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Title: The Effect of Heating Time of Soybean on Vitamin B-6 and Folacin Retention, Trypsin Inhibitor Activity, and Microstructure Changes

Abstract approved: Zoe Ann Holmes

Four different heating treatments with two different methods of cooking were applied to soybeans. The treatments were boiling 20 min, autoclaving 5 min, 10 min, or 20 min, after soaking the beans for 10 hr at 25°C.

Vitamin B-6 and free folacin in cooking water were significantly ($P \leq 0.05$) affected by the heat treatments. Treatments also significantly influenced vitamin B-6, free folacin, trypsin inhibitor activity, water absorption, moisture content; and blue, green, and amber values, in the cooked soybeans. Treatments decreased the definition of the soybean cell structures. Boiling method caused relatively more losses of vitamin B-6 into cooking water, inactivated trypsin inhibitor at the same level, and caused a lighter color of cooked soybean than autoclaving methods.

Analysis of covariance showed relationships ($P \leq 0.05$) between water absorption after cooking with total folacin in cooked soybeans, and water absorption after cooking with blue color value. Other relationships ($P \leq 0.05$) were observed in cooked soybeans between texture and total folacin, moisture content and trypsin inhibitor activity and free folacin.

The Effect of Heating Time of Soybean on Vitamin B-6 and Folacin Retention Trypsin Inhibitor Activity, and Microstructure Changes

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to:

beloved-memet and tris-

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THE EFFECT OF HEATING TIME OF SOYBEAN ON VITAMIN B-6 AND FOLACIN RETENTION, TRYPSIN INHIBITOR ACTIVITY, AND MICROSTRUCTURE CHANGES

INTRODUCTION

Soybeans (Glycine max (L) Merrill) have always been popular as human food, especially in oriental countries (Bailey et al., 1935; Goodman, 1976). In the last 30 years, the increasing popularity of soybean products in the United States is reflected by the tremendous expansion in planted acreage (McArthur, 1980). These soybeans are used in a variety of ways and in many different products (Willet, 1976; Nelson et al., 1978). These may range from consumption of the cooked beans in soups or casseroles to eating products such as bean curd, fermented beans, soybean concentrates, or soybean isolates. Soybean flour, or defatted flour, has been widely used in improving the nutritional status of children and adults in many developing countries (Sabin, 1961), as well as in the United States.

Because of its extensive use as a high quality protein supplement in human diets, an investigation into the influence of selected preparation and processing procedures on other select nutrients, vitamin B-6 and folacin, would be pertinent. Soybeans are rich in these two vitamins (Bailey et al., 1935; Harris and Karmas, 1975; Perloff and Butrum, 1977). A review of the literature indicates that vitamin B-6 and folacin are water soluble and heat labile vitamins (Storvick et al., 1964; Herbert and Bertino, 1967). The conditions of soybean preparation for consumption would permit the leaching of the vitamins in the soaking and cooking water. Studies by a number of workers have reported vitamin B-6, thiamine, niacin and folacin retention in pinto beans

(Miller et al., 1973), and in lima, blackeye and pink beans (Rockland et al., 1977), or thiamine retention in soybeans (Perry et al., 1976) upon soaking or cooking, either in plain water or salt solutions.

Although there is decreased vitamin retention with soaking and cooking soybeans, it may enhance the nutritive value of the beans due to destruction of both an antinutritional factor and undesirable factors upon digestion. Trypsin inhibitors, present in soybeans, reduce the nutritional quality of the protein (Jansen et al., 1978). Other components such as urease and hemaglutinin have been reported to contribute toxic effects to animals (National Research Council, 1973). These three substances, trypsin inhibitors, urease, and hemaglutinin, are heat labile. The oligosaccharides, such as raffinose and stachyose, which are causative factors for flatulence, can be leached out or partially converted to monosaccharides upon soaking and cooking (Steggerda et al., 1970; Hymowitz, 1976; Ku et al., 1976).

A nutritionally important aspect to any food is its edibility. Soaking of beans such as cowpeas (Sefa-Dedeh et al., 1978) or soybeans (Perry et al., 1976; Wang et al., 1979) prior to cooking has been reported to improve both color and texture of cooked beans. One-hundred percent hydration of soybeans, equal to soaking 5.5 hr at 20°C, gave better results than unsoaked beans, while complete hydration (140% hydration) did not give further improvement in cooking quality of the beans. Wang et al. (1979) reported the texture of cooked soybeans which had been soaked prior to cooking gave more tender beans at the same cooking time than the soybeans without soaking.

The objectives of the experiment reported in this research were to

study the effect of four selected heat treatments on vitamin B-6 and folacin retention. Additionally, the effect of the heat treatments on soybean trypsin inhibitor activity, texture, color, and microstructure, were studied. The possible relationship and/or interaction of vitamin B-6 or folacin retention and color or texture or microstructure changes were evaluated.

REVIEW OF LITERATURE

Composition of Soybeans

The acreage planted in soybeans has expanded tremendously in the last 30 years (McArthur, 1980). Because of its impact on the food chain, a review of factors affecting the soybeans' nutritive value and quality is pertinent.

Soybeans as the whole seed are composed of the cotyledon, which accounts for 90% of the dry weight, the embryo, two percent, and the seed coat, eight percent (Markley and Goss, 1944; Markley, 1950; Harris and Karmas, 1975). The principle constituents of protein, oil, carbohydrates, minerals and vitamins are affected by the genetic factor, agriculture methods, climate, soil condition and other environmental factors. A review of the composition is reported in Table 1.

Vitamins

Soybeans do not contain vitamin A (Table 1; Markley and Goss, 1944), but they contain carotene. Soybeans contain about 10 to 20 μg carotene /100 g which is equal to 16 to 32 IU vitamin A/100 g. These values are lower than that in the U.S.D.A. Handbook #8 (Watt and Merrill, 1975) which has a value of 360 IU. Vitamin D is also found in soybeans with about 13.4 to 16.5 IU/100 g. However, those values indicate that the soybeans are not a reliably high source of the vitamins.

Other vitamins present in the soybeans are water soluble vitamins such as thiamine, 4.99 mg, riboflavin, 1.41 mg, niacin, 10 mg, per 100 g soybeans (Watt and Merrill, 1975); pyridoxine (vitamin B-6), 640 μg

TABLE 1. Protein, fat, carbohydrate, minerals, and vitamins of raw and processed whole soybeans.

Nutrient	Process	Amount	Reference
Protein[1]:			
	Whole bean, raw, dry	34.1	Watt and Merrill, 1975
		36.9	Street and Bailey, 1915
		40.0	Bailey et al., 1935
		40.0	Markley and Goss, 1944
		37.6	Markley, 1950
		40.0	Harris and Karmas, 1975
		39.41	Krivoruchco et al., 1979
		38.0	Schmitt, 1979
		39.0	Omosaiye and Cheryan, 1979
		39.0	Bair and Snyder, 1980
		38.2	Kylen and McCready, 1975
	Whole bean, raw, fresh	38.8	Bair and Snyder, 1980
	Whole bean, raw, green	38.1-40.9	Kylen and McCready, 1975
	Flake, full-fat	40.9	Bair and Snyder, 1980
	Flour, full-fat, roasted	36.6	Jansen et al., 1978
	Flour, full-fat, boiled: 0 min	32.3	Collins and Beaty, 1980
	1 min	32.7	Collins and Beaty, 1980
	2 min	33.1	Collins and Beaty, 1980
	3 min	33.4	Collins and Beaty, 1980
	6 min	33.5	Collins and Beaty, 1980
	9 min	33.7	Collins and Beaty, 1980
	Whole bean, cooked	11.0	Watt and Merrill, 1975
	Whole bean, autoclaved, 15 min	36.6	Sgarbieri et al., 1978
Fat[1]:			
	Whole bean, raw, dry	17.7	Watt and Merrill, 1975
		18.0	Bailey et al., 1935
		18.0	Markley and Goss, 1944
		18.9	Markley, 1950
		21.0	Harris and Karmas, 1975
		19.51	Krivoruchco et al., 1979
		21.6	Omosaiye and Cheryan, 1979
		22.1	Bair and Snyder, 1980
		18.6-20.3	Perry et al., 1976
		20.1	Kylen and McCready, 1975
	Whole bean, raw, fresh	21.8	Bair and Snyder, 1980
	Whole bean, raw, green	14.6-17.1	Islam and Lean, 1979
	Flake, full-fat	22.3	Bair and Snyder, 1980
	Flour, full-fat, roasted	20.5	Jansen et al., 1978
	Flour, full-fat, boiled: 0 min	21.6	Collins and Beaty, 1980
	1 min	22.8	Collins and Beaty, 1980

Table 1. continued.

Nutrient	Process	Amount	Reference
Fat[1]:	Flour, full-fat, boiled: 2 min	23.0	Collins and Beaty, 1980
	3 min	23.7	Collins and Beaty, 1980
	6 min	25.7	Collins and Beaty, 1980
	9 min	26.3	Collins and Beaty, 1980
	Whole bean, cooked	5.7	Watt and Merrill, 1975
		8.4-9.2	Perry et al., 1976
	Whole bean, autoclaved 15 min	22.7	Sgarbieri et al., 1978
Carbohydrate[1]:			
	Whole bean, raw, dry	33.5	Watt and Merrill, 1975
		31.08	Street and Bailey, 1915
		20.5	Bailey et al., 1935
		20.5	Markley and Goss, 1944
		44.6	Markley, 1950
		34.0	Harris and Karmas, 1975
		24.06	Omosaiye and Cheryan, 1979
		34.1	Bair and Snyder, 1980
	Whole bean, raw, fresh	34.5	Bair and Snyder, 1980
	Flake, full-fat	32.2	Bair and Snyder, 1980
	Flour, full-fat, roasted	2.5	Jansen et al., 1978
	Whole bean, cooked	10.8	Watt and Merrill, 1975
	Whole bean, autoclaved, 15 min	33.1	Sgarbieri et al., 1978
Minerals[1]:			
P	Whole bean, raw, dry	0.55	Watt and Merrill, 1975
		1.7	Bailey et al., 1935
		0.66	Markley, 1950
		0.78	Harris and Karmas, 1975
	Whole bean, raw, green	0.5-0.6	Islam and Lea, 1979
	Whole bean, cooked	0.18	Watt and Merrill, 1975
K	Whole bean, raw, dry	1.68	Watt and Merrill, 1975
		2.3	Bailey et al., 1935
		1.67	Markley, 1950
		1.83	Harris and Karmas, 1975
		0.54	Watt and Merrill, 1975
	Whole bean, raw, green	2.0-2.2	Islam and Lea, 1979
Ca	Whole bean, raw, dry	0.22	Watt and Merrill, 1975
		0.5	Bailey et al., 1935
		0.24	Harris and Karmas, 1975
		0.22	Kylen and McCready, 1975
	Whole bean, raw, green	0.22	Islam and Lea, 1979
	Whole bean, cooked	0.002	Watt and Merrill, 1975
Na	Whole bean, raw, dry	0.05	Watt and Merrill, 1975
	Whole bean, raw, green	0.01	Islam and Lea, 1979
	Whole bean, cooked	0.002	Watt and Merrill, 1975

Table 1. continued.

Nutrient	Process	Amount	Reference
Minerals[1]:			
Mg	Whole bean, raw, dry	0.5	Bailey et al., 1935
	Whole bean, raw, green	0.10-0.12	Islam and Lea, 1979
S	Whole bean, raw, dry	0.24	Harris and Karmas, 1975
Cl	Whole bean, raw, dry	0.03	Harris and Karmas, 1975
Fe	Whole bean, raw, dry	0.008	Watt and Merrill, 1975
		0.002	Kylen and McCready, 1975
Vitamins[2]:			
B-6	Whole bean, raw, dry	0.64	Harris and Karmas, 1975
Total folacin			
	Whole bean, raw, dry	0.171	Perloff and Butrum, 1977
Free folacin			
	Whole bean, raw, dry	0.075	Perloff and Butrum, 1977
Thiamine			
	Whole bean, raw, dry	1.1	Watt and Merrill, 1975
		0.87-1.02	Perry et al., 1976
		1.19	Kylen and McCready, 1975
		1.1-1.75	Harris and Karmas, 1975
	Whole bean, cooked	0.21	Watt and Merrill, 1975
		0.14-0.20	Perry et al., 1976
Niacin			
	Whole bean, raw, dry	2.2	Watt and Merrill, 1975
		2.0-2.59	Harris and Karmas, 1975
		3.0	Kylen and McCready, 1975
	Whole bean, cooked	0.6	Watt and Merrill, 1975
β-carotene			
	Whole bean, raw, dry	70.3 IU	Watt and Merrill, 1975
		0.02-0.24	Harris and Karmas, 1975
		0.02-0.24	Sherman and Salmon, 1939
	Whole bean, raw, green	0.74-1.04	Islam and Lea, 1979
	Whole bean, cooked	30 IU	Watt and Merrill, 1975
	Whole bean, green, canned, salt brine	0.54-0.97	Islam and Lea, 1979
Vitamin C			
	Whole bean, raw, green	18.2-28.7	Islam and Lea, 1979
	Whole bean, green, canned, salt brine	3.6-11.6	Islam and Lea, 1979
Riboflavin			
	Whole bean, raw, dry	0.3	Watt and Merrill, 1975
		0.23	Kylen and McCready, 1975
	Whole bean, cooked	0.09	Watt and Merrill, 1975

[1] Nutrient content on g/100 g

[2] Nutrient content on mg/100 g, unless specified

(Harris and Karmas, 1975); total folacin, 171 μg, and free folacin, 75 μg, per 100 g soybeans (Perloff and Butrum, 1977). Vitamin B-6 and thiamine content have been reported to increase with increased soybean maturation (Markley, 1950).

Fat

Fat content has been reported to be negatively correlated with the protein content in soybeans (Krivoruchco et al., 1979). It has an average value (Table 1) of 20% on dry weight basis. Eighty-eight percent of the fat is unsaturated fatty acids consisting of linoleic, oleic and linolenic, in arrangement of the highest to the lowest percentage (Markley, 1950; Collins and Sedgwick, 1959). The remaining 12% are saturated fatty acids which consist of palmitic, stearic, myristic, and arachidic acids.

Carbohydrates

Soybeans contain about 34% total carbohydrates (Harris and Karmas, 1975). This includes polysaccharides, oligosaccharides, and sucrose. Polysaccharide components of soybeans have been studied by Aspinal et al. (1967). They found that soybeans contain four types of polysaccharides. These are galactomannans, acidic polysaccharides of the pectic acid type, xylan hemicellulose, and cellulose. Fourteen to seventeen percent of the polysaccharides are found in the cotyledon, while the other percentages are mostly located in the hulls.

Bils and Howell (1963) reported that mature soybeans did not contain starch due to its metabolism and use in lipid, protein, and

other syntheses in the cotyledon during maturity. But in 1976, Boonvisut and Whitaker found that soybeans contain 0.52% starch when analyzed by the digestion method using alpha-amylase. Also, Wilson et al. (1978) have isolated and characterized the starch from eleven different cultivars of soybeans. They reported that mature soybeans have 0.08 $\pm$ 0.03% starch of the dry weight, with 15 to 20% as amylose. They have a gelatinization temperature in the range of 73 to 83°C. This is higher than that of pinto, navy, faba, mung beans, or chick peas and horse beans (Lineback and Ke, 1975; Naivikul and D'Appolonia, 1979). The starch granules disappeared after heating due to the rupture of the granules, as the indication of complete gelatinization.

Starch in soybeans affects the digestibility of soybean protein (Boonvisut and Whitaker, 1976), as shown by the in vitro digestibility of soybean protein fractions by trypsin. Its digestibility is increased after treating the fractions by alpha-amylase.

Low molecular weight carbohydrate fractions or oligosaccharides such as stachyose and raffinose, which are found in soybeans, appear to be the gas producing factors which cause flatulence (Steggerda et al., 1970). This is due to the lack of alpha-galactosidase in the human digestive tract. The lack of alpha-galactosidase brings about the breakdown of the oligosaccharides by the anaerobic type bacteria in the large intestine.

Minerals

The total ash (Table 1) of soybeans is higher than that of wheat, with four times as much potassium and sodium, five times as much calcium, three times as much magnesium, two times as much phosphorus, and about

the same amount of sulfur (Bailey et al., 1935). Soybeans have only one-third as much chlorine. The values of 1.9, 0.3, 0.2, 0.2, 0.6, 0.4, and 0.02% were reported for potassium, sodium, calcium, magnesium, phosphorus, sulfur, and chloride, respectively. The phosphorus content is distributed as 77% phytin, 14% phosphatides, 5% inorganic phosphorus and 4% residual phosphorus (Markley, 1950; Harris and Karmas, 1975).

Protein

Soybean protein is the best quality and quantity among other legumes such as peas, lentils, or other beans (Schmitt, 1979). The protein content (Table 1) averages 40% of dry weight and consists of 20 amino acids including all essential amino acids. There is a high lysine content but it is deficient in the sulfur containing amino acids, i.e. methionine and cystine. Cystine is not an essential amino acid, but it can supplement methionine to a limited extent (Krober and Cartter, 1966). There is a tendency towards increased methionine content with the increased protein in soybeans. Krivoruchco et al. (1979) reported that the positive correlation with protein content is valid only with the total sulfur amino acids content, but it is not valid with the methionine content by itself. The average value of 1.16 g and 1.62 g per 16 g nitrogen were reported for methionine and cystine, respectively.

Antinutritional Factor

The most prevalent antinutritional factor in soybeans is the trypsin inhibitor (TI). This TI has been isolated from soybeans in crystalline form (Kunitz, 1946, 1947). Trypsin inhibitor is a stable protein of the globulin type; it has a molecular weight of about 24,000.

The TI crystal is stable at pH 1 to 12 in dilute buffer at temperatures below 40°C. The inhibiting action of TI is due to its combination with trypsin at equal weight, which is irreversible, and this combination is apparently instantaneous.

At higher temperature and in stronger acid or alkaline solution this protein is gradually denatured, concurrent with a loss in its ability to inhibit trypsin action (Kunitz, 1947). This was explained by Wu and Scheraga (1962) who reported that TI in native form contains a mixture of random coils and left and right handed helices. These helices are transformed to random coils in high temperatures as the indication of its denaturation.

The relative activity of TI to inhibit growth, reduce protein efficiency ratio, lower nitrogen digestibility, and enlarge the pancreas was studied by Rackis and McGhee (1975). They used defatted soybean flour diets which contained graded levels of TI activity. The result indicated that the diet which contained 282 mg TI/100 g diet or more significantly lowered body weight and protein efficiency ratio compared to the maximum values obtained with 119 mg TI/100 g diet. The enlargement of pancreas and low nitrogen digestibility occurred only in rats fed diets with 532 to 887 mg TI/100 g. Another study was carried out by Collins and Beaty (1980) on the physiological response of rats fed raw green soybeans (51.9 TIU/mg dry soybean). The weight gain of rats fed these raw soybeans was less than those fed casein, with protein efficiency ratio values of 1.20 and 2.50 for the raw soybeans and casein, respectively.

Color

Soybeans contain four different types of pigments which contribute to a range of colors, depending on the soybean variety. These pigments are distributed throughout the various parts of the seed (Markley and Goss, 1944). The pigments are: the red-yellow carotenoids, the yellow isoflavone glycosides, the blue-purple anthocyanins, and chlorophyll. The carotenoid pigments are present in the oil droplets of the cotyledon, while the chlorophyll occurs in the plastids.

Microstructure of Soybeans

To be able to evaluate the microstructure changes of soybeans upon soaking or heating, the microstructure of soybeans themselves in the dry or initial condition has been investigated. This microstructure of soybeans has been studied by Wolf and Baker (1972) using a scanning electron microscope and by Saio et al. (1973) using both a light microscope and transmission electron microscope.

The soybean seed coat consists of a cuticular layer with numerous pits or porelike identations. These pits are due to the depression of cuticular into palisade layer; palisade cells, columnar or hourglass cells (subepidermis); several layers of parenchyma (spongy parenchyma) cells; a layer of rectangular aleurone cells, and the compressed fiber-like cells (Wolf and Baker, 1972; Saio et al., 1973; Wolf and Baker, 1980). The aleurone cells and compressed cells are part of the endosperm or cotyledon. The cotyledon surface has appreciable texture with ribbed membrane-like material. The internal structure of cotyledon was observable with the cell walls slightly ridged. The protein bodies

are surrounded by protein networks which enclose the oil-containing spherosomes (Wolf and Baker, 1972).

Effect of Preparation

Vitamin Losses

Water blanching before cooking the garbanzo and lima beans has been reported to cause higher losses of vitamin B-6 than steam blanching (Raab et al., 1973; Daoud et al., 1977). In this study, there was an indication that steam blanching improved the retention of vitamin B-6 during cooking lima beans. The increased temperature and time of soaking or cooking the small white beans or processed pinto beans caused increased of vitamin B-6 leaching to the soaking or cooking water (Kon et al., 1973; Miller et al., 1973: Kon, 1979), while the splitting of beans did not significantly affect the vitamin B-6 retention in small white beans.

The addition of $NaHSO_3$ at 800 or 1600 ppm into soaking water for garbanzo or pinto and pink beans caused decreased retention of vitamin B-6, but it did not affect the vitamin B-6 in lima or blackeye beans (Daoud et al., 1977; Rockland et al., 1977). Addition of etylenediamine-tetraacetic acid (EDTA) into the brine did not have a significant effect on vitamin B-6 retention in garbanzo beans (Daoud et al., 1977).

The folacin losses during cooking of the processed pinto beans were observed to be mostly in the discarded cooking water with large increases in losses due to increased time or temperature (Miller et al., 1973). Splitting the small white beans before cooking did not affect the folacin retention (Kon et al., 1973).

Thiamine loss through the leaching into cooked water of pinto beans, and its breakdown due to the higher temperature and longer process, is higher than pyridoxine or folacin losses (Miller et al., 1973). A difference in the effect of salt solution in soaking water on thiamine retention in cooked beans was observed by Rockland et al. (1977). There was increased thiamine retention in lima and blackeye beans, but decreased thiamine retention in pinto and pink beans, compared to when the beans were soaked in plain water.

Higher soaking temperature increased the extraction of niacin into the soaking water when compared to that in a lower soaking temperature using small white beans (Kon, 1979). The same occurrence when pinto beans were processed at a longer heating time (Miller et al., 1973).

Protein Losses

Soaking soybeans overnight caused leaching of soluble protein, about 7 to 16% of total solids losses (Wang et al., 1979). The protein losses into the soaking water increased steadily as the soaking temperature and time increased over the first 18 hr; then it reached equilibrium as the soaking continued to 24 hr. In the case of small white beans which were soaked at different soaking temperatures, only the nonprotein nitrogen was found in the soaking water (Kon, 1979). Kon suggested that higher soaking temperatures caused denaturation of protein and it became insoluble and did not diffuse out.

Ku et al. (1976) evaluated the protein loss due to cooking at different soybean to water ratios, and different pH solutions. They reported more loss of protein when cooked with beans at ratios 1:10 than

at lower ratios. The alkaline pH (0.5% $NaHCO_3$) of cooking water caused more leaching of protein than when the beans were cooked in acid pH or in tap water. The increase in cooking time also increased protein loss for all soaking solutions. These greater losses were due to the increased protein solubility in alkaline conditions and the increased permeability of the seed coat.

Antinutritional Factor Changes

Trypsin inhibitor activity was destroyed during steaming at 100°C (atmospheric steaming) or during immersion cooking of soybeans (Albrecht et al., 1966). The initial moisture content is a major factor influencing the cooking rate. For example, in immersion cooking of whole soybeans with 62 or 65% moisture, five or seven minutes, respsectively, were needed to destroy the same level of the trypsin inhibitor. In atmospheric steaming, the whole soybeans with 20% moisture content needed 15 min, or the half beans with eight percent moisture content, needed 30 min to get the same level of deactivation of trypsin inhibitor, i.e. about 99% of the original trypsin inhibitor activity.

The trypsin inhibitor activity was decreased rapidly within the first ten minutes of moist heat treatment of soy flour (Rackis and McGhee, 1975). This inactivated 79 to 87% of the TI activity. Beyond that time the rate of inactivation was lower, and only 92% of the original activity was destroyed after 30 min of heating.

The protein efficiency ratio (PER) of full-fat soy flour produced by heat roasting was associated with the inactivation of TI (Jansen et al., 1978). Roasting beans at 178°C for 15 to 24 sec only slightly

increased the PER from 1.49 in raw beans to 1.62 to 1.67 in roasted beans. The TI activity only decreased 36 to 47%. Increasing temperatures to 206°C for 22 sec for 234°C for 15 sec gave a PER 2.20 and 2.31, respectively, while TI activity lost 75 to 90% of its original activity.

Carbohydrate Losses

Soluble sugars such as fructose, sucrose, raffinose, and stachyose were measured in the soaking water of soybeans (Wang et al., 1979). About 30 to 50% of the soluble sugars were leached out after soaking the beans overnight at 25°C. The total losses were higher as the soaking temperature and time increased. The total recovery of fructose was higher, while other sugars were very low. This was explained by the fact that glucose and fructose appeared to be derived mainly from hydrolysis of raffinose and stachyose by invertase. About 48% of the original raffinose and 32% of the original stachyose was leached out from soybeans after soaking at 25°C for 18 hr.

The removal of 50 to 59% of stachyose and raffinose from small white beans after soaking at 60°C or above was reported (Kon, 1979). This was compared to about five percent removal of these oligosaccharides at room temperature soaking. While 33% of the oligosaccharides in the whole soybeans was removed by boiling them for 20 min with 1:10 ratio of bean to water (Ku et al., 1976), it was higher than that with lower ratio. The extending of time to 60 min caused 59% removal of oligosaccharides. The alkaline pH also increased the removal of stachyose and raffinose, while the acid pH had the same effect as that of tap water.

Mineral Losses

The losses of total phosphorus, calcium, and magnesium from small white beans have been reported to be higher at higher soaking temperatures (Kon, 1979). The extraction was more greatly increased at 60°C or above than that at 50°C or lower. Organic phosphorus was hydrolyzed and leached as inorganic phosphorus at lower temperatures due to the phytase activity in the beans which has optimum activity at temperature around 40°C. Yet there was more leaching at higher temperatures. More calcium and magnesium were also extracted at 60°C or above. This extraction was almost four times that at lower temperatures.

Color Changes

Soaking the soybeans prior to cooking gave lighter and brighter color to the soybeans than that of unsoaked cooked soybeans (Wang et al., 1979). Salt solutions of $NaHSO_3$ were used at different concentrations to soak garbanzo beans before cooking or canning (Daoud et al., 1977). The physical and sensory evaluation resulted with data indicating that the canned beans were significantly improved by the addition of salt in the soaking water, while the effect of blanching alone did not give any significant improvement.

Addition of EDTA into brine as the bleaching agent improved the color of the canned garbanzo beans significantly, but in lesser extent than that affected by $NaHSO_3$ solution (Daoud et al., 1977). In another study (Junek et al., 1980) the addition of EDTA to the soaking water tended to increase the "a" value of pinto and kidney beans as measured by the Gardner Color Difference Meter.

Malic or citric acid in soaking water has been used to improve the color of cooked beans (Junek et al., 1980). The results indicate that the "b" values of navy and pinto beans were higher than the control soaking water. The higher "a" value was also reported for kidney and pinto beans.

Texture Changes

Wang et al. (1979) studied the effect of soaking time on the texture of cooked soybeans. The increase in tenderness in the beans due to the hydration prior to cooking has been found to give the same degree of tenderness for beans soaked for one hour as for those cooked for 1.5 hr. There was evidence that the unsoaked beans could not reach the tenderness of soaked and cooked soybeans even with longer cooking times. The complete hydration (140% hydration) did not increase the tenderness when compared to partially hydrated (100% hydration) cooked soybeans.

Sefa-Dedeh et al. (1978) used cowpeas to study the effect of soaking time and cooking condition on texture. They found that the rate of cooking relative to texture followed first order kinetics. According to their experiment, the texture of cooked cowpeas could be predicted from the texture of the corresponding soaked peas.

Malic or citric acid in soaking water of pinto, kidney, and navy beans caused the increase of force required to shear navy beans (Junek et al., 1980), but there was no effect on the other beans' tenderness. Even though a prolonged soaking time of six hours was used, the shear press values for navy beans were slightly increased while that for kidney and pinto beans were decreased. All the cooked beans became

more tender when soaked at 35°C as compared to the tenderness of those soaked at 15 or 25°C.

Swelling Power

Soybeans absorbed an equal weight of water after soaking for 5.5 hr at 20°C, or for 2.5 hr at 37°C (Wang et al., 1979). The complete hydration (140%) was reached after six hours at 37°C or after 16 hr at 20°C.

Junek et al. (1980) reported that prolonged soaking time to 14 hr for navy, kidney, and pinto beans caused greatest drained weight of a canned product of the beans when compared to those which were soaked for three or six hours. The malic or citric acid in soaking water at concentration of 0.2 or 0.3% caused lower drained weight of navy beans than that if they were soaked in water. Soaking temperature of 35°C gave higher drained weight and more split beans than that of 15 or 25°C.

The effect of soaking time on water absorption was reported by Sefa-Dedeh et al. (1978). They used cowpeas in their experiment and soaked them at 25°C for 1, 3, 6, 12, 18 and 24 hr. This water absorption was accomplished by swelling of the beans. The amount of water absorbed may be significantly affected by the protein and starch matrix of the cotyledon as well as the anatomical characteristics such as seed coat thickness and size of micropyle.

Microstructure Changes

The change of soybean structure during soaking or cooking has been studied (Saio et al., 1973; Sefa-Dedeh and Stanley, 1979). During soaking, parenchymal cells in hulls swelled and elongated faster than

cells in cotyledon. This caused splitting between the parenchyma and overlying hourglass cells. This taking up of water in the soybean seed was higher than in other beans such as cowpeas, white, pinto, adzuki, or blackeye beans (Sefa-Dedeh and Stanley, 1979). This might be due to higher protein content in the soybeans than in the other beans.

The effect of salt solution on the cell's changes have been studied on the lima beans (Rockland and Jones, 1974; Hahn et al., 1977). The soaked raw beans which were examined using a light microscope and a scanning electron microscope showed the rupture in the cell wall when they were fractured.

Heating soybeans at 100°C caused separation of intact cells, but there was less breakdown of the middle lamella compared to that in cowpeas, white, pinto, adzuki, or blackeye beans (Sefa-Dedeh and Stanley, 1979). In cooked or partially cooked lima beans the cell wall separated readily along the surface of individual intact cell walls. This was due to the loosened intercellular matrix of the middle lamella during heat treatment. The same occurrence was seen in the microstructure changes during heating of soaked cowpeas (Sefa-Dedeh et al., 1978). When the peas were heated at 100°C there was separation of cell walls on their middle lamella, while broken tissues occurred when peas were heated at 25 to 90°C. These were explained by the fact that in the raw condition or at low temperature, the middle lamella is stronger than the cell wall and thus the cell wall tissue broke under stress. The heat will soften the middle lamella and cause the separation of the cell instead of breaking the cell.

Water washing of undefatted, freeze-fractured cotyledon surface dissolved lipid and protein bodies from cooked soybean cotyledon cell (Wolf and Baker, 1980). It left empty and wrinkled surfaces on cells. Cooking soybeans at 115°C for 30 min resulted in rupturing and bursting of the protein bodies, together with curdling of the proteins.

MATERIALS AND METHODS

Samples and Sampling

Pacific Coast soybeans (Walla Walla, WA 99362) used in this experiment were selected for soundness and wholeness (Want et al., 1979). One-hundred grams of selected beans were weighed and placed and sealed in each of 32 plastic bags. The bags were randomly assigned to the four treatments with eight replications per treatment.

For each treatment, the 100 g portion of soybeans were soaked in 500 mL redistilled water (25°C) in 1000 mL-glass beaker covered with a watch glass for 10 hr in a 25°C incubator (Freas 815, Precision Scientific, subsidiary of GCA Corporation, Chicago, IL). The soaked beans were drained for 10 min, gently blotted dry with Kim Wipes (Kimberly-Clark, Stock Number 34155), and weighed.

Blotted, soaked beans were cooked according to treatment designation in a 1000 mL-glass beaker with 500 mL redistilled water covered with a watch glass. The boiling method (B-20) was done on top of a hot plate (Pyro Magnestir King Size, Lab-Line Instruments, Melrose Park, IL: stirring speed, off; heating set, maximum) which was preheated for 30 min. Boiling time was counted 20 min after boiling was initiated. The pressure cooking was carried out in a Precision Surgical Supply Sterilizer (American Sterilizer Company, Erie, PA) at 102 kPa (15 lb/in^2; 117°C) for 5 min, 10 min, or 20 min, which will be referred to as A-5, A-10, or A-20, respectively.

Cooked soybeans were cooled (30 to 36°C) for 20 min, drained and blotted dry as for soaked beans. After weighing the soybeans for

determination of percent water absorption, evaluation of color, determination of texture and evaluation with the scanning electron microscopy, samples of cooked soybeans were frozen in liquid nitrogen (-196°C). The frozen samples were ground to a fine powder using Osterizer Dual Range-10 (Oster Corporation, Milwaukee, WI) at grind speed for vitamin B-6, folacin, trypsin inhibitor, and moisture analysis.

Vitamin B-6 Determination

Total vitamin B-6 of cooked beans, cooking water and soaking water was determined by the method of Storvick et al. (1964) after hydrolyzing two gram of samples with 0.44N HCl. Hydrolyzed samples were kept frozen (-14°C) in a walk-in freezer (Kalt, Portland, OR) until assay.

Saccharomyces carlsbergensis ATCC #9080 (American Type Culture Collection, Rockville, MD) was used as test organism (Storvick et al., 1964). Vitamin Assay Casamino Acids, dehydrated (difco #0288-02, Difco Laboratories, Detroit, MI) substituted for the acid hydrolyzed casein in making the basal medium. Dilutions of 3000X, 1500X, and 750X were used for cooked beans, cooking water, and soaking water samples, respectively.

Vitamin B-6 value of the sample was calculated utilizing a pyridoxine monohydrochloride (#P-97557; Sigma Chemical Company, St. Louis, MO) standard curve and using a power curve-fit program (Hewlett-Packard Calculator Model 41-C, Loveland, CO 80537; Surjadi, 1980). Values are expressed as ng/g of soybeans on a wet and dry weight basis. Soaking water and cooking water vitamin B-6 values are expressed as ng/g wet weight basis.

Folacin Determination

Folacin activity was assayed (Herbert and Bertino, 1967; Hurdle et al., 1968) microbiologically with *Lactobacillus casei* ATCC #7469. *L. casei* was cultured in Lactobacillus Broth AOAC (Difco #DF-0900-15, Difco Laboratories, Detroit, MI) for 18 hr at 37°C (Thelco Model 6 Incubator, Precision Scientific Company, Chicago, IL 60647), then 0.5 mL mixed culture was cultured for another eight hours. One-half milliliter of rinsed eight-hour culture were diluted with 10 mL rinse solution, then used for assay.

Free folacin was measured in samples without conjugase treatment, while total folacin was assayed in samples treated with chicken pancreas (Difco #0459-12, Difco Laboratories, Detroit, MI) as a conjugase (Hurdle et al., 1968). The chicken pancreas solution was added to the sample supernatant as suggested by Butterfield and Calloway (1972). This was then incubated (Thelco Model 6 Incubator, Precision Scientific Company, Chicago, IL 60646) for six hours at 37°C. L-ascorbic acid powder (#B581, J. T. Baker, Phillipsburg, NJ 08865) was added to both the buffer used for extracting the vitamin from food and for the assay growth medium at a concentration of 150 mg/100 mL buffer (Herbert and Bertino, 1967; Hurdle et al., 1968). Dilutions of 1250X and 1000X were used for free folacin in cooked beans and cooking water or soaking water, respectively. A dilution of 3000X was used for total folacin in all samples.

The folacin values of cooked beans, cooking water, or soaking water samples were calculated utilizing a pteroylglutamic acid (#6133, National Biochemical Corporation, Chicago, IL) standard curve and using

a power curve-fit program (Hewlett-Packard Calculator Model 41-C, Loveland, CO 80537; Surjadi, 1980). Values are expressed as ng/g of soybeans on a wet and dry weight basis. Soaking water and cooking water total or free folacin values are expressed as ng/g samples on wet weight basis.

Trypsin Inhibitor Activity (TIA) Determination

The enzyme, trypsin (#-8253, 2X crystallized, salt free, Sigma Chemical Company, St. Louis, MO), and the substrate, benzoyl-DL-arginine -p-nitroanalide (BAPNA) hydrochloride (#B-4875, Sigma Chemical Company, St. Louis, MO) were used in the assay (Kadade et al., 1969; Kadade et al., 1974) of trypsin inhibitor activity (TIA).

Reactions were carried out for ten minutes at 37°C (Thelco Model 84 Waterbath; GCA/Precision Scientific Company, Chicago, IL 60647). The mixtures were then centrifuged for 20 min at speed 30 (IEC International Centrifuge, Size 2, Model K, International Equipment Company, Needham Heights, MA) and the resulting supernatant was read at 410 nm in a Spectronic-20 colorimeter (Bausch & Lomb, Rochester, NY 14625). Results are expressed as trypsin inhibitor units (TIU). One trypsin unit is arbitrarily defined as an increase of 0.01 absorbance units at a 410 nm wavelength per ten milliliters of the reaction mixture.

Texture Measurement

The Instron Universal Testing Machine (Model 1132, Instron Corporation, Canton, MA) and Kramer cell attachment were used (crosshead speed, 12 in/min; chart speed, 20 in/min; range 10) to measure the force required to shear triplicate samples of 25 g each of cooked soybeans.

The resulting values were averaged and presented as lb/25 g.

Color Measurement

Color of cooked soybeans was determined as percent reflectance using Photovolt Reflection Meter (Model 670, Photovolt, New York, NY 10010). The search unit was standardized in an upward position using a standard white enamel plaque (blue, (B), 51.5; green, (G), 50.0; amber, (A), 48.5). Triplicate samples of 50 g cooked soybeans in an identical 100 mL-pyrex glass beaker were evaluated for each treatment replication. The average value is reported.

Water Absorption

Measurement of water absorption was determined as the percentage increase in weight from initial soybean weight. Calculations of percent water absorption were made after soaking as well as after cooking of soybean samples.

Moisture Determination

Moisture content of five grams ground cooked soybeans was determined using a vacuum oven (20 to 25 lb negative pressure; Model 58301, National Appliance Company, Portland, OR) at 60°C for eight hours. Percent moisture was calculated (Equation 1) and done in duplicate and an average reported.

Equation 1. $$\text{Percent moisture} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Scanning Electron Microscopy

Freeze-drying. Cooked soybean samples were freeze-dried in preparation for scanning electron micrographs. Duplicate samples of each treatment replication were prepared. Soaked or cooked soybean samples were cut crosswise at the middle portion of the bean and cut lengthwise at the inner part of the 1.5 mm thick using a scapel blade. Raw soybean fragments were also prepared.

The sample was put in a five-milliliter vial (Wheaton 223738 S-104 E serum bottle, Wheaton Scientific, Millville, NJ 08332) and fully covered with liquid nitrogen. The samples were frozen in liquid nitrogen and stored at -80°C (Ultra Low Freezer, Revco Incorporated, West Columbia, SC 29169). Frozen samples were later freeze-dried overnight (12 hr) in an Atmo-Vac Table Freeze Drier (model 50000 Refrigeration for Science Incorporated, Island Park, NY) before final preparation of the samples for scanning electron micrographs.

Mounting and Microscopy. After drying, clean glass cover slips 13 mm in diameter were fastened to aluminum planchets with colloidal silver paint (Pelco Cat. #1603-4, Pelco, Incorporated, Tustin, CA). One small drop of polyvinyl chloride adhesive solution (Pelco, "Mikrostik" #1603-3, Pelco, Incorporated, Tustin, CA) was placed on each mounted cover glass, and the solvent was allowed to completely evaporate. The freeze-dried samples were then transferred and dusted onto the adhesive with toothpicks.

The prepared planchets were fastened in a rotation tilting device (Fullam #1253, Ernest Fullam, Incorporated, Schenectady, NY) in a Varian

VE-10 vacuum evaporator (Varian Vacuum Division, Los Altos, CA) and brought to a pressure of approximately $1 \times 10^{-5.5}$ Torr (Holmes and Soeldner, 1981). Two hundred Å of 60:40 gold/palladium alloy was then deposited on the samples by vacuum evaporation. Microscopy was done at 150X and 1000X using an AMRAY 1000 A scanning electron microscope (Advanced Metals Research Corportion, Bedford, MA). Images were recorded on type 55 Polaroid film (Polaroid Corporation, Cambridge, MA).

Statistical Analysis

An incomplete block design for the eight replications of the four treatments was used (Federer, 1955). The total blocks were 16 with two treatments each laboratory period. Data were statistically analyzed by standard deviation (Hebbler, 1979) and least square analysis of variance and covariance and t-test (Niess, 1980).

RESULTS AND DISCUSSION

The heating treatments in this experiment tended to affect vitamin B-6 and folacin retention, trypsin inhibitor activity, color values, texture, percent water absorption, and moisture content. These results are discussed in the following pages. Detailed replication data are recorded in the appendix tables.

Vitamin B-6 Retention

The values of vitamin B-6 in the beans, in the cooking water, or in the soaking water are shown in Table 2. These values of vitamin B-6 in the cooked soybeans are lower than those reported by other researchers (Kabir, 1981; Miller, 1981). All heat treatments reduced the vitamin B-6 content approximately 40% from that in the raw beans. This is to be expected as other workers have indicated that vitamin B-6 is destroyed by heat (Storvick et al., 1964; Daoud et al., 1977; Perera et al., 1979). Kabir (1981) found the value of 6,887 ng/g cooked soybean on wet weight basis, when he autoclaved soybeans without soaking, for 15 min. On the other hand, Miller (1981) autoclaved soaked beans in their soaking water for 20 min, and reported 2,700 ng/g vitamin B-6. Those differences from the current experimental results could be due to the differences in cooking water concentration or to the differences in moisture content.

Although there is no significant difference (Table 2; Appendix, Table 17) among the means of the bean vitamin B-6 of each heating treatment, there is a tendency for higher retention in the beans of A-5, followed by A-10, B-20, and A-20. The heating treatment did

TABLE 2. Vitamin B-6 (ng/g) in raw, cooked soybeans, cooking water, and soaking water.

Treatment[1]	Soybean		Cooking water[2,4]	Soaking water[2]
Raw	6266.1 ± 275.9[2]	(7044.9 ± 269.0)[3]	-	-
B-20	1312.8 ± 109.6	(4018.4 ± 386.4)	927.5 ± 260.1[a]	97.0 ± 5.2
A-5	1478.8 ± 185.9	(4243.6 ± 540.0)	503.2 ± 105.3[b]	101.4 ± 5.2
A-10	1373.6 ± 169.7	(4134.0 ± 523.1)	591.4 ± 148.1[b]	104.4 ± 13.2
A-20	1267.7 ± 403.9	(4106.5 ± 1428.7)	691.7 ± 202.1[a]	100.2 ± 9.4

[1] B-20 represents 20 min boiling. A-5, A-10, A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

[2] Value on wet weight basis.

[3] Value on dry weight basis.

[4] Letters not in common denote statistical significance ($P \leq 0.05$).

significantly influence the vitamin B-6 content in their respective cooking water. Vitamin B-6 content in A-20 or B-20 cooking water is significantly higher than all other treatments; the vitamin B-6 content in the B-20 cooking water is higher than in those from the autoclaved heat treated beans (A-5, A-10, A-20). The vitamin B-6 content in the soaking water, as expected, did not differ significantly. The stability of vitamin B-6 has been frequently reported for a number of food items. Gregory and Kirk (1978) reported roasting at 180°F for 25 min resulted in a 50 to 70% degradation of the vitamin B-6 added to breakfast cereal as the model system. Only slight losses (0 to 15%) of vitamin B-6 occurred during baking of the enriched-flour bread (Perera et al., 1979). Vitamin B-6 was relatively stable in enriched flour when stored at low temperatures (-5°C).

Actual destruction of vitamin B-6 by heat has been evaluated by totalling the content in soaked water, cooked water and the beans and then comparing the totals to the value in the raw beans. Miller et al. (1973) reported that during the processing and canning of pinto and lima beans, loss of vitamin B-6 was mostly in the cooking water. In the current experiment the total recovery of vitamin B-6 from beans, cooking water and soaking water were 71.6, 68.8, 68.6, and 69.5% for B-20, A-5, A-10, and A-20, respectively.

Folacin Retention

Similar results have been reported (Leichter et al., 1978) for folacin loss in cooking water of vegetables. In the current experiment the total and free folacin values (Table 3; Appendix, Table 18) in the

TABLE 3. Total folacin (ng/g) and free folacin (ng/g) in raw and cooked soybeans, cooking water, and soaking water.

Treatment[1]	Soybean	Cooking water[2,4]	Soaking water[2]
Total folacin:			
Raw	1676.2 ± 0.0[2] (1883.6 ± 21.2)[3]	-	-
B-20	504.3 ± 90.1 (1543.2 ± 282.3)	347.1 ± 107.2	184.8 ± 47.2
A-5	603.6 ± 174.0 (1732.8 ± 501.8)	266.0 ± 118.0	194.6 ± 52.6
A-10	571.6 ± 84.0 (1721.4 ± 265.4)	228.1 ± 39.3	199.6 ± 47.2
A-20	477.0 ± 59.9 (1534.5 ± 203.0)	347.3 ± 103.1	197.7 ± 34.6
Free Folacin:			
Raw	1272.2 ± 216.6[2] (1691.9 ± 259.5)[3]	-	-
B-20	399.9 ± 80.0 (1223.0 ± 243.9)	192.5 ± 38.4[a]	62.9 ± 14.1
A-5	526.1 ± 169.2 (1506.9 ± 477.2)	110.3 ± 19.5[b]	56.6 ± 13.0
A-10	450.9 ± 79.0 (1355.9 ± 237.7)	125.1 ± 26.6[a,b]	64.6 ± 16.2
A-20	373.2 ± 135.8 (1204.3 ± 441.1)	171.0 ± 62.9[a,b]	60.4 ± 15.1

[1] B-20 represents 20 min boiling. A-5, A-10, A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

[2] Value on wet weight basis.

[3] Value on dry weight basis.

[4] Letters not in common denote statistical significant ($P \leq 0.05$).

beans and soak water did not differ significantly ($P \leq 0.05$). However, as with vitamin B-6 values, as the samples were autoclaved for greater lengths of time, the total and free folacin content decreased in the bean samples. De Ritter (1976) has indicated that total folacin does decrease during cooking. In preparing soybeans, there were some losses of folacin into soaking water and cooking water, since folacin is water soluble. Miller et al. (1973) reported more losses due to leaching of folacin to soaking and cooking water than losses due to prolonged heating of canned and powdered pinto beans. Cooking decreased total and free folacin content of large lima, blackeye and pink beans for all beans soaked in water or salt solution (Rockland et al., 1977). Actually, heat treatment may be advantageous in folacin determination. Holmes et al. (1979) obtained data from blanching of green beans that suggests that the heating in water may increase the availability of total folacin to *L. casei*, the assay organism. These results are supported by work reported by Leichter et al. (1978) who noted similar trends in their experiment on asparagus, broccoli, brussels sprouts, cabbage, cauliflower and spinach. The recovery in the current experiment is greater than 100% total folacin.

Although total folacin values did not differ significantly ($P \leq 0.05$, Table 3) in the cooking water, free folacin did show some differences. B-20 had a significantly ($P \leq 0.05$) higher free folacin value than A-5 but was not significantly different with A-10 or A-20. The reason for the differences in significancy with treatment of total folacin versus free folacin could possibly be attributed to the ease of leaching. Free folacin is more easily leached out than the conjugated

form (Herbert and Bertino, 1967). However, values obtained in the current experiment were similar to those reported by Perloff and Butrum (1977). They reported 171 μg of total folacin per 100 g edible portion of raw soybeans.

Trypsin Inhibitor Activity (TIA)

The heat sensitivity of the legume trypsin inhibitor (TI) has been frequently reported (Kunitz, 1947; Wu and Scheraga, 1962; Rackis, 1966; Rackis and McGhee, 1975; Jansen et al., 1978). Albrecht et al. (1966), using soybeans (20% moisture), reported that 99% of its TIA was destroyed when steamed at 100°C for 15 min. Inactivation was accelerated to five to seven minutes when the soaked whole soybeans (62 to 65% moisture) were immersed and boiled in water. In the current experiment, over 98% (Table 4) of the TIA, expressed as trypsin inhibitor units (TIU), was inactivated. As Albrecht et al. (1966) observed, the boiling method can be as effective as pressure cooking in destroying the TI. B-20 and A-10 were significantly ($P \leq 0.05$) different from the values for A-5 or A-20. A-5 was significantly ($P \leq 0.05$) higher in TIA than B-20, A-10 or A-20. While B-20 and A-10 are not significantly different, they were higher ($A \leq 0.05$) than A-20. As has been reported in many experiments, the TIA is decreased by heating. Collins and Beaty (1980) reported that boiling whole soybeans for nine minutes inactivated 96.1% of TIA. There was evidence that TIA decreased drastically in the first three minutes. Wang et al. (1979) found that soaking the soybeans for 18 hr at 25°C leached only four to six percent of TIA and suggested that soaking without cooking was not efficient in removing TIA.

TABLE 4. Trypsin inhibitor activity, texture, and color values of raw and cooked soybeans.

Treatment[1]	Trypsin inhibitor activity (TIU/g)[2,3]	Texture (lb/25 g)[5]	Color[3,6]		
			Blue	Green	Amber
Raw	61,349.7 ± 418.9	-	13.6 ± 0.8	26.2 ± 1.0	30.0 ± 0.5
B-20	828.0 ± 99.2[a]	121.3 ± 4.2	15.8 ± 0.4[a]	31.8 ± 0.7[a]	35.3 ± 1.0[a]
A-5	1,168.5 ± 85.6[b]	160.3 ± 18.4[4]	15.0 ± 1.0[a,b]	31.1 ± 1.4[a,b]	34.6 ± 1.4
A-10	766.5 ± 87.8[a]	115.4 ± 40.8	15.4 ± 0.7[a,b]	30.6 ± 1.0[b]	34.1 ± 1.0
A-20	602.5 ± 57.0[c]	107.4 ± 42.5	14.9 ± 0.7[b]	29.4 ± 1.6[b]	32.9 ± 1.6

[1]B-20 represents boiling 20 min. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

[2]TIU = trypsin inhibitor units per gram of sample on wet weight basis.

[3]Letters not in common denote statistical significant ($P \leq 0.05$).

[4]Average value of 6 replications instead of 8 replications.

[5]Pounds per 25 grams of samples on wet weight basis.

[6]Average value of percent reflectance.

Texture Measurement

There were not significant ($P<0.05$) differences in the texture (Table 4), as measured by shear pressure, of the soybeans from the four heat treatments. In comparing the pressure cooked beans, the greatest decrease of approximately 28% occurred during the five minute to ten minute cooking periods; however, a seven percent further increase in tenderness occurred from A-10 to A-20. These results concur with those reported by Sefa-Dedeh et al. (1978) who observed, using an Instron Testing Machine (Model TM-M, texture test attachment of the Ottawa Texture Measuring System), that the longer the cooking time of a soaked bean the more tender the bean. This is likely because the longer cooking time of soaked beans permitted the greater tenderization of the bean as more water was absorbed into the beans. Additionally, this tenderization of the soybeans during cooking may be due to the breakdown of middle lamella (Rockland and Jones, 1974; Sefa-Dedeh et al., 1978), or due to the softening of the protein matrix and gelatinization of starch (Sefa-Dedeh and Stanley, 1979) in the cotyledon. It also might be due to the bursting of the hulls after absorption of water.

Color Measurement

Color of legumes has been used to evaluate their quality (Daoud et al., 1977; Wang et al., 1979; Junek et al., 1980). In the current experiment, blue (B), green (G), and amber (A) percent reflectance values were obtained using the Photovolt Reflectance Meter. The blue values (Table 4) appear to be the color value of greatest discrimination. The mean blue value of B-20 was significantly ($P\leq0.05$) higher

than that of A-20. However, the mean blue value for B-20 was not significantly different from those of A-5 or A-10. The green value of B-20 was significantly ($P \leq 0.05$) higher than that of A-10 or A-20, but was not significantly different from that of A-5; and amber value of B-20 was also significantly ($P \leq 0.05$) higher than any of the pressure heat treated samples.

The higher blue, green, and amber values indicated a whiter, lighter brown soybeans with the boiling heat treatment. Possibly this type of heating caused less occurrence of the browning reaction due to lower temperature (100°C) employed as compared to that in the autoclave (117°C), and due to more leaching of protein and reducing sugar during boiling. Values which used the Photovolt Reflection Meter for description of the color of soybeans were not located in the literature; however, a number of workers have used other instrumentation. For example, Junek et al. (1980) and Daoud et al. (1977) used a Gardner Color Difference Meter to measure the color of cooked pinto, kidney, navy, and garbanzo beans.

Water Absorption and Moisture Content

The relationship of moisture content and/or water absorption has been related to these legumes' quality (Burr et al., 1968; Wang et al., 1979). In the current experiment (Table 5), the beans' moisture content was significantly ($P \leq 0.05$) affected by the heat treatment. However, if compared to values cited in Table 1, the values are within the range of those reported in other experiments (Albrecht et al., 1966; Watt and Merrill, 1975). As noted in Table 5, the moisture content of the

TABLE 5. Moisture content and percent water absorption after soaking and after cooking.

Treatment[1]	Moisture[2] %	Water absorption	
		After soaking %	After cooking %
B-20	67.3 ± 0.95[a]	126.8 ± 0.95	143.1 ± 2.39[ab]
A-5	65.1 ± 0.85[b]	126.8 ± 1.02	141.4 ± 2.11[a]
A-10	66.8 ± 0.78[a]	127.4 ± 1.62	146.5 ± 2.87[b]
A-20	68.9 ± 1.07[c]	127.4 ± 1.18	154.0 ± 2.88[c]

[1]B-20 represents 20 min boiling. A-5, A-10, A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

[2]Letters not in common denote statistical significance ($P \leq 0.05$).

pressure cooked samples differed significantly from each other.

Water absorption (Table 5) was evaluated at the end of the ten hour soaking period (25°C) and upon completion of cooking. Beans at the completion of the soak period increased an average of 127%, with no differences ($P \leq 0.05$) due to treatment. The type of heat treatment significantly influenced percent water absorption. The value for A-20 is significantly higher with treatment A-5 having the lowest value. B-20 was not significantly different from A-5 or A-10. The higher value of A-20 in the pressure heat treatment comparisons is likely to be due to the longer cooking time, which gave more time for water absorption (Junek et al., 1980).

Microstructure Appearance

An evaluation of a number of scanning electron micrographs (SEM) for each treatment is shown by the representative photographs in Figure 1. The SEM of the raw soybean cotyledon revealed the cell structure because of their slight rigidity. As noted by Figure 1A, the cell contains round bodies. These are likely to be either protein bodies emeshed in a protein network or starch or lipid. The SEM's appear similar to those of soybeans described by Wolf and Baker (1972; 1980). A comparison of treatment in the current experiment would indicate that soaking and increasing the cooking stress decreased the definition of the soybean cell structure. Note the insert in the SEM's from Figure 1A to 1E. The protein network ridges surrounding the protein bodies are apparent in 1A whereas they are almost absent in 1F. Wolf and Baker (1972) indicated that these ridges likely enclosed oil-containing

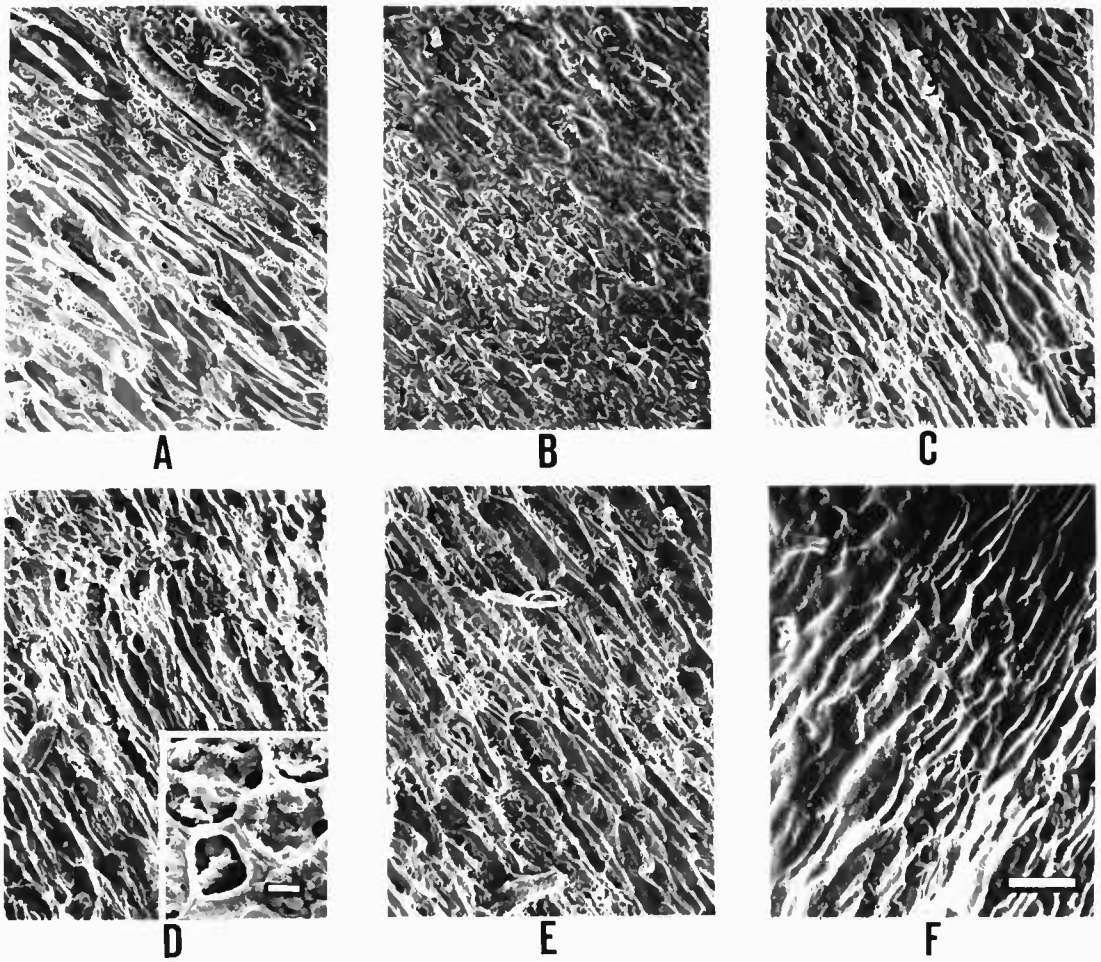

Figure 1. Microstructure appearance of longitudinal cross section of raw, soaked, and cooked soybeans (150X magnification; with molecule size: 100 μm).

A. SEM of raw soybean.
B. SEM of soaked soybean (10 hr at 25°C).
C. Boiled soybean (B-20).
D. Autoclaved soybean (A-5); with insertion of 1000X magnification, with molecule size: 10 μm.
E. Autoclaved soybean (A-10).
F. Autoclaved soybean (A-20), with molecule size: 100 μm.

spherosomes. The meshlike network on the protein bodies is also present in the current experiment.

Interrelationships of Objective Tests

Many studies have related the influence of nutrient retention and the treatment variable or stress. For example, the effect of heat upon vitamin retention is well documented (Hein and Hutchings, 1974; De Ritter, 1976). A review of the literature would indicate only a minor portion of the investigations have evaluated the extent of the relationships or influence of secondary factors to vitamin retention. This thesis investigation, using a covariance type of analysis, has attempted to evaluate the interactive effect or associative causative relationships of texture or color changes, trypsin inhibitor inactivation or moisture or water absorption changes to vitamin B-6 or folacin retention. These interrelationships are defined in tabular (Table 6; Appendix, Table 25-52) and figure (Figures 2-4) forms.

Texture had a significant negative ($P \leq 0.05$) relationship to the total folacin content (Figure 2). Although not significant, texture values and free folacin or vitamin B-6 were positively related (Figure 2). A possible critical factor in this relationship was the correlation (Table 6, $P \leq 0.05$) between texture and water absorption. Texture values, indicating decreased firmness, decreased as water absorption and moisture content increased. Total folacin content was significantly ($P \leq 0.05$) increased with increasing water absorption (Figure 3). Both free folacin and vitamin B-6 tended to decrease as percent water absorption and moisture content increased (Figure 3).

TABLE 6. Some relationships between the physical and the nutritional evaluations of the cooked soybeans, as affected by four different heating treatments.

Independent variable (X)	Dependent variable (Y)	Equation of the regression[a]	t-values of the X
Water absorption after cooking	Color:		
	Blue value	-7.39 + 0.15x	1.937*
	Green value	12.20 + 0.13x	0.958
	Amber value	18.70 + 0.11x	0.851
	Texture	702.51 + 4.00x	-0.761
	Trypsin inhibitor activity	3062.12 - 15.13x	-1.775
Moisture content	Trypsin inhibitor activity	4515.22 - 54.83x	-2.345*
Color:			
Blue value	Vitamin B-6	4430.35 - 19.97x	-0.066
	Total folacin	1282.49 + 22.97x	0.197
	Free folacin	-1683.54 + 197.00x	1.573
Green value	Vitamin B-6	7640.19 - 114.27x	-0.572
	Total folacin	2638.00 - 32.68x	-0.422
	Free folacin	-2496.91 + 124.22x	1.472
Amber value	Vitamin B-6	9304.78 - 151.36x	-0.713
	Total folacin	1620.72 + 0.36x	0.004
	Free folacin	-3554.30 + 142.52x	1.600

[a]Equation line: $y = \mu + bx$

*Significant at $P \leq 0.05$.

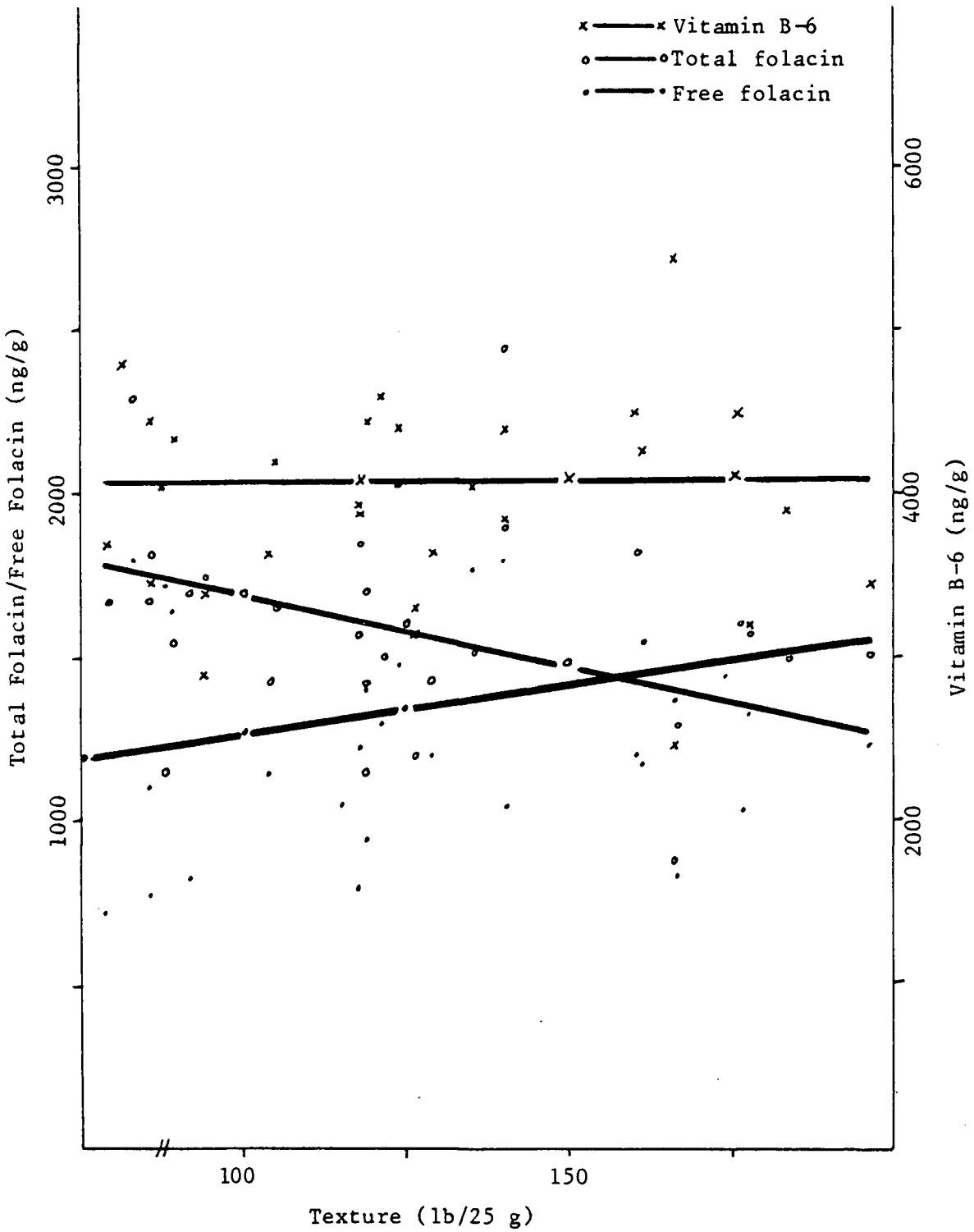

Figure 2. Relationships of vitamin B-6, total folacin and free folacin to texture of cooked soybeans.

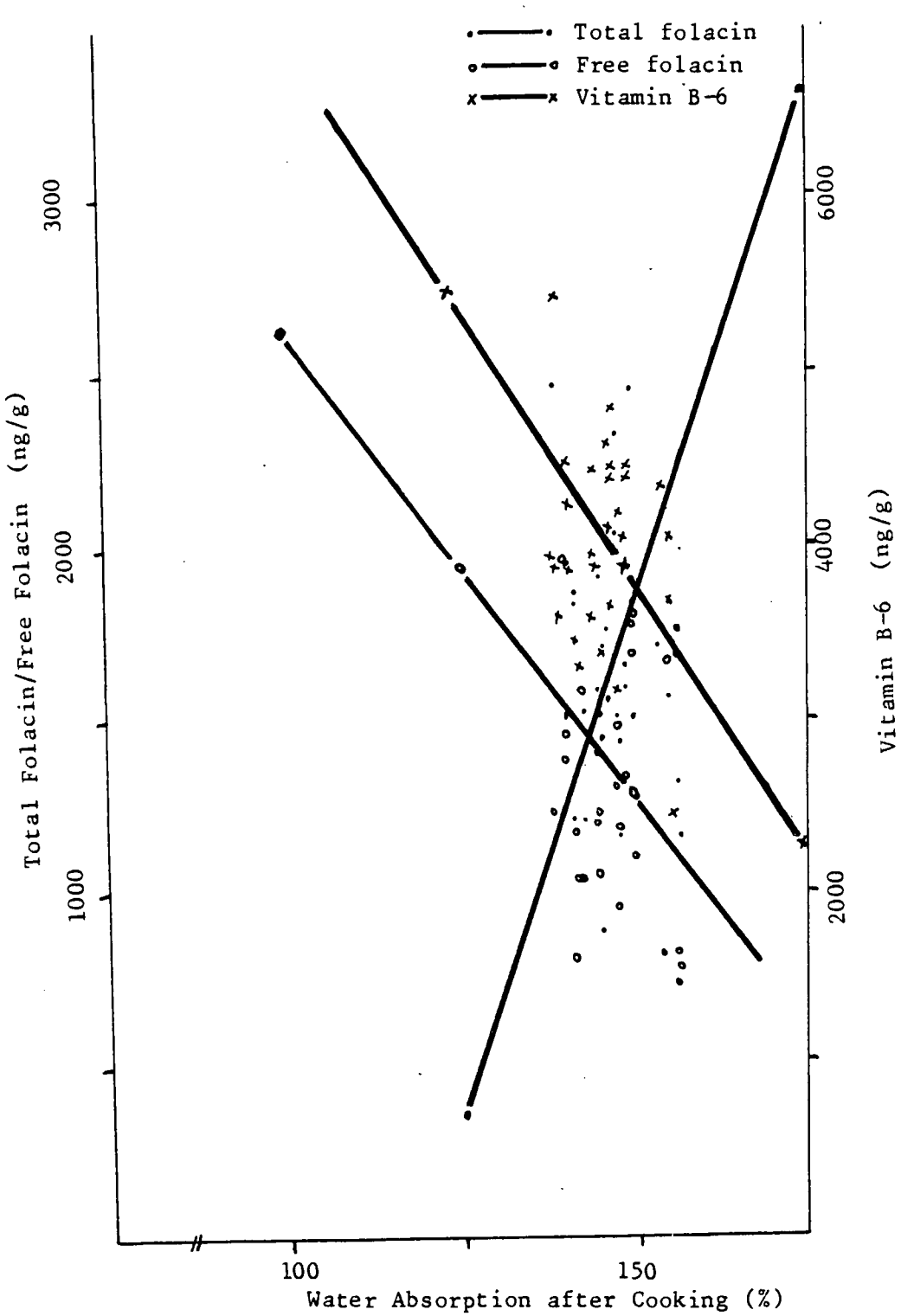

Figure 3. Relationships of vitamin B-6, total folacin and free folacin to the percent water absorption after cooking.

These relationships may occur due to the disruption of the soybean cell integrity. As the cell's integrity decreases, it is likely to be more easily sheared, indicating a more tender product. Additionally, with possible loss of integrity the cell exudes more of the free folacin and vitamin B-6 and/or makes it more susceptible to heat destruction and/or to more easy analysis.

Analysis of the relationship of folacin or vitamin B-6 retention and color appeared to show a relationship (Table 6). With the exception of the green values to total folacin content, all color values (blue, green, amber) had a positive relationship to total and free folacin. As the color values increased, the free and total folacin values also increased. This generally positive relation between color values and folacin could possibly be attributed to the relationship of color to water absorption after cooking (Table 6). The negative relationship between color values and vitamin B-6 retention also appeared; however, there is no statistical significance. Compared to that of green or amber values, the blue value has less effect on the regression of vitamin B-6. The blue values were evaluated to have a significant ($P \leq 0.05$) relationship with percent water absorption. Although not significant, the other two color values, green and amber, also had a positive relationship. Blue values generally are considered to be analogus to hue in perception. Thus, the significance of blue values to water absorption is expected.

Free folacin has a significant ($P \leq 0.05$) negative relationship with TIA (Figure 4). Evidently, the more moisture in the beans, the more media available for increasing the effectiveness to inactivate the TI (Table 6). As shown in Figure 4, there also is a negative

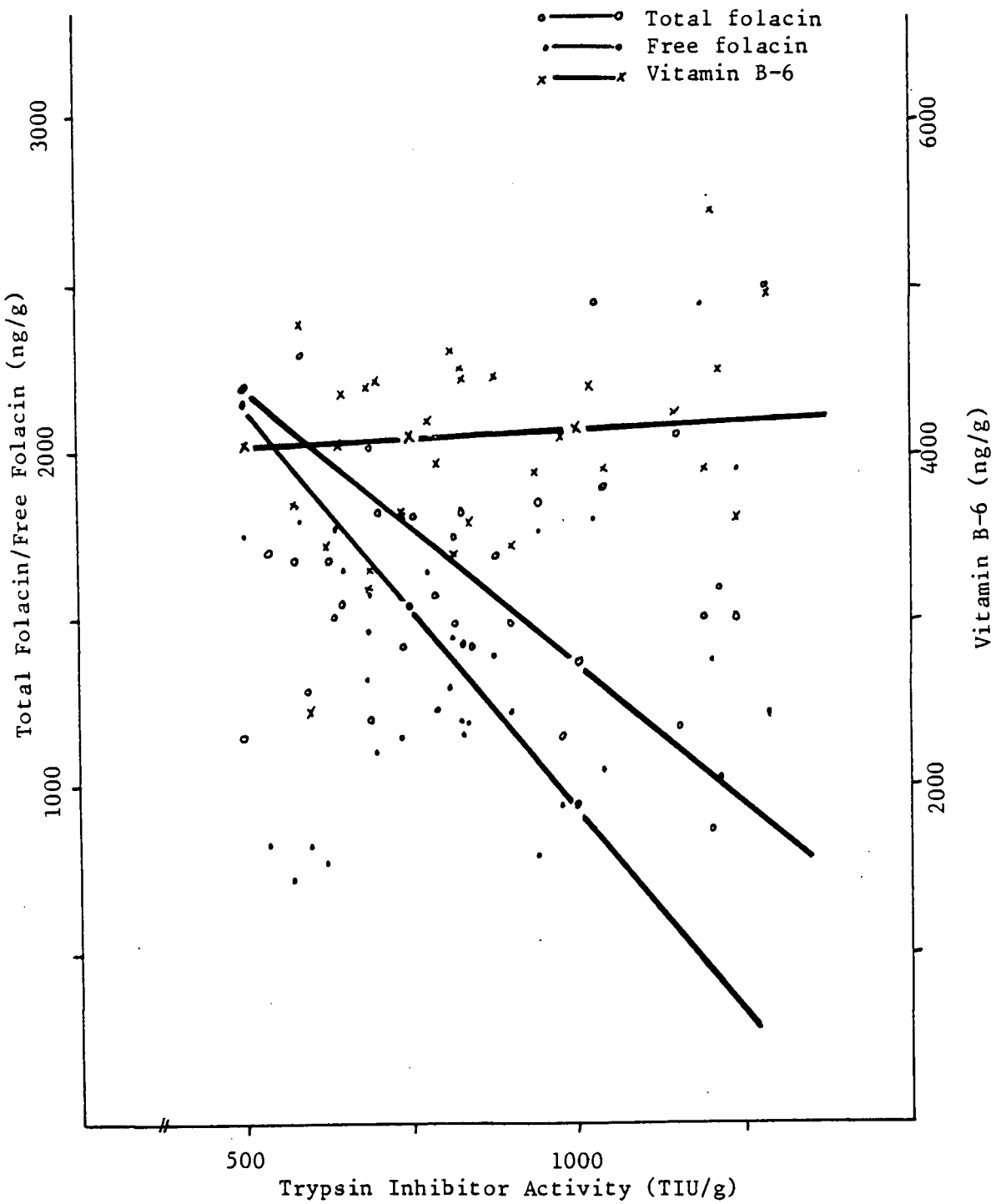

Figure 4. Relationships of vitamin B-6, total folacin and free folacin to the trypsin inhibitor activity of the cooked soybeans.

relationship between TIA with free and total folacin contents; in this case, free folacin has significant ($P \leq 0.05$) negative relationship to TIA. On the other hand, vitamin B-6 (Figure 4) is not significantly ($P \leq 0.05$) increased as the TIA increased; however, there is tendency to have positive relationship.

FUTURE RESEARCH

The four different heating treatments did not statistically influence the vitamin B-6 or folacin retention in the soybeans. The leaching out of both vitamins to the cooking water did indicate a significant ($P \leq 0.05$) influence of treatment.

As the percent water absorption after cooking increased, the vitamin B-6 and free folacin decreased. Total folacin was observed to increase ($P \leq 0.05$) by increasing the water absorption after cooking.

However, vitamin B-6 and total and free folacin were observed to have a number of relationships with the other objective tests. With all these relationships, it must be recognized that they only indicate that the values are correlated. They could be interrelated or could only be similarly influenced by an identical stress.

Future research should focus on attempting to discriminate as to the causative interrelationships of the many factors functioning in nutrient retention. This might further define some of the mechanisms of vitamin destruction in complex food products.

REFERENCES

Albrecht, W. J., Mustakas, G. C., and McGhee, J. E. 1966. Rate studies atmospheric steaming and immersion cooking of soybeans. Cereal Chemistry 43:400.

Aspinall, G. O., Begbie, R., and McKay, J. E. 1967. Polysaccharide components of soybeans. Cereal Science Today 12(6):223.

Bailey, L. H., Capen, R. G., and LeClerc, J. A. 1935. The composition and characteristics of soybeans, soybean flour, and soybean bread. Cereal Chemistry 12(5):441.

Bair, C. W., and Snyder, H. E. 1980. Ultrastructural changes in soybeans during processing to yield desolventized-toasted meal. J. Food Science 45:529.

Bils, R. F., and Howell, R. W. 1963. Biochemical and cytological changes in developing soybean cotyledons. Crop Science 3:304.

Boonvisut, S., and Whitaker, J. R. 1976. Effect of heat, amylase, and disulfide bond cleavage on the in vitro digestibility of soybean proteins. J. Agriculture and Food Chemistry 24(6):1130.

Burr, H. K., Kon, S., and Morris, H. J. 1968. Cooking rates of dry beans as influenced by moisture content and temperature and time of storage. Food Technology 22:336.

Butterfield, S., and Calloway, D. H. 1972. Folacin in wheat and selected foods. J. American Dietetic Association 60:310.

Collins, F. I., and Sedgwick, V. E. 1959. Fatty acid composition of several varieties of soybeans. J. American Oil Chemistry Society 36:641.

Collins, J. L., and Beaty, B. F. 1980. Heat inactivation of trypsin inhibitor in fresh green soybeans and physiological responses of rats fed the beans. J. Food Science 45:542.

Daoud, H. N., Luh, B. S., and Miller, M. W. 1977. Effect of blanching, EDTA and $NaHSO_3$ on color and vitamin B-6 retention in canned garbanzo beans. J. Food Science 42(2):375.

De Ritter, E. 1976. Stability characteristics of vitamin in processed foods. Food Technology 30:48.

Federer, W. T. 1955. Experimental Design. First Edition, MacMillan Company, New York, NY, p. 415.

Gregory, J. F., and Kirk, J. R. 1978. Assessment of roasting effects on vitamin B-6 stability and bioavailability in dehydrated food systems. J. Food Science 43:1585.

Goodman, R. M. 1976. Expanding the use of soybeans. International Soybean Program. Series No. 10. International Agricultural Publications, College of Agriculture, University of Illinois at Urbana. Champaign, IL. p. 122-154.

Hahn, D. M., Jones, F. T., Akhawan, I., and Rockland, B. 1977. Light and scanning electron microscope studies on dry beans: intercellular gelatinization of starch in cotyledon of large lima beans (P. Lunatus). J. Food Science 42:1208.

Harris, R. S., and Karmas, E. 1975. Nutritional Evaluation of Food Processing. AVI Publishing Company., Incorporated, Westport, Connecticut.

Hebbler, S. W. 1979. Advanced Statistical Analysis (For level II Basic TRS-80 Microcomputer System). Radio Shack, Tandy Corporation. Fort Worth, Texas 76102.

Hein, R. E., and Hutchings, I. J. 1974. Influence of processing on vitamin-mineral content and bioavailability in processed foods. IN: "Nutrients in Processed Foods". American Medical Association. Publishing Sciences Group, Inc., Massachusetts. p. 59.

Herbert, V., and Bertino, J. R. 1967. Folic Acids. IN: "The Vitamin Chemistry, Physiology, Pathology, Methods," vol. 7, second edition. p. 243. P. Gyorgy and W. N. Pearson. Academic Press, NY.

Holmes, Z. A., Miller, L. T., Edwards, M., and Benson, E. 1979. Vitamin retention during home drying of vegetables and fruits. Home Economic Research J. 7:258.

Holmes, Z. A., and Soeldner, A. 1981. Macrostructure of selected raw starches and selected heated starch dispersions. J. American Dietetic Association 78(2):153.

Hurdle, A. D. P., Barton, D., and Searles, I. H. 1968. A method for measuring folate in food and its application to a hospital diet. American J. Clinical Nutrition 21(10):1202.

Hymowitz, T. 1976. Extraction of oligosaccharides during cooking of whole soybeans. J. Food Science 41:361.

Islam, M. I., and Lea, R. A. 1979. Effect of maturity on the nutrient content and the canning quality of Emerald soybean. J. Food Science 44:204.

Jansen, G. R., Harper, J. M., and O'Deen, L. A. 1978. Nutritional evaluation of full-fat soy flour produced by dry heat roasting. J. Food Science 43:1350.

Junek, J. J., Sistrunk, W. A., and Neely, M. B. 1980. Influence of processing methodology on quality attributes of canned dry bean. J. Food Science 45:821.

Kabir, H. 1981. Personal Communication. Unpublished Data. Foods and Nutrition Department, Oregon State University, OR 97331.

Kakade, M. L., Rackis, J. J., McGhee, J. E., and Puski, G. 1974. Determination of trypsin inhibitor activity of soy product: a Collaborative analysis of an improved procedure. Cereal Chemistry 51:376.

Kakade, M. L., Simons, N., and Leiner, I. E. 1969. An evaluation of natural vs. synthetic substrates for measuring the antitryptic activity of soybean samples. Cereal Chemistry 46:518.

Kon, S., Brown, A. H., Ohanneson, J. G., and Booth, A. N. 1973. Split peeled beans: Preparation and some properties. J. Food Science 38:496.

Kon, S. 1979. Effect of soaking temperature on cooking and nutritional quality of beans. J. Food Science 44:1329.

Krober, O. A., and Cartter, J. L. 1966. Relation of methionine content to protein levels in soybeans. Cereal Chemistry 43:320.

Krivoruchco, D., Kaba, H., Sambucetti, M. E., and Sanahuja, J. C. 1979. Maturation time and some seed composition characters affecting nutritive value in soybean varieties. Cereal Chemistry 56(4):217.

Ku, S., Wei, L. S., Steinberg, M. P., Nelson, A. I., and Hymowitz, T. 1976. Extraction of oligosaccharides during cooking of whole soybeans. J. Food Science 41:361.

Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. J. General Physiology 30:291.

Kunitz, M. 1946. Crystalline soybean trypsin inhibitor. J. General Physiology 29:149.

Kylen, A. M., and McCready, R. M. 1975. Nutrients in seeds and sprouts of alfalfa, lentils, mung beans and soybeans. J. Food Science 40:1008.

Leichter, J., Switzer, V. P., and Landymore, A. F. 1978. Effect of cooking on folate content of vegetables. Nutrition Report International 18(4):475.

Lineback, D. R., and Ke, C. H. 1975. Starches and low-molecular-weight carbohydrates from chickpea and horse bean flours. Cereal Chemistry 52:334.

Markley, K. S. 1950. Soybeans and Soybean Products. Interscience Publisher, Incorporated, NY. p. 142.

Markley, K. S., and Goss, W. H. 1944. Soybean Chemistry and Technology Chemical Publishing Company, Incorporated. Brooklyn, NY.

McArthur, W. C. 1980 October. Soybean Production Practices and Costs in the United States. Research Report 360. Agricultural Experiment Station, University of Georgia, Athens, Georgia 30602.

Miller, C. F., Guadagni, D. G., and Kon, S. 1973. Vitamin retention in bean products: cooked, canned and instant bean powders. J. Food Science 42:25.

Miller, L. T. 1981. Personal Communication. Unpublished Data. Foods and Nutrition Department, Oregon State University, OR 97331.

Naivikul, O., and D'Appolonia, B. L. 1979. Carbohydrates of legume flours compared with wheat flour. II. Starch. Cereal Chemistry 56(1):24.

National Research Council, Committee on Food Protection. 1973. Toxicants Occurring Naturally in Foods. National Academy of Sciences, Washington, D. C. p. 109.

Nelson, A. I., Steinberg, M. P., and Wei, L. S. 1978. Whole soybean foods for home and village use. International Agricultural Publications. IN: "Soy Series," No. 14. Urbana, IL.

Niess, D. G. 1980. Personal Communication. December 16. Computer Center, Oregon State University, OR 97331.

Omosaiye, O., and Cheryan, M. 1979. Ultrafiltration of soybean water extracts: Processing characteristics and yields. J. Food Science 44:1027.

Perera, A. D., Leklem, J. E., and Miller, L. T. 1979. Stability of vitamin B-6 during bread making and storage of bread and flour. Cereal Chemistry 56:577.

Perloff, B. P., and Butrum, R. R. 1977. Folacin in selected foods. J. American Dietetic Association 70:161.

Perry, A. K., Peters, C. R., and Van Duyne, F. O. 1976. Effect of variety and cooling method on cooling times, thiamin content and palatability of soybeans. J. Food Science 41:1330.

Raab, D. A., Luh, B. S., and Schweigh, B. S. 1973. Effects of heat processing on the retention of vitamin B-6 in lima beans. J. Food Science 38:544.

Rackis, J. J. 1966. Soybean trypsin inhibitor: their activation during meal processing. Food Technology 20:1482.

Rackis, J. J., and McGhee, J. E. 1975. Biological threshold levels of soybean trypsin inhibitors by rats bioassay. Cereal Chemistry 52:85.

Rockland, L. B., and Jones, F. T. 1974. Scanning electron microscope studies on dry beans. Effect of cooking on the cellular structure of cotyledons in rehydrated large lima beans. J. Food Science 39:342.

Rockland, L. B., Jones, F. T., and Hahn, D. M. 1977. Light and scanning microscope studies on dry beans: Extracellular gelatinization of lima bean starch in water and a mixed salt solution. J. Food Science 42(5):1204.

Rockland, L. B., Miller, C. F., and Hahn, D. M. 1977. Thiamine, pyridoxine, niacin, and folacin in quick-cooking beans. J. Food Science 42:25.

Sabin, D. R. 1961. Implementation of the WHO/FAO/UNICEF Protein Rich Foods Program. IN: Conference on Soybean Products for Protein in Human Foods. Peoria, IL.

Saio, K., Watanabe, T., and Arai, K. 1973. Fine structure of soybean seed coat and its changes on cooking. Cereal Science Today 18(7):197.

Schmitt, B. A. 1979. Protein, Calories and Development. Westview Press/Boulder, CO. p. 39-74.

Sefa-Dedeh, S., Stanley, D. W., and Voisey, P. W. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas (Vigna unquilata). J. Food Science 43:1832.

Sefa-Dedeh, S., Stanley, D. W., and Voisey, P. W. 1979. Effect of storage time and conditions on the hard-to-cook defect in cowpeas (Vigna unquilata). J. Food Science 44:790.

Sefa-Dedeh, S., and Stanley, D. W. 1979. Textural implications of the microstructure of legumes. Food Technology 33(10):77.

Sgarbieri, V. C., Garruti, R. S., Moraes, M. A. C., and Hartman, L. 1978. Nutritional and sensory evaluation of mixtures of soybean (Glycine max L.) and common bean (Phaseolus vulgaris L.), for direct use as human food. J. Food Science 43:208.

Sherman, W. C., and Salmon, W. D. 1939. Carotene content of different varieties of green and mature soybeans and cowpeas. Food Research 4:371.

Steggerda, F. R., Shimizu, T., Anderson, J., and Pearl, S. L. 1970. Soybean factors relating to gas production by intestinal bacteria. J. Food Science 35:634.

Storvick, C. A., Benson, E. M., Edwards, M. A., and Woodring, M. J. 1964. Chemical and microbiological determination of vitamin B-6. IN: Methods of Biochemical Analysis. Vol. XII. Palo Alto, CA. p. 183.

Street, J. P., and Bailey, E. M. 1915. The carbohydrates and the enzymes of the soybean. Industrial Engineering Chemistry 7:853.

Surjadi, A. 1980. Personal Communication. November 17. Civil Engineering Department, Oregon State University, Corvallis, OR. 97331.

Wang, H. L., Swain, E. W., Hesseltine, C. W., and Heath, H. D. 1979. Hydration of whole soybeans affects solids losses and cooking quality. J. Food Science 44:1510.

Watt, B. K., and Merrill, A. L. 1975. Composition of Foods. Raw, processed, prepared. Agriculture Handbook No. 8. U.S.D.A.

Willet, J. W. 1976. Research to meet U.S. and world food needs. IN: The World Food Situation. Problems and Prospects to 1985. Oceana Publications, Incorporated. Dobbs Ferry, NY. p. 857.

Wilson, L. A., Birmingham, V. A., Moon, D. P., and Snyder, H. E. 1978. Isolation and characterization of starch from mature soybeans. Cereal Chemistry 55(5):661.

Wolf, W. J., and Baker, F. L. 1972. Scanning electron microscopy of soybeans. Cereal Science Today 17:125.

Wolf, W. J., and Baker, F. L. 1980. Scanning electron microscopy of soybeans and soybean protein products. Scanning Electron Microscopy iii:621.

Wu, Y. V., and Scheraga, H. A. 1962. Studies of soybean trypsin inhibitor. I. Physiochemical properties. Biochemistry 1(4):698.

APPENDICES

Table 1. Vitamin B-6 (ng/g wet basis) in cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	1189.2	1314.7	1544.6	2251.5
2	1345.8	1488.9	1426.4	1046.4
3	1308.4	1608.7	1192.4	1343.9
4	1288.6	1292.0	1404.1	1221.2
5	1445.2	1901.9	1532.7	801.1
6	1342.3	1450.8	1110.5	1053.5
7	1458.0	1366.3	1575.6	1138.3
8	1115.3	1407.3	1202.8	1285.5

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 2. Vitamin B-6 (ng/g dry basis) in cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	3609.1	3912.8	4617.6	7593.6
2	4441.6	4385.6	4457.5	3453.5
3	3941.0	4525.2	3646.5	4355.2
4	3893.0	3574.0	4191.3	4030.4
5	4392.7	5457.4	4521.2	2457.4
6	4110.3	4242.1	3375.4	3216.8
7	4445.1	3914.9	4796.4	3707.8
8	3314.4	3936.5	3466.3	4035.1

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 3. Vitamin B-6 (ng/g wet basis) in cooking water as inluenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	913.5	540.0	857.6	948.9
2	442.9	684.2	617.6	1071.4
3	1062.1	543.0	645.6	575.2
4	828.2	367.5	519.2	658.7
5	1195.0	551.1	616.0	725.7
6	661.1	541.5	327.0	591.0
7	1278.8	331.7	685.5	502.1
8	1038.1	466.6	462.9	458.5

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclve time of 5, 10, or 20 min, respectively.

Table 4. Vitamin B-6 (ng/g wet basis) in soaking water.

REPLICATION	B-20	A-5	A-10	A-20
1	104.4	104.4	101.3	88.0
2	96.1	101.3	88.0	96.1
3	101.4	104.1	101.4	97.7
4	104.1	97.7	89.1	94.5
5	89.1	103.4	98.0	103.4
6	94.5	98.0	117.4	117.4
7	97.0	101.1	120.0	104.0
8	97.0	101.1	120.0	104.0

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 5. Free folacin (ng/g wet basis) and total folacin (ng/g wet basis) in cooked soybeans as influenced by four heating treatments.

REPLICATION	Free folacin B-20	A-5	A-10	A-20	Total folacin B-20	A-5	A-10	A-20
1	391.40	351.40	430.50	246.60	469.50	640.60	501.10	505.30
2	423.10	614.70	348.10	229.00	518.10	827.60	587.20	504.10
3	407.40	362.00	373.20	510.90	522.60	571.40	462.90	478.60
4	266.10	704.70	549.00	529.40	608.20	540.70	553.60	348.40
5	487.40	479.60	408.80	272.10	664.50	303.40	616.60	426.50
6	315.80	402.70	475.80	431.30	379.90	526.00	580.00	512.70
7	376.50	853.50	593.60	220.30	466.20	526.70	755.90	559.60
8	531.60	440.40	428.00	547.10	405.10	892.70	515.90	481.00

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 6. Free folacin (ng/g dry basis) and total folacin (ng/g dry basis) in cooked soybeans as influenced by four heating treatments.

REPLICATION	Free folacin B-20	A-5	A-10	A-20	Total folacin B-20	A-5	A-10	A-20
1	1187.9	1045.8	1287.0	828.3	1424.9	1906.6	1498.1	1704.2
2	1396.4	1810.6	1087.8	755.8	1709.9	2437.7	1835.0	1663.7
3	1227.1	1018.3	1141.3	1656.1	1574.1	1607.3	1415.6	1551.4
4	803.9	1949.4	1638.8	1747.2	1837.5	1495.7	1652.5	1149.8
5	1481.5	1376.2	1205.9	834.7	2019.8	870.6	1818.9	1308.3
6	959.9	1177.5	1446.2	1317.0	1154.7	1538.0	1762.9	1565.5
7	1147.9	2445.6	1807.0	717.6	1421.3	1509.1	2301.1	1822.8
8	1579.8	1231.9	1233.4	1777.7	1203.9	2497.1	1486.7	1510.2

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 7. Free folacin (ng/g wet basis) and total folacin (ng/g wet basis) in cooking water as influenced by four heating treatments.

REPLICATION	Free folacin B-20	A-5	A-10	A-20	Total folacin B-20	A-5	A-10	A-20
1	237.30	129.70	112.80	141.90	282.50	240.80	188.10	243.20
2	216.90	123.30	143.40	169.00	373.50	178.50	232.70	374.10
3	198.60	105.90	163.40	142.80	200.70	569.70	193.20	293.80
4	170.10	85.90	129.40	167.60	312.50	226.60	266.10	319.30
5	215.90	145.30	127.40	161.50	562.40	179.30	312.00	211.50
6	106.20	98.00	108.30	108.00	239.60	263.50	212.80	366.60
7	214.90	105.60	145.70	330.20	390.70	234.60	218.10	566.00
8	179.80	88.60	70.70	147.10	414.90	235.10	202.00	403.50

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 8. Free folacin (ng/g wet basis) and total folacin (ng/g wet basis) in soaking water.

REPLICATION	Free folacin B-20	A-5	A-10	A-20	Total folacin B-20	A-5	A-10	A-20
1	47.90	47.90	47.00	47.50	182.4	182.4	232.9	174.6
2	46.40	47.00	47.50	46.40	151.5	232.9	174.6	151.5
3	63.90	47.60	63.90	80.00	149.4	105.8	149.4	228.5
4	47.60	80.00	79.80	80.50	105.8	228.5	190.0	229.9
5	79.80	74.40	54.60	74.40	190.0	212.6	123.6	212.6
6	80.50	54.60	54.70	54.70	229.9	123.6	243.0	243.0
7	68.40	50.70	84.60	49.80	234.5	235.5	241.8	170.8
8	68.40	50.70	84.60	49.80	234.5	235.5	241.8	170.8

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 9. Trypsin inhibitor activity (TIU/g) in cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	842.5	1041.1	808.2	541.9
2	868.3	1028.5	700.3	628.5
3	790.2	1216.8	742.9	549.1
4	942.3	1237.8	771.6	497.0
5	685.4	1197.7	822.0	604.6
6	977.6	1147.5	807.9	685.7
7	830.2	1192.6	583.6	580.2
8	687.6	1285.7	895.6	633.1

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min.

Table 10. Texture measurement (lb/25 g) of cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	128.8	139.8	120.7	66.7
2	120.3	132.9	59.7	60.6
3	117.4	176.2	103.4	89.2
4	117.1	-	105.5	87.1
5	123.9	166.7	160.5	135.1
6	118.5	161.8	94.3	177.7
7	118.3	184.3	83.1	78.5
8	126.4	-	195.7	134.7

B-20 represents 20 min boiling. A-5, A-10, or A20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 11. Blue color values (% reflectance) of cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	15.6	15.5	15.7	13.6
2	16.3	15.7	15.4	14.1
3	16.1	15.0	14.8	15.3
4	16.2	14.6	15.3	14.8
5	15.3	15.3	15.3	15.6
6	15.7	15.9	15.4	15.7
7	16.0	15.2	16.3	15.4
8	15.3	12.4	14.2	14.3

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, 20 min, respectively.

Table 12. Green color values (% reflectance) of cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	32.4	31.5	32.2	27.1
2	33.1	32.1	29.9	28.0
3	32.3	29.9	29.5	30.8
4	31.4	32.7	30.3	29.0
5	30.8	30.6	31.4	30.7
6	31.5	32.5	30.2	32.3
7	32.0	31.7	32.0	29.3
8	31.2	28.1	29.4	28.3

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 13. Amber color values (% reflectance) of cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	37.0	35.0	35.1	30.2
2	36.9	36.0	33.0	31.7
3	35.4	32.7	33.1	33.8
4	34.8	35.8	33.8	32.8
5	34.3	34.0	35.3	34.0
6	34.8	35.9	33.9	36.0
7	34.8	35.0	35.6	33.0
8	34.3	32.3	33.0	31.7

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 14. Moisture content (%) of cooked soybeans as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	67.0	66.4	66.5	70.4
2	69.7	66.0	68.0	69.7
3	66.8	64.4	67.3	69.2
4	66.9	63.8	66.5	69.7
5	67.1	65.2	66.1	67.4
6	67.1	65.8	67.1	67.2
7	67.2	65.1	67.2	69.3
8	66.4	64.2	65.3	68.2

B-20 represents 20 min boiling. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 15. Water absorption (%) after soaking.

REPLICATION	B-20	A-5	A-10	A-20
1	124.6	125.2	125.8	125.3
2	126.7	126.6	125.3	125.5
3	127.3	127.0	127.0	127.1
4	127.0	127.7	126.4	127.7
5	126.5	127.9	127.4	127.7
6	127.2	127.4	130.2	129.4
7	127.5	127.3	128.8	128.1
8	127.5	125.4	128.0	127.8

B-20 represents 20 min boiling time. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min respectively.

Table 16. Water absorption (%) after cooking as influenced by four heating treatments.

REPLICATION	B-20	A-5	A-10	A-20
1	145.0	144.4	147.0	153.7
2	145.3	144.9	150.3	155.7
3	145.4	141.6	147.0	154.9
4	141.4	140.3	149.1	156.7
5	148.1	140.2	141.6	156.6
6	147.0	141.6	146.8	148.8
7	146.9	139.7	147.8	156.0
8	141.9	138.5	142.2	149.9

B-20 represents 20 min boiling time. A-5, A-10, or A-20 represents an autoclave time of 5, 10, or 20 min, respectively.

Table 17. Analysis of variance for water absorption (%) after cooking.

Source of variation	d.f	Mean square	F-value
Regression:	19	36316.19	4679.57*
Mean	1	689226.00	88811.19*
Blocks	15	7.06	0.91
Treatments	3	100.26	12.92*
Error	13	7.76	

*Significant at ($P \leq 0.05$).

Table 18. Analysis of variance for blue, green and amber color values (% reflectance).

Source of variation	d.f	Mean square	F-value
Blue values			
Regression:	19	392.80	506.28*
Mean	1	7451.15	9603.67*
Blocks	15	0.49	0.63
Treatments	3	1.11	1.43
Error	13	0.78	
Green values			
Regression:	19	1595.90	914.81*
Mean	1	30170.30	17351.66*
Blocks	15	1.81	1.04
Treatments	3	6.08	3.49*
Error	13	1.74	
Amber values			
Regression:	19	1975.10	1295.72*
Mean	1	37469.53	24581.03*
Blocks	15	2.21	1.45
Treatments	3	6.46	4.24*
Error	13	1.52	

*Significant at ($P \leq 0.05$).

Table 19. Analysis of variance for moisture content (%), and texture (lb/25 g).

Source of variation	d.f	Mean square	F-value
Moisture content			
Regression:	19	7565.72	8472.43*
Mean	1	143674.80	-
Blocks	15	1.05	1.18
Treatments	3	6.19	6.93*
Error	13	0.89	
Texture			
Regression:	19	24510.42	9.10*
Mean	1	431265.06	160.05*
Blocks	15	2231.68	0.83
Treatments	3	604.10	0.22
Error	13	2694.52	

*Significant at ($P \leq 0.05$).

Table 20. Analysis of variance for vitamin B-6 (ng/g), on wet and dry weight basis, in cooked soybeans and cooking water.

Source of variation	d.f	Mean square	F-value
In the beans on wet basis			
Regression:	19	3163587.60	40.02*
Mean	1	59007545.00	746.79*
Blocks	15	589692.11	0.74
Treatments	3	6189.36	0.78
Error	13	79015.32	
In the beans on dry basis			
Regression:	19	29250008.00	34.05*
Mean	1	54662540.00	634.16*
Blocks	15	725403.64	0.84
Treatments	3	338499.77	0.39
Error	13	858871.55	

Table 20. (Continued).

Source of variation	d.f	Mean square	F-value
In cooking water			
Regression:	19	858200.49	31.14*
Mean	1	14727793.00	535.15*
Blocks	15	51619.74	1.88
Treatments	3	157855.00	5.74*
Error	13	27520.83	

*Significant at ($P \leq 0.05$).

Table 21. Analysis of variance for total and free folacin (ng/g),on wet or dry weight basis, in cooked soybeans and in cooking water.

Source of variation	d.f	Mean square	F-value
Total folacin:			
In the beans on wet basis			
Regression:	19	504784.34	35.38*
Mean	1	930163.15	65.20*
Blocks	15	13795.91	0.96
Treatments	3	3882.81	0.27
Error	13	14265.71	
In the beans on dry basis			
Regression:	19	4605350.00	36.11*
Mean	1	85330455.00	669.08*
Blocks	15	125804.60	0.97
Treatments	3	21372.42	0.17
Error	13	127553.36	
In cooking water			
Regression:	19	164172.18	23.09*
Mean	1	2825005.10	397.30*
Blocks	15	13890.20	1.95
Treatments	3	6038.83	0.85
Error	13	7110.51	

Table 21. (Continued).

Source of variation	d.f	Mean square	F-value
Free folacin:			
In the beans on wet basis			
Regression:	19	340987.33	18.93*
Mean	1	6126750.10	340.19*
Blocks	15	16233.72	0.90
Treatments	3	14432.69	0.80
Error	13	18009.92	
In the beans on dry basis			
Regression:	19	3076901.90	17.48*
Mean	1	55972168.00	317.90*
Blocks	15	134471.60	0.76
Treatments	3	66907.48	0.38
Error	13	176065.67	
In cooking water			
Regression:	19	40921.19	19.36*
Mean	1	717362.42	339.36*
Blocks	15	1641.61	0.78
Treatments	3	3445.47	1.63
Error	13	2113.88	

*Significant at ($P \leq 0.05$).

Table 22. The t-values for testing the differences between treatment effects on some physical and nutritional evaluations.

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Vitamin B-6						
Wet basis	-0.770	0.578	0.524	1.349	1.294	-0.054
Dry basis	-0.384	0.685	0.179	1.069	0.563	-0.506
In cooking water	-2.573*	2.733*	-0.511	0.160	-3.084*	-3.244*
Total folacin:						
Wet basis	-0.337	0.502	0.319	0.838	0.656	-0.183
Dry basis	-0.045	0.585	0.105	0.630	0.150	-0.480
In cooking water	-0.105	0.876	-0.704	0.981	-0.600	-1.581
Free folacin:						
Wet basis	-0.972	-0.129	0.560	0.843	1.532	0.689
Dry basis	-0.713	-0.108	0.331	0.606	1.044	0.439
In cooking water	2.091*	1.465	0.751	-0.626	-1.340	-0.714
Trypsin inhibitor activity	-3.811*	0.831	4.572*	4.642*	8.384*	3.742*
Texture	0.195	0.646	-0.114	0.451	-0.310	-0.760
Color:						
Blue values	1.686	1.024	1.866*	-0.662	0.181	0.843
Green values	1.727	2.262*	3.132*	0.535	1.405	0.870
Amber values	2.105*	2.534*	3.436*	0.430	1.332	0.902

Table 22. (Continued).

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A- 5 vs A-20	A-10 vs A-20
Water absorption after cooking	0.711	-1.371	-5.000*	-2.081*	-5.711*	-3.630*
Moisture	2.264*	0.449	-2.264*	-1.815*	-4.527*	-2.713*

*Significant at ($P \leq 0.05$).

Table 23. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by water absorption (%) after soaking.

Source of variation	d.f	Mean square[a]	F-value
In the beans on wet basis			
Regression:	20	3007.3769	37.25*
Mean	1	48.4589	0.60
Blocks	15	59.2538	0.73
Treatments	3	34.5703	0.43
Covariate	1	58.3732	0.72
Error	12	80.7355	
In the beans on dry basis			
Regression:	20	27843.0170	33.23*
Mean	1	976.7957	1.17
Blocks	15	739.6140	0.88
Treatments	3	144.7997	0.17
Covariate	1	1109.9993	1.32
Error	12	837.9442	
In cooking water			
Regression:	20	816.9727	30.25*
Mean	1	37.6948	1.40
Blocks	15	44.4990	1.64
Treatments	3	149.7699	5.54*
Covariate	1	33.6449	1.25
Error	12	27.0105	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 24. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by water absorption (%) after cooking.

Source of variation	d.f	Mean square[a]	F-value
In the beans on wet basis			
Regression:	20	3007.8413	37.62*
Mean	1	124.6315	1.56
Blocks	15	63.1593	0.79
Treatments	3	30.2672	0.38
Covariate	1	67.6624	0.85
Error	12	79.9614	
In the beans on dry basis			
Regression:	20	27808.1950	31.04*
Mean	1	856.3237	0.95
Blocks	15	741.9390	0.83
Treatments	3	317.6963	0.36
Error	12	895.9808	
In cooking water			
Regression:	20	815.8358	28.22*
Mean	1	3.3641	0.12
Blocks	15	46.5279	1.61
Treatments	3	109.5461	3.79*
Covariate	1	10.9070	0.38
Error	12	28.9053	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 25. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by moisture content (%).

Source of variation	d.f	Mean square[a]	F-value
In the beans on wet basis			
Regression:	20	3010.2644	39.65*
Mean	1	73.8265	0.97
Blocks	15	49.5143	0.65
Treatments	3	99.7490	1.31
Covariate	1	116.1241	1.53
Error	12	75.9229	
In the beans on dry basis			
Regression:	20	27894.4040	37.07*
Mean	1	1568.1851	2.09
Blocks	15	526.6756	0.70
Treatments	3	938.3819	1.25
Covariate	1	2137.7469	2.84*
Error	12	752.2986	
In cooking water			
Regression:	20	815.8202	28.20*
Mean	1	18.8838	0.65
Blocks	15	52.3137	1.81
Treatments	3	114.7753	3.97*
Covariate	1	10.5941	0.37
Error	12	28.9314	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 26. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by trypsin inhibitor activity.

Source of variation	d.f	Mean square[a]	F-value
In the beans on wet basis			
Regression:	20	3004.8395	35.37*
Mean	1	201.9932	2.38
Blocks	15	56.2315	0.66
Treatments	3	15.5788	0.18
Covariate	1	7.6264	0.09
Error	12	84.9644	
In the beans on dry basis			
Regression:	20	27787.9170	29.89*
Mean	1	2374.5630	2.55
Blocks	15	557.1875	0.72
Treatments	3	231.6963	0.25
Covariate	1	7.9900	0.01
Error	12	929.7784	
In cooking water			
Regression:	20	815.3208	27.39*
Mean	1	85.6276	2.88
Blocks	15	36.1467	1.21
Treatments	3	94.6132	3.18
Covariate	1	0.6064	0.02
Error	12	29.7637	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 27. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by texture (lb/25 g).

Source of variation	d.f	Mean Square[a]	F-value
In the beans on dry basis			
Regression:	20	27787.5240	29.85*
Mean	1	41057.8280	44.13*
Blocks	15	701.3637	0.75
Treatments	3	333.8247	0.36
Covariate	1	0.1329	0.00
Error	12	930.4331	
In the beans on wet basis			
Regression:	20	3004.4585	35.10*
Mean	1	4422.5552	51.67*
Blocks	15	57.7544	0.68
Treatments	3	61.7834	0.72
Covariate	1	0.0063	0.00
Error	12	85.5994	
In cooking water			
Regression:	20	819.1661	35.08*
Mean	1	1741.3239	74.56*
Blocks	15	56.4706	2.42
Treatments	3	177.0719	7.58
Covariate	1	77.5124	3.32
Error	12	23.3549	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 28. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by blue color values (% reflectance).

Source of variation	d.f	Mean square[a]	F-value
In the beans on wet basis			
Regression:	20	3004.6450	35.23*
Mean	1	117.9961	1.38
Blocks	15	57.5650	0.68
Treatments	3	61.5086	0.77
Covariate	1	3.7351	0.04
Errror	12	85.2887	
In the beans on dry basis			
Regression:	20	27787.7180	29.88*
Mean	1	849.0738	0.91
Blocks	15	710.4954	0.76
Treatments	3	334.4556	0.36
Covariate	1	4.0226	0.00
Error	12	930.1090	
In cooking water			
Regression:	20	816.1844	28.81*
Mean	1	75.4732	2.66
Blocks	15	52.2027	1.84
Treatments	3	162.5971	5.74*
Covariate	1	17.8794	0.63
Error	12	28.3243	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 29. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by green color values (% reflectance).

Source of variation	d.f	Mean square[a]	F-value
In the beans on wet basis			
Regression:	20	3006.0883	35.27*
Mean	1	152.6239	1.84
Blocks	15	52.2019	0.63
Treatments	3	72.6819	0.88
Covariate	1	32.6013	0.39
Error	12	82.8832	
In the beans on dry basis			
Regression:	20	27802.3240	30.70*
Mean	1	1398.4122	1.54
Blocks	15	641.9562	0.70
Treatments	3	426.5861	0.47
Covariate	1	296.1402	0.33
Error	12	905.7658	
In cooking water			
Regression:	20	817.1657	30.62*
Mean	1	89.1583	3.34
Blocks	15	52.1613	1.95
Treatments	3	166.2690	6.23*
Covariate	1	37.5050	1.40
Error	12	26.6888	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 30. Analysis of covariance for vitamin B-6 (ng/g), on wet and dry weight basis, controlled by amber color values (% reflectance).

Source of variation	d.f	Mean square[a]	F-value
In the beans on wet basis			
Regression:	20	3006.9422	36.91*
Mean	1	159.5527	1.96
Blocks	15	47.6937	0.59
Treatments	3	77.6034	0.95
Covariate	1	49.6805	0.61
Error	12	81.4599	
In the beans on dry basis			
Regression:	20	27810.2150	31.16*
Mean	1	1464.4545	1.64
Blocks	15	594.7189	0.67
Treatments	3	465.5983	0.52
Covaraite	1	453.9549	0.51
Error	12	892.6146	
In cooking water			
Regression:	20	817.7160	31.73*
Mean	1	95.1246	3.70
Blocks	15	50.8412	1.97
Treatments	3	170.8635	6.63*
Covariate	1	48.5097	1.88
Error	12	25.7718	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 31. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by water absorption (%) after soaking.

Source of variation	d.f	Mean square[a]	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	479.7515	31.75*
Mean	1	5.2955	0.35
Blocks	15	13.3033	0.88
Treatments	3	5.0720	0.34
Covariate	1	4.1276	0.27
Error	12	15.1106	
In the beans on dry basis			
Regression:	20	4375.3878	31.79*
Mean	1	10.8058	0.08
Blocks	15	116.3738	0.84
Treatments	3	23.3658	0.17
Covariate	1	6.1046	0.04
Error	12	137.6524	
In cooking water			
Regression:	20	155.9901	20.37
Mean	1	0.7694	0.10
Blocks	15	13.8287	1.81
Treatments	3	6.1497	0.80
Covariate	1	0.5309	0.07
Error	12	7.6588	
Free folacin:			
In the beans on wet basis			
Regression:	20	327.8448	25.22*
Mean	1	74.3158	5.72*
Blocks	15	18.0176	1.39
Treatments	3	5.9460	0.46
Covariate	1	78.1363	6.01*
Error	12	12.9994	

Table 31. (Continued).

Source of variation	d.f	Mean square[a]	F-value
In the beans on dry basis			
Regression:	20	2961.6768	23.44*
Mean	1	736.0755	5.82*
Blocks	15	159.4638	1.26
Treatments	3	21.6711	0.17
Covariate	1	772.4000	6.11*
Error	12	126.3711	
In cooking water			
Regression:	20	39.0129	18.93*
Mean	1	2.5121	1.22
Blocks	15	1.7563	0.89
Treatments	3	3.8996	1.89
Covariate	1	2.7551	1.34
Error	12	2.0604	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 32. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by water absorption (%) after cooking.

Source of variation	d.f	Mean square[a]	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	480.9608	36.73*
Mean	1	17.2541	1.32
Blocks	15	15.6258	1.19
Treatments	3	13.1387	1.00
Covariate	1	28.3127	2.16
Error	12	13.0951	
In the beans on dry basis			
Regression:	20	4392.4381	40.22*
Mean	1	227.8776	2.09
Blocks	15	145.5097	1.33
Treatments	3	116.0631	1.06
Covariate	1	347.1108	3.18
Error	12	109.2352	
In cooking water			
Regression:	20	156.1360	21.16*
Mean	1	1.4738	0.20
Blocks	15	14.1116	1.90
Treatments	3	4.8183	0.65
Covariate	1	3.4490	0.46
Error	12	7.4156	
Free folacin:			
In the beans on wet basis			
Regression:	20	324.5349	17.53*
Mean	1	19.3777	1.05
Blocks	15	16.2944	0.88
Treatments	3	4.1334	0.22
Covariate	1	11.9393	0.64
Error	12	18.5158	

Table 32. (Continued).

Source of variation	d.f	Mean square[a]	F-value
In the beans on dry basis			
Regression:	20	2926.9058	15.88*
Mean	1	135.3808	0.73
Blocks	15	136.2585	0.74
Treatments	3	23.5575	0.13
Covariate	1	76.9799	0.42
Error	12	184.3228	
In cooking water			
Regression:	20	39.3111	25.14*
Mean	1	6.9103	4.42
Blocks	15	1.7140	1.10
Treatments	3	4.7165	3.02
Covariate	1	8.7201	5.58*
Error	12	1.5634	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 33. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by moisture content (%).

Source of variation	d.f	Mean square[a]	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	479.7130	31.61*
Mean	1	0.9323	0.06
Blocks	15	13.9078	0.92
Treatments	3	4.8941	0.32
Covariate	1	3.3581	0.22
Error	12	15.1747	
In the beans on dry basis			
Regression:	20	4380.9598	34.13*
Mean	1	67.4985	0.53
Blocks	15	123.6938	0.96
Treatments	3	47.9640	0.37
Covariate	1	117.5454	0.92
Error	12	128.3657	
In cooking water			
Regression:	20	155.9764	20.30*
Mean	1	0.9685	0.13
Blocks	15	13.8401	1.80
Treatments	3	5.5740	0.73
Covariate	1	0.2565	0.03
Error	12	7.6817	
Free folacin:			
In the beans on wet basis			
Regression:	20	324.1145	16.87*
Mean	1	1.3810	0.07
Blocks	15	15.5527	0.81
Treatments	3	10.5552	0.55
Covariate	1	3.5299	0.18
Error	12	19.2166	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$)

Table 33. (Continued).

Source of variation	d.f	Mean square	F-value
In the beans on dry basis			
Regression:	20	2928.2898	16.09*
Mean	1	65.6655	0.36
Blocks	15	139.6531	0.77
Treatments	3	95.2914	0.52
Covariate	1	104.6603	0.58
Error	12	182.0161	
In cooking water			
Regression:	20	39.2520	23.62*
Mean	1	6.2721	3.77
Blocks	15	1.6920	1.02
Treatments	3	2.3115	1.39
Covariate	1	7.5365	4.54
Error	12	1.6620	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 34. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by trypsin inhibitor activity (TIU/g).

Source of variation	d.f	Mean square[a]	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	480.7713	35.35*
Mean	1	136.3418	10.17*
Blocks	15	14.3906	1.07
Treatments	3	11.9752	0.89
Covariate	1	24.5231	1.83
Error	12	13.4109	
In the beans on dry basis			
Regression:	20	4390.8357	39.24*
Mean	1	1452.4037	12.98*
Blocks	15	130.9625	1.17
Treatments	3	118.6682	1.06
Covariate	1	315.0622	2.82
Error	12	111.9060	
In cooking water			
Regression:	20	156.2366	21.56*
Mean	1	36.5273	5.04*
Blocks	15	14.0415	1.94
Treatments	3	6.3137	0.87
Covariate	1	5.0615	0.75
Error	12	7.2479	
Free folacin:			
In the beans on wet basis			
Regression:	20	326.2948	20.94*
Mean	1	151.6888	9.73*
Blocks	15	18.3147	1.18
Treatments	3	26.7280	1.72
Covariate	1	47.1373	3.02
Error	12	15.5826	

Table 34. (Continued).

Source of variation	d.f	Mean square[a]	F-value
In the beans on dry basis			
Regression:	20	2950.0919	20.25*
Mean	1	1577.7849	10.83*
Blocks	15	156.0554	1.07
Treatments	3	243.2569	1.67
Covariate	1	540.7027	3.71
Error	12	145.6792	
In cooking water			
Regression:	20	39.2408	23.35*
Mean	1	20.8724	12.42*
Blocks	15	1.5050	0.90
Treatments	3	3.5230	2.91
Covariate	1	7.3139	4.35
Error	12	1.6805	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$)

Table 35. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by texture (lb/25 g).

Source of variation	d.f	Mean square[a]	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	483.5821	55.42*
Mean	1	1230.2786	140.99*
Blocks	15	15.8135	1.81
Treatments	3	6.7806	0.78
Covariate	1	80.7389	9.25*
Error	12	8.7263	
In the beans on dry basis			
Regression:	20	4409.2301	54.27*
Mean	1	11066.2060	136.20*
Blocks	15	138.1895	1.70
Treatments	3	56.7543	0.70
Covariate	1	682.9517	8.41*
Error	12	81.2485	
In cooking water			
Regression:	20	156.0421	20.61*
Mean	1	178.5543	23.58*
Blocks	15	13.8000	1.82
Treatments	3	5.0821	0.67
Covariate	1	1.5708	0.21
Error	12	7.5722	
Free folacin:			
In the beans on wet basis			
Regression:	20	325.8965	20.06*
Mean	1	238.2234	14.66*
Blocks	15	18.8361	1.16
Treatments	3	17.2058	1.06
Covariate	1	39.1706	2.41
Error	12	16.2465	

Table 35. (Continued).

Source of variation	d.f	Mean square[a]	F-value
In the beans on dry basis			
Regression:	20	2941.4960	18.38*
Mean	1	2150.6793	13.44*
Blocks	15	159.0080	0.99
Treatments	3	86.6579	0.54
Covariate	1	368.7838	2.30
Error	12	160.0058	
In cooking water			
Regression:	20	38.9830	18.47*
Mean	1	76.6249	36.31*
Blocks	15	1.7332	0.82
Treatments	3	3.7973	1.80
Covariate	1	2.1576	1.02
Error	12	2.1102	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 36. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by blue color value (% reflectance).

Source of variation	d.f	Mean square[a]	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	479.5505	31.05*
Mean	1	14.9963	0.97
Blocks	15	13.2352	0.85
Treatments	3	3.8872	0.25
Covariate	1	0.1067	0.01
Error	12	15.4456	
In the beans on dry basis			
Regression:	20	4375.3486	31.77*
Mean	1	71.1503	0.52
Blocks	15	123.3919	0.90
Treatments	3	21.9483	0.16
Covariate	1	5.3207	0.04
Error	12	137.7178	
In cooking water			
Regression:	20	155.9843	20.34*
Mean	1	1.7196	0.22
Blocks	15	13.9148	1.82
Treatments	3	6.1461	0.80
Covariate	1	0.4138	0.05
Error	12	7.6686	
Free folacin:			
In the beans on wet basis			
Regression:	20	325.8097	19.88*
Mean	1	10.4736	0.64
Blocks	15	18.5971	1.14
Treatments	3	18.2777	1.12
Covariate	1	37.4339	2.28
Error	12	16.3913	

Table 36. (Continued).

Source of variation	d.f	Mean square[a]	F-value
In the beans on dry basis			
Regression:	20	2942.6284	18.61*
Mean	1	122.6064	0.78
Blocks	15	158.5873	1.00
Treatments	3	108.7650	0.69
Covariate	1	391.4333	2.48
Error	12	158.1184	
In cooking water			
Regression:	20	39.1054	20.51*
Mean	1	1.3448	0.70
Blocks	15	1.7772	0.93
Treatments	3	2.1579	1.13
Covariate	1	4.6048	2.42
Error	12	1.9063	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 37. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by green color value (% reflectance).

Source of variation	d.f	Mean square[a]	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	479.7724	31.82*
Mean	1	22.7558	1.51
Blocks	15	13.8077	0.92
Treatments	3	5.3117	0.35
Covariate	1	4.5464	0.30
Error	12	15.0757	
In the beans on dry basis			
Regression:	20	4376.2934	32.14*
Mean	1	166.7154	1.22
Blocks	15	124.8536	0.92
Treatments	3	28.5057	0.21
Covariate	1	24.2165	0.19
Error	12	136.1431	
In cooking water			
Regression:	20	155.9763	20.30*
Mean	1	0.9015	0.12
Blocks	15	13.2414	1.72
Treatments	3	6.1103	0.80
Covariate	1	0.2550	0.03
Error	12	7.6818	
Free folacin:			
In the beans on wet basis			
Regression:	20	325.8523	19.97*
Mean	1	16.3505	1.00
Blocks	15	15.7586	0.97
Treatments	3	15.5361	0.95
Covariate	1	38.2859	2.35
Error	12	16.3203	

Table 37. (Continued).

Source of variation	d.f	Mean square[a]	F-value
In the beans on dry basis			
Regression:	20	2940.5533	18.20*
Mean	1	149.4789	0.92
Blocks	15	131.9895	0.82
Treatments	3	94.9559	0.59
Covariate	1	349.9311	2.17
Error	12	161.5769	
In cooking water			
Regression:	20	38.9490	17.97*
Mean	1	0.2326	0.11
Blocks	15	1.7392	0.80
Treatments	3	2.7718	1.28
Covariate	1	1.4776	0.68
Error	12	2.1669	

[a]Value at 10^3.

*Significant at ($P \leq 0.05$).

Table 38. Analysis of covariance for total and free folacin (ng/g), on wet and dry weight basis, controlled by amber color value (% reflectance).

Source of variation	d.f	Mean square	F-value
Total folacin:			
In the beans on wet basis			
Regression:	20	479.5540	31.06*
Mean	1	6.9574	0.45
Blocks	15	13.8075	0.89
Treatments	3	3.7704	0.24
Covariate	1	0.1768	0.11
Error	12	15.4398	
In the beans on dry basis			
Regression:	20	4375.0827	31.67*
Mean	1	44.4302	0.32
Blocks	15	125.8039	0.91
Treatments	3	20.0107	0.14
Covariate	1	0.0025	0.00
Error	12	138.1609	
In cooking water			
Regression:	20	155.9757	20.30*
Mean	1	0.5321	0.07
Blocks	15	13.0978	1.70
Treatments	3	6.1015	0.79
Covariate	1	0.2427	0.03
Error	12	7.6828	
Free folacin:			
In the beans on wet basis			
Regression:	20	326.1348	20.58*
Mean	1	23.3006	1.47
Blocks	15	16.3538	1.03
Treatments	3	17.9045	1.13
Covariate	1	43.9358	2.77
Error	12	15.8494	

Table 38. (Continued).

Source of variation	d.f	Mean square[a]	F-value
In the beans on dry basis			
Regression:	20	2943.1820	18.72*
Mean	1	213.6834	1.35
Blocks	15	136.6947	0.87
Treatments	3	114.5572	0.73
Covariate	1	402.5045	2.56
Error	12	157.1958	
In cooking water			
Regression:	20	39.0488	19.52*
Mean	1	1.5567	0.78
Blocks	15	1.8643	0.93
Treatments	3	2.5678	1.28
Covariate	1	3.4743	1.74
Error	12	2.0005	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 39. Analysis of covariance for blue, green, and amber values (% reflectance), controlled by water absorption (%) after soaking.

Source of variation	d.f	Mean square	F-value
Blue color value			
Regression:	20	373.3243	654.48*
Mean	1	2.4308	4.62
Blocks	15	0.5746	1.00
Treatments	3	1.2102	2.12
Covariate	1	3.2413	5.68*
Error	12	0.5704	
Green color value			
Regression:	20	1516.2928	961.46*
Mean	1	2.1060	1.34
Blocks	15	1.7562	1.11
Treatments	3	5.1359	3.26
Covariate	1	3.7540	2.38
Error	12	1.5771	
Amber color value			
Regression:	20	1876.4911	1332.50*
Mean	1	1.3618	0.97
Blocks	15	2.1802	1.55
Treatments	3	5.6810	4.03*
Covariate	1	2.9173	2.07
Error	12	1.4082	

*Significant at ($P \leq 0.05$).

Table 40. Analysis of covariance for blue, green, and amber values (% reflectance), controlled by water absorption (%) after cooking.

Source of variation	d.f	Mean square	F-value
Blue color value			
Regression:	20	373.2823	583.00*
Mean	1	0.2558	0.40
Blocks	15	0.6002	0.94
Treatments	3	1.9076	2.98
Covariate	1	1.2403	3.75
Error	12	0.6403	
Green color value			
Regression:	20	1516.6186	863.64*
Mean	1	0.6977	0.40
Blocks	15	1.9190	1.09
Treatments	3	5.1480	2.93
Covariate	1	1.6120	0.92
Error	12	1.7556	
Amber color value			
Regression:	20	1876.4016	1204.86*
Mean	1	1.6379	1.05
Blocks	15	2.2827	1.47
Treatments	3	5.3361	3.42
Covariate	1	1.1279	0.72
Error	12	1.5574	

*Significant at ($P \leq 0.05$).

Table 41. Analysis of covariance for blue, green, and amber values (% reflectance), controlled by moisture content (%).

Source of variation	d.f	Mean square	F-value
Blue color value			
Regression:	20	373.2734	569.69*
Mean	1	0.5115	0.78
Blocks	15	0.6120	0.93
Treatments	3	1.6423	2.51
Covariate	1	2.2236	3.39
Error	12	0.6552	
Green color value			
Regression:	20	1516.1277	818.54*
Mean	1	0.7949	0.43
Blocks	15	1.8179	0.98
Treatments	3	5.9460	3.21
Covariate	1	0.4520	0.24
Error	12	1.8522	
Amber color value			
Regression:	20	1876.3684	1163.56*
Mean	1	1.1200	0.69
Blocks	15	2.2257	1.38
Treatments	3	6.4242	3.98
Covariate	1	0.4649	0.29
Error	12	1.6126	

*Significant at ($P \leq 0.05$).

Table 42. Analysis of covariance for blue, green, and amber color value (% reflectance), controlled by water absorption (%) after soaking and after cooking.

Source of variation	d.f	Mean square	F-value
Blue color value			
Regression:	21	355.6186	732.55*
Mean	1	2.5799	5.31*
Blocks	15	0.5508	1.14
Treatments	3	1.6881	3.48
Covariate (1)	1	2.3433	4.83*
(2)	1	1.5049	3.10
Error	11	0.4855	
Green color value			
Regression:	21	1444.1281	878.17*
Mean	1	2.2085	1.34
Blocks	15	1.7553	1.07
Treatments	3	4.3782	2.66
Covariate (1)	1	2.9775	1.81
(2)	1	0.8355	0.51
Error	11	1.6444	
Amber color value			
Regression:	21	1787.1609	1203.10*
Mean	1	1.4292	0.96
Blocks	15	2.1993	1.48
Treatments	3	4.7609	3.20
Covariate (1)	1	2.3480	1.58
(2)	1	0.5587	0.38
Error	11	1.4855	

*Significant at ($P \leq 0.05$).

Table 43. Analysis of covariance for texture (lb/25 g), controlled by water absorption (%) after soaking.

Source of variation	d.f	Mean square	F-value
Regression:	20	23.4046	8.61*
Mean	1	2.2182	0.82
Blocks	15	2.0303	0.75
Treatments	3	0.6110	0.22
Covariate	1	2.3945	0.88
Error	12	2.7195	

*Significant at ($P \leq 0.05$).

Table 44. Analysis of covariance for texture (lb/25 g), controlled by water absorption (%) after cooking.

Source of variation	d.f	Mean square	F-value
Regression:	20	23.3654	8.39*
Mean	1	2.3114	0.83
Blocks	15	2.0505	0.74
Treatments	3	1.1208	0.40
Covariate	1	1.6108	0.58
Error	12	2.7848	

*Significant at ($P \leq 0.05$).

Table 45. Analysis of covariance for texture (lb/25 g), controlled by moisture content (%).

Regression:	20	23.2859	7.98*
Mean	1	0.1059	0.04
Blocks	15	2.0006	0.69
Treatments	3	0.5496	0.19
Covariate	1	0.0192	0.01
Error	12	2.9175	

*Significant at ($P \leq 0.05$).

Table 46. Analysis of covariance for texture (lb/25 g), controlled by water absorption (%) after soaking, after cooking, and moisture content (%).

Source of variation	d.f	Mean square	F-value
Regression:	22	21.4013	7.16*
Mean	1	2.2148	0.74
Blocks	15	2.0586	0.69
Treatments	3	1.4457	0.48
Covariates (1)	1	3.1443	1.05
(2)	1	1.3664	0.45
(3)	1	0.1878	0.06
Error	10	2.9897	

*Significant at ($P \leq 0.05$).

Table 47. Analysis of covariance for trypsin inhibitor activity (TIU/g), controlled by texture (lb/25 g).

Source of variation	d.f	Mean square[a]	F-value
Regression:	20	1206.5274	136.52*
Mean	1	1528.8805	172.98*
Blocks	15	7.8595	0.89
Treatments	3	203.6999	23.05*
Covariate	1	5.0077	0.57
Error	12	8.8379	

*Significant at ($P \leq 0.05$).

[a]Values at 10^3.

Table 48. Analysis of covariance for trypsin inhibitor activity (TIU/g) controlled by water absorption (%) after soaking, after cooking, and moisture content (%).

Source of variation	d.f	Mean square[a]	F-value
By water absorption after soaking			
Regression:	20	1207.7730	178.61*
Mean	1	34.6983	5.13*
Blocks	15	9.4483	1.40
Treatments	3	210.2824	31.10*
Covariate	1	29.9194	4.12
Error	12	6.7619	
By water absorption after cooking			
Regression:	20	1207.7432	164.72*
Mean	1	43.9144	5.99*
Blocks	15	5.1021	0.70
Treatments	3	44.3063	6.04*
Covariate	1	23.1007	3.15
Error	12	7.3302	
By moisture content			
Regression:	20	1208.0219	190.33*
Mean	1	52.7081	8.30*
Blocks	15	7.2029	1.14
Treatments	3	44.2577	6.97*
Covariate	1	34.8977	5.50*
Error	12	6.3471	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 49. Analysis of covariance for trypsin inhibitor activity (TIU/g) controlled by blue, green, and amber color value (% reflectance).

Source of variation	d.f	Mean square[a]	F-value
By blue color value			
Regression:	20	1207.6298	172.50*
Mean	1	115.1697	16.45*
Blocks	15	8.6594	1.24
Treatments	3	208.3356	29.76*
Covariate	1	27.0550	3.86
Error	12	7.0006	
By green color value			
Regression:	20	1206.7174	141.61*
Mean	1	50.1920	5.89*
Blocks	15	8.1443	0.96
Treatments	3	193.1480	22.67*
Covariate	1	8.8066	1.03
Error	12	8.5213	
By amber color value			
Regression:	20	1206.9170	147.39*
Mean	1	49.5185	6.04*
Blocks	15	8.4212	1.03
Treatments	3	197.9184	24.17*
Covariate	1	12.7988	1.56
Error	12	8.1886	

[a]Values at 10^3.

*Significant at ($P \leq 0.05$).

Table 50. The t-values for testing the differences between treatment effects on some physical and nutritional evaluations.

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Vitamin B-6 (wet basis)						
Controlled by:						
-Water absorption after soaking	-0.628	0.404	0.379	1.015	0.974	-0.046
-Water absorption after cooking	-0.573	0.210	-0.441	0.701	-0.091	-0.691
-Moisture content	-1.323	0.433	1.110	1.785	1.790	0.699
-Trypsin inhibitor activity	-0.293	0.476	0.078	0.561	0.218	-0.252
-Texture	-0.739	0.545	0.504	1.285	1.240	-0.049
-Blue color value	-0.583	0.593	0.544	1.279	1.255	-0.033
-Green color value	-0.407	0.812	0.798	1.395	1.405	0.096
-Amber color value	-0.261	0.915	0.912	1.411	1.466	0.138
Vitamin B-6 (dry basis)						
Controlled by:						
-Water absorption after soaking	-0.215	0.494	-0.001	0.684	0.206	-0.502
-Water absorption after cooking	-0.238	0.385	-0.449	0.567	-0.280	-0.831
-Moisture content	-1.244	0.518	1.058	1.778	1.693	0.581
-Trypsin inhibitor activity	-0.187	0.621	0.033	0.557	0.129	-0.404
-Texture	-0.368	0.650	0.171	1.021	0.538	-0.478
-Blue color value	-0.307	0.651	0.183	0.999	0.544	-0.459
-Green color value	-0.091	0.869	0.506	1.114	0.718	-0.345
-Amber color value	0.034	0.960	0.619	1.126	0.765	-0.309

Table 50. (Continued).

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Vitamin B-6 (in cooking water)						
Controlled by:						
-Water absorption after soaking	2.404*	2.905*	-0.335	0.491	-2.647*	-3.284*
-Water absorption after cooking	2.345*	2.711*	0.206	0.442	-1.087	-1.795*
-Moisture content	2.447*	2.720*	-0.744	-0.133	-2.348*	-2.892*
-Trypsin inhibitor activity	1.597	2.593*	-0.192	0.207	-1.041	-2.062
-Texture	2.888*	3.241*	-0.612	0.398	-3.492*	-0.822
-Blue color value	2.634*	2.808*	-0.082	0.011	-2.997*	-2.933*
-Green color value	2.869*	2.981*	0.385	0.334	-2.488*	-2.924*
-Amber color value	2.988*	3.099*	0.564	0.326	-2.515*	-2.919*
Total folacin (wet basis)						
Controlled by:						
-Water absorption after soaking	-0.401	0.567	0.388	0.934	0.763	-0.182
-Water absorption after cooking	-0.629	1.102	1.387	1.493	1.609	0.909
-Moisture content	-0.527	0.425	0.512	0.938	0.764	0.141
-Trypsin inhibitor activity	-1.221	0.808	1.266	1.598	1.509	0.843
-Texture	-0.265	1.168	0.311	1.441	0.575	-0.856
-Blue color value	-0.258	0.487	0.310	0.778	0.633	-0.152
-Green color value	-0.058	0.705	0.594	0.887	0.794	-0.044
-Amber color value	-0.225	0.456	0.296	0.813	0.628	-0.145

Table 50. (Continued).

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Total folacin (dry basis)						
Controlled by:						
-Water absorption after soaking	-0.074	0.590	0.132	0.642	0.200	-0.464
-Water absorption after cooking	-0.393	1.224	1.512	1.481	1.594	0.899
-Moisture content	-0.547	0.460	0.597	0.991	0.842	0.193
-Trypsin inhibitor activity	1.252	0.985	1.387	1.738	1.605	0.852
-Texture	0.100	1.233	0.039	1.143	-0.061	-1.187
-Blue color value	-0.123	0.488	-0.001	0.632	0.134	-0.495
-Green color value	0.143	0.704	0.353	0.665	0.288	-0.353
-Amber color value	-0.040	0.457	0.070	0.601	0.134	-0.449
Total folacin (in cooking water)						
Controlled by:						
-Water absorption after soaking	-0.139	0.876	-0.629	0.981	-0.475	-1.525
-Water absorption after cooking	-0.233	1.044	0.150	1.173	0.263	-0.607
-Moisture content	0.012	0.859	-0.671	0.761	-0.502	-1.325
-Trypsin inhibitor activity	-0.702	1.041	0.250	1.282	0.563	-0.461
-Texture	-0.126	0.756	-0.668	0.887	-0.540	-1.405
-Blue color value	-0.190	0.748	-0.709	0.971	-0.588	-1.535
-Amber color value	-0.177	0.587	-0.613	0.916	-0.663	-0.518

Table 50. (Continued).

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Free folacin (wet basis)						
Controlled by:						
-Water absorption after soaking	-0.769	-0.554	0.267	0.206	1.002	0.833
-Water absorption after cooking	-0.785	-0.404	-0.328	0.319	0.128	-0.091
-Moisture content	-1.025	-0.177	0.687	0.922	1.259	0.791
-Trypsin inhibitor activity	-1.982*	0.255	1.738	1.930*	2.248*	1.756
-Texture	-1.106	-0.408	0.638	0.688	1.740	1.030
-Blue color value	-1.563	-0.543	-0.174	1.142	1.528	0.359
-Green color value	-1.583	-0.929	-0.560	0.651	0.943	0.344
-Amber color value	-1.734	-1.070	-0.717	0.695	0.955	0.308
Free folacin (dry basis)						
Controlled by:						
-Water absorption after soaking	-0.467	-0.533	-0.000	-0.065	0.451	0.540
-Water absorption after cooking	-0.559	-0.328	-0.335	0.189	-0.189	-0.156
-Moisture content	-0.997	-0.199	0.679	0.873	1.233	0.801
-Trypsin inhibitor activity	-1.938*	0.318	1.738	1.930*	2.223*	1.722
-Texture	-0.829	-0.379	0.395	0.442	1.221	0.1722
-Blue color value	-1.348	-0.539	-0.413	0.913	1.022	0.093
-Green color value	-1.307	-0.877	-0.704	0.409	0.481	0.100
-Amber value	-1.459	-1.013	-0.850	0.447	0.483	0.062

Table 50. (Continued).

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Free folacin (in cooking water)						
Controlled by:						
-Water absorption after soaking	2.266*	1.273	0.570	-0.954	-1.638	-0.713
-Water absorption after cooking	1.929*	2.431*	2.416*	0.550	1.165	1.090
-Moisture content	0.865	1.376	1.849*	0.327	0.724	0.636
-Trypsin inhibitor activity	0.096	2.070*	2.159*	1.217	1.323	0.946
-Texture	2.144*	1.622	0.719	-0.496	-1.423	-0.908
-Blue color value	1.336	1.060	-0.013	-0.368	-1.487	-1.806
-Green color value	1.506	0.787	0.018	-0.733	-1.533	-0.880
-Amber color value	1.192	0.474	-0.351	-0.795	-1.749	-1.032
Blue color value						
Controlled by:						
-Water absorption after soaking	2.297*	0.784	1.777	-1.456	-0.500	1.004
-Water absorption after cooking	1.446	1.742	2.773*	0.337	1.744	2.028
-Moisture content	0.574	0.878	2.700*	0.184	1.563	1.840
-Water absorption after soaking and after cooking	2.037*	1.460	2.548*	-0.414	1.206	2.020*

Table 50. (Continued).

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Green color value						
Controlled by:						
-Water absorption after soaking	2.024*	2.092*	3.012*	0.071	0.959	0.929
-Water absorption after cooking	1.503	2.448*	2.603*	0.941	1.558	1.291
-Moisture content	1.156	2.117*	2.837*	0.686	1.236	0.972
-Water absorption after soaking and after cooking	1.775	2.166*	2.277*	0.435	1.102	1.145
Amber color value						
Controlled by:						
-Water absorption after soaking	2.379*	2.363*	3.306*	-0.008	0.901	0.951
-Water absorption after cooking	1.878*	2.646*	2.679*	0.794	1.423	1.233
-Moisture content	1.448	2.379*	3.115*	0.614	1.227	1.024
-Water absorption after soaking and after cooking	2.122*	2.361*	2.350*	0.316	0.985	1.086
Texture						
Controlled by:						
-Water absorption after soaking	0.311	0.480	-0.259	0.144	-0.571	-0.748
-Water absorption after cooking	0.336	0.324	-0.683	0.004	-0.806	-1.067
-Moisture content	0.202	0.626	-0.136	0.350	-0.249	-0.633

Table 50. (Continued).

Evaluation	B-20 vs A-5	B-20 vs A-10	B-20 vs A-20	A-5 vs A-10	A-5 vs A-20	A-10 vs A-20
Texture						
Controlled by:						
-Water absorption after soaking and after cooking and moisture content	0.556	0.131	-0.915	-0.440	-1.070	-1.182
Trypsin inhibitor activity						
Controlled by:						
-Water absorption after soaking	-4.547*	1.268	5.406*	5.609*	9.625*	4.187*
-Water absorption after cooking	-3.693	0.208	1.448	3.453*	3.331*	1.588
-Moisture content	-2.497*	1.247	3.247*	3.756*	4.226*	2.060*
-Texture	-3.782*	0.672	4.518*	4.435*	8.277*	3.755*
-Blue color value	-2.981*	1.420	5.390*	4.688*	9.348*	4.473*
-Green color value	-3.002	1.245	4.124*	4.747*	8.191*	3.881*
-Amber color value	-2.731	1.414	4.244*	4.856*	8.466*	4.011*

*Significant at ($P \leq 0.05$).