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FROM AIR CLASSIFIED BEAN FLOUR FRACTIONS

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By

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ABSTRACT

PHYTIC ACID AND MINERAL
PARTITIONING AND IRON BIOAVAILABILITY
FROM AIR CLASSIFIED BEAN FLOUR FRACTIONS

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Hull flours and air classified intermediate starch, high starch, and high protein navy, pinto, and black bean flours were analyzed for calcium, copper, iron, magnesium, sodium, phosphorus, and zinc by plasma emission spectroscopy and for phytic acid by the method of Wheeler and Ferrel (1971).

Phytic acid content ranged from 4.29 to 8.72 mg/g in the high starch flours to 23.74 to 30.22 mg/g in the protein flours. Partitioning of phosphorus, zinc, iron, potassium, and magnesium into the protein flours was also noted. Strong and significant correlation coefficients were obtained between phytic acid content and phosphorus, iron, zinc, magnesium, and potassium content, and between the content of these minerals and protein content.

As measured by hemoglobin regeneration in anemic rats, iron bioavailability from navy hull, high starch, and protein flours did not appear to be influenced by endogenous phytic acid.

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INTRODUCTION

Although legumes contain approximately twice as much protein as cereal grains, it is well recognized that they remain an underutilized food source for man, particularly in the United States. Several reasons for the low level of consumption have been proposed, including flatulence associated with legume consumption, the presence of antinutritional factors in unheated legumes, and especially, the long soaking and cooking times required to adequately soften legumes.

Seeking alternative uses for dry beans in order to increase their utilization, researchers have experimented with flours prepared from different bean varieties in various baked and extruded products. Air classification has permitted the separation of dehulled whole bean flours into high protein and starch fractions which offer more desirable functional and sensory characteristics than the whole bean flours for making specific products.

While the composition of whole beans, and therefore whole bean flours, has been established, less is known about the nutritional contribution made by various air classified and hull flour fractions. Specifically, little information exists regarding the distribution of nutritionally important elements and the heat stable antinutritional factor, phytic acid, in these flours. Phytic acid has the potential to bind mono and divalent cations, most notably zinc and iron,

possibly decreasing their bioavailability.

This study was undertaken to determine the quantity and partitioning pattern of eight important dietary minerals as well as phytic acid in each of five flour fractions of navy, pinto, and black beans. For each bean type, hull, intermediate starch, high starch, high protein, and dehulled whole flour fractions were produced. After obtaining data on mineral content, phytic acid content, and proximate compostion of each flour fraction of each bean type, it was possible to attempt to correlate the individual microconstituents with macroconstituents, thus providing an indication of with which macrocomponents the microcomponents partitioned. To demonstrate whether or not iron bioavailability was affected by any phytic acid-iron chelates which might have been present, hemoglobin regeneration in anemic rats fed diets containing three of the navy bean flour fractions (high starch, high protein, and hull) was assessed relative to rats fed standard diets containing ferrous sulfate.

REVIEW OF LITERATURE

The *Phaseoleae* subfamily of the Leguminosae contains 47 genera including *Phaseolus* with 150 species. Despite this large number, only a select few of these species are actually cultivated. Food legumes belonging to the *Phaseolus* genus are often referred to by various locally recognized names including common, kidney, field, garden, or haricot beans. The best known and most widely cultivated species is *Phaseolus vulgaris*, to which the majority of legumes grown in America belong. Dry beans which may be classified into this group include pinto, black, small white or navy, red, yellow eye, and great northern (Deschamps, 1958).

Dry Bean Composition

Proteins

Dry beans contain substantial quantities of several important macro and micronutrients. Legumes have a high protein content, averaging between 20 and 25% on a dry weight basis. The average protein content of legumes is twice that found in cereal crops on a per serving basis (Bressani, 1975). However this is only about half as much protein as is contained in a serving of lean meat (Charley,

1970). The predominant class of proteins present in seeds of Phaseolus are salt-soluble globulins of which three distinct proteins have been identified; phaseolin, phaselin, and conphaseolin (Bressani, 1975; Kay, 1979). Analysis of the seed coat or testa, which comprises between 6.6 and 9.2% of the total weight of the bean (Kay, 1979), shows that the hull has a crude protein content of approximately 6.0% (Tobin and Carpenter, 1978).

Amino Acids

Legumes as a group have been shown to contain relatively large amounts of lysine, making them an excellent choice to complement cereals in order to achieve complete amino acid patterns. Legumes are also better sources of isoleucine, leucine, phenylalanine, threonine, and valine than are cereals (Charley, 1970; Bressani, 1975; Tobin and Carpenter, 1978). Legumes, like cereals, tend to be somewhat deficient in methionine. The exact amino acid content of legumes depends on the species, varieties, localities, and management practices. The application of minor element fertilizers has been found to influence the amino acid composition of certain legume varieties. Studied separately, uptake of both zinc and sulfur by Pisum sativum caused increases in the methionine content of mature peas (Bressani, 1975). Many studies have been conducted to assess the quality of legume protein both with and without supplementation or complementation by various cereal grains, seeds, and nuts (Rockland and Radke, 1981; Sgarbieri et al., 1978; Baloorforooshan and Markakis, 1979; Kakade and Evans, 1965). Determining and improving the protein quality of legumes is considered particularly important in parts of Africa, Asia, and Latin America where cereals and tubers are major

components of the diet (Sinha, 1977). In countries such as the United States, however, meat, eggs, and milk provide the majority of the protein in the diet such that the protein quality of legumes is of little importance except for those individuals following strict vegetarian diets.

Fats

Legumes of the Phaseolus vulgaris species have a low fat content, generally below 2.0%, with the majority (63.3%) of the fatty acids being unsaturated (Deschamps, 1958; Watt and Merrill, 1979).

Carbohydrates

The average carbohydrate content of Phaseolus seeds is approximately 60% of the dry weight of the bean (Kay, 1979). Of this 60%, starch is typically the carbohydrate present in greatest abundance, reported to account for about 35.2% of the dry weight. As for other carbohydrates, Kay (1979) reported the following percentages: pentosans, 8.4%; dextrins, 3.7%; cellulose, 3.1%; sugars, 1.6%, and galactans, 1.3%. Walker and Hymowitz (1972) found a considerably higher sugar content in the 28 varieties of Phaseolus vulgaris they analyzed, ranging from 4.4 to 9.2%. Of the total sugar present, on average, sucrose accounted for 46.4%; raffinose, 10.4%; and stachyose, 43.0%. Raffinose, stachyose, and verbascose are the three, four, and five unit oligosaccharides considered to be, at least in part, responsible for the flatulence associated with bean consumption (Tobin and Carpenter, 1978).

Crude fiber values of less than 6.0% have been noted for the more common varieties of the genus Phaseolus (Deschamps, 1958; Tobin and

Carpenter, 1978; Kay, 1979). According to many nutritionists, however, crude fiber, which is defined as the residual insoluble organic matter after digestion for a set period of time first in acid and then in alkali, does not provide an accurate indication of the total amount of unavailable carbohydrate. As such, more recently the amount of dietary fiber of Phaseolus vulgaris, defined as the plant polysaccharides plus lignin which are resistant to hydrolysis by man's digestive enzymes, has been reported as 225 g/kg or 22.5% (Tobin and Carpenter, 1978).

Ash and Minerals

It is generally agreed that the total ash content of P. vulgaris falls between 3.5 and 4.1% (Fordham et al., 1975; Tobin and Carpenter, 1978; Kay, 1979). The content of specific minerals in mature, raw legumes has been reported by several researchers in recent years, however most values have pertained to beans grown in countries other than the United States. Since the soil composition, growing conditions, and varieties of legumes grown in North America differ somewhat from those in other parts of the world, only findings for beans grown in the U.S. are discussed.

The range in content of several minerals for various classes of raw P. vulgaris which have been reported in the literature appear in Table 1. All researchers except Walker and Hymowitz (1972) and Augustin et al. (1981) used atomic absorption (AA) spectrometry to measure the content of all minerals except phosphorus. Walker and Hymowitz (1972) employed emission spectroscopy in their analyses, and both Augustin et al. (1981) and Meiners et al. (1976) measured phosphorus colorimetrically. Although Augustin et al. (1981) used AA

Table 1. Range in content of selected mineral elements of raw, mature Phaseolus vulgaris classes reported in the literature.

Reference	ppm								
	Ca	Cu	Fe	Mg	Mn	P	K	Na	Zn
Augustin et al. (1981)[1]	700-2100	5-14	33-80	1600-2300	10-20	3800-5700	13200-17800	40-210	19-65
Fordham et al. (1975)[2]	1030-1666	-	13-83	1620-2222	173-232	3650-5090	10850-15260	-	-
Koehler and Burke (1981)[3]	1380-2020	-	78-93	-	-	-	15570-17120	-	38-40
Meiners et al. (1976)[4]	595-1812	7-8	53-75	1230-1783	10-15	3740-5100	8208-13007	17-36	21-25
Walker and Hymowitz (1972)[5]	1100-2600	8-12	69-135	1300-1800	6-20	2800-5000	11200-19400	-	17-29
Watt and Merrill (1963)[6]	1110-1440	-	64-78	-	-	4060-4570	9840-11960	100-190	-

[1]Black, cranberry, great northern, navy, red kidney, pink, pinto, small red, and small white beans were analyzed.

[2]Great northern, navy, red kidney, and pinto beans were analyzed.

[3]Small red, pink, pinto, and red kidney beans were analyzed.

[4]Navy, great northern, pinto, and red kidney beans were analyzed.

[5]Navy, black, white, and red kidney beans were analyzed.

[6]White, red, pinto, calico, and red Mexican beans were analyzed.

to measure calcium, magnesium, zinc, copper, and iron; sodium and potassium were measured by flame emission spectrometry. In general, the data reported by all these workers indicate that dry beans contain substantial amounts of calcium, iron, magnesium, phosphorus, and potassium. Overall, the mean values for each mineral determined by the various research teams are in close agreement except for sodium and manganese. In addition to high interlaboratory variability of manganese content, intralaboratory variability was also found to be high by both Fordham et al. (1975) and Augustin et al. (1981). Although the beans analyzed by Fordham and coworkers (1975) were purchased in a local Kentucky market and the precise growing regions were not known, these researchers pointed out that the concentration of manganese in vegetables has been shown to vary by as much as 14 fold depending on the locations from which the samples were obtained. This may in part explain the large range of manganese values appearing in the literature.

Augustin et al. (1981) noted large variability both within and between bean types for sodium content and attributed it to the inherently low sodium content of beans. However, Meiners et al. (1976) found lower sodium values with much smaller variability in their work, perhaps reflecting a difference due to the method of analysis. Since sodium and other elements such as iron, zinc, and calcium must be considered ubiquitous, errors which could possibly result from contamination should not be overlooked when considering sources of variability.

In terms of other minerals, Meiners et al. (1976) found significant differences among the four P. vulgaris bean types

purchased in a local Virginia market for all minerals except phosphorus, however no one bean was consistently higher or lower in any one mineral than the other three types. Similarly, Augustin et al. (1981), in studying nine classes of raw P. vulgaris grown in six locations (CA, CO, ID, NE, MI, and ND) found low variability for both phosphorus and magnesium, whereas higher variability was noted for all other minerals analyzed. These workers also found that while potassium showed low variability both between and within bean classes when grown in the same location, differences within classes grown in different areas were much larger. Perhaps the somewhat high potassium content reported by Koehler and Burke (1981) for beans grown in the state of Washington may also be due to a location or soil difference. The zinc content of these beans was also slightly higher than the values reported by the other workers. Whereas Meiners et al. (1976) noted the least variability for zinc content, Augustin et al. (1981) found the overall variability of this element to be high. It may be hypothesized that the variation in zinc content and other minerals analyzed is likely the result of soil or location differences, stage of maturity at harvest, and the analytical technique employed in the laboratory.

Walker and Hymowitz (1972) found significant negative correlations between fat content and zinc, iron, and calcium contents of 28 varieties of P. vulgaris studied, perhaps suggesting that these elements are not associated with the lipid material present in beans. Significant correlation coefficients were also produced between raffinose content and phosphorus and potassium contents, and between stachyose and protein content. Although these correlation

coefficients were statistically significant, all were less than 0.60, suggesting that the relationships between the organic components and the inorganic elements of beans are not directly proportional.

Vitamins

There is no evidence in the literature which indicates that dry beans contain appreciable amounts of fat soluble vitamins. The quantity of vitamin A reported for P. vulgaris classes is less than 30 International Units per 100 grams of raw beans (Watt and Merrill, 1963; Kay, 1979). The tocopherol content of four varieties of P. vulgaris seed averaged only 1.2 mg/100 g (Fordham et al., 1975). In terms of water-soluble vitamins, dry beans have been found to contain high levels of thiamin, riboflavin, niacin, and folic acid, but they contain very little (less than 5 mg/100 g) ascorbic acid (Watt and Merrill, 1963; Fordham et al., 1975; Tobin and Carpenter, 1978). A summary of the range in content of several water-soluble vitamins contained in raw, mature P. vulgaris seeds is presented in Table 2. The data are in close agreement for the majority of these nutrients. Average thiamin content showed the highest interlaboratory variability of all the vitamins studied, with values ranging from 0.64 to 1.07 mg/100 g. Augustin et al. (1981) suggested that the high variability in values reported in their laboratory for this nutrient might be the result of different growing locations and possibly different sampling times following harvest. Values reported for various vitamins analyzed were expected to decrease as the amount of time between harvest and analysis increased.

In assessing both the vitamin and mineral content of dry beans it is important to recognize that soaking and cooking by conventional

Table 2. Range in content of selected vitamins of raw, mature Phaseolus vulgaris classes reported in the literature.

Reference	mg per 100 g				
	Thiamin	Riboflavin	Niacin	Folic Acid	Pyridoxine
Augustin et al. (1981)[1]	0.81-1.32	0.11-0.41	0.85-3.21	0.15-0.68	0.30-0.66
Fordham et al. (1975)[2]	0.88-1.39	0.14-0.25	1.05-3.14	-	-
Rockland et al. (1977)[3]	0.90	-	1.9	0.28	0.70
Watt and Merrill (1963)[4]	0.51-0.84	0.20-0.22	2.20-2.40	-	-

[1]Black, cranberry, great northern, navy, red kidney, pink, pinto, small red, and small white beans were analyzed.

[2]Great northern, navy, red kidney, and pinto beans were analyzed.

[3]Pinto beans only were analyzed.

[4]White, red, pinto, calico, and red Mexican beans were analyzed.

methods will result in losses of the water soluble forms of these nutrients. The extent of losses due to heat treatment and leaching will depend on the specific nutrient, the length of soaking, the amount of water used, and the severity of the cooking process (Miller et al., 1973). In the study conducted by Augustin et al. (1981), retention values exceeded 70% for water-soluble viatamins and 80% for all minerals except sodium, for which retention was in the 40% range. In the case of dry roasted bean flours being incorporated into baked products, no soaking takes place, thus the losses due to leaching would presumably be eliminated.

Antinutritional Factors

Although some vitamins and minerals may be lost by soaking and cooking, these processes have been found to result in the inactivation or removal of certain antinutritional factors present in P. vulgaris. Bressani (1975) categorized the toxic substances present in legumes into the following seven groups: trypsin inhibitors, hemagglutinins or lectins, goitrogenic factors, cyanogenic glucosides, lathyric factors, compounds that cause favism, and other factors about which much less information is known. Of these factors, trypsin inhibitors and hemagglutinins have been considered primarily responsible for causing the growth retardation observed in laboratory animals fed raw P. vulgaris (Honavar et al., 1962; Liener, 1962; Kakade and Evans, 1965, 1966; Jaffe, 1969; Liener and Kakade, 1969; Liener, 1975).

Trypsin inhibitors, which can inhibit the action of the enzyme trypsin, are believed to stimulate increased synthesis and flow of pancreatic digestive enzymes into the intestine (Stein, 1976). As a

result of increased protein synthesis, the pancreas may become enlarged, with a subsequent loss of essential amino acids (Liener, 1962). It has been demonstrated that approximately 40% of both the pancreatic hypertrophy and growth depression effects associated with raw bean consumption may be accounted for by trypsin inhibitor activity (Stein, 1976). Kakade and Evans (1965) reported that autoclaving navy beans at 121^{o} C for five minutes destroyed 80% of the trypsin inhibitor activity and the growth performance of rats improved markedly. Honavar et al. (1962) found that protein fractions of kidney beans which contained no antitryptic activity but which were high in hemagglutinating activity also inhibited the growth of rats when incorporated into basal diets containing 10% casein at levels as low as 0.5%. When the purified hemagglutinin was boiled for 30 minutes before being incorporated into the diet at 0.5%, no growth inhibition was observed.

The findings of several workers including Kakade and Evans (1965,1966), Honavar et al. (1962), Iyer et al. (1980), and Thompson et al. (1983) indicate the importance of adequate heat treatment in order to eliminate the toxicity of trypsin inhibitors and hemagglutinins in P. vulgaris. However, there is some disagreement as to whether or not soaking, as well as heating, is required to completely inactivate these factors. Honavar et al. (1962) found that feeding weanling rats diets containing black or kidney beans which had been autoclaved caused the rats to grow very slowly or actually lose weight, whereas beans which had first been soaked and then autoclaved produced a growth rate comparable to that obtained when casein was fed. Although Kakade and Evans (1966) found that soaking navy beans

for 1 to 4 days decreased the trypsin inhibitory activity and especially the hemagglutinating activity, rats fed a diet of unsoaked, heat treated navy bean meal supplemented with methionine grew as well as rats fed a casein diet. More recently, Iyer et al. (1980) reported that soaking alone decreased the trypsin inhibitor activity of P. vulgaris by only 15% or less, whereas soaking followed by convential cooking reduced the activity by approximately 90% of that present in the raw state. Thompson et al. (1983) found that presoaking red kidney beans followed by heating at 100° C decreased hemagglutinin activity below detectable levels, as did pressure cooking without presoaking. Unlike the findings of Kakade and Evans (1966), these workers found that merely soaking the beans did not result in any loss of hemagglutinating activity. Thus, while heat treatment without prior soaking may not result in complete destruction of these antinutritional factors, it appears that any toxic effects are substantially reduced by heating alone. In general, the extent of the enhancement of nutritive value which results from heat treatment will depend on the temperature, length of heating, and moisture conditions (Liener, 1975).

Phytic Acid

In addition to the antinutritional factors already mentioned, legumes may contain relatively large amounts of phytic acid, up to 5% by weight (deBoland et al., 1975), which has the ability to bind nutritionally important mono- and divalent cations to form the complex phytate. Each phytic acid molecule contains 12 replaceable hydrogen atoms with which it may form insoluble salts with various elements.

The results of many studies conducted with several species indicate that the minerals present in phytate complexes become unavailable or only partially available (Sathe and Krishnamurthy, 1953; O'Dell and Savage, 1960; Roberts and Yudkin, 1960; O'Dell, 1969; O'Dell et al., 1972; Davies and Nightingale, 1975; Maga, 1982).

The exact chemical structure of phytic acid, or myo-inositol hexaphosphoric acid [1,2,3,4,5,6-hexakis (dihydrogen phosphate)] has been debated for some time. The structure proposed by Anderson (1914) shown in Figure 1, suggests a symmetrical hexaorthophosphate arrangement which has more recently been supported by ^{31}P nuclear magnetic resonance (Johnson and Tate, 1969). However, there is some evidence that phytic acid is present as the asymmetrical hydrated triphosphate structure proposed by Neuberg in 1908 (Oberleas, 1973). As such, Erdman (1979) hypothesized that both forms may exist, however it appears that the Anderson form predominates in plants. While several isomers of inositol hexaphosphate are known to exist and have been isolated from soils, only the myo form has been isolated from plants (Cosgrove, 1966).

Approximately 70% of the total phosphorus in dry beans is reported to be present as phytic acid or its calcium, magnesium, and potassium salts (Makower, 1969; Lolas and Markakis, 1977). Although several possible roles have been suggested for phytic acid, it is generally recognized that its primary function is to serve as a storage form of phosphorus to be used during germination (Oberleas, 1971; Lolas and Markakis, 1975; Erdman, 1979). It is thought that the formation of phytic acid prevents the accumulation of excessively high levels of inorganic phosphate (Erdman, 1979). The existence and

concentration of phytic acid or phytate vary substantially depending on which part of the plant is considered and its stage of maturity (Oberleas, 1973).

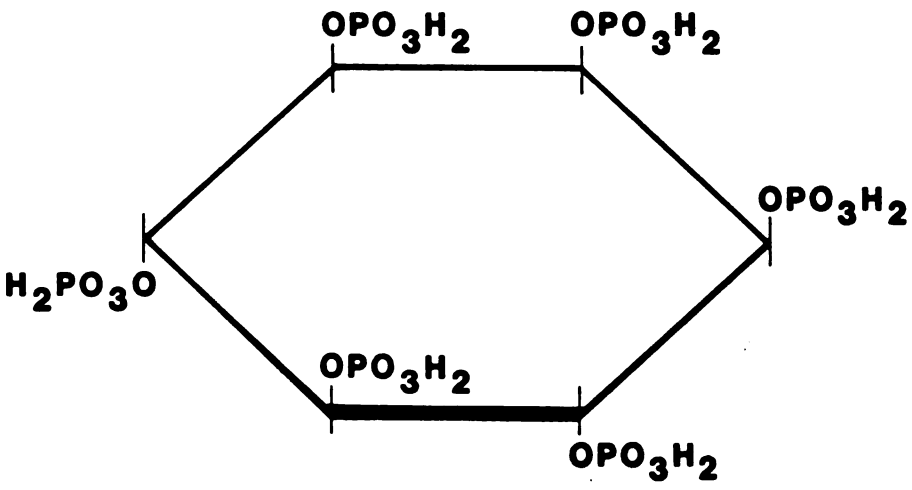

Figure 1. Structure of phytic acid proposed by Anderson (1914).

Using the increasing percentage of total solids as an indicator of increasing maturity, Makower (1969) measured changes in phytic acid in maturing pinto beans. During the early period of growth, characterized by enlarging cotyledons and pods, only a 14% change in total solids occurred, but phytic acid content increased from 0.13 to 0.77% of the dry bean weight. Most of the phytic acid was found to accumulate towards the end of maturation but before the rapid increase in the percent total solids. The increase in phytic acid phosphorus as a percentage of total phosphous was small after the midpoint in seed dry weight was obtained, indicating that little synthesis of

phytic acid occurred just prior to and during desiccation. Walker (1974) found that 90% of the total phytic acid in P. vulgaris cotyledon was laid down between days 24 and 30 of the embryogeny process which normally requires 36 days. Rapid cotyledon enlargement was noted between days 12 and 30 and dehydration became apparent around day 32. Thus the findings of both workers indicate that most of the phytic acid accumulates towards the end of maturation, but before desiccation. In addition, Walker (1974) demonstrated that 90% of the phytic acid of P. vulgaris is lost within the first 10 days of germination.

Although the precise location of phytate in legume seeds has not been determined, it is believed to be associated with protein (Oberleas, 1973). The phytate of most plant seeds is primarily contained in the bran and germ (Oberleas, 1973). When phytic acid is associated with the specific components as such, it may be preferentially extracted with them (Erdman, 1979). Ferrel et al. (1969) pointed out that in milling, phytate may become concentrated in high protein flours by the remilling of selected by-products. Wheat protein concentrate was found to contain approximately 20 times more phytic acid than wheat flour (Ranhotra, 1972).

The phytic acid content of mature P. vulgaris has been measured by Lolas and Markakis (1975), Iyer et al. (1980), and Deshpande et al. (1982). For the fifty varieties of mature dry beans analyzed by Lolas and Markakis (1975), phytic acid content was found to range from 0.54 to 1.58%. Total phosphorus, which ranged from 0.259 to 0.556%, was found to correlate well (r=0.9847) with phytic acid content. Additionally it was found that over 99% of the total phytic acid

present in the beans analyzed existed in a water soluble form. The phytate phosphorus content of raw great northern, kidney, and pinto beans was reported to be 4.6, 5.8, and 5.5 mg/g, respectively, by Iyer et al. (1980). Based on the assumption that phosphous accounts for 28.2% of the weight of phytic acid, that represents an average of 1.8% phytic acid for the three bean types. Deshpande et al. (1982) measured the phytic acid content of 10 cultivars of dry beans for whole intact beans and dehulled and hull bean fractions to determine the effect of seed coat removal on phytic acid concentration. The phytic acid content of whole beans ranged from 1.16 to 2.93%, values almost twice that reported by Lolas and Markakis (1975). The authors attributed the discrepancies to differences in varieties of beans grown, climate, soil, location, irrigation conditions, and year. Except for pinto beans, dehulling increased the percentage of phytic acid by an average of 31% as compared to the whole beans. The researchers conjectured that phytic acid may be characteristically present in the cotyledon fractions and also, since the seed coat contributes a relatively large portion of the whole seed weight, dehulling may result in an increase in the phytic acid concentration on a unit weight basis. It was suggested that the large increase in phytic acid coincident with hull removal might reflect an improvement in the extraction efficiency of this compound.

Phytase

Phytase enzymes which hydrolyze phytic acid to inositol and phosphoric acid have been identified in both plants and the intestinal tracts of several animal species (Cosgrove, 1966; Johnson

and Tate, 1969; Bitar and Reinhold, 1972). While phytase is present in dry, dormant, mature seeds, the findings of several workers including Gibbons and Norris (1963), Makower (1969), and Walker (1974) indicate that it does not become active until germination. Lolas and Markakis (1977), Chang and Schwimmer (1977), and Gibbins and Norris (1963) isolated the phytase enzyme from P. vulgaris and demonstrated optimal activity at pH 5.2 or 5.3. Whereas Lolas and Markakis (1977) reported 50^{o} C to be the optimum temperature for phytase activity of navy beans, Chang and Schwimmer (1977) reported 60^{o} C as optimal for California small white beans. In addition, Lolas and Markakis (1977) noted that almost complete inactivation of phytase occurred at 80^{o} C. Previously, optimal pH values of plant phytases have been reported to range from 4.5 to 5.5 with optimal temperatures between 45 and 56^{o} C (Sloane-Stanley, 1961). Phytase activity has been found to be inhibited by high substrate concentrations (Gibbins and Norris, 1963; Lolas and Markakis, 1977; Chang and Schwimmer, 1977) and by phytate-precipitants such as Cu^{+2}, Zn^{+2}, Fe^{+3}, and Ca^{+2}(Sloane-Stanley, 1961). Phytase activity has been demonstrated in the small intestine of the rat, chicken, calf, and man (Bitar and Reinhold, 1972), however little is known about the role of these intestinal phytases in phytate-phosphorus metabolism.

Unlike trypsin inhibitors and hemagglutinins, phytates are relatively heat stable. Rackis (1974) reported that four hours of autoclaving at 115^{o} C were necessary to destroy the majority of the phytic acid present in soy isolate. Such a long heat treatment would not be feasible since it would result in amino acid destruction as well. Using approximately the same temperature and time conditions,

Lease (1966) determined that only 20% of the phytate associated with sesame meal was destroyed. The findings of these workers support the conclusion reached by deBoland et al. (1975), that the thermal destruction rate of phytate appears to be product specific. Destruction of phytate has been reported for food systems in which phytase has been activated. Both rye flour and yeast are known to have relatively high phytase activities (Hoff-Jorgensen et al., 1946). During the fermentation of bread doughs containing rye flour and/or yeast, the phytase becomes activated, resulting in baked bread with a reduced phytate content (Reinhold, 1971).

Metallic-Phytates

Whether or not a specific mineral binds with phytic acid depends on pH as well as on the presence of secondary cations (Oberleas, 1973). Ferric and scandium phytates are least soluble in dilute acid and dissociate in concentrated acid or dilute alkali. Zinc and copper phytates are most stable between pH values of 4 and 7, and calcium, magnesium, and barium phytates form most readily under slightly alkaline conditions.

Vohra et al. (1965) studied the titration curves of phytate as the free acid and as the sodium salt in the presence of various single polyvalent cations. These workers found that at pH 7.4, phytate formed complexes with metals in the following decreasing order: Cu^{+2} > Zn^{+2} > Co^{+2} > Mn^{+2} > Fe^{+3} > Ca^{+2}. Zinc and copper formed the most stable metal-phytate complexes.

When two or more cations are present simultaneously, they may act in unison to increase the quantity of metallic phytate precipitated

(Oberleas, 1973). Most notably this has been observed for zinc and calcium, and for copper and calcium. For example, doubling the molar concentration of calcium increased the percentage of calcium and zinc recovered in the phytate precipitate from 63 to 84% and from 67 to 97%, respectively.

The ability of metallic phytates to depress absorption of the chelated minerals depends on many factors including the intestinal and food/meal phytase activities, previous food processing conditions (especially pH), digestibility of the food eaten, and the physiological status of the individual consuming the food (Erdman, 1979).

The major adverse effect of phytic acid on mineral availability has been demonstrated in relation to zinc. The work of several researchers including O'Dell and Savage (1960) and Likuski and Forbes (1965) has indicated that an inverse relationship exists between the level of phytic acid in the diet and zinc bioavailability. In addition, high levels of calcium associated with phytate have been shown to decrease zinc bioavailability (Oberleas et al., 1966). Davies and Nightingale (1975) found similar interactions betwen zinc and copper.

Several reports which appeared in the literature during the 1930s, 1940s, and early 1950s indicated that cereals had anticalcifying and rachitogenic properties. Phytates were implicated as the causative agent based on findings of research conducted on dogs, however, in humans these results could not be substantiated (Maga, 1982). Oberleas (1973) noted that calcium absorption is influenced by vitamin D, lipids, and other dietary factors in addition

to dietary phytate. Therefore foods having a calcium:inorganic phosphate ratio between 1:1 and 2:1 and that contain adequate amounts of calcium, phosphate, and vitamin D, in all liklihood will not be rachitogenic even if calcium is bound by phytate (Oberleas, 1973).

Roberts and Yudkin (1960) cited dietary phytate as a possible cause of magnesium deficiency and later it was shown by Likuski and Forbes (1965) that calcium in conjuction with phytic acid may also depress magnesium absorption. In addition, phytate phosphorus is generally considered to be unavailable for monogastric animals including man (Oberleas, 1973).

Next to zinc, iron is the nutritionally important mineral most frequently associated with phytate binding. Although iron availability in relation to phytic acid has been studied by several researchers, the data are not in agreement as to whether phytate inhibits iron absorption or has no effect.

Nutritional Significance of Iron-Phytate

Iron Absorption, Transfer and Storage

The basis for phytic acid inhibiting iron absorption in humans relates to the insolubility of iron phytate at the exceptionally low pH provided by the gastric juice of the stomach. It is thought that since iron phytate is not solubilized by the gastric juice, it does not ionize, and therefore cannot be absorbed. As such, the effect of phytic acid on mineral availability, if any, is as an intraluminal factor which inhibits solubilization of iron before the ingested food leaves the stomach (Prasad, 1978).

Normally, the iron from foods and iron salts is solubilized and

ionized by the gastric juice and passes from the stomach to the duodenum where the majority of iron absorption occurs (Underwood, 1977; Narasinga Rao, 1981). In the duodenum, the pH increases from approximately 1.5 to 7.0 as a result of duodenal secretions, causing the precipitation of ferric (Fe^{+3}) ions, but maintaining the solubility of the ferrous (Fe^{+2}) form. Thus it is generally accepted that ferrous iron is better absorbed than ferric forms (Narasinga Rao, 1981).

The amount of iron which is transferred from the gut lumen to the mucosa depends on the number of receptors on the brush border; the number increasing during iron deficiency. While the inorganic iron supplied by food and/or inorganic iron salts (e.g. iron supplements) must be taken up by the brush border receptors, heme iron (ingested as such or made available by the removal of nonviable red blood cells) enters the mucosal cells directly without first having to be released from the bound form. Once in the mucosal cell, xanthine oxidase liberates iron from the heme complexes (Prasad, 1978; Narasinga Rao, 1981).

From the mucosal cell, iron is transferred to plasma transerrin which carries ferric iron to the bone marrow for hemoglobin (Hb) synthesis, or to reticuloendothelial cells for storage as the two nonheme compounds ferritin and hemosiderin (Underwood, 1977; Beutler, 1980; Narasinga Rao, 1981). Once in the bone marrow, ferric iron is reduced to the ferrous form and detached from transferrin, facilitating its transfer to protoporphyrin such that it becomes stabilized in Hb, a complex of globin and four ferroprotoporphyrin moities, where it can reversibly bind to oxygen, permitting Hb to

serve as an oxygen carrier (Underwood, 1977; Prasad, 1978; Beutler, 1980). A small amount of iron is also incorporated in various enzymes as iron-porphyrin complexes, iron-flavoproteins, or it may serve as a cofactor. The total amount of circulating Hb, approximately 800 to 900g in an average man, is synthesized and catabolized every 120 days. The breakdown of red blood cells releases about 20 mg of endogenous iron each day, which is reutilized for Hb synthesis (Prasad, 1978). Over 90% of body iron is conserved and reutilized (Beutler, 1980).

Ferritin, the water soluble storage form of iron, constitutes a slowly exchangeable pool of iron which can be mobilized by exchanging with the carriers. As the level of iron to be stored increases, more iron is stored as the insoluble hemosiderin. Both storage forms of iron serve as reserves to protect against sudden losses due to unanticipated blood loss (Prasad, 1978; Tyler, 1979; Narasinga Rao, 1981).

Other Factors Influencing Iron Absorption

Since the body has only a limited capability to excrete iron, homeostasis of this nutrient is maintained primarily by regulation of the amount absorbed. Healthy individuals are estimated to absorb 5 to 10% of dietary iron whereas for iron-deficient individuals it is believed that this amount ranges from 10 to 20%. Iron deficiency may result in the development of hypochromic, microcytic anemia accompanied by a normoblastic, hyperplastic bone marrow that contains little or no hemosiderin. The existence of iron deficiency without anemia has also been confirmed by several investigators and is believed to be two to three times more prevalent than true iron

deficiency anemia (Prasad, 1978). Evidence exists which suggests that some of the symptoms of anemia may actually be due to decreased activity of intracellular enzymes, and not to low levels of Hb, as previously thought (Tyler,1979).

In addition to phosphates and phytic acid, several factors are known to influence iron absorption. The presence of other foods and food components in the diet, particularly meat and fish, but also tea, dietary fiber, fat, and ascorbic acid have been reported to influence iron absorption (Young and Janghorbani, 1981). Layrisse et al. (1969) investigated the availability of iron from various food sources with ^{55}Fe or ^{59}Fe in both iron adequate and deficient human subjects. These workers found absorption to range from 2% for lettuce to 20% for veal. Relatively low mean absorption values of 1.7 to 7.9% were reported for wheat, corn, black beans, lettuce, and spinach. Martinez-Torres and Layrisse (1970) observed that iron absorption in human subjects from either corn or black beans administered as a test meal was enhanced when these foods were mixed with veal, fish, or a mixture of amino acids similar to that present in fish. Based on the percentage transferrin saturation values, iron absorption in 18 healthy adults from black beans alone was 1.3%, but this amount increased to 3.1% when the beans were administered with amino acids or fish. Of the amino acids fed, only cysteine was found to enhance absorption.

Although it is a common notion that iron from animal foods is better utilized than that from plant sources, the findings of Fritz et al. (1970) could not confirm this. These workers conducted repletion tests with both weanling rats and chicks to compare the availability of iron from animal sources including blood meal, egg yolk, and fish

protein concentrate with plant sources including biscuits, corn meal, corn germ, cereal, and various flours. Perhaps the specific selection of animal and plant foods used in the investigation was not representative of all animal and plant foods which are consumed.

Amine and Hegsted (1971), in working with iron deficient rats, found that as the level of salt/mineral mix in the diet was increased, iron absorption decreased. Iron retention, as measured by the difference in total body count two hours and nine days after ingestion of ^{59}Fe, was greatest in rats fed diets containing no salt/mineral mix. Retention was significantly lower from the diet supplying 2% salt/mineral mix than from the diet containing no salt/mineral mix. Similarly, iron retention was significantly higher from the diet containing 2% salt mix than the one containing 4% salt mix. When the carbohydrate source of the diets was changed, iron retention was significantly lower from diets containing glucose than from those containing 60% sucrose, but significantly higher from glucose than from diets containing 60% starch. In terms of the carbohydrate source, maximum retention was observed with diets containing 60% lactose or 20% lactose and 40% starch.

Reducing agents such as cysteine, fructose, and glutathione are thought to enhance iron absorption by reducing Fe^{+3} to Fe^{+2}, thus preventing precipitation at the neutral or alkaline pH of the duodenum (Narasinga Rao, 1981). The absorption of inorganic iron is much more sensitive to changes in the intestinal environment than is the absorption of heme iron. Unlike nonheme forms of iron, heme iron absorption is not increased by ascorbic acid or decreased by phytates (Underwood, 1977; Beutler, 1980).

Feeding Studies Relating Iron Absorption to Phytic Acid Intake

Sathe and Krishnamurthy (1953) placed anemic young rats (Hb = 7-8 g/100mL) into three groups of five rats each and fed them semi-purified diets containing rice which received no polishing, slight polishing, or much polishing, corresponding to high phytate, moderate phytate, and low phytate diets. Iron absorption as measured by hemoglobin gain was determined after one, two, three, and four weeks. These workers attributed the differences in hemoglobin levels obtained for the three groups to differences in the phytic acid content of the diets, such that hemoglobin levels were higher for rats fed diets containing less phytic acid.

Using healthy adolescent boys as their subjects, Sharpe et al. (1950) measured iron absorption as retention of ^{55}Fe or ^{59}Fe from seven different breakfasts chosen to contain various amounts of phytic acid. The iron content of each breakfast was standardized using ferric chloride. One breakfast consisted solely of water and ferric chloride, another of milk plus added ferric chloride and one of milk, sodium phytate, and ferric chloride. The other four breakfasts contained one or more of the following in varying amounts: cooked rolled oats, white bread, egg omelet, and tomato juice. The authors found iron absorption to be greatest from the distilled water, whereas milk alone, which contained no phytate, appeared to decrease iron absorption appreciably. It was expected that the rolled oats would have a high phytate content and therefore would depress iron availability. However, the rolled oats and milk together reduced absorption by only twice as much as milk alone, and no correlation was

found between the phytate content of the oats and the reduction of iron absorption. Therefore these workers, unlike Sathe and Krishnamurthy (1953), concluded that endogenous phytate was not an important factor in decreasing iron absorption. Sharpe et al. (1950) hypothesized that the calcium in milk may have preferentially combined with phytate, thus iron availability was not affected to a greater extent. Added sodium phytate, however, was found to decrease the absorption of iron by 15 fold, indicating that added soluble phytates could interfere with iron absorption.

Similarly, studies conducted by Hussain and Patwardhan (1959) with four healthy male subjects revealed that sodium phytate added to otherwise adequate diets inhibited iron absorption. Diets were prepared to contain 8% and 40% of the total phosphorus from phytate, and equivalent amounts of endogenous iron. Little differences in individual and average iron intakes were noted on both diets, however iron retention of subjects on the diet containing 40% phytate phosphorus was markedly reduced. On the 8% phytate phosphorus diet, more than 10% of the dietary iron was absorbed. At the 40% level, absorption was less than 3%.

Unlike the findings with human subjects which demonstrated inhibition of iron absorption from added sodium phytate, Cowan et al. (1966) found that varying levels of added sodium phytate had no effect on iron absorption by anemic rats. Anemic Sprague-Dawley rats (Hb < 7 g/100 mL) were distributed into groups of eight and for four weeks were fed purified diets containing 10 or 20 ppm iron as ferous sulfate with either 0, 45, or 75% of the phosphorus derived from sodium phytate. Expressed in terms of milligrams of iron consumed, there

were no significant differences at either the 10 or 20 ppm level of iron between control values and those of the diets containing sodium phytate. The authors concluded that high levels of phytate have no effect on iron absorption in the rat.

Hunter (1981) confirmed the findings of Cowan et al. (1966) in a similar experiment. Hunter (1981) fed iron deficient rats one of 16 diets containing either 0, 4, 8, or 12 ppm iron as ferrous sulfate and 0, 0.25, 1.0, or 4.0% sodium phytate. As measured by Hb regeneration after two weeks, iron absorption from any of the variable phytate diets was not significantly different from the iron absorption by rats fed the phytate free diet. Hunter (1981) also force fed both iron deficient and iron adequate rats slurries containing an iron deficient diet, ^{59}Fe, and sodium phytate to compare the amount of iron absorbed by the iron adequate and iron depleted rats. Iron absorption, measured as the percentage of the ^{59}Fe dose retained after nine days, was greater for the iron deficient rats. The addition of sodium phytate depressed iron absorption by about 30% in the iron adequate rats but by only 18% in the iron deficient rats. This finding is consistent with the basic principle of maintaining iron homeostasis by regulating iron absorption.

Instead of studying the effects of added phytate, Welch and VanCampen (1975) were interested in determining the effects of endogenous phytic acid in soybean seeds on the bioavailability of ^{59}Fe to iron-depleted rats. Both immature and mature soybean seeds containing Fe were fed in a single dose to young male rats (Hb averaging 8.3 g/100 mL). As measured by whole-body counting, ^{59}Fe from the mature soybean seeds was more available than the ^{59}Fe present

in the immature seeds even though the phytic acid content of the mature seeds was almost three times that of the immature seeds. The authors concluded that the availability of ^{59}Fe from soybean seeds was not directly correlated to their phytic acid content. In addition they hypothesized that the immature seeds might contain another factor which could account for the reduced iron availability.

Also studying the effect of endogenous phytate on iron absorption, Ifon (1981) fed young iron depleted rats (Hb averaging 9 g/100 mL) semipurified diets containing 20 ppm iron from millet, guinea corn, maize, soybeans or bambara nuts. No correlation was found between the level of ingested phytate and the proportion of iron utilized for Hb synthesis. Maize and bambara nuts, both of which had high phytic acid contents, showed high levels of iron utilization. Thus it was concluded that the effect of phytic acid on iron absorption was minor or nonexistent.

Morris and Ellis (1976) isolated monoferric phytate from wheat bran and found it to be a water soluble complex of iron which had no negative effect on iron absorption and was actually of high biological availability. Iron availability as measured in a hemoglobin repletion test with iron depleted rats revealed that the monoferric phytate afforded the same biological availability as ferrous ammonium sulfate, which was the reference compound used. In contrast, ferric phytate, which contains three to four moles of iron per mole of phytate was found to be insoluble and of low bioavailability. These researchers considered monoferric phytate to be the predominant form of iron in wheat, being bound to cationic sites of protein or other cellular constituents of the wheat bran. The authors conjectured that seemingly

conflicting reports in the literature on the effect of phytate on iron absorption might be due to the formation or use of ferric phytates of differing degrees of saturation.

Other recent research indicates that decreased iron availability once attributed to phytate may be due to binding with fiber or other factors. Reinhold et al. (1975) dephytinized wholemeal breads and separated zinc, calcium, and iron and found that the binding of these minerals increased after phytate removal due to increased fiber concentration. These workers concluded that fiber and not phytate was the primary determinant of the availability of the divalent minerals in wholemeal bread.

Morris et al. (1980) prepared muffins with dephytinized wheat bran (DWB), whole wheat bran (WWB), and without bran (NBM) and fed them with a standard meal (STD) of cooked beef and a milk shake to healthy human subjects. WWB decreased nonheme iron absorption by 2.4 fold when the STD included ascorbic acid and four fold when meat or ascorbic acid were omitted. The absorption ratio of STD and NBM to STD and WWB or DWB was 2.4 and 1.8, respectively, indicating that the inhibitory effect of bran was not solely due to the phytate content. Insoluble, high fiber fractions of DWB were also found to inhibit absorption of inorganic iron.

Thus, it can readily be seen that the complex interaction of many factors makes the prediction of iron bioavailability difficult. As such, the possible significance of only phytic acid in relation to iron nutrition in humans is difficult to determine. When reviewing the findings of research or planning investigations relating phytic acid to iron availability, it is important to consider the composition

of the total diet and to remember that animal models, especially in iron deficient states, may not necessarily provide precise estimates of the true availability of minerals from actual diets consumed by healthy human beings under normal living conditions.

MATERIALS AND METHODS

Raw Material Procurement and Processing

Source

Prime, handpicked navy, pinto, and black beans (Phaseolus Vulgaris L.) from the 1981 Michigan crop were used in the production of dry roasted fractionated bean flours. Ninety-one kilograms of each type of bean were shipped to the Food Protein Research and Development center at Texas A&M University for processing.

Roasting and Dehulling

Forty-five kilograms of each type of bean were roasted in a particle-to-particle heat exchanger (custom built by Food Processes Inc., Saginaw, MI). The heat transfer medium of the exchanger consisted of 1.6 mm (1/16") diameter,type A, 90% aluminum oxide ceramic beads (Coors Ceramic Co., Golden City, CO) with a specific gravity of 3.6 g/cm^3. The beads were heated to 240^{o} C and were maintained in the chamber with the raw beans for 100 seconds in a 1:5 ratio of beans to beads. These processing conditions resulted in an exit temperature of the beans of 113^{o} C. Roasted beans were cracked through a corrugated roller mill (Ferrell Ross; Oklahoma City, OK) into 6 to 8 pieces. The hulls, or seed coats, were removed using a zig-zag aspirator (Kice Metal Products; Wichita, KS). A hull flour fraction was produced by grinding the hull pieces through a swinging

blade Model D6 Fitzmill (W.J. Fitzpatrick Co., Chicago, IL) using the impact surfaces for pulverization through a 0.69 mm (1/37 inch) round hole screen.

Grinding and Air Classification

After hull removal was completed at the Food Protein Research and Development Center, the cracked cotyledons were sent to the Alpine American Corporation; Natick, MA for milling and air classification. The cracked cotyledons were finely ground in a Model 250 CW Stud Impact Mill at a speed of 11,789 rpm and a door speed of 5,647 rpm. The resulting flours were air classified in a Model 410 MPVI Air Classifier at a rotor speed of 2,200 rpm and a brake ring setting of 3, using a 7.62 cm (3 inch) screw feeder operating at 25 rpm. At this point, two flour fractions were obtained: an intermediate starch fraction (coarse I) and an intermediate protein fraction (fines I). The intermediate protein fraction was reclassified under the following conditions: rotor speed of 2,200 rpm; brake ring setting of 0; 7.62 (3 inch) screw feeder operating at 25 rpm. As a result of this second air classification step, a high starch fraction (coarse II) and a high protein fraction (fines II) were obtained.

Sample Materials

The final materials produced as a result of the processes described included the hull flour fraction and three air classified fractions: intermediate starch (starch I), high starch (starch II), and high protein (protein II). A flow diagram showing the flour processing scheme and the fractions produced appears in Figure 2. The percentage yield of each fraction of each bean type is shown

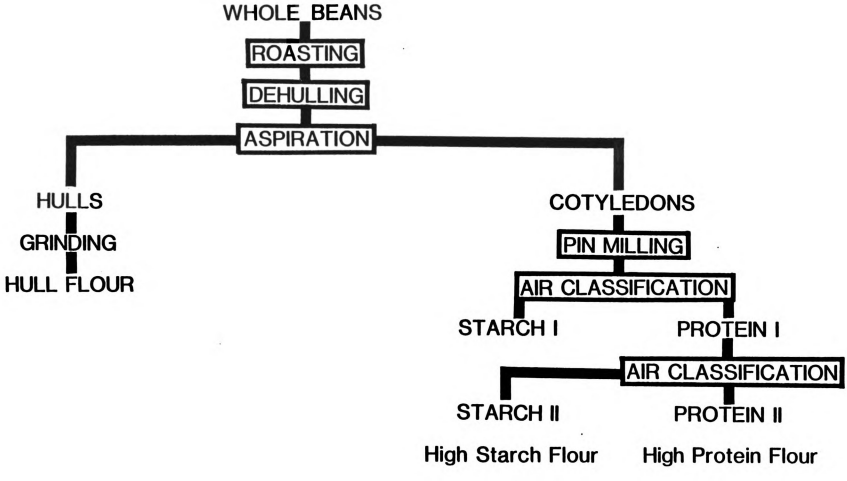

Figure 2. Flow diagram of flour processing scheme.

diagramatically in Figure 3. In addition to these four flour fractions, a small amount of dehulled, ground cotyledon material, referred to as the whole-dehulled fraction, was reserved and later analyzed. All flour fractions were packaged in polyethylene bags and shipped to Michigan State University in fiber drums at ambient temperature. Upon arrival at Michigan State, within two weeks after shipping, the flour fractions were maintained in the shipping containers at room temperature. The five flour fractions of each of the three bean types were employed in all subsequent analyses. Proximate analyses were begun immediately upon arrival. Mineral and phytic acid analyses were conducted within the following four months.

Proximate Analyses

Moisture

AACC Method 44-32 (1962) was modified slightly for determining moisture content in duplicate. Five g flour samples were weighed into previously dried, cooled, and weighed 50 ml porcelain crucibles. Samples were dried under a partial vacuum (ca. 25 mm Hg) between 95 and 100^{o} C for 6 hours. After cooling in a desiccator, samples were reweighed and percentage moisture was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Wet sample wt.} - \text{Dry sample wt.}}{\text{Wet sample wt.}} \times 100$$

Ash

Ash content was determined for each flour fraction in triplicate by AACC Method 08-01(1962). The dried flour samples obtained from the moisture determinations were incinerated at 525^{o} C for 24 hours in a

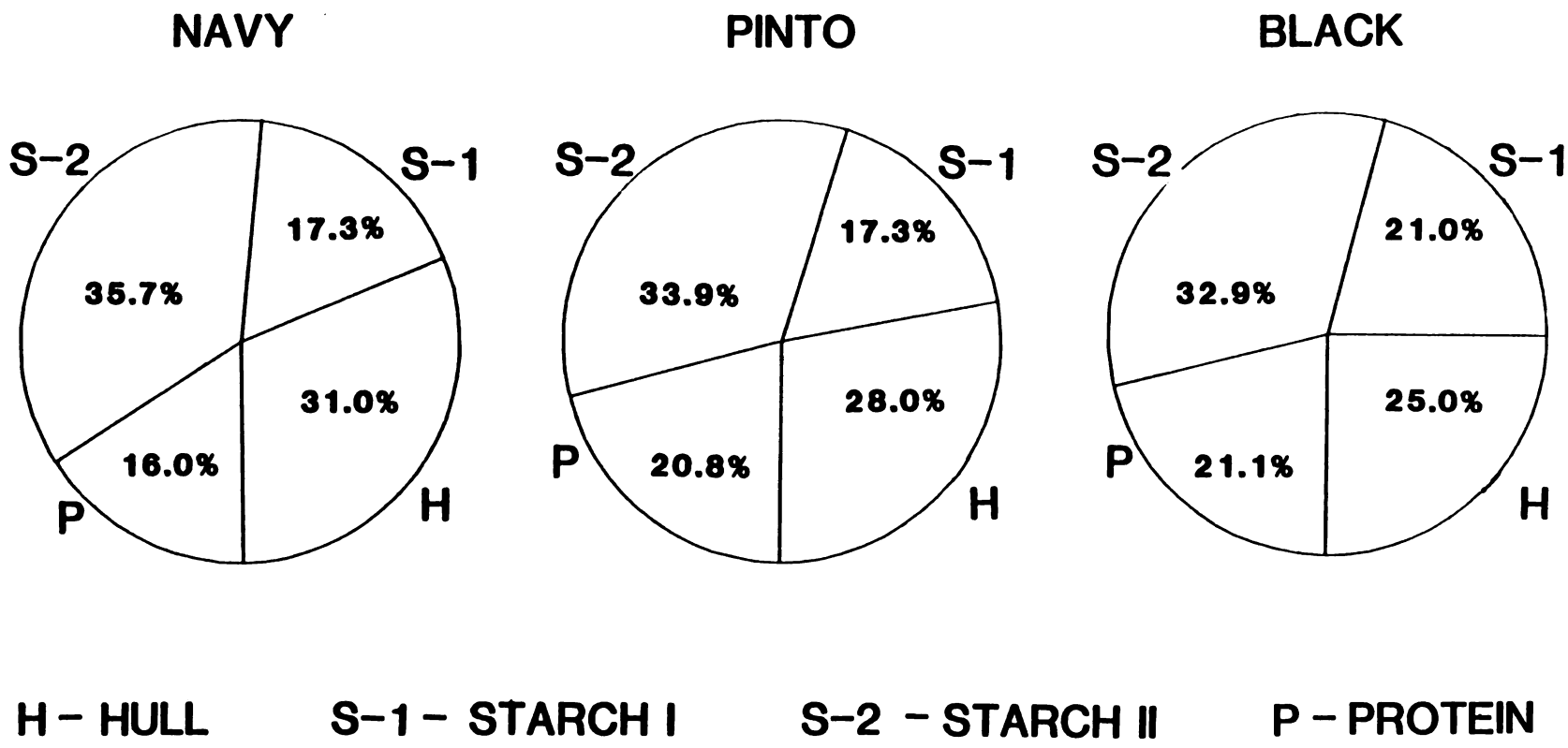

Figure 3. Percentage yield of flour fractions obtained from navy, pinto, and black beans.

Barber-Coleman muffle furnace Model No. 293C (Thermolyne Corp.; Dubuque, Iowa). The uniform white ash was allowed to cool to room temperature in a desiccator and was then weighed. Percentage ash was calculated on a dry weight basis.

$$\% \text{ Ash} = \frac{(\text{Wt. crucible + ash}) - (\text{Wt. crucible})}{\text{Sample wt.}} \times 100$$

Protein

Protein content was determined for each flour fraction in duplicate according to AACC Method 46-23 (1962), a standard micro-Kjeldahl procedure. Percentage nitrogen was calculated as follows:

$$\% \text{ N} = \frac{(\text{ml HCl - ml blank}) \times \text{normality} \times \text{equiv. wt. N}}{\text{Sample wt. (mg)}} \times 100$$

Percentage nitrogen was then multiplied by 6.25 to provide percentage total protein.

Crude Fat

Crude fat content was determined for each flour fraction in triplicate using a modification of AACC Method 30-25 (1962), a Soxhlet extraction procedure. Approximately 20 g of undried flour were weighed into a Whatman cellulose extraction thimble, 33 x 80 mm. A small wad of glass wool was firmly placed over the sample material to prevent its escape. The thimble was placed in a Soxhlet extraction tube and approximately 300 ml of crude, reagent grade hexanes were refluxed through the sample for 6 hr to remove the lipid material present. After the hexanes cooled, the contents of the flask and condenser were passed through a fine (F), stemmed sintered glass Gooch

crucible with suction to remove any residual flour particles. The total volume of filtrate was measured. A 50 ml aliquot of this filtrate was placed in a previously dried and weighed 125 ml Erlenmeyer flask. The contents of the flask were evaporated on a steam bath until all but 5-10 ml of the solvent-fat mixture remained. The flask was then dried at 70^{o} C overnight in a Labline vacuum oven (Labline Inc.; Chicago, IL). After cooling to room temperature in a desiccator, the flask was reweighed. Crude fat content (wet basis) was calculated as follows:

$$\% \text{ Crude Fat} = \frac{\text{Wt. fat + flask(g)}}{\text{Sample wt. (g)}} \times \frac{\text{Filtrate vol. (ml)}}{\text{50 ml}} \times 100$$

Corrections to dry weight basis were made using moisture content value determined by the formerly described method.

Dietary Fiber

Enzyme neutral detergent fiber (ENDF) was determined for each flour fraction in triplicate by the method of Robertson and VanSoest (1977). This procedure was modified to include 1 mg of amyloglucosidase to provide for complete starch digestion.

Starch

The percentage starch content (dry weight basis) of each flour fraction was estimated by subtracting the sum of the mean values for all other macroconstituents, on a dry weight basis, from 100.

% Starch = 100 - (% protein + % crude fat + % ENDF + % ash)

Mineral Analysis

Equipment Handling

All crucibles, volumetric flasks, funnels, and culture tubes used in preparing samples for mineral analyses were specially cleaned. After being washed with detergent and rinsed with distilled water, they were soaked in a 3N Baker Instra-analyzed nitric acid solution for a minimum of 2 hr, rinsed three times with water of double distilled quality, allowed to air dry, and were stored separately from other laboratory glassware. Several samples of the double distilled quality water were analyzed for mineral content and found to contain below detectable levels of the eight minerals studied in this investigation. The muffle furnace was washed and thoroughly rinsed with the double distilled quality water before each use.

Sample Preparation

Triplicate samples of all five flour fractions of each bean type were analyzed for mineral content. One g samples of previously dried flour were weighed into 50 ml porcelain crucibles and heated on a Labconco micro-Kjeldahl burner unit (Labconco Corp., Kansas City, MO) on the low setting for approximately 20 to 30 min until all smoke dissipated and the samples appeared charred. Samples were then placed in a Barber-Coleman muffle furnace Model No. 293C (Thermolyne Corp.;Dubuque, Iowa) at room temperature. Between 20 and 24 crucibles were incinerated in the muffle furnace at one time. Samples were ashed at 500^{o} C for approximately 16 hr. When cool, the residue remaining in each crucible was dissolved in 2 ml concentrated Baker Instra-analyzed nitric acid for approximately 1 hr. The dissolved

residues were transferred to 10 ml volumetric flasks and brought to volume with deionized water. The mineral solutions were transferred to labeled polypropylene culture tubes which were capped and refrigerated overnight. If the solutions contained visible sediment the next day, the solutions were decanted into clean culture tubes so that only clear solutions were introduced to the argon gas constituting the plasma. For approximately every 10 flour samples, one sample of standard reference material (SRM) #1572, citrus leaves (National Bureau of Standards, Washington,DC), was prepared, as well as a procedural blank, which contained no sample material. The standard reference material and procedural blank samples received the same treatment as flour samples. All flour sample, SRM, and procedural blank solutions were stored at -20^{o} F until the day of analysis.

ICP Emission Spectrometer

An inductively coupled plasma (ICP) emission spectrometer (Jarrell-Ash Model 955 Plasma Atomcomp; Fisher Scientific Co., Pittsburgh, PA) was employed to measure the Zn, Fe, Ca, Cu, Na, K, Mg, and P content of each sample. The plasma emission unit, located in the Department of Pharmacology and Toxicology at Michigan State University, was operated by a trained technician of that Department. All samples were analyzed on the same day.

Phytic Acid Analysis

Phytic acid was determined by a combination of two methods. The procedure of Wheeler and Ferrel (1971) was followed for the extraction

and precipitation of phytic acid, whereas the iron of the precipitate was measured by Makower's method (1970). The phytic acid content of each flour fraction was analyzed in triplicate. Two g samples of previously dried flour were analyzed for all fractions except protein, for which 1 g samples were used.

Phytic acid was extracted from each flour sample into 40 ml of a 3% trichloroacetic acid solution with the aid of mechanical shaking (Model No. 62105 Mechanical Shaker; New Brunswick Scientific Co., New Brunswick, NJ). After centrifugation of the resulting suspension at 20,000 X g in an IEC Model B-20A centrifuge (Daman/IEC Division; Needham Hts., MA), a 10 ml aliquot was removed, added to an excess of ferric chloride and heated for 1 hr in a boiling water bath to precipitate the phytic acid present. The resulting mixture containing ferric phytate was centrifuged at 2,000 rpm in a Sorvall Model GLC-1 centrifuge (Ivan Sorvall, Inc.; Norwalk, CN) and decanted. The ferric phytate precipitate was washed with 20 - 25 ml of a 3% TCA solution, centrifuged and decanted. This washing procedure was repeated again with 3% TCA and then with deionized water. Ferric hydroxide was formed by heating the ferric phytate precipitate with 5 ml deionized water and 5 ml 0.6 N NaOH for approximately 45 min. The washed ferric hydroxide precipitate was dissolved in 0.5 ml 0.5 N HCl by heating in boiling water for 10 min and transferred to a 100 ml volumetric flask. Each flask was brought to volume with 0.1 N HCl. Two ml of that solution were transferred to a 25 ml volumetric flask. One ml 10% hydroxylamine HCl was added, the flask was rotated and permitted to stand for approximately 5 minutes. Then 9.5 ml of 2M sodium acetate solution and 1.0 ml of 0.1% orthophenanthroline

solution were added. The flasks were brought to volume with 0.1N HCl, rotated, and allowed to sit for at least 5 minutes before absorbance was read at 510 nm from a Beckman Model DB-G grating spectrophotometer (Beckman Instruments, Inc.; Fullerton, CA). The iron concentration of each sample was calculated using the linear regression equation, $A_{510} = 0.019 + 6.697x$, which was determined from absorbance values obtained with standard iron solutions containing between 0.2 and 3.5 mg Fe/100 ml.

Phytate phosphorus content was calculated from iron concentration data by assuming a 4:6 atomic ratio of iron to phosphorus. Phytic acid content was estimated on the basis that phosphorus accounts for 28.20% of the compound's weight.

Feeding Study for Determining Iron Bioavailability

A hemoglobin regeneration assay was conducted with young rats according to AOAC Method 43.219 (1980). The basal diet, including both the vitamin and mineral premixes, was prepared in accordance with the recommendations of Fritz et al. (1978). Hemoglobin determinations were made as outlined in AOAC Method 43.218 (1980).

Animals and Depletion Treatment

Weanling male Sprague-Dawley rats (Harlan Breeding Laboratories; Indianapolis, IN) less than 21 days old weighing approximately 50 g were housed in stainless-steel cages with wire-mesh floors in a temperature controlled room (22^{o} C) with a 12 hr light-dark cycle. All rats were provided with double distilled quality water and basal

diet ad libitum. The composition of the basal diet containing no added iron is given in Table 3.

During the depletion period, the rats were weighed weekly and food consumption per two rats was recorded every 2 to 3 days. After 26 days, the hemoglobin concentration of each rat was determined in duplicate from 0.02 ml samples of whole blood obtained by amputating the tip of the tail. The quantitative colorimetric determination of total hemoglobin was made by the cyanmethemoglobin method using Drabkin's reagent and a stable, human hemoglobin standard. All solutions used in this procedure were prepared from Sigma Chemical Company kit No. 525-A (Sigma Chemical Company; St. Louis, MO). A Beckman Model DB-G spectrophotometer set at 540 nm was used in making these determinations.

Experimental Diets and Treatments

Rats having hemoglobin levels less than 6 g/100 ml were considered satisfactorily depleted and were divided into treatment groups so that the initial mean and standard deviation for hemoglobin concentration were approximately the same for each group. In total, 13 groups consisting of eight rats per group, each housed individually in a stainless-steel cage, were established. Each group was fed one of the 13 diets described below.

In addition to the basal diet, three standard diets were prepared by substituting 6, 12, and 24 ppm iron in the form of ferrous sulfate for equal weights of cerelose in the basal diet. Bean flour test diets were prepared by incorporating amounts of navy hull, navy high starch (starch II) and navy protein flours which supplied 6, 12, and

Table 3. Composition of the basal low iron diet.

Ingredient	Percentage
Cerelose[1]	69.38
Vitamin-free casein[2]	20.00
Corn oil[3]	5.00
Monosodium phosphate	2.00
Calcium carbonate	2.00
Potassium chloride	0.50
Iodized salt	0.50
Trace mineral premix[4]	0.27
Choline chloride	0.15
Vitamin premix[5]	0.10
dl-Methionine	0.10

[1]CPC International, Englewood Cliffs, NJ.

[2]United States Biochemical Corp., Cleveland, OH.

[3]Capri corn oil, Anderson Clayton Foods, Dallas, TX.

[4]Composed of (in g/kg diet): $MgSO_4$, 1.982; $ZnSO_4 \cdot 7H_2O$, 0.528; $MnSO_4 \cdot H_2O$, 0.154; $CuSO_4 \cdot 5H_2O$, 0.020, and KIO_3, 0.002.

[5]Composed of (in mg/kg diet): alpha tocopherol succinate, 41.32; menadione, 0.4; thiamin HCl, 10.0; calcium pantothenate, 10.0; niacin, 30.0; folic acid, 1.0; vitamin B_{12}, 0.02; vitamin D, 0.0375; vitamin A acetate, 10.0, and cerelose to 1.0 g.

24 ppm iron in addition to the iron inheretly present in the other diet ingredients. Bean flour test diet formulations were such that the casein content was decreased by the amount of protein contributed by the bean flour. Cerelose was decreased in all test diets by the amount necessary to maintain component percentage levels and yield the predetermined quantity of diet. All other diet ingredients were used at the same percentage levels as in the basal diet. Navy flour fractions were chosen for the feeding study since they contained higher levels of phytic acid than the corresponding black and pinto flours. The composition of each experimental diet is provided in the Appendix. Standard and bean flour test diets were fed ad libitum for 14 days and samples for hemoglobin assay were obtained as before. Food intake during repletion was recorded every 3 days and animal weights were taken weekly. Test diets were analyzed for iron content by the procedure described in the Mineral Analysis section.

Repletion Parameters

From data obtained during the repletion period, total food consumption and weight gain during repletion were calculated. The change in hemoglobin concentration from the end of depletion to the end of repletion was calculated for each rat, which enabled a group mean to be obtained. Following the assumptions that 7% of a rat's body weight is blood (Cartland and Koch, 1928) and that hemoglobin contains 0.34% iron, total initial and total final iron hemoglobin levels and total change in iron hemoglobin levels were calculated.

Statistical Analyses of the Data

Using the Statistical Package for the Social Sciences (SPSS), one way analyses of variance were performed to determine if any significant differences existed in the mean levels of the eight minerals analyzed and phytic acid between the various flour fractions within each bean type. Significance was determined by Tukey's test at the 0.05 alpha level. Additionally, correlation coefficients were produced for each bean type between the level of each of the eight minerals analyzed and protein content, dietary fiber content, crude fat content, and starch content. Similarly, correlation coefficients were also generated for each bean type between phytic acid content and the level of each of the eight minerals analyzed, protein content, dietary fiber content, crude fat content, and starch content.

RESULTS AND DISCUSSION

Proximate Composition

The moisture, ash, protein, fat, fiber, and starch contents of each fraction of navy, pinto, and black bean flour is shown in Tables 4-6. These same values are also provided in the form of bar graphs in Figures 4-6.

Moisture

The moisture content for the flour fractions of any one bean type varied by less than 1.5%. The moisture contents of the flour fractions of all three bean types were less than 10%. In general, the navy bean flour fractions had the highest moisture values and the pinto flours, the lowest. No trends could be seen among bean types in terms of any particular fraction containing more or less moisture than any other fractions.

Ash

The range in ash values for the various flour fractions of the three bean types were as follows: navy flours, 3.63 to 5.72%; pinto flours, 2.95 to 6.02%, and black flours, 3.74 to 8.04%. For all three bean types, the starch II flour fraction possessed the lowest ash content, and except for navy bean flours, the protein fraction had the highest ash content. The ash content of the navy starch I fraction

Table 4. Proximate composition of various navy bean flour fractions.

Flour fraction	% Moisture[1]	% dry basis				
		Ash[1]	Protein[1]	Fat[2]	Fiber[2]	Starch
Whole-dehulled	9.81	4.40	24.33	2.23	4.44	64.60
Hull	8.96	4.85	22.21	1.87	13.40	57.67
Starch I	9.64	5.72	26.09	2.40	8.67	57.12
Starch II	9.79	3.63	15.51	1.47	1.76	77.63
Protein	9.18	5.41	44.06	3.68	1.78	45.07

[1] n=2

[2] n=3

Table 5. Proximate composition of various pinto bean flour fractions.

Flour fraction	% Moisture[1]	% dry basis				
		Ash[1]	Protein[1]	Fat[2]	Fiber[2]	Starch
Whole-dehulled	6.23	4.16	24.50	1.22	3.98	66.14
Hull	7.64	4.29	22.81	1.43	11.31	60.16
Starch I	7.08	4.91	27.60	1.82	7.47	58.20
Starch II	7.76	2.95	12.21	0.87	1.50	82.47
Protein	6.58	6.02	44.23	1.14	4.31	44.30

[1] n=2

[2] n=3

was slightly higher than that of the navy protein fraction. The ash contents of the whole-dehulled and hull flours fell between those determined for starch II and protein flour fractions for all three bean types. Overall, the black bean flour fractions had higher ash contents than the corresponding pinto and navy flour fractions.

Table 6. Proximate composition of various black bean flour fractions.

		% dry basis				
Flour fraction	Moisture[1]	Ash[1]	Protein[1]	Fat[2]	Fiber[2]	Starch
Whole-dehulled	8.40	5.54	27.14	1.34	4.95	61.03
Hull	8.70	6.26	22.54	1.53	13.84	55.83
Starch I	8.42	6.27	29.96	2.14	6.40	55.23
Starch II	8.14	3.74	12.42	0.83	1.72	81.29
Protein	7.29	8.04	45.52	1.16	3.67	41.61

[1] n=2

[2] n=3

Protein

The protein content of the flours ranged from 15.51 to 44.06% for navy fractions, from 12.21 to 44.23% for pinto fractions, and from 12.42 to 45.52% for black fractions. The values were lowest for the starch II fractions and highest for the protein fractions of all bean types. The protein values for the whole-dehulled, hull, and starch I flour fractions were very similar to each other, being about half way between those reported for the starch II and protein flour fractions.

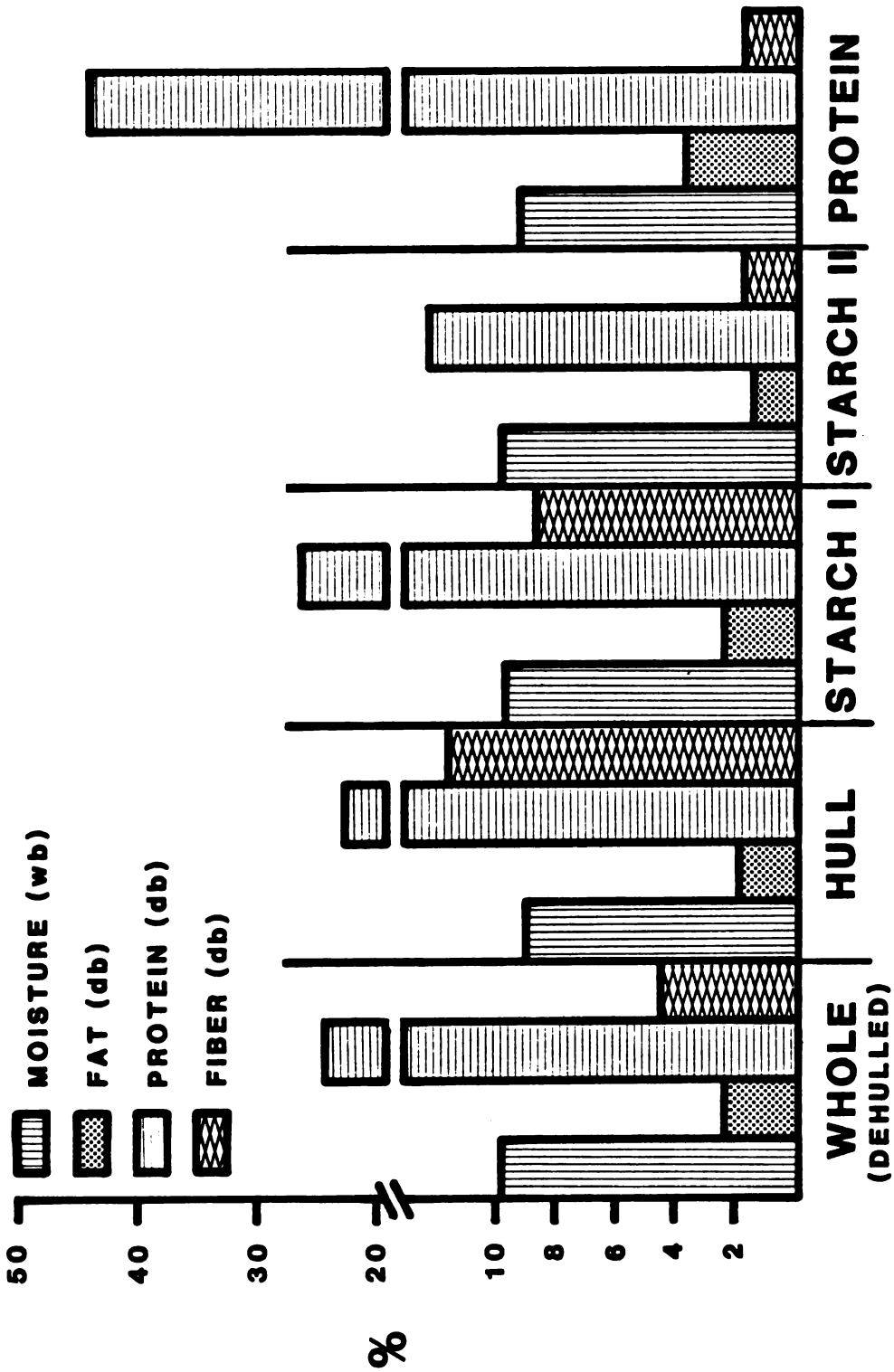

Figure 4. Proximate composition of various navy bean flour fractions.

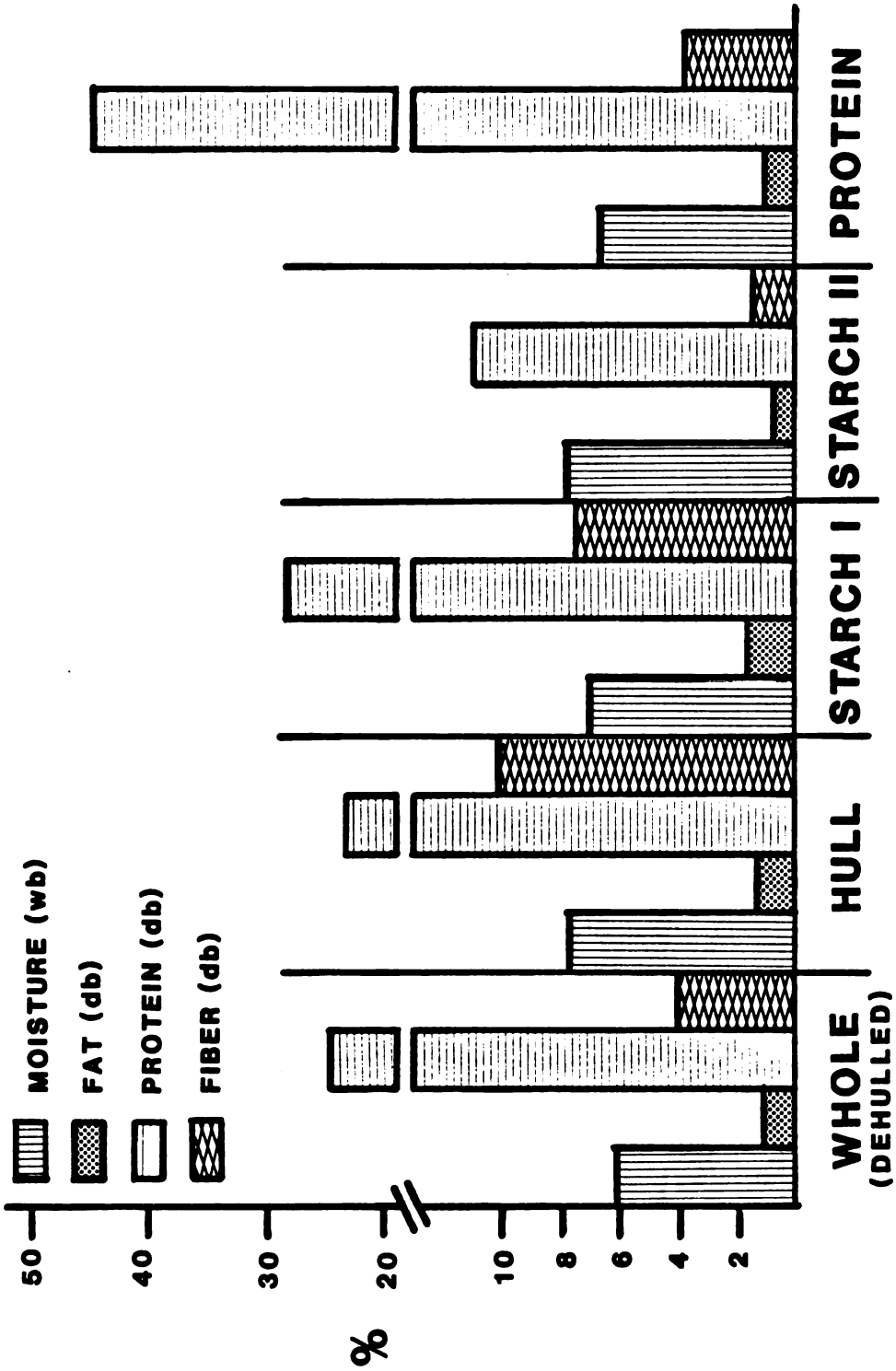

Figure 5. Proximate composition of various pinto bean flour fractions.

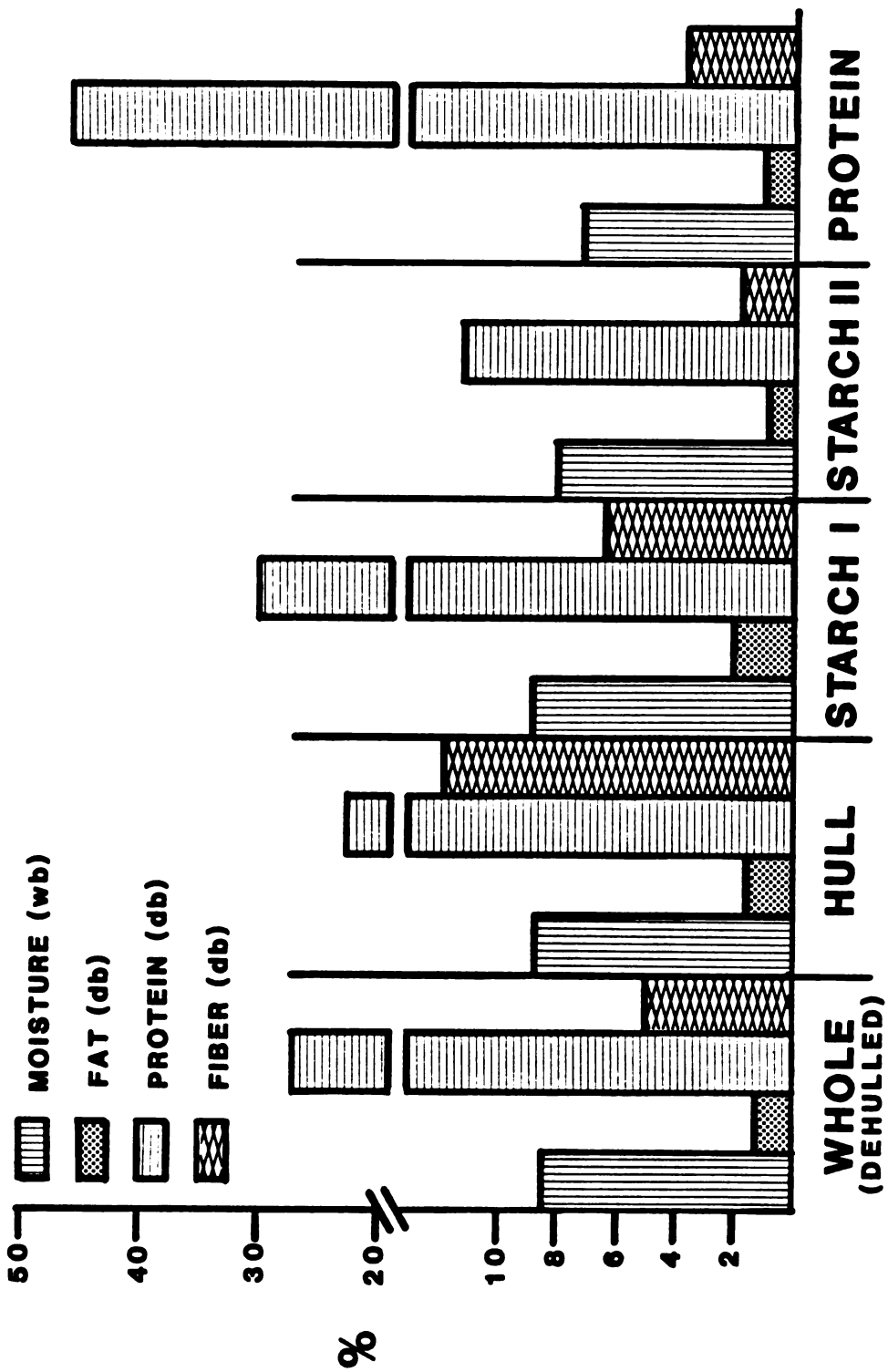

Figure 6. Proximate composition of various black bean flour fractions.

Fat

The navy bean flour fractions contained more crude fat than either the pinto or black flour fractions. No trend was found among bean types in terms of partitioning of fat into particular fractions. The crude fat content of each flour fraction of all bean types was less than 4.0%.

Dietary Fiber

The dietary fiber values varied greatly among fractions of any given bean type, being consistently highest for the hull flour and lowest for the starch II flour fraction. For all bean types, the dietary fiber content of the starch I fraction was exceeded only by that of the hull fraction. Dietary fiber values ranged from 1.76 to 13.40% for navy flours, from 1.50 to 11.31% for pinto flours, and from 1.72 to 13.84% for black flours.

Although the hull flours contained more dietary fiber than the other flour fractions, the values were considered low in comparison to those obtained in a previous study. Aguilera et al. (1982), using the same process as was used in this investigation, produced navy hull flours with dietary fiber contents ranging from 31.2 to 50.2%. The discrepancy may be traced to the separation process as indicated by the materials balance data obtained in the two studies. Hull flour accounted for an average of 10% of the initial weight of the beans in the experiments described by Aguilera et al. (1982), whereas this figure was approximately 29% in the current study. Difficulty in separating the hull and cotyledon material in the latter investigation resulted in a sizable amount of cotyledon being incorporated into the

hull fraction. Inclusion of cotyledon in the hull flour fraction presumably had the effect of "diluting" the hull component, thereby decreasing the dietary fiber content of this flour.

Starch

As was expected based on the results of the ash, protein, fat, and fiber analyses, the calculated starch content of all three bean types was highest in the starch II fraction and lowest in the protein fraction: Starch content exceeded 77% for starch II flour fractions and was less than 46% for protein flour fractions. The starch contents of the whole-dehulled, hull, and starch I flours were intermediate, ranging between approximately 55 and 66% for the three bean types.

Mineral Analysis

The content of the eight minerals measured in each navy, pinto, and black flour fraction are shown in Tables 7-9. Significant differences in the content of each mineral among the various flour fractions are indicated in these tables. Figures 7-14 provide bar graphs showing the relative amounts of each of the eight minerals for the flour fractions of the three bean types. Overall, the large number of significant differences among the fractions of all three bean types, except for sodium, reveals that the various minerals studied were not equally distributed among the fractions, but rather, that partitioning occurred. The protein flour fractions contained higher amounts of most minerals studied whereas the starch II fractions contained lower amounts than the other flour fractions.

Table 7. Average content of eight minerals in various navy bean flour fractions (n=3).[1]

Mineral	parts per million (ppm)				
	Whole-dehulled	Hull	Starch I[2]	Starch II	Protein
Ca	1019 ± 28c	4173 ± 5d	3780 ± 184e	273 ± 4a	709 ± 9b
Cu	52.6 ± 5.2b	12.2 ± 0.2a	121.0 ± 25.5c	9.6 ± 0.1a	22.6 ± 0.5a
Fe	67.1 ± 0.4a	86.4 ± 1.3b	91.1 ± 2.3b	71.6 ± 6.0a	139.7 ± 2.3c
Mg	1677 ± 12b	2237 ± 15c	2350 ± 42d	1103 ± 42a	2597 ± 31e
P	5233 ± 40c	4617 ± 159b	5210 ± 28c	3997 ± 25a	8833 ± 67d
Zn	32.1 ± 0.5b	35.9 ± 0.3c	39.0 ± 1.4d	19.9 ± 0.3a	52.5 ± 1.0e
Na	117.3 ± 50.6a	104.8 ± 97.3a	34.3 ± 10.8a	34.0 ± 14.2a	62.8 ± 52.6a
K	17033 ± 231a	17100 ± 200a	19350 ± 212b	16867 ± 153a	19800 ± 361b

[1]For each mineral, those values with the same letter are not significantly different from each other as judged by Tukey's test at alpha = 0.05.

[2]n=2

Table 8. Average content of eight minerals in various pinto bean flour fractions (n=3).[1]

Mineral	parts per million (ppm)				
	Whole-dehulled	Hull	Starch I	Starch II	Protein
Ca	710 ± 5b	1027 ± 23c	2433 ± 133d	216 ± 3a	675 ± 4b
Cu	12.3 ± 4.1a	43.5 ± 7.8b	67.2 ± 20.3b	8.5 ± 0.3a	17.1 ± 0.5a
Fe	79.8 ± 2.6b	71.6 ± 3.4ab	100.0 ± 3.5c	61.9 ± 9.3a	119.3 ± 2.9d
Mg	1416 ± 8b	1487 ± 32b	2023 ± 42c	726 ± 5a	2223 ± 29d
P	4933 ± 21b	5073 ± 31c	5273 ± 29d	2807 ± 15a	8440 ± 70e
Zn	31.6 ± 1.1b	31.3 ± 0.9b	37.8 ± 1.2c	15.9 ± 0.1a	51.0 ± 0.7d
Na	79.2 ± 70.9a	53.0 ± 14.7a	91.2 ± 36.8a	134.7 ± 6.7a	45.8 ± 24.1a
K	16967 ± 306b	17500 ± 458b	18733 ± 115c	12900 ± 0a	24267 ± 577d

[1]For each mineral, those values with the same letter are not significantly different from each other as judged by Tukey's test at alpha = 0.05.

Table 9. Average content of eight minerals in various black bean flour fractions (n=3).[1]

Mineral	parts per million (ppm)				
	Whole-dehulled	Hull	Starch I	Starch II	Protein
Ca	1447 ± 38b	4270 ± 21d	2777 ± 21d	696 ± 13a	1760 ± 10c
Cu	7.0 ± 0.9b	16.5 ± 1.2d	12.4 ± 0.4c	4.9 ± 0.1a	15.8 ± 0.3d
Fe	281.0 ± 5.6c	44.0 ± 0.4a	168.0 ± 9.6b	168.7 ± 4.0b	533.3 ± 23.1d
Mg	2083 ± 15b	2833 ± 15d	2613 ± 29c	1033 ± 15a	3280 ± 46e
P	5347 ± 15	4453 ± 25b	5413 ± 32c	2747 ± 38a	9163 ± 74d
Zn	32.3 ± 0.3a	40.1 ± 0.2ab	40.2 ± 0.1ab	23.9 ± 14.2a	52.6 ± 0.5b
Na	177.3 ± 102.8a	121.7 ± 47.3a	47.8 ± 16.0a	119.0 ± 1.7a	83.7 ± 45.4a
K	18367 ± 551b	17500 ± 200b	19957 ± 530c	13667 ± 115a	25567 ± 306d

[1]For each mineral, those values with the same letter are not significantly different from each other as judged by Tukey's test at alpha = 0.05.

This finding was consistent with the generally higher ash values reported for the protein fractions, and consistently low ash values reported for the starch II fractions.

Calcium

The average calcium content of the pinto flour fractions was consistently lower than for the corresponding navy and black flour fractions. Values ranged from 216 to 2433 ppm for pinto flours, from 273 to 4173 ppm for navy flours, and from 696 to 4270 ppm for black flours. For all bean types, the starch II fraction contained the least calcium. For both navy and black bean flours, the hull fraction contained the most calcium, whereas the starch I fraction of the pinto flour had the highest calcium content.

Copper

Average copper values ranged from 9.6 to 52.6 ppm for navy flours, from 8.5 to 67.2 ppm for pinto flours, and from 4.9 to 16.5 ppm for black flours. The lowest values were found for the starch II fraction of each bean type. The starch I fraction of navy and pinto flours contained the most copper. For the black flours, the hull fraction had the highest copper value, however, this amount was not significantly different from the copper content of the protein fraction, which differed from the hull fraction by less than one ppm. Each fraction of black flour contained less copper than the corresponding navy and pinto flours.

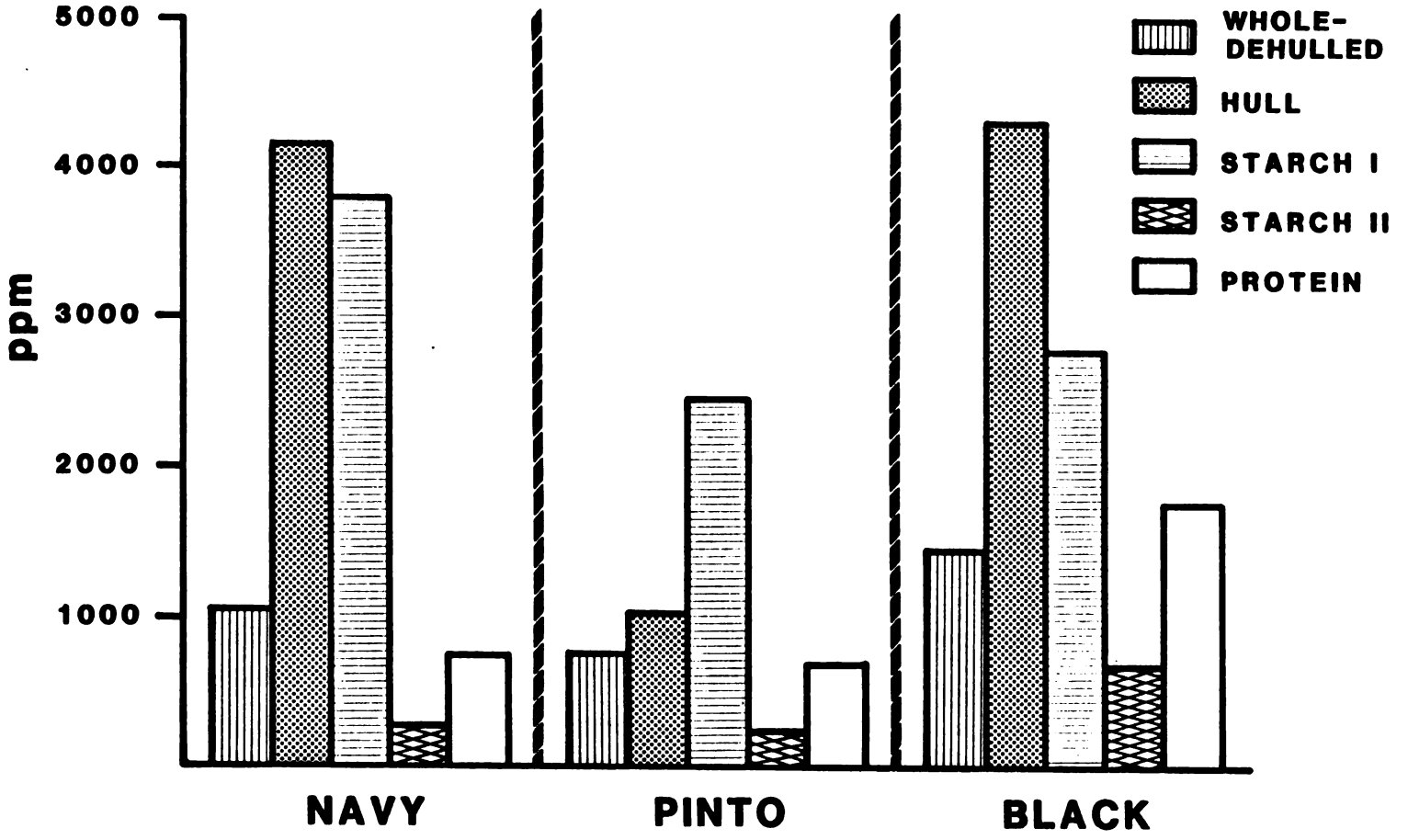

Figure 7. Average calcium content of various navy, pinto and black bean flour fractions.

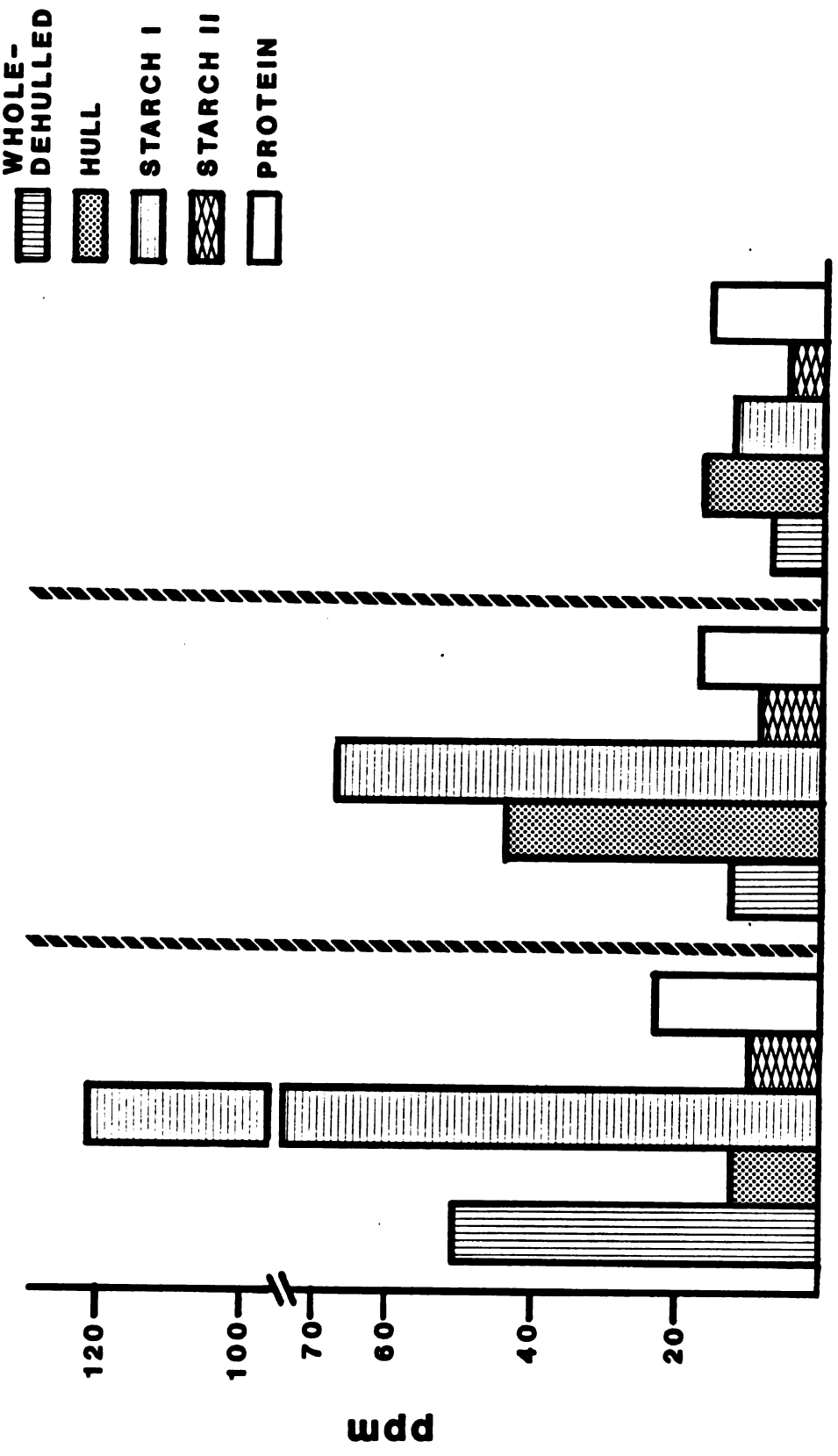

Figure 8. Average copper content of various navy, pinto and black bean flour fractions.

Iron

The average iron content of the protein flour fraction of each of the three bean types was significantly higher than the iron content of the other fractions. For navy flours values ranged from 67.1 to 139.7 ppm, for pinto flours from 61.9 to 119.3 ppm, and for black flours from 44.0 to 533.3 ppm. With the exception of the hull fraction, all black flours had a substantially higher iron content than the corresponding navy and pinto flours. Upon comparing the three bean types, no one fraction could be described as consistently having the lowest iron content.

Magnesium

As was true for iron, the average magnesium content of the protein flour fractions of the three bean types was significantly higher than the magnesium content of the other fractions. The starch II fraction of each bean type contained significantly less magnesium than the other fractions. Overall, each pinto flour fraction contained less magnesium than the corresponding navy and black bean flours. The magnesium content of pinto flours ranged from 726 to 2223 ppm, whereas this range was 1103 to 2597 ppm for navy flours and 1033 to 3280 ppm for black flours.

Phosphorus

The protein flour fraction of each bean type contained significantly more phosphorus while the starch II fraction contained significantly less phosphorus than the other three flour fractions. Values ranged from 3997 to 8833 ppm for navy flours, from 2807 to 8440 ppm for pinto flours, and from 2747 to 9163 ppm for black flours.

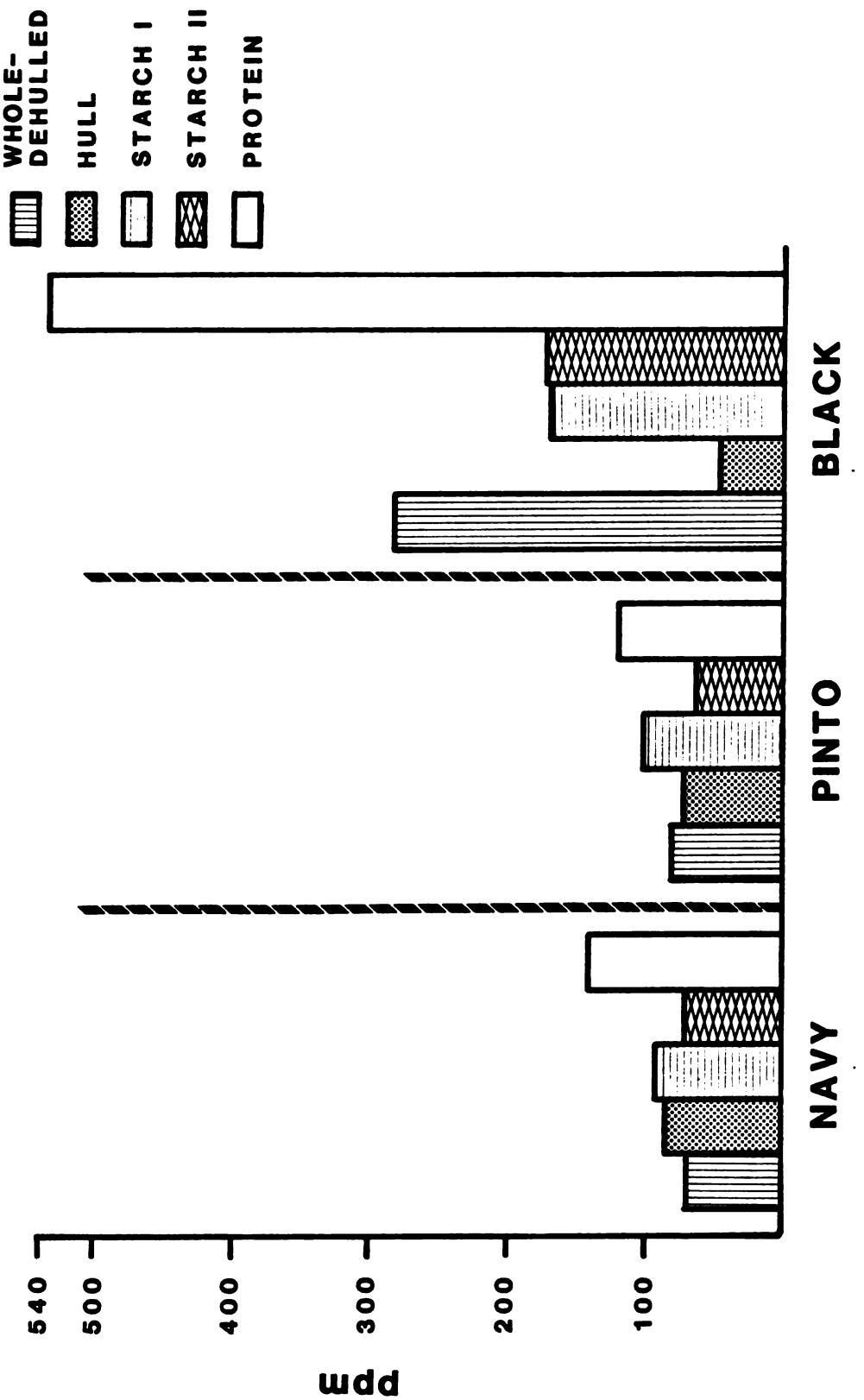

Figure 9. Average iron content of various navy, pinto and black bean flour fractions.

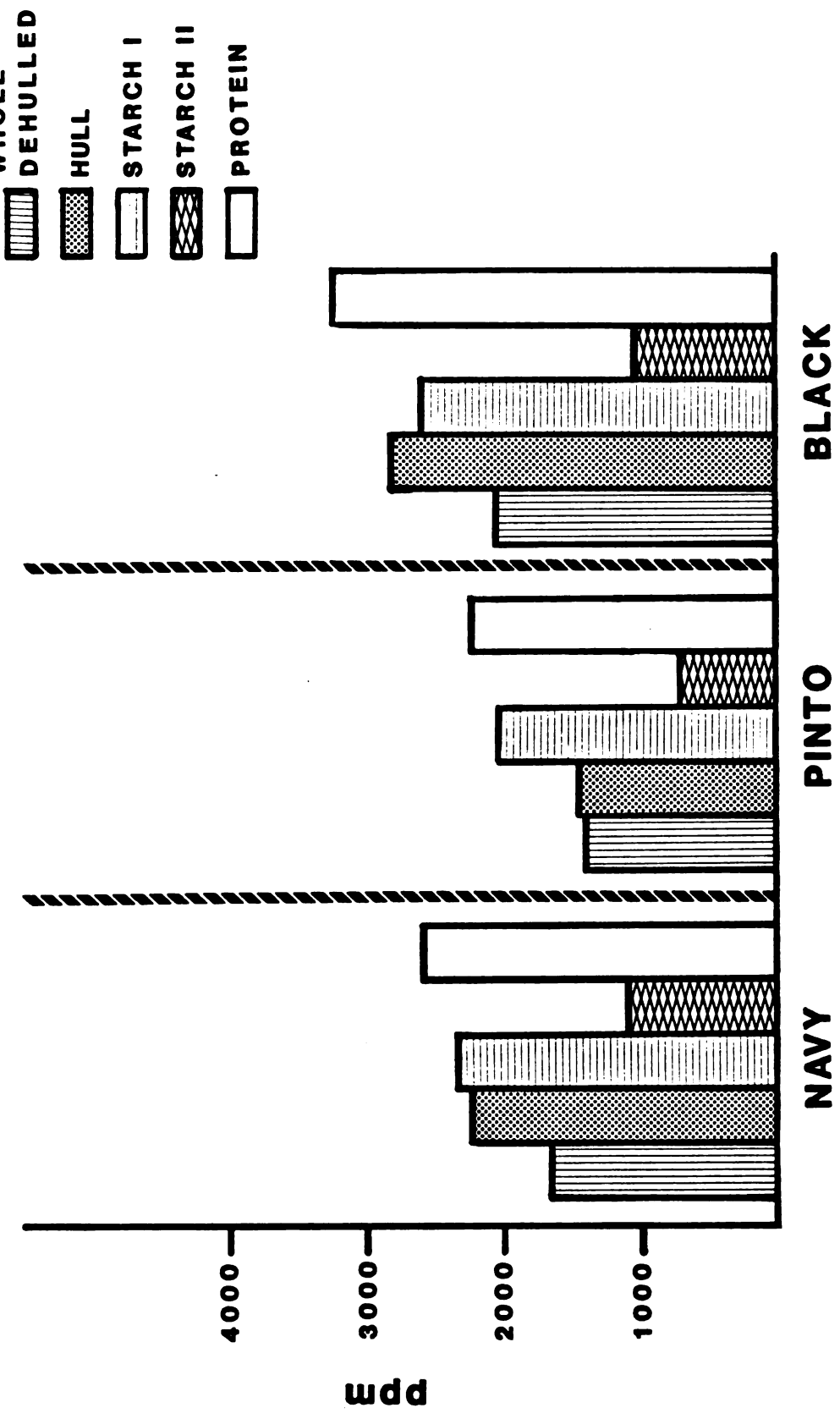

Figure 10. Average magnesium content of various navy, pinto and black bean flour fractions.

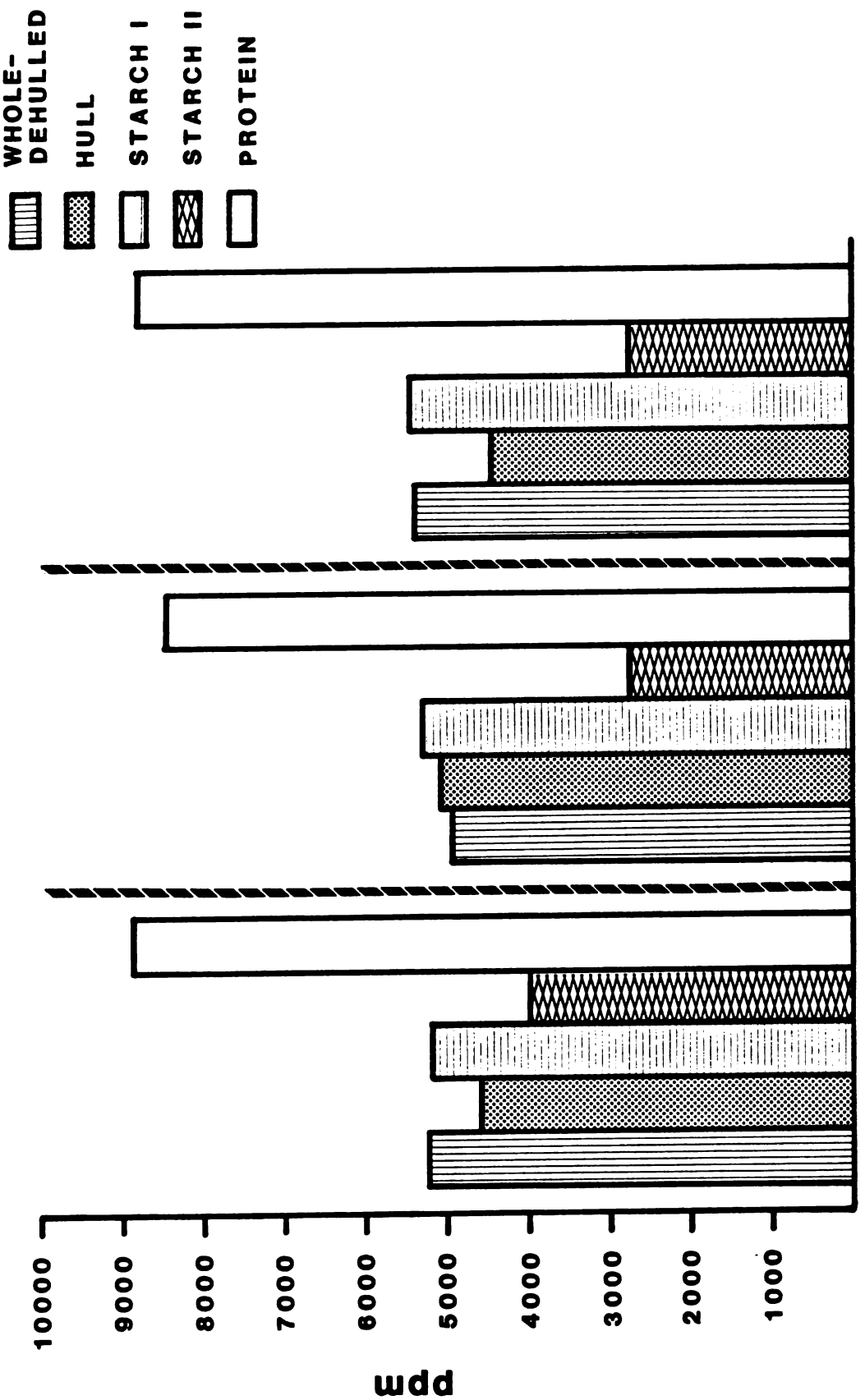

Figure 11. Average phosphorus content of various navy, pinto and black bean flour fractions.

Zinc

The same trend of highest values being found for the protein fraction and lowest for the starch II fraction were also observed for the zinc content of the navy, pinto, and black flours. However, in the case of the black flours, the high and low values were not significantly different from values reported for the other three fractions. Zinc content ranged from 19.9 to 52.5 ppm for navy, from 15.9 to 51.0 ppm for pinto, and from 23.9 to 52.6 ppm for black flours. Corresponding flour fractions of the three bean types contained very similar amounts of zinc.

Sodium

Sodium was the only element studied which was not present in significantly greater amounts in any one fraction than another. No trends were found across bean types in terms of sodium content being highest or lowest in a particular fraction. Values ranged from 34.0 to 117.3 ppm for navy flours, from 53.0 to 134.7 ppm for pinto flours, and from 47.8 to 177.3 ppm for black flours. The relatively high standard errors in relation to the mean sodium values indicated poor precision in the analysis. This undoubtedly contributed to the failure to obtain significant differences and/or partitioning trends.

Potassium

Average potassium content ranged from 16867 to 19800 ppm for navy flours, from 12900 to 24267 ppm for pinto flours, and from 13667 to 25567 ppm for black flours. The protein flour fractions of all three bean types contained the highest levels of potassium whereas the starch II fractions contained the lowest levels. For both the pinto

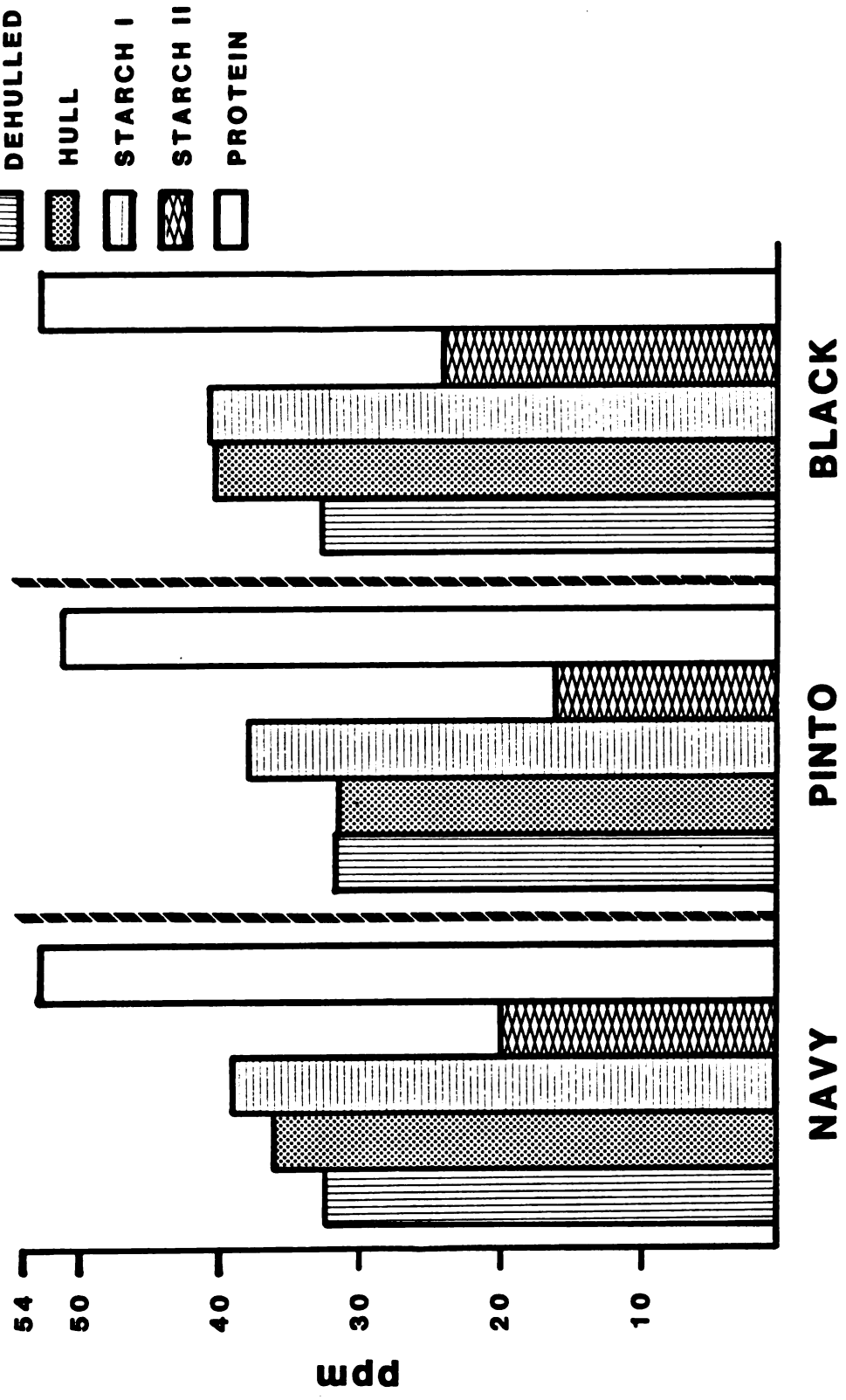

Figure 12. Average zinc content of various navy, pinto and black bean flour fractions.

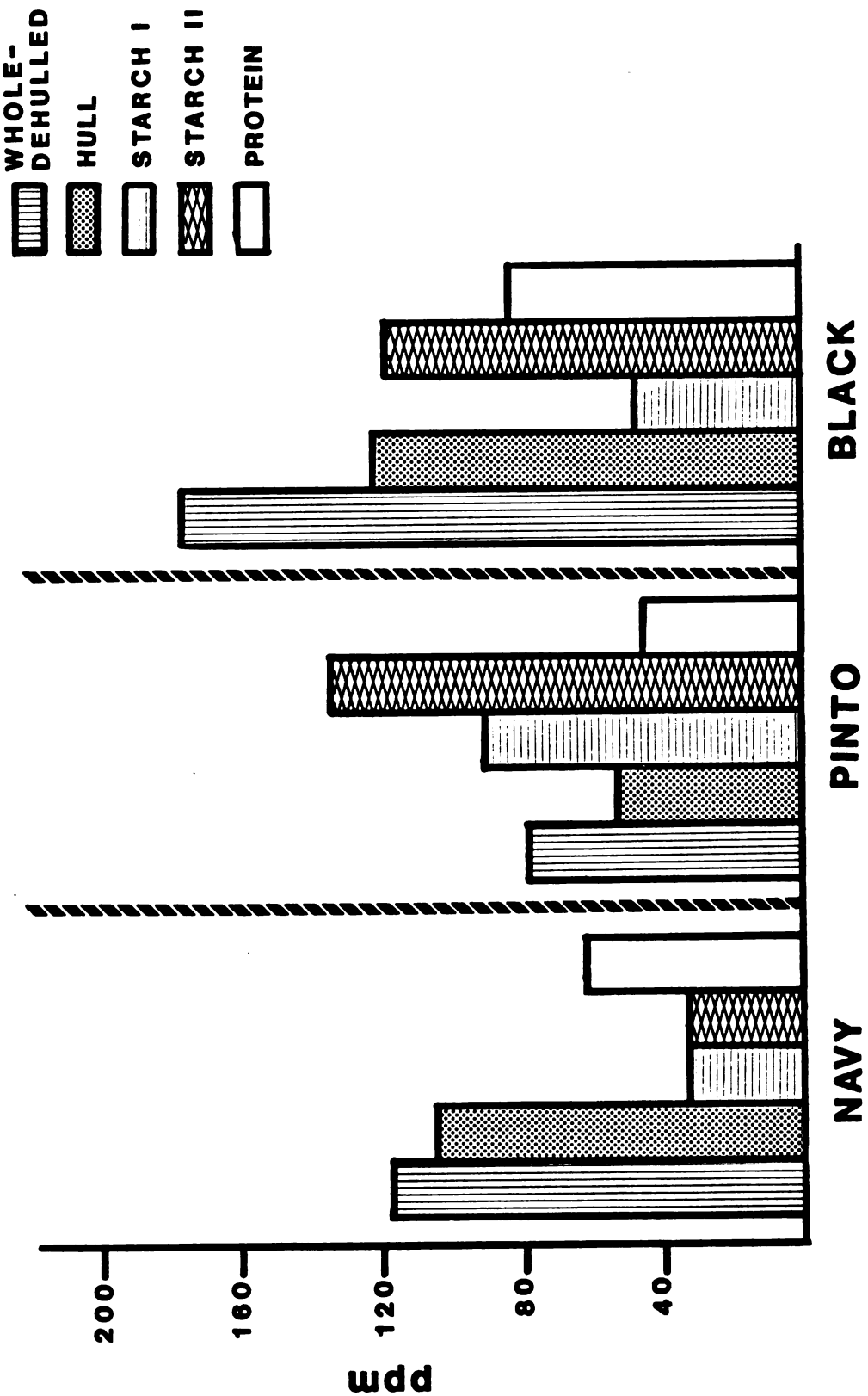

Figure 13. Average sodium content of various navy, pinto and black bean flour fractions.

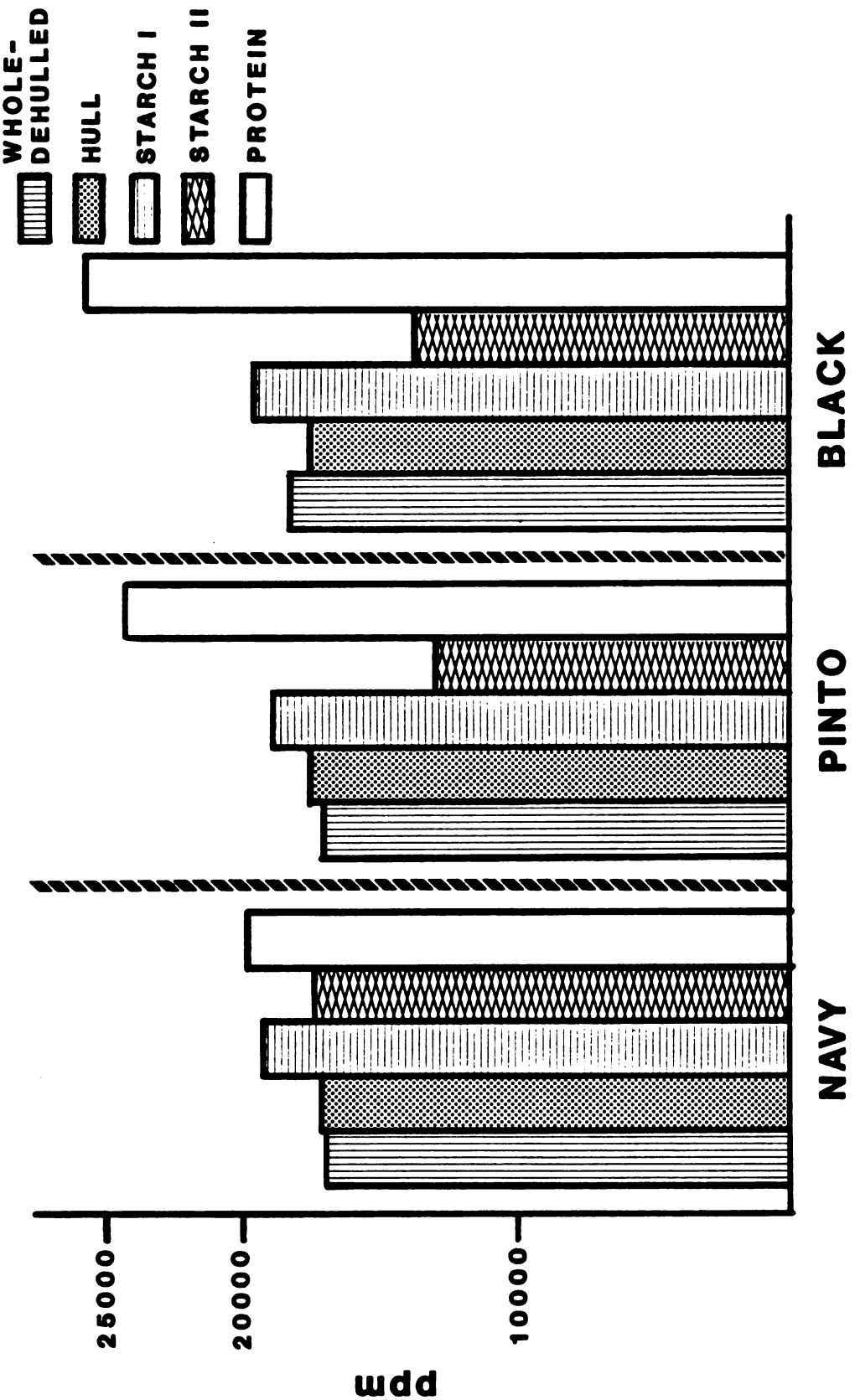

Figure 14. Average potassium content of various navy, pinto and black bean flour fractions.

and black bean flours, the highest and lowest values were significantly different from the values reported for the other fractions. This was not true for the navy flours.

Related Research Findings

Using a slightly different air classification scheme, Patel et al. (1980) separated navy bean flour into a high protein fraction and a starch residue fraction and measured the content of 11 minerals in each. Similar to the findings of the current investigation, these workers found that there was a pronounced shift of all elements measured (potassium, magnesium, phosphorus, aluminum, boron, calcium, copper, iron manganese, zinc, and sodium), except calcium, into the protein fraction. Based on the finding of the present study indicating that calcium was concentrated in the hull fraction, it may be conjectured that the partitioning of calcium into the starch residue fraction observed by Patel et al. (1980), was a result of seed coat incorporation in this flour fraction.

Materials Balance Analysis of Mineral Content

Using the information obtained on the percentage yield of the hull, starch I, starch II, and protein fractions (Figure 2) and the mineral contents determined for each of those fractions, it was possible to calculate the approximate content of each mineral in the original navy, pinto, and black beans. These calculations can only be considered approximate since they assume that no gain of minerals due to contamination or loss for other reasons occurred as a result of the handling or processing conditions employed. The results of the materials balance calculations for mineral content appear in Table 10.

The values obtained by calculation may be compared with similar data from the literature which was previously summarized in Table 1. When the calculated calcium values are averaged for the navy, pinto, and black beans, the amount obtained, 1777 ppm, is comparable to the values reported by Koehler and Burke (1981) and Walker and Hymowitz (1972).

Table 10. Calculated mineral content of original navy, pinto, and black beans based on materials balance information.

	parts per million		
Mineral	Navy	Pinto	Black
Ca	2158	922	2251
Cu	31.8	30.2	11.7
Fe	90.5	83.1	214.3
Mg	1909	1475	2289
P	5173	5040	5087
Zn	33.4	31.3	37.4
Na	60.6	85.8	97.3
K	17838	17561	18373

The calculated copper content of black beans was slightly higher than the literature values, however the calculated values for navy and pinto beans were more than three times as high. While it is possible that differences in the varieties of the beans and/or growing region

may account for some of the discrepancy between the calculated copper content and the values appearing in the literature, it is unlikely that these factors could produce differences of this magnitude. Since the values obtained for the samples of standard reference material fell within the certified values, it appears more likely that the beans became contaminated with copper at some stage of processing. It is not unusual for food processing equipment to contain brass fittings or other parts which could transfer copper to food. Another possible explanation is that the navy and pinto samples which were prepared for mineral analysis became contaminated with copper.

The calculated iron content of the navy and pinto beans is consistent with the literature values reported by Koehler and Burke (1981) and Walker and Hymowitz (1972). The calculated iron content of black beans was more than twice as high as both the literature values and those calculated for the navy and pinto beans. It is interesting to note that the black hull fraction contained only 44 ppm iron (Table 10), which was actually less iron than the amount found in either the navy or pinto hull flours. This suggests that either the cotyledons of the specific black beans utilized in flour production had an inherently higher iron content than the hull portion of the beans, in which case the exceptionally high calculated iron value is the result of naturally occurring iron, or that iron contamination of the whole-dehulled beans occurred after seed coat removal.

The calculated phosphorus contents of the navy, pinto, and black beans were slightly higher than values appearing in the literature. The calculated values were in closest agreement with the value reported by Fordham et al. (1975). Since the calculated and

literature values did not differ by much, it is likely that differences may be attributable to variety, location, and/or method of analysis.

The calculated potassium values for the three bean types were also somewhat higher than those reported in the literature. As was concluded for phosphorus, this is likely to have been due to differences in variety of beans grown, the growing location, and/or the method of analysis.

In the literature, Augustin et al. (1981) and Meiners et al. (1976) have reported sodium content for P. vulgaris. The former authors reported a sodium content of 103 ppm; the latter reported 26 ppm. The calculated sodium values for the three bean types ranged from 60.6 to 97.3 ppm. It should be noted that Augustin et al. (1981) measured sodium by flame emission and Meiners et al. (1976) used atomic absorption in comparison to the samples analyzed by plasma emission in the current study. The calculated values fell within the range of the previously reported values. The sodium values obtained for the standard reference material analyzed in this study were slightly higher than the certified values. In view of this information and other factors including the large standard errors obtained for the triplicate sodium values of each flour fraction analyzed (Tables 7-9) and the low value of 26 ppm reported by Meiners et al. (1976), it appears likely that contamination of these samples may have occurred.

The literature values for magnesium and those calculated for the three bean types were in close agreement. The calculated zinc values

were consistent with those reported by Augustin et al. (1981) and Koehler and Burke (1981).

Phytic Acid Content

The mean phytic acid content of the five fractions of navy, pinto, and black bean flours are shown in Table 11. Significant differences in the phytic acid content of the fractions within each bean type are indicated in this table. The bar graph shown in Figure 15 provides a means of visualizing the relative phytic acid content of the different beans and fractions.

Table 11. Average phytic acid content (mg/g) of various navy, pinto and black bean flour fractions (n=3).[1]

	mg/g		
Flour fraction	Navy	Pinto	Black
Whole-dehulled	16.58 ± 0.46c	13.92 ± 0.36c	16.19 ± 0.27c
Hull	13.56 ± 0.75b	10.27 ± 0.27b	10.22 ± 0.18b
Starch I	15.32 ± 0.57c	13.03 ± 0.40c	15.56 ± 0.63c
Starch II	8.72 ± 0.48a	4.29 ± 0.25a	7.54 ± 0.12a
Protein	30.22 ± 0.72d	23.74 ± 0.91d	27.36 ± 0.20d

[1]For each bean type, those values with the same letter are not significantly different from each other as judged by Tukey's test at alpha = 0.05.

Phytic acid content ranged from 8.72 to 30.22 mg/g for the navy flours, from 4.29 to 23.74 mg/g for the pinto flours, and from 7.54 to 27.36 mg/g for the black flours. For all but the hull fraction, the

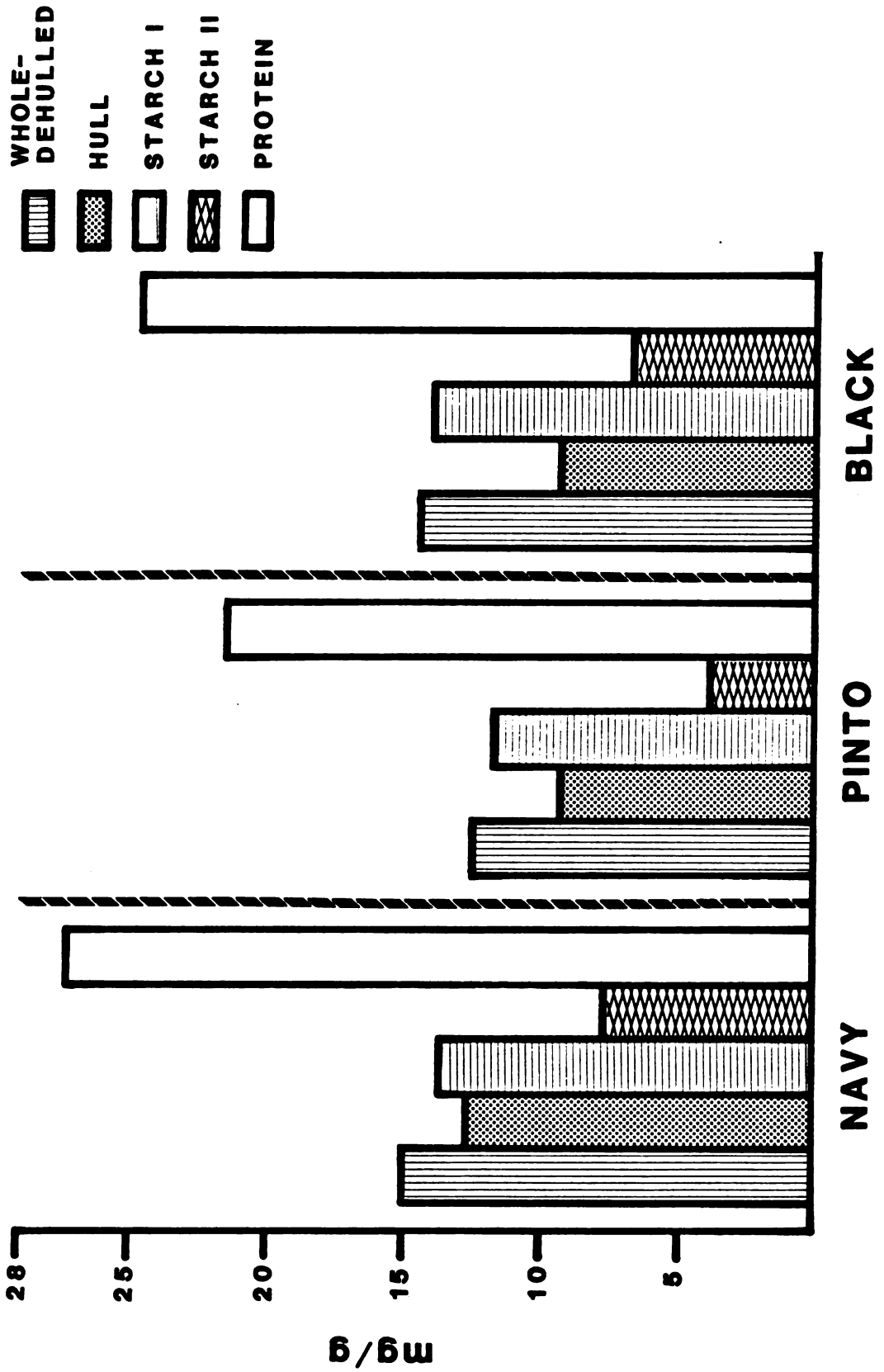

Figure 15. Average phytic acid content of various navy, pinto and black bean flour fractions.

pinto flours contained somewhat less phytic acid than the corresponding navy and black flours. Except for the whole-dehulled and starch I fractions of all three bean types, the amount of phytic acid in each fraction was significantly different from the phytic acid content of all other fractions. The trend in phytic acid partitioning observed for all three bean types, from lowest value to highest value, was as follows: starch II, hull, starch I, whole-dehulled, and protein.

The partitioning of the largest amount of phytic acid into the protein fraction suggests that phytic acid may be associated with the protein components of dry beans. This notion is consistent with the views expressed by other workers including Oberleas (1973). This theory provides an explanation for why the high starch fraction, starch II, which had the lowest protein content of all the flour fractions, also contained the least phytic acid. Additionally, this theory is consistent with the partitioning of phosphorus among the various flour fractions. Since up to 70% of the phosphorus of beans may be present in phytic acid, it is not surprising to note that the protein fractions, which had the highest phytic acid content, also contained more phosphorus than the other flour fractions.

Materials Balance Analysis of Phytic Acid Content

The phytic acid content of the original navy, pinto, and black beans was calculated using the materials balance information as was done for the eight minerals. The values calculated for the phytic acid content of the three types of beans are given in Table 12. Navy beans showed a slightly higher phytic acid content than black beans.

As expected based on the phytic acid data obtained on individual fractions, the pinto beans contained notably less phytic acid than the other two varieties. When converted to percentages, the calculated values for the three bean types translate into 1.48, 1.15, and 1.41% for navy, pinto, and black beans, respectively. These values are consistent with those obtained by Lolas and Markakis (1975). These researchers found the phytic acid content of 50 varieties of raw P. vulgaris to range from 0.54 to 1.58%. The calculated values obtained in the current investigation were just slightly lower than the average phytic acid value of 1.8% obtained by Iyer et al. (1980) for great northern, pinto, and kidney beans. More recently, Deshpande et al. (1982) reported that the phytic acid content of 10 cultivars of P. vulgaris ranged from 1.16 to 2.93%, values which are much higher than those obtained not only in the current study, but also by Lolas and Markakis (1975) and Iyer et al. (1980). Since all investigators, except Iyer, used the same analytical procedure, a discrepancy this large cannot readily be explained.

Table 12. Calculated phytic acid content of original navy, pinto, and black beans based on materials balance information.

	mg/g		
	Navy	Pinto	Black
Phytic acid	14.76 (1.48%)	11.52 (1.15%)	14.07 (1.41%)

Deshpande et al. (1982) also studied the effect of seed coat removal on the phytic acid content of beans and found that hand

removal of the hull caused the phytic acid content to increase by an average of 31.0%. Additional calculations carried out with the previously calculated phytic acid values for the three bean types (Table 12) studied in this investigation indicate that a mean increase in phytic acid content of 16.1% occurred upon hull removal. The increase might have been larger, closer to the value obtained by Deshpande et al. (1982), if the hulls had been removed by hand. The percentage yield data (Figure 3) indicates that the hull flour fractions accounted for between 25 and 31% of the initial weight of the beans, yet it has been reported that the seed coat accounts for less than 10% of the bean weight (Kay, 1979). It is probable that the incorporation of cotyledon into the hull material served to incorporate additional phytic acid into this fraction as well.

Correlations Between Mineral Content, Phytic Acid Content, and Macroconstituents

Many very strong and highly significant correlation coefficients were obtained between the mineral content, phytic acid content, and macroconstituents of all three bean types studied. These correlation coefficients are provided in Tables 13-15.

The strong (0.98 and 0.99), highly significant, positive correlation coefficients generated between phytic acid content and protein content for all three bean types substantiate the data which suggest that phytic acid is associated with protein. The generally strong, highly significant negative correlation coefficients (-0.87, -0.92, and -0.76 for navy, pinto, and black beans, respectively) between phytic acid content and starch content provide further support

Table 13. Correlation coefficients between various macro and micro-constituents of navy bean flour (n=14).

	Protein	Starch	Fat	Fiber	Phytic Acid
Phytic acid[1]	0.99**	-0.87**	1.00**	-0.31	---
P	0.99**	-0.84**	0.99**	-0.38	0.99**
Fe	0.92**	-0.85**	0.89**	-0.21	0.89**
Zn	0.95**	-0.99**	0.91**	0.04	0.92**
K	0.84**	-0.79**	0.79**	-0.20	0.79**
Mg	0.79**	-0.97**	0.72**	0.35	0.74**
Ca	-0.15	-0.28	-0.25	0.96**	-0.22
Cu	0.06	-0.16	0.00	0.15	0.00
Na	0.01	-0.11	0.03	0.25	0.03

[1] n=15

*$p \leq 0.05$

**$p \leq 0.01$

Table 14. Correlation coefficients between various macro and micro-constituents of pinto bean flour (n=15).

	Protein	Starch	Fat	Fiber	Phytic Acid
Phytic acid	0.99**	-0.92**	0.16	0.05	---
P	0.99**	-0.96**	0.19	0.18	0.98**
Fe	0.93**	-0.87**	0.33	0.03	0.91**
Zn	0.98**	-0.98**	0.38	0.25	0.96**
K	0.99**	-0.97**	0.27	0.21	0.97**
Mg	0.91**	-0.96**	0.59**	0.36	0.87**
Ca	0.19	-0.31	0.89**	0.70**	0.13
Cu	0.09	-0.31	0.89**	0.70**	0.01
Na	-0.55*	0.63**	-0.22	-0.42	-0.56*

*$p \leq 0.05$

**$p \leq 0.01$

Table 15. Correlation coefficients between various macro and micro-constituents of black bean flour (n=15).

	Protein	Starch	Fat	Fiber	Phytic Acid
Phytic acid	0.98**	-0.76**	0.07	-0.25	---
P	0.99**	-0.82**	0.11	-0.11	0.99**
Fe	0.80**	-0.43	-0.31	-0.58*	0.90**
Zn	0.81**	-0.81**	0.29	0.22	0.73**
K	0.99**	-0.87**	0.20	-0.04	0.97**
Mg	0.84**	-0.94**	0.48*	0.48*	0.71**
Ca	0.09	-0.49*	0.63**	0.96**	-0.11
Cu	0.62**	-0.79**	0.42	0.64**	0.46*
Na	0.25	0.37	0.35	0.01	-0.19

*$p \leq 0.05$

**$p \leq 0.01$

for the association between phytic acid and protein since the flour fractions with higher protein contents necessarily contained less starch.

The coefficients generated for all three bean types also showed very strong, highly significant correlations between phosphorus content and protein content (0.99), and between total phosphorus content and phytic acid content (0.98 and 0.99). Working with 50 varieties of P. vulgaris, Lolas and Markakis (1975) similarly found that the total phosphorus content of whole beans correlated well with phytic acid content. The current work demonstrates that this is true for various bean flour fractions as well. Assuming that phytic acid does partition with protein, it is also reasonable to expect a high degree of correlation between phosphorus and protein content.

The strong, highly significant negative correlation coefficients produced between phosphorus content and starch content (-0.84 for navy, -0.96 for pinto, and -0.82 for black) may be viewed as indicators of the partitioning of phosphorus (much in the form of phytic acid) with the protein, and consequently, away from the starch.

In terms of the correlation coefficients obtained for the content of various minerals with phytic acid content, and the macroconstituents, several similar trends were observed for the three bean types. The iron, zinc, potassium, and magnesium content of the navy, pinto, and black bean flours showed very or fairly strong, highly significant, positive correlations with both protein content and phytic acid content. The correlation coefficients with phytic acid suggest that these minerals may become bound to phytic acid, forming metallic phytates. As such, the correlations are not actually

between individual metals and macroconstituents, but rather, between the metallic-phytates and macroconstituents. Obviously, if iron, zinc, potassium, and magnesium are associated with phytic acid, and phytic acid is associated with protein, these minerals must also be associated with protein. This explains the strong, significant correlation coefficients produced between the content of these metals and protein content. The strong, highly significant negative correlation coefficients between the minerals and starch content should be interpreted using the same line of reasoning.

Calcium, unlike the minerals discussed thus far, did not correlate at all well with protein content, phytic acid content, or starch content. However, for navy and black beans, and to a much lesser extent for pinto beans, calcium content did correlate well with dietary fiber content. These findings suggest that instead of being bound by phytic acid, calcium may be bound by fiber. It should be noted that the hull flour fractions of navy and black beans had both the highest dietary fiber contents and the highest calcium contents of all fractions studied.

Although a few isolated significant correlation coefficients were produced between copper or sodium content and phytic acid content and the contents of some macroconstituents, no trends could be found across bean types. As such, the importance of some of these individual correlation coefficients is not readily apparent. In addition, although a number of these coefficients were judged significant, their absolute values are too small to warrant further attention. It is of interest to note that these same minerals, copper and sodium, were the only ones which were not reconciled in the

materials balance analyses. One trend which was observed only for the navy bean flours was that of strong correlations between the crude fat content, phytic acid content, phosphorus content, and the contents of several minerals. It appears that it is no coincidence that the minerals involved are those (iron, zinc, potassium, and magnesium) that were found to correlate well with phytic acid, protein, and starch. Presumably the fat content of the navy bean is associated with phytic acid. This appears logical since the fat content correlated extremely well with both phytic acid content and phosphorus content. Interestingly, Walker and Hymowitz (1972) found significant negative correlations between the fat content and the zinc, iron, and calcium content of 28 varieties of P. vulgaris. Unfortunately there does not appear to be any rational theory to explain these discrepancies.

Feeding Study for Determining Iron Bioavailability

Rationale

The feeding study conducted as part of this investigation was done in an attempt to determine if the phytic acid present in the bean flour fractions affected the bioavailability of iron. While it is possible to conclusively establish whether or not particular metal phytates exist in foods by chemical methods of analysis, it is not possible to determine if the metals in these chelates can be absorbed unless feeding trials are conducted. Navy flour fractions were chosen for the feeding trial rather than pinto or black flours because of their higher phytic acid contents. Iron is a controversial mineral in terms of the effect of phytic acid influencing its availability, and

it is also considered to be a nutrient of great concern world-wide in terms of its adequacy in man's diet.

Repletion Data

The data presented in Table 16 summarize the measurements made during the repletion period and provide the parameters necessary to compare Hb regeneration in rats fed test diets with those fed standard diets containing ferrous sulfate. When interpreting these data it is important to recognize that iron intake has been calculated based on the estimated iron content of the diets, and not on the actual, measured amount of iron present. Several repeated analyses conducted to determine the true iron content of the various diets produced values which showed great variation between replicates of the same sample and quite often, less variation between samples. Examples of this variability are provided in Table 17. It is likely that the small amounts of iron incorporated into the diets, especially those containing ferrous sulfate, were not uniformly distributed. As a result, the analysis of very small quantities of diet for iron content did not produce values truly representative of the amount of iron consumed by the rats based on normal intakes of between 10 and 30 grams per day.

Based on the feed intake data and the phytic acid content of the diets, it is apparent that the amount of phytic acid consumed differed substantially both for groups fed different iron levels of the same flour and for groups fed different flours. For those groups fed different levels of iron from the same flour, the ratio of iron intake to phytic acid intake remained constant, whereas this was not so for

Table 16. Repletion parameters of anemic rats fed basal low iron diet and test diets containing navy hull, starch II, and protein flour fractions for 14 days.

Iron Source	Estimated Fe Added (mg/kg)	Estimated Phytic Acid Content(mg/kg)	Feed Intake (g)	Estimated Fe Intake (mg)	Estimated Phytic Acid Intake (mg)	Weight Gain (g)	Final Hb (g/100 ml)
Basal diet[1]	0	0	157 ± 28	0	0	13.6 ± 12.9	3.9 ± 0.79
Ferrous sulfate[1]	6	0	198 ± 17	1.2	0	43.6 ± 8.5	5.5 ± 0.53
Ferrous sulfate[1]	12	0	188 ± 28	2.3	0	49.9 ± 12.6	7.0 ± 1.30
Ferrous sulfate[1]	24	0	215 ± 33	5.2	0	61.1 ± 12.3	10.6 ± 0.53
Navy hull[2]	6	987	164 ± 13	1.0	161.9	32.2 ± 9.0	5.1 ± 0.64
Navy hull[1]	12	1975	197 ± 30	2.4	389.1	54.4 ± 13.4	6.6 ± 0.89
Navy hull[1]	24	3950	218 ± 23	5.2	861.1	75.9 ± 16.3	9.0 ± 0.65
Navy starch II[2]	6	774	175 ± 29	1.1	135.5	28.3 ± 8.1	4.8 ± 0.51
Navy starch II[2]	12	1548	216 ± 39	2.6	334.4	41.9 ± 11.4	5.8 ± 0.33
Navy starch II[2]	24	3096	168 ± 15	4.0	520.1	52.3 ± 20.7	8.2 ± 0.48
Navy protein[3]	6	1336	183 ± 26	1.1	244.5	43.0 ± 13.3	6.5 ± 0.49
Navy protein[1]	12	3563	222 ± 46	2.7	791.0	70.5 ± 7.2	9.4 ± 1.07
Navy protein[1]	24	5345	206 ± 32	4.9	1101.1	71.6 ± 11.8	11.1 ± 0.81

[1] n=7

[2] n=6

[3] n=5

Table 17. Iron content of test and standard diets determined in repeated analyses.

Diet	parts per million (ppm)		
	Analysis 1[1]	Analysis 2[2]	Analysis 3[3]
Basal low iron	6.3	1.80	9.23
	7.8	1.90	9.92
	-	-	-
Ferrous sulfate-6 ppm	5.7	3.40	14.40
	6.4	3.20	14.89
	9.2	-	13.09
Ferrous sulfate-12 ppm	14.9	4.40	26.86
	7.3	4.40	19.31
	4.4	-	15.85
Ferrous sulfate-24 ppm	18.2	6.70	57.83
	16.0	12.20	49.27
	11.3	-	29.19
Navy hull flour-6 ppm	5.5	7.70	19.75
	3.2	6.60	21.03
	5.7	-	18.29
Navy hull flour-12 ppm	9.3	11.50	28.42
	9.2	11.41	34.68
	7.3	-	26.24
Navy hull flour-24 ppm	10.5	20.79	52.58
	12.1	21.90	71.55
	7.9	-	49.62
Navy starch II flour 6 ppm	10.3	5.60	14.30
	5.8	5.20	15.84
	7.6	-	16.75
Navy starch II flour 12 ppm	11.0	8.70	24.32
	11.4	6.50	26.80
	12.8	-	23.06
Navy starch II flour 24 ppm	25.0	17.70	29.68
	22.4	15.11	34.54
	21.6	-	35.01
Navy protein flour-6 ppm	15.0	7.20	22.42
	10.4	7.30	24.03
	12.3	-	22.77
Navy protein flour-12 ppm	21.1	20.10	42.70
	25.2	21.29	46.69
	23.4	-	44.02
Navy protein flour-24 ppm	35.6	23.89	57.66
	35.4	21.60	58.53
	41.2	-	63.07

[1]Values were determined for dry ashed samples by plasma emission.

[2]Values were determined for dry ashed samples by atomic absorption.

[3]Values were determined for wet ashed samples by atomic absorption.

groups consuming diets containing different flours, even at the same iron level. Per milligram of iron consumed, groups fed protein flour diets consumed more phytic acid than groups fed hull flour diets. Groups fed hull flour diets consumed more phytic acid than groups fed starch II flour diets per milligram of iron consumed.

The weight gain data reveals that groups fed diets containing higher levels of iron gained more weight, however the total food intake values of these groups of animals were not substantially different from intake values of groups fed the basal diet or diets containing the lowest level of added iron. Presumably the reason for this relates to the accuracy of recording food consumption and not the actual amount of food eaten. The difficulty in recovering spilled feed could account for the discrepancy.

The final Hb concentrations of the 13 groups indicate that true differences did exist both between groups fed different amounts of iron and those fed iron from different sources. The group fed the basal, low iron diet showed the lowest final Hb value (3.9 g/100 ml). Comparison of groups fed ferrous sulfate with groups fed navy hull flour shows similar responses in terms of final Hb concentration at all levels. As such the effect of phytic acid on iron absorption from navy hull flour does not appear to be major.

The final hemoglobin concentrations were markedly lower for the groups fed navy starch II than for the groups fed ferrous sulfate. Since the estimated iron intake of the group fed navy starch II at a level of 24 ppm was lower than the corresponding group consuming ferrous sulfate, it is difficult to establish to what extent, if any, phytic acid influenced iron absorption. A statistical procedure such

as a slope ratio or parallel lines assay should be conducted in order to ascertain this.

Groups fed protein flour diets showed greater final Hb concentrations than groups fed ferrous sulfate diets. One possible explanation for this finding is that the protein flour diets contained more iron than was estimated. If that assumption is correct, unless the actual amount of iron present was much greater than the estimated amount, a reduction in iron absorption might still be expected owing to the relatively high phytic acid content of this flour fraction. Taking this into consideration, it appears likely that the phytic acid present did not inhibit iron absorption from navy protein flour. If the actual iron contents of these protein flour diets were very similar to the estimated values, the possibility of there being some factor present which could have enhanced iron absorptin might deserve investigation.

Without having conducted the statistical analyses appropriate for this type of study, it is not correct to conclusively state that phytic acid did or did not influence iron absorption from the various bean flour fractions. However, it is within reason to state that a preliminary examination of the data suggests that the absorption of iron from navy bean flours by anemic rats does not appear to be adversely affected by the presence of endogenous phytic acid.

SUMMARY AND CONCLUSIONS

A major objective of this study was to demonstrate the partitioning of several nutritionally important minerals and phytic acid among hull, intermediate starch, high starch, high protein, and whole-dehulled flour fractions derived from navy, pinto, and black beans. To determine if endogenous phytic acid influenced the availability of iron from any of the flours, hemoglobin regeneration in anemic rats fed diets containing various bean flour fractions as the iron source was compared to the hemoglobin regeneration in rats fed standard diets containing ferrous sulfate.

Results of the mineral analysis indicated that phosphorus, zinc, iron, potassium, and magnesium shifted into the protein flour fractions of all three bean types. For both navy and black bean flours, calcium became concentrated in the hull flour, presumably in association with the fiber component of this fraction. Copper and sodium showed no partitioning trends across bean types. Except copper and sodium, all minerals were present in the smallest amounts in the high starch flours. Small quantities of all minerals in the high starch fractions of all bean types indicated that these flours could not be considered good dietary sources of the minerals studied.

Phytic acid content of the three bean types ranged from low values of 4.29 - 8.72 mg/g for the high starch flours to high values of 23.74 - 30.22 mg/g for the protein flour fractions. Thus, partitioning of phytic acid with the protein flour fraction was noted.

Correlation coefficients generated between phytic acid content and protein content, between phytic acid content and phosphorus, iron, zinc, magnesium, and potassium content, and between the content of these minerals and protein content were very strong and highly significant ($p \leq .01$) for all three bean types. Based on these findings it was concluded that the phytic acid present in dry beans is associated with protein. As such, any processing techniques implemented to isolate protein will also serve to increase the concentration of phytic acid. The high degree of correlation achieved between protein content and the content of zinc, iron, potassium, and magnesium, and between phytic acid content and these minerals suggests that these elements were present as metallic phytates. Total phosphorus content, of which phytate phosphorus is a major component, appeared to be a reliable indicator of the phytic acid content of various bean flour fractions.

The data obtained from the feeding study indicates that rats fed navy hull, high starch, and protein flours showed changes in hemoglobin concentration comparable to those of groups fed the standard ferrous sulfate diets. Overall, without having conducted the appropriate statistical analyses, it appears that although iron may have been bound to phytic acid, its absorption by anemic rats was not hindered by the presence of endogenous phytic acid.

In future investigations, the bioavailability of other minerals, especially zinc, from various bean flour fractions should be considered. Other nutritional aspects of air classified bean flours which warrant further study include the partitioning of water-soluble vitamins and amino acids among the various flour fractions.

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APPENDIX

Composition of Standard and Test Diets Estimated To Contain 6, 12, and 24 ppm Added Iron

Table 18. Composition of standard diets estimated to contain 6, 12, and 24 ppm added iron from ferrous sulfate.[1]

	grams		
Ingredient	6 ppm	12 ppm	24 ppm
Cerelose	2081.22	2081.04	2080.68
Casein	600.00	600.00	600.00
Corn oil	150.00	150.00	150.00
$NaH_2PO_4 \cdot H_2O$	60.00	60.00	60.00
$CaCO_3$	60.00	60.00	60.00
KCl	15.00	15.00	15.00
NaCl	15.00	15.00	15.00
Mineral premix	8.10	8.10	8.10
Choline chloride	4.50	4.50	4.50
Vitamin premix	3.00	3.00	3.00
$FeSO_4 \cdot 7H_2O$ mix	0.18	0.36	0.72
dl-Methionine	3.00	3.00	3.00

[1]All ingredients were obtained from the same sources as those in the basal diet (p. 45).

Prepared with cerelose such that 1 g of mix contained 0.1 g Fe.

Table 19. Composition of test diets estimated to contain 6, 12, and 24 ppm added iron from navy hull flour.[1]

	grams		
Ingredient	6 ppm	12 ppm	24 ppm
Cerelose	1911.47	1741.54	1401.68
Casein	551.48	502.97	405.93
Corn oil	150.00	150.00	150.00
$NaH_2PO_4 \cdot H_2O$	60.00	60.00	60.00
$CaCO_3$	60.00	60.00	60.00
KCl	15.00	15.00	15.00
NaCl	15.00	15.00	15.00
Mineral premix	8.10	8.10	8.10
Choline chloride	4.50	4.50	4.50
Vitamin premix	3.00	3.00	3.00
Bean flour	218.45	436.89	873.79
dl-Methionine	3.00	3.00	3.00

[1]All ingredients were obtained from the same sources as those in the basal diet (p. 45).

Table 20. Composition of test diets estimated to contain 6, 12, and 24 ppm added iron from navy starch II flour.[1]

	grams		
Ingredient	6 ppm	12 ppm	24 ppm
Cerelose	1856.43	1631.46	1181.51
Casein	558.70	517.40	434.80
Corn oil	150.00	150.00	150.00
$NaH_2PO_4 \cdot H_2O$	60.00	60.00	60.00
$CaCO_3$	60.00	60.00	60.00
KCl	15.00	15.00	15.00
NaCl	15.00	15.00	15.00
Mineral premix	8.10	8.10	8.10
Choline chloride	4.50	4.50	4.50
Vitamin premix	3.00	3.00	3.00
Bean flour	266.27	532.54	1065.09
dl-Methionine	3.00	3.00	3.00

[1] All ingredients were obtained from the same sources as those in the basal diet (p. 45).

Table 21. Composition of test diets estimated to contain 6, 12, and 24 ppm added iron from navy protein flour.[1]

Ingredient	grams		
	6 ppm	12 ppm	24 ppm
Cerelose	2007.19	1883.53	1784.59
Casein	541.56	444.15	366.23
Corn oil	150.00	150.00	150.00
$NaH_2PO_4 \cdot H_2O$	60.00	60.00	60.00
$CaCO_3$	60.00	60.00	60.00
KCl	15.00	15.00	15.00
NaCl	15.00	15.00	15.00
Mineral premix	8.10	8.10	8.10
Choline chloride	4.50	4.50	4.50
Vitamin premix	3.00	3.00	3.00
Bean flour	132.65	353.72	530.58
dl-Methionine	3.00	3.00	3.00

[1]All ingredients were obtained from the same sources as those in the basal diet (p. 45).