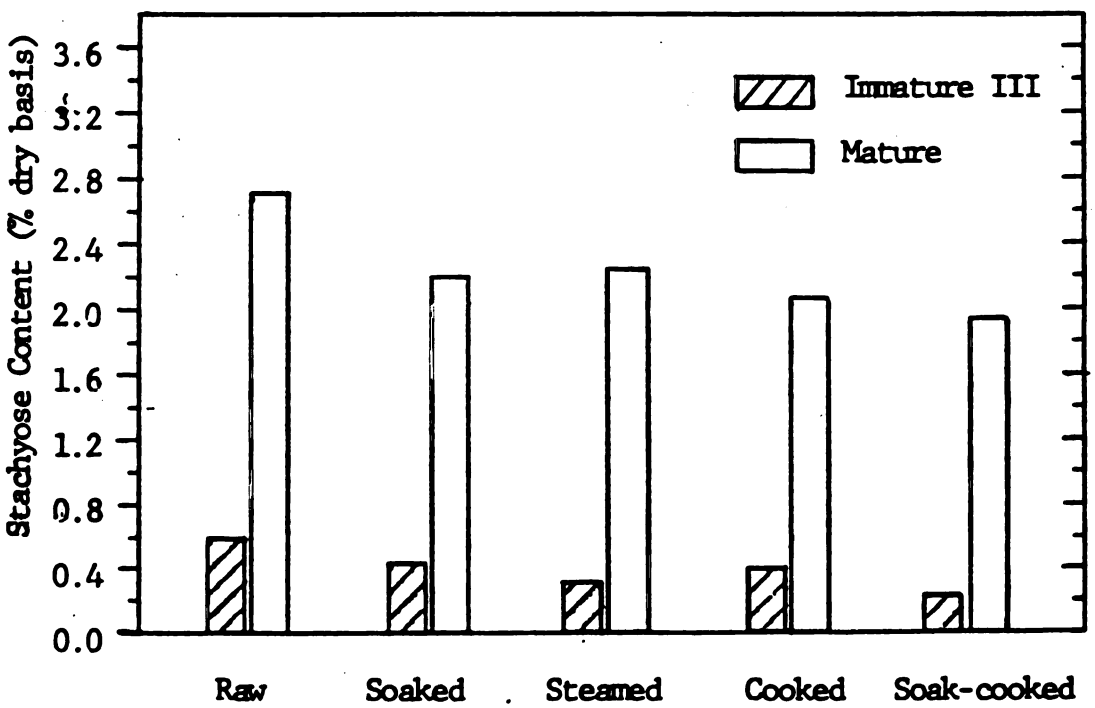


Figure 13. Effect of processing on stachyose in Pella soybeans of different maturity

soybeans for 24 hr and then cooking for 20 min in boiling water tended to give the greatest reduction (28.7-49.4%), while the remaining three treatments seemed to cause approximate equal reduction in stachyose content (12.9-34.7%).

From Figures 12 and 13, it was also found that these treatments could not completely eliminate the stachyose in Immature III seeds although at this stage the original content was very low.

5. Effect of processing on raffinose in different maturity soybeans

Raffinose content of raw and processed soybeans of two cultivars harvested at the final three maturity stages is given in Figures 14 and 15. Like the other two sugars in soybeans, raffinose appeared to decrease to some extent under various treatments. Soaking-cooking caused the largest reduction in raffinose content.

The effects of various processing treatments on oligosaccharides in mature soybeans have been studied by several investigators although so far no data are available concerning immature soybeans. Bianchi and Silva (1983) reported that soaking promoted no reduction in oligosaccharide contents regardless of the soaking time (3, 6, 12, 18, and 24 hr); cooking in an autoclave at 120C reduced the oligosaccharides but soaking plus cooking resulted in

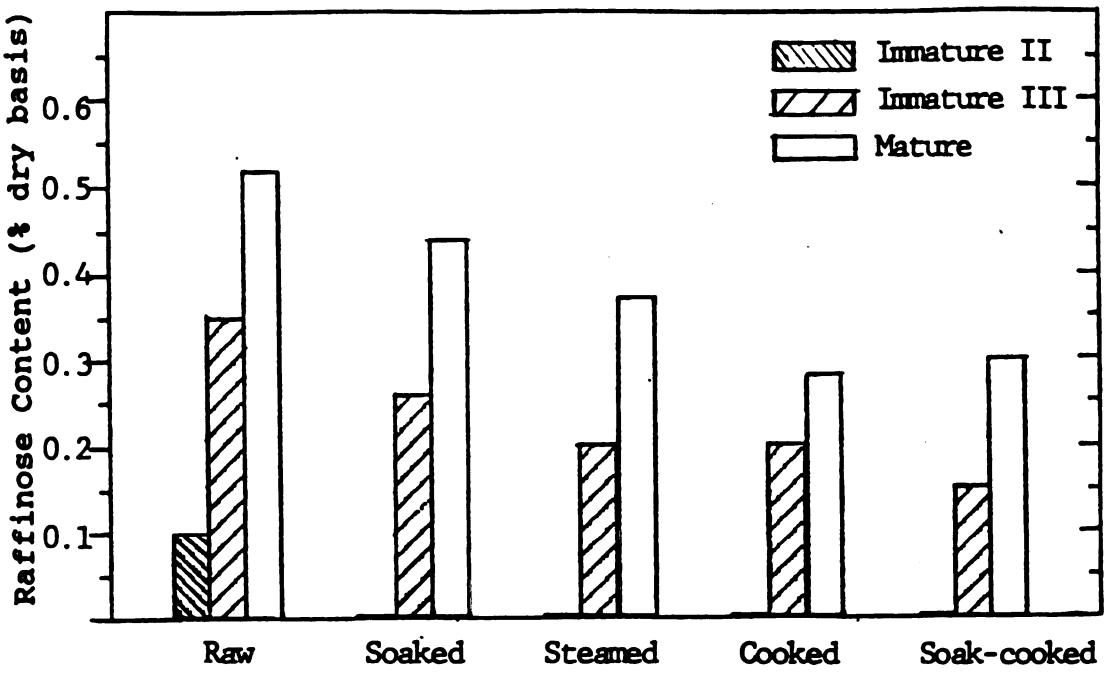


Figure 14. Effect of processing on raffinose in Beeson 80 soybeans of different maturity

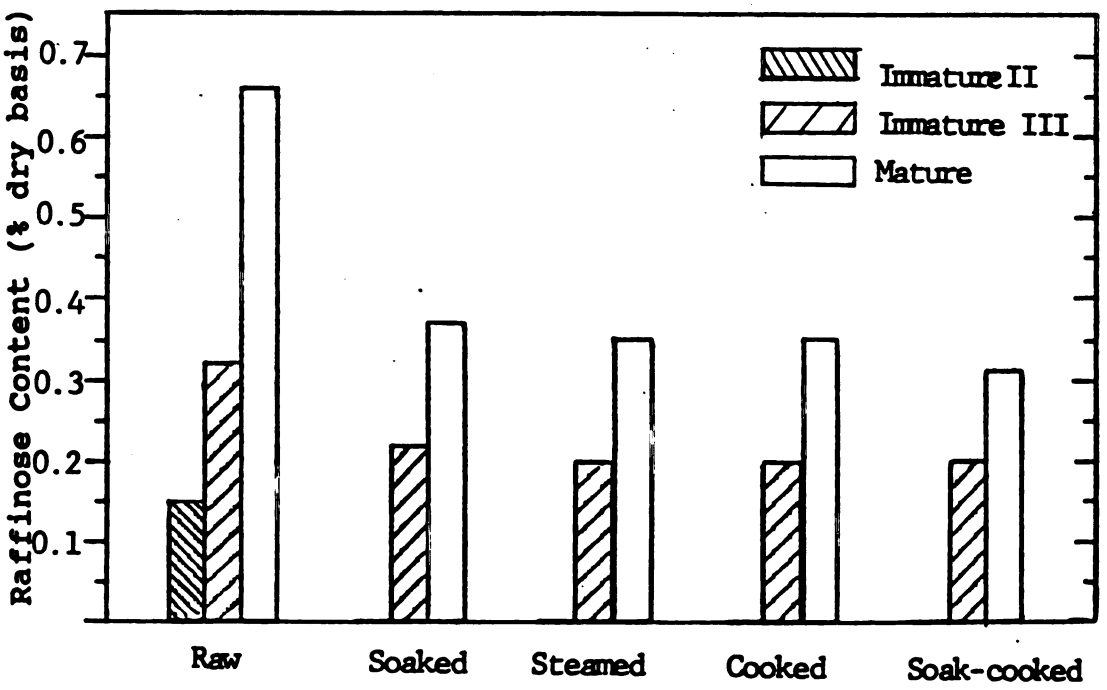


Figure 15. Effect of processing on raffinose in Pella soybeans of different maturity

the greatest removal of oligosaccharides.

Kim et al. (1973) found that soaking soybeans in water for 15 hr reduced oligosaccharide content by about 7%; Lo et al. (1968) also found that as the soaking time for soybeans increased, large quantities of water-soluble solids including oligosaccharides leached into the soak water. Recently, Savitri and Desikachar (1985) reported that there was an apparent increase in the measurable total oligosaccaharides in Kalatur soybeans after cooking.

These divergent results might not be suitable for comparison with each other because of the different varieties of soybeans and different processing conditions used in each study.

The fact that similar reduction patterns of oligosaccharides upon various processing methods were found between immature and mature soybeans in this study might indicate that higher moisture content and softer texture of young seeds could not provide the more favorable condition for the reduction and elimination of their oligosaccharide content. Nevertheless, the amount of these oligosaccharides in young green soybeans might be too low to cause digestive disorders in humans.

IV. Phytic Acid

1. Comparison of two assay methods for phytic acid

In method I, 3% TCA was used as an extractant and the 4:6 ratio of Fe:P was used for calculation of phytic acid content. In method II, 3% TCA + 10% Na_2SO_4 was used as an extractant and the 6:6 ratio of Fe:P was introduced in calculating the phytic acid content. The results obtained by the two methods were not statistically different (Table 13). However, by using the modified procedure (method II), the sample extracts were clearer and therefore were more amenable to decantation of the supernatant. Furthermore, the results by the modified method were more reproducible; the average relative difference of 9 samples was only 2.3% as compared with that of 6.9% by method I. (For the standard iron curve used in this colorimetric measurement refer to Appendix, Figure A1).

Thompson and Erdman (1982) hypothesized the structure shown in Figure 16 for the ferric phytate precipitate and the assumption of 4:6 molar ratio of Fe:P. According to this model, eight ferric irons interact with one phytate molecule.

Now for the assumption of 6:6 molar ratio of Fe:P introduced herein, it is proposed that in the presence of excess Na^+ and $SO_4^=$, the Neuberg-based structure dominate. As a result, additional four ferric iron could be incorporated

Table 13. Comparison of two assay methods for phytic acid content in soybeans

No. of Samples	Method I				Method II				difference between methods
	1st run	2nd run	average	relative difference (%)	1st run	2nd run	average	relative difference (%)	I and II
1	0.93	1.01	0.97	8.2	0.98	0.96	0.97	2.1	0.00
2	1.28	1.40	1.34	9.0	1.35	1.36	1.36	0.8	-0.02
3	1.17	1.27	1.22	8.2	1.30	1.29	1.30	0.8	-0.07
4	1.45	1.57	1.51	8.0	1.48	1.52	1.50	2.7	+0.01
5	1.30	1.34	1.32	3.0	1.39	1.33	1.36	4.4	-0.04
6	1.43	1.57	1.50	9.3	1.35	1.39	1.37	2.9	+0.13
7	1.22	1.32	1.27	7.8	1.34	1.38	1.36	2.9	-0.09
8	1.20	1.27	1.24	5.7	1.31	1.26	1.28	3.9	-0.04
9	1.09	1.11	1.10	1.8	1.16	1.16	1.16	0.0	-0.06
Average	1.230	1.318	1.274	6.9	1.296	1.294	1.295	2.3	-0.02

[a] Phytic acid content % dry basis.

[b] Method I: using 3% TCA solution as extractant with assumption of 4:6 Fe:P molar ratio.

[c] Method II: using 3% TCA plus 10% Na_2SO_4 solution as extractant with assumption of 6:6 Fe:P molar ratio.

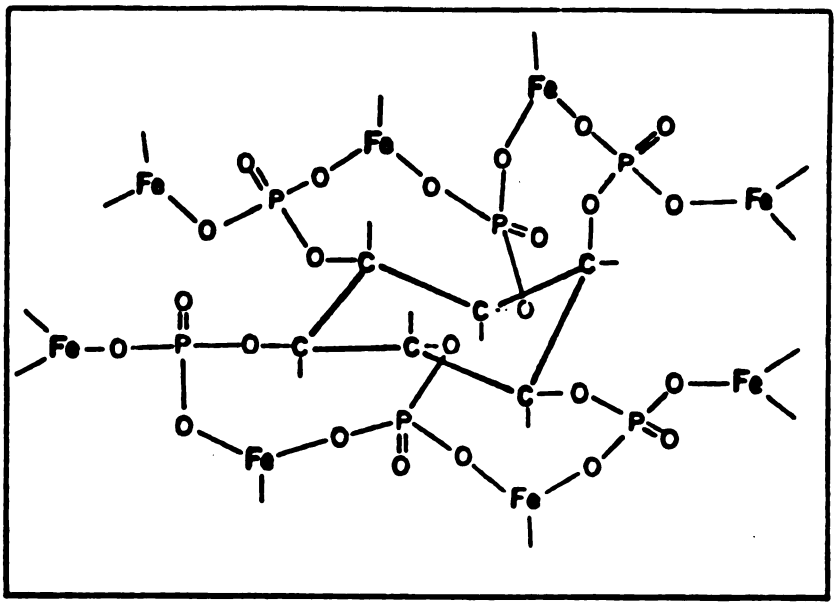


Figure 16. Proposed structure for ferric phytate (4:6 Fe:P) (From Thompson and Erdman, 1982)

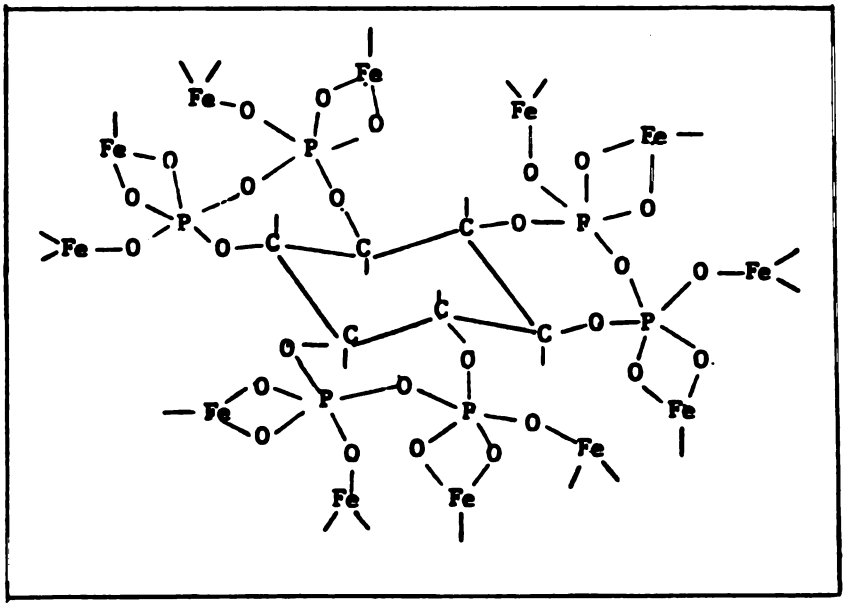


Figure 17. Proposed structure for ferric phytate (6:6 Fe:P)

into the precipitate (Figure 17).

2. Change of phytic acid during maturation

Data about the effect of maturity and processing on phytate content in both soybean cultivars are given in Tables 14 and 15. The results indicated that from Immature I to Mature stage, phytic acid content increased from 0.84-1.36% for Beeson 80 and from 0.86-1.39% for Pella. These results agree with findings of Yao et al. (1983); they reported that the phytate content in Williams soybeans increased from 0.866 to 1.26% during the final three harvest stages (Sept. 20, 26 and Oct. 3).

Welch and House (1982) studied the effect of maturity of soybeans on availability to rats of zinc from the seeds. They found that phytic acid concentration in seeds of several species increased with seed maturation and zinc availability to rats decreased, as determined by the absorption of radiozinc.

As for the availability of iron, a study by Welch et al. (1975) showed that iron from the mature seeds was more available than that from immature seeds even through the mature seeds contained approximately three times as much phytate. So immature soybeans might contain a factor that depressed iron availability.

Therefore, mineral availability in beans may depend not only on stage of maturity and their content of phytic acid, but also on the individual mineral and other factors.

Table 14. Effect of maturity and processing on phytic acid content in Beeson 80 soybeans [a,b]

Date of Harvest	Stage of maturity	Phytic acid content (%)				
		Raw	Soaked	Steamed	Cooked	S-cooked
9/9	Imm. I	0.84 c				
9/17	Imm. II	0.91 c	0.92 c	0.88 c	0.89 c	1.28 c
9/26	Imm. III	1.16 b	1.17 b	1.12 b	1.14 b	1.28 b
10/18	Mature	1.36 a	1.37 a	1.32 a	1.36 a	1.50 a

a. Means in the same vertical column bearing different subscripts differ significantly ($P < 0.05$).

b. Means with a common underline in the horizonal row do not differ significantly ($P > 0.05$).

Table 15. Effect of maturity and processing on phytic acid content in Pella soybeans [a,b]

Date of harvest	Stage of maturity	Phytic acid content (%) Raw	Soaked	Steamed	Cooked	S-cooked
9/9	Imm. I	0.86 d				
9/22	Imm. II	0.97 c	0.98 c	0.98 c	0.97 c	1.04 c
9/30	Imm. III	1.16 b	1.16 b	1.17 b	1.16 b	1.29 b
10/23	Mature	1.39 a	1.41 a	1.34 a	1.36 a	1.57 a

a. Means in the same vertical column bearing different subscripts differ significantly ($P < 0.05$).

b. Means with a common underline in the horizonal row do not differ significantly ($P > 0.05$).

3. Effect of processing on phytic acid in different maturity soybeans

As shown in Tables 14 and 15, changes in phytic acid content upon processing differed with individual treatment. In general, soaking for 24 hours had little or no effect on phytic acid content, while cooking or steaming in boiling water for 20 min had a slight decreasing effect. All these changes were not significant at 5% level. However, soaking plus cooking appeared to increase phytic acid content significantly.

Several reports regarding effects of various processing treatments on phytate contents in soybeans have appeared in the literature. Sudarmadji and Markakis (1977) reported that overnight soaking did not reduce the phytic acid content of soybeans while boiling for 30 min reduced it by 14%. Ologhobo and Fetuga (1984) stated that apart from germination, soaking soybeans for 3 days, or autoclaving at 105C for 15 min. were not very effective in lowering their phytate content. It seems that our results agree with these findings.

The fact that a significant increase in phytic acid content was observed upon soaking-cooking might indicate that this treatment resulted in higher extractability of phytic acid from bound forms of the latter.

The statistical analysis of data showed that no interactive effect of processing and maturity on changes in

phytic acid content existed. This might indicate that the higher moisture content in immature soybeans could not possibly facilitate in lowering their phytic acid contents upon various methods of processing.

CONCLUSIONS

Studies with two soybean cultivars, Beeson 80 and Pella showed that, on a dry basis, immature soybeans contained similar amounts of protein, oil and ash, but considerably lower amounts of oligosaccharides (sucrose, raffinose and stachyose) and phytic acid than mature soybeans. The trypsin inhibitory activity (TIA) of immature Beeson 80 soybeans was slightly lower than that of mature ones. The opposite was true for the Pella cultivar.

The effects of a) soaking (24 hr), b) cooking (20 min), c) steaming (20 min), and d) soaking (24 hr) plus cooking (20 min) on the antinutritional factors of soybeans were as follows: Cooking or steaming completely inactivated the trypsin inhibitor of immature soybeans, but only partly in mature soybeans. For the latter, both soaking and cooking was necessary for complete inhibition. Regarding oligosaccharides and phytic acid, the aforementioned treatments had similar effects on both immature and mature soybeans. Soaking, cooking, or steaming alone significantly reduced the oligosaccharide content, but had little or no effect on phytic acid content. Soaking plus cooking resulted in even greater reduction in oligosaccharides, but in a slight

increase in phytic acid content. Nevertheless, because immature soybeans contain much lower amounts of these antinutritional factors as compared with mature ones, the traditional Chinese cooking of immature soybeans for direct consumption is sufficient to avoid the detrimental effects of these antinutrients.

APPENDIX

APPENDIX

Table A1. Colorimetric assay for trypsin inhibitory activity [a]

sample	level of suspension	A_{410}	TU[b]	TUI/level[c]	TUI/ml[d]	average	inhibition[f] %
	0.0	0.824	82.4				
	0.4	0.602	60.2	22.2	55.5		26.9
A	0.8	0.426	42.6	39.8	**49.8**		**48.3**
	1.2	0.096	9.6	72.8	60.6		88.4
	1.4	0.009	0.9	81.5	58.2		98.9
						56.0	
	0.0	0.824	82.4				
	0.4	0.593	59.3	23.1	57.8		28.0
B	0.8	0.383	38.3	44.1	**55.1**		**53.5**
	1.2	0.056	5.6	76.8	64.0		93.2
	1.4	0.008	0.8	81.6	58.3		99.0
						58.8	

a. Final dilution of soybean suspension: **1mg/ml.** 0.8 ml of this diluted raw soybean suspension inhibited trypsin in the range of 40-60%.

b. One trypsin unit (TU) is arbitrarily defined as an increase of 0.01 absorbance unit at 410 nm per 10 ml of the reaction mixture (2ml water and/or soy suspension; 2ml trypsion solution; 5 ml BAPNA solution and 1 ml acetic acid solution) under condition herein.

c. Trypsin units inhibited per level of suspension. Example of 0.4 ml of sample A: **82.4-60.2 = 22.2 TUI/0.4ml**

d. Trypsin units inhibited per 1 ml of suspension, eg. **22.2 TUI/0.4ml = 55.5 TUI/ 1 ml.**

f. Percentage of trypsin inhibited, eg. for 0.4ml level of sample A, $\frac{0.824-0.602}{0.824} \times 100 = 26.9\%$.

APPENDIX

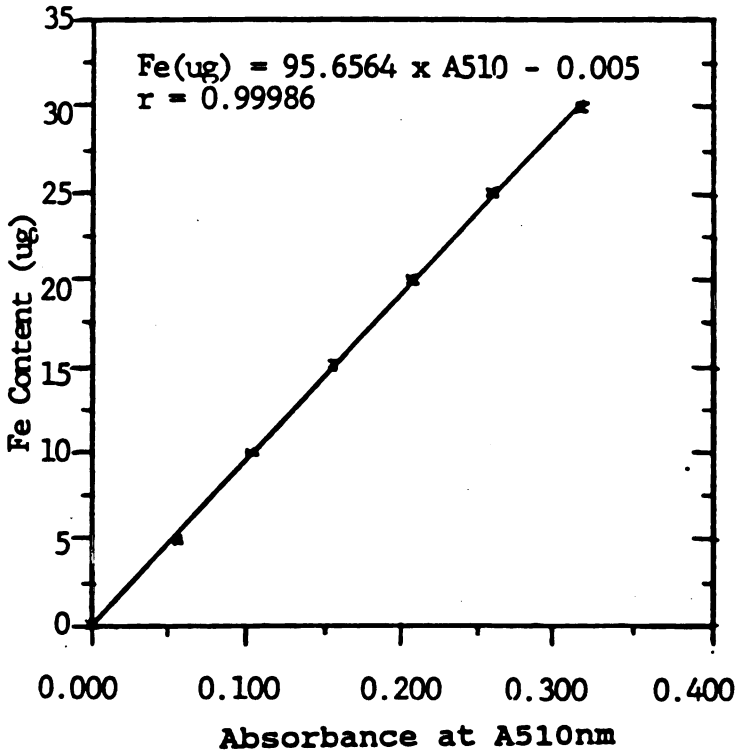


Figure A1. Standard curve for Fe measurement

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