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NAVY BEAN PHYSICO-CHEMICAL CHARACTERISTICS AND CANNED PRODUCT QUALITY

By

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ABSTRACT

NAVY BEAN PHYSICO-CHEMICAL CHARACTERISTICS AND CANNED PRODUCT QUALITY

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Canning quality of navy beans can be assessed by processing these bean samples using a 202 x 214 can. This method required smaller sample size (28.5 g dry bean solids) as compared to the conventional 303 x 406 (100 g dry bean solids) method. Whole flour pasting characteristics (torque values) of navy beans had high correlation ($R^2 = 0.97$) with texture of corresponding canned beans and yet required smaller sample size (< 2 g, db).

Distinct differences in canned bean texture (compression/shear) were found among the cultivars as follows: Fleetwood, 26.6/41.5; C-20 28.1/29.3; Seafarer 28.0/28.9 and 84004 19.1/22.5 kg force/50 g canned beans. Apparent seed density was highly correlated ($R^2 = 0.98$) with compression texture of canned beans. Highest seed coat and cotyledonary parenchyma cell wall thickness of Fleetwood may contribute to its highest shear texture (Type A textural configuration). Cotyledonary proteins of these navy beans were isolated and differentiated into six solubility fractions, ranked by content from high to low as: globulins (G I + G II), albumin, non-extractable protein, glutelin and prolamin. Nitrogen distribution of cotyledon proteins varied among cultivars. Albumin SDS-PAGE peptide patterns demonstrated similarity of the following pairs: C-20 and 84004, and Seafarer and Fleetwood. Larger differences in amino acid composition were found among protein fractions than among cultivars. In addition, the mineral content of seed coats and cotyledons were analyzed and correlated with canned bean quality.

In-vitro protein digestibility of selected navy beans varied with the differences in genetic background and growing location. Globulin I (G I) possesses the highest percent digestibility in contrast to albumin and globulin II (G II). Heat treatment (autoclave: 121^{o}C for 20 min) resulted in enhancing the digestibility of G II fraction to that of G I and casein. Heat induced improvement was not substantially demonstrated for albumin, suggesting the presence of a heat stable protein coagulation structure and/or an active protease inhibitor.

To my parents and family
for their love, patience and moral support

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The common bean (*Phaseolus vulgaris*) is the most advanced species of the genus in terms of domestication and cultivation (Hidalgo, 1988). Although common beans are similar botanically, they vary in color, size, shape and flavor characteristics. In many countries, the differences among seed characteristics are formalized into market classes. Eleven market classes of common beans are recognized in the U.S.A. Seven classes are grown in Michigan and account for nearly one-third of the annual U.S. production of beans. The commercial bean classes grown in Michigan are navy, cranberry, dark red kidney, light red kidney, pinto, black turtle soup and yellow eye. Navy beans, the single largest class, are produced on bush or indeterminate, short-vine plants that have white flowers. The navy bean seeds are chalky white, roundish to ovoid in shape, and weigh between 17 to 19 g per 100 seeds.

Legumes contribute a good source of several important nutrients to diets and provide variety to the human diet. Legumes are an economical source of supplementary protein for many populations lacking animal proteins, and especially in young or developing countries. Food legumes are rich in lysine but limiting in methionine. Legumes complement well with lysine deficient-methionine rich cereals in terms of amino acid content, thereby complementing the amino acid pattern found in cereal grains. Legumes are also recognized as as a major source of complex carbohydrates, including dietary fiber, which has been demonstrated to have an effect of lowering blood serum cholesterol (Anderson and Bridges, 1988).

Although dry beans possess high nutrient levels, several factors are found to specially limit biological utilization. In addition to low sulfur amino acid content (Bressani, 1975), beans are limited nutritionally because of a low digestibility of proteins (Chang and

Satterlee, 1981), presence of anti-nutrients (Gomes et al., 1979 and Tyler et al., 1981), high level of phytic acid (Sathe and Krishnamurthy, 1953; Roberts and Yudkin, 1960; O'Dell et al., 1972; and Maga, 1982), flatulence contributing factors (Fleming, 1981 a & b). In addition, beans often develop hard shell and hard-to-cook defects which often develop during dry bean storage and limit food preparation and reduce palatability (Gloyer, 1921; Bourne, 1967; Stanley and Aguilera, 1985 and Srisuma et al., 1989).

Dry beans are not a staple in the U.S. diet, and thus, their consumption has been declining since 1962. This decline in bean consumption is directly related to changes in the consumers' food preferences. Rising incomes, urbanization, single adult households and an ever increasing number of women in the labor force have adversely affected bean consumption. Consumer preferences are shifting toward convenience foods and commodities with a short preparation time. Standard dry bean products in today's food markets do not align themselves with these emerging consumer changes and thus require a significant development of innovative technology or a modified genetic base to affect increased utilization.

Recently, consumers have become increasingly aware of the food that they eat and are making food selections according to diet/health information. This awareness has led to the consumer eating less saturated fat, cholesterol, sugar and salt and eating more complex carbohydrates such as fiber. Protein quality is not a nutritional concern for consumers who have mixed diets containing both vegetable and animal proteins. However, utilization of dry beans can be promoted since a good source of dietary fiber in beans may attract consumer attention.

The research reported in this dissertation was directed and conducted toward the development and assessment of improved quality methodology and to attain a greater understanding of fundamental compositional relationships within the seed. Both approaches are necessary for implementing biological and technological strategies for improvement of dry bean quality.

This research was conducted as a series of studies reported in three independent Chapters. Chapter titles and their respective Null hypotheses (H_o) are presented:

Chapter 1: Quality Assessment Methodology of Early Generation Bean Breeding Lines for Optimum Canning Quality

H_o: Canned bean quality can only be assessed through direct full scale evaluation techniques.

Chapter 2: Interrelationships between Physico-chemical Characteristics (Seed Microstructure, Proteins and Minerals) and Canned Product Quality of Four Selected Navy Bean Cultivars

H_o: Canned bean quality is not related to its physico-chemical characteristics.

Chapter 3: In-vitro Protein Digestibility of Selected Dry Navy Beans: Canned Beans, Cotyledon Flours and Isolated Protein Fractions

H_o: Protein digestibility is not different among canned products obtained from selected bean cultivars and for various cotyledon flours/isolated protein fractions.

REVIEW OF LITERATURE

Dry Bean Breeding Program at Michigan State University

The major objective in most bean breeding programs has been to increase and stabilize seed yield over a range of environmental conditions. This can be accomplished through the selection of a) plant physiological and morphological characteristics which lead to high yield and environmental adaptability, b) plants with improved insect pest/disease resistance and c) chemical tolerance, especially herbicides. In addition, bean breeding programs should also focus on improving food quality which includes the characteristics that have direct impact on human nutrition as well as on consumer palatability and commercial utilization. Consumers are most conscious of bean quality characteristics such as color and appearance, ease of preparation, wholesomeness, texture, flavor and digestibility. Processors, while restricted by the expectations of consumers, have put an emphasis on characteristics that are related to cookability and on developing the most efficient methods of processing to obtain maximum and uniform product quality. Thus, breeding programs should be concerned with one or more of these desired qualities from both agronomic and food standpoints.

Selection in segregating generations following hybridization is the current practice to make genetic advances for traits in beans (Figure 1). At Michigan State University, hybridizations are carried out in the greenhouse during fall and early winter months. Since the seed from the initial cross (F_1) is hybrid, all genetic variability is masked. It will be necessary to grow out the F_1 hybrid seed as quickly as possible to unmask the genetic variability necessary for selection. To maximize efficiency in bean breeding in terms of time, the initial crossed seed (F_1) is sent to a Winter nursery near Mayaguez Puerto Rico. This seed is planted to form the second generation (F_2) after crossing. By producing the

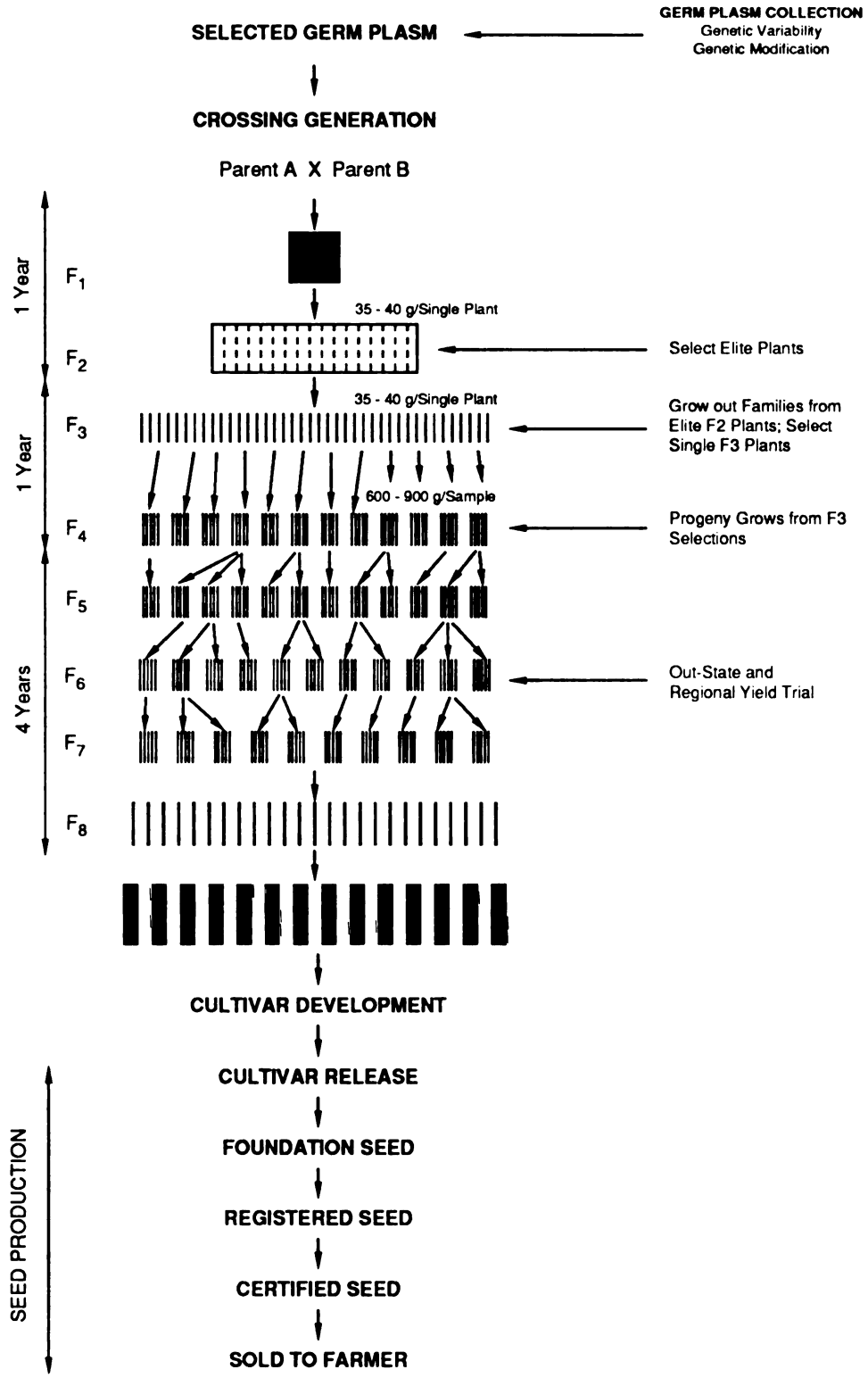

Figure 1. A Breeding Program Illustrating the Initial Parent Crossing Following with Successive Generations to Develop Commercial Cultivars

F_2 seed in the winter nursery, the F_3 or first generation where family structure is obviated can be grown in the field for selection in Michigan. This procedure allows the first cycle of selection to be initiated in one year rather than the second year after hybridization which would be the case if the winter nursery was not utilized. The seeds from elite selections are planted in the greenhouse to be used as parents for crossing among each other to recycle favorable genes for the traits under selection. This procedure forms the basis for the first recurrent cycle of selection. The recurrent selection procedure is practiced for several cycles and elite materials are selected after each cycle. Furthermore, the desirable parents for creating breeding populations can be supplied from various diverse genetic resources such as the international collections of the Centro International de Agricultura Tropical (CIAT), Cali Columbia and other active bean breeding agencies. CIAT has actively performed the evaluation of bean germplasm (accessions) serving as a battery of characteristics such as seed color, size, plant growth habit, flower color and set date, reaction to a variety of diseases and insect pests and maturity date. This information is then used to select parents carrying a specific characteristic useful in the genetic improvement for new cultivars. The stress tolerance and lodging resistance characteristics of tropical bean germplasm may be of potential use and could be transferred to commercially acceptable temperate-climate genotypes while the breeder is simultaneously breeding to maintain or improve their food quality.

Physico-Chemical Composition of Dry Beans

The physical and chemical properties of dry beans are primary factors in determining processing parameters and subsequent final product quality. Dry bean seeds are comprised of the seed coat, cotyledon and embryonic tissue. Structurally, the seed coat, cell wall, middle lamella and other cellular membranes greatly influence dry bean product quality. Furthermore, dry bean chemical constituents such as carbohydrates,

proteins, phytate, polyphenols and lignin also have profound effects on bean product quality.

Structural Characteristics

The seed coat is the outermost layer and serves to protect the embryonic structure. Two external anatomical features include the hilum and micropyle which are thought to have a role in water absorption. The seed coat consists of approximately 7-8 % of the total dry weight of the mature bean and has a protein content of 5% on a dry weight basis (Powrie et al., 1960 and Ott and Ball, 1943). The major structures in the seed coat of legumes include the waxy cuticle layer, palisade cell layer, hourglass cells and thick cell-walled parenchyma cells. The waxy cuticle layer is the outermost portion of the seed coat and its prime function is to prevent water penetration due to its hydrophobic property although it does allow permeation of some polar and non-polar compounds (Bukovac et al., 1981). Sefa-Dedeh and Stanley (1979 a and b) found that seed coat thickness, seed volume, and hilum size of cowpeas along with their protein contents were all factors in regulating water uptake.

The cotyledon contributes a valuable component to the functional (appearance, texture, flavor, etc.) and nutritive value of the bean. The cotyledon portion including the embryonic tissues makes up 91% of the total bean on a dry weight basis (Powrie et al., 1960). Parenchyma cells make up the major portion of the cotyledon (Sgarbieri and Whitaker, 1982 and Stanley and Aguilera, 1985). These cells are bound by a distinct cell wall and middle lamella with a few vascular bundles. The parenchyma cell walls are mainly comprised of an organized phase of cellulose microfibrils surrounded by a continuous matrix of hemicellulose, pectin and lignin. These cell walls function to give rigidity to the cotyledon tissue. Within each parenchyma cell, starch granules are embedded in a protein matrix. The secondary walls, found only in mature parenchyma cells are very thick and contain pits which facilitate the diffusion of water during soaking. The middle lamella is composed primarily of pectic substances which provides adhesion to

adjacent cells resulting in the integrity of the total tissue. In addition, pectic substances also allow divalent cation cross-linking, thus forming intercellular polyelectrolyte gels which contribute significantly to the textural quality (Dull and Leeper, 1975 and Van Buren, 1979 & 1980).

Legumes contain appreciable amounts of crude fiber ranging from 1.2 to 13.5 % (Deschamps, 1958; Tobin and Carpenter, 1978; Kay, 1979 and Reddy et al., 1984), a significant proportion of which (80-93%) is localized in the seed coat (Reddy et al., 1984 and Salunkhe et al., 1985).

Plant cell walls vary greatly in their composition, depending partly on the role the cells play in the structure of plant and partly on the age of individual cells. In general, cell wall components include cellulose, hemicellulose, pectic polysaccharides, lignin and cell wall protein (Goodwin and Mercer, 1983). Cellulose has a characteristic fibrous structure (partially crystalline) and provides basic structural strength to the cell wall. The cellulose fibers are embedded with an amorphous matrix of pectic polysaccharides and hemicelluloses. The roles of these amorphous matrix components are thought to give the flexibility to the cell wall (Raven et al., 1986). Pectin molecules have a backbone of linear chains of galacturonic acid residues with α-1,4-glycosidic linkages. In higher plants, the galacturonic polymers are accompanied by the less abundant and neutral arabinans and galactans. Rees and Wight (1971) studied the major conformational characteristics of polygalacturonate chains. They proposed that the uronic acid residues have a helical arrangement with exactly three monomers per turn of the helix.

Powell et al. (1982) proposed that gel-forming polysaccharides should feature the structural irregularities which in turn reduce the regularity of interchain associations. Without this irregularity characteristic, polysaccharides such as pectin or carageenan would probably form condensed, insoluble precipitates rather than hydrated gels with variable firmness and rigidity. Irregularities in the structure of pectin are caused by variable methylation of the carboxyl groups of galacturonic acid residues, neutral sugar side chains,

and occasional rhamnose residues in the main chain of the molecule. The molecular weight of pectin has been reported to vary from 6100 for a commercial sample of citrus polypectate (Fishman et al., 1984) to 366,000 for pectin isolated from unripe peaches (Shewfelt et al., 1971). Pectin molecular weight estimation has been limited since pectin is tightly held in the cell wall matrix and therefore, any extracted pectin for molecular weight determinations is partially degraded. Furthermore, pectins tend to aggregate in aqueous solution (Sorochan et al., 1971 and Fishman et al., 1984). The extent of aggregation can be affected by the extent of methylation, presence of neutral sugars, pH, and the ionic strength of the medium. This aggregation effect will result in over-estimating molecular weights using viscometry, ultracentrifugation, light scattering or osmometry.

The extent of pectin methyl esterification varies widely among fruit and vegetable tissues. It is not known whether galacturonic acid residues are methylated randomly or by some defined pattern. DeVries et al. (1983) studied the statistical distribution of methyl groups in apple pectin and concluded that the esterified carboxyl groups probably occur randomly. A random distribution of methyl groups was also found in lemon pectin (DeVries et al., 1984).

In recent years, plant cell wall material, defined as a dietary fiber, has become an important area of research. Many methods have been developed and used to extract and fractionate cell wall polysaccharides from various edible plant tissues. However, the results vary greatly among the laboratories due to different methods used. Knowledge of the composition of cell wall materials and their structure/organization is necessary for an understanding of their functional characteristics. There is also increased need to establish a standard method for the isolation and fractionation of these cell wall materials. Furthermore, cell wall isolation from legume cotyledon requires additional treatments to remove storage starch and protein prior to cell wall fractionation.

Previous research on bean cell wall was conducted using different preparation procedures which cause difficulty in comparison of the results (Monte and Maga, 1980;

Salimath and Tharanathan, 1982; and Champ et al., 1986). Most techniques used in detailed cell wall analysis are very time consuming and require elaborate and expensive equipment. Monte and Maga (1980) separated the fiber portion of the pinto bean into 13 fractions. Their cell wall extraction method is quite tedious but overcomes many problems caused by high starch and high protein in the samples. They found that cooked pinto beans contained more than twice the amount of soluble fiber than the raw beans. The two-hour boiling of pinto beans reduced approximately one-third of the extractable hemicellulose A and completely depleted the hemicellulose B. They concluded that water-soluble fractions were lost with the cooking water even though the bean was intact, and that this cooking process may have caused modifications in certain constituents of other fiber fractions. Cotyledon cell walls from kidney bean, lentil and chickpea were reported to contain 67, 73 and 42 % pectic polysaccharides, associated with 16, 12 and 10% cellulose, respectively (Champ et al., 1986). Further, hulls were mainly composed of cellulose (29 - 41%) with small amount of lignins (1.2 - 1.7%). Anderson and Bridges (1988) measured dietary fiber content (g/100 g, db) of various foods including raw and canned legumes (10 cultivars) and reported that raw *Phaseolus* legumes, pinto and white beans, contain 3.73 and 4.14% cellulose, and 1.58 and 1.04% lignin respectively. In addition, they observed the higher total dietary fiber in cooked beans compared to raw beans.

Compositional Characteristics

Carbohydrates

Starch. Within each parenchyma cell, starch granules are embedded within a protein matrix (Powrie et al., 1960; Sefa-Dedeh and Staley, 1979 and McEwen et al., 1974). Legumes contain 24% (winged beans) to 68% (cowpeas) total carbohydrate on a dry basis of which starch makes up a larger portion ranging from 24 to 56% (Reddy et al., 1984). These variations in starch contents are due to different cultivars and analytical procedures (Pritchard et al., 1973 and Cerning-Beroard et al., 1975). Starches contain two types of glucose polymers: amylose, a linear chain (α-1,4 linkages) and amylopectin, a

branched form (α-1,4 and α-1,6 linkages). The amylopectin molecules are highly branched and in general, larger than amylose molecules. The starch found in legumes has oblong granules which vary in size by species. The dry bean starch granule is resistant to swelling and rupture and generally contains high amylose content (30 - 37%) (Hoover and Sosulski, 1985). Starch granules can greatly influence the cooking characteristics of legumes. Gelatinization temperatures ranging from 60°C to over 75°C are relatively high compared to cereals and may contribute to processing variability (Hahn et al., 1977).

Sugars. Total sugars comprised of mono- and oligosaccharides represent only a small portion of total carbohydrate content in legumes. Among the sugars, oligosaccharides of the raffinose family are most prevalent ranging from 31 to 76% (Nene et al., 1975; Hymowitz et al., 1972; Cerning-Beroard and Filiatre, 1976; Naivikul and D'Appolonia, 1978; Becker et al., 1974; Kon, 1979; Rockland et al., 1979; Akpapunam and Markakis, 1979; Ekpenyong and Borchers, 1980; Reddy and Salunkhe, 1980; Fleming, 1981 a & b; and Sathe and Salunkhe, 1981). The oligosaccharides include raffinose, stachyose, verbascose, and ajugose with stachyose being predominate in most varieties of *Phaseolus vulgaris*.

Proteins

Legumes are excellent sources of plant protein and range from 20 to 40% on a dry weight basis. Researchers of dry beans (*Phaseolus vulgaris*) have reported protein content ranging from 18.8 to 29.3% (Meiners et al., 1976; Varriano-Marston and DeOmana, 1979; Hosfield and Uebersax, 1980 and Chang and Satterlee, 1982). Deshpande and Nielsen (1987) investigated the nitrogenous constituents of four legume species and their varieties. The total nitrogen of whole seeds ranged from 3.2-4.2% of which 8.3-14.5% was non-protein nitrogen. Water and salt soluble proteins were 72-94% of the total seed protein with mean protein contents of 67 and 87% and carbohydrate contents of 17-30% and 4-14% respectively.

Protein bodies, contained within a lipoprotein membrane, are generally spherical and relatively smaller than starch granules. The primary components of protein bodies include storage proteins (70-80 % db), salts of phytic acid (10% db), hydrolytic enzymes (protease and phytase), cations, ribonucleic acids and oxalic acid salts (Lott and Buttrose, 1978 and Prattley and Staley 1982). The proteins in beans can be classified into two categories: metabolic and storage proteins. The storage proteins tend to be found in the globulin fractions while the metabolic (enzymatic or non-storage) proteins are primarily found in the albumin fraction (Deshpande and Nielsen, 1987). The storage proteins are more important in the study of protein functionality since they make up a higher percentage of the protein in the seed and are responsible for many of the physical characteristics of the seed protein fraction (Boulter, 1981). The storage (reserve) material serves to provide a source of nitrogen and carbon compounds for the seedling. The nitrogen is provided to the germinating seedling as protein while the carbon is yielded in the form of oil and/or carbohydrates (specifically oligosaccharides and starch for dry beans) (Derbyshire et al., 1976). Several recent studies have shown that the reserve protein of dry beans are synthesized on the rough endoplasmic reticulum and later accumulated in the protein bodies (Bollini and Chrispeels, 1979; Bollini et al., 1982). It has been arbitrarily established that extracted protein which are more than 5% of the total protein in a seed is storage protein (Derbyshire et al., 1976). Beachy (1982) extensively reviewed molecular studies which helped define the factors controlling the biosynthesis of seed protein in soybeans, peas, french beans, and several varieties of legumes while methods used for the isolation and characterization of legume storage protein were extensively reviewed by Derbyshire et al. (1976).

The isolation and characterization of legume protein in general and dry bean proteins in particular has been the subject of a great deal of research in recent years (Osborne and Campbell, 1898; Pusztai and Watt, 1970; McLeester et al. 1973; Ishino and Ortega, 1975; Barker et al. 1976; Derbyshire and Boulter, 1976; Hall et al., 1977; Ma and

Bliss, 1978; Chang and Satterlee, 1982; Sgarbieri and Whitaker, 1982; Sathe et al., 1984). Millerd (1975) has extensively reviewed the biochemistry of legume seed proteins, while Higgins (1984) reviewed the synthesis and regulation of the major proteins in seeds including legumes.

Hall et al. (1979) reported that French bean seed contains 60% globulin (salt soluble), 20% albumin (water soluble), 10% glutelin (alkali soluble) and 3% prolamin (alcohol soluble). Free amino acids account for about 7% of total seed nitrogen. The globulin fraction which is a major storage protein, can be subdivided into Globulin I (G I: high salt solubility) and Globulin II (G II: low salt solubility). The ratio of G I and G II is about 6 to 1. The G I fraction is also reported to be vicilin which is 6.9S proteins and can aggregate to form an 18S tetramer at pH 4.5. Phytohemagglutinin (PHA) is identified as G II with a characteristic of 6.4S protein. This PHA has the ability to agglutinate red blood cells, disrupt the internal mucosa and reduce nutrient absorption. Thus, PHA is considered an anti-nutritional factor that limits the use of dry bean.

Liener and Thompson (1980), Geervani and Theophilus (1982) and Sathe et al. (1981) reported that the Great Northern (*Phaseolus vulgaris* L.) bean protein, albumins and protein isolates were characterized by high acidic amino acid content, while globulins and protein concentrates had a high proportion of hydrophobic amino acids. They also found that the bean proteins were resistant to in-vitro enzymatic attack; however, heating improved in-vitro susceptibility to enzymatic hydrolysis.

Phaseolin (vicilin) is a 6.9S globulin which forms an 18S configuration at pH 4.5 and has been reported to have between three to five subunits ranging in size from 23,000 to 56,000 (Pusztai and Watt, 1970; Derbyshire et al., 1976; Bollini and Chrispeels, 1978; Brown et al., 1981a). Dieckert and Dieckert (1985) reviewed the available literature for seed storage proteins in general and concluded that the 6.9S proteins appear "to be dimers or trimers of the fundamental subunits" and that disulfide bridging between the subunits generally does not occur. However, "the native monomers seem to form associating-

dissociating systems depending on the milieu." Very little amino acid sequencing data has been published for these proteins so that it is very difficult to determine inter-species homology. The 6.9S globulin in dry beans has been determined to be a glycoprotein which contains 4.5% neutral sugars and 1.1% hexosamine (Pusztai and Watt, 1970). Chang and Satterlee (1981) isolated and characterized the major protein of Great Northern Beans using classical isolation techniques. The major protein subunits were found in the globulin fraction and had molecular weights of 51 and 45 kd (kilodaltons) with a total molecular weight that was estimated to be 186 kd is equivalent to a 6.9S protein. This protein was found to account for 37% of the protein present in the crude bean extract and 31% of the total seed protein. This globular protein was a glycoprotein which contained 6.5% sugar (as glucose) and about 50% alpha-helix. The protein was most stable at pH values between 4.0 and 6.0 and had a compact structure that was resistant to proteolysis. Nesser et al. (1985) investigated the carbohydrate moieties of isolated glycoasparagines from glycoprotein II of white kidney beans. They showed that the structures were similar to oligomannoside-type chains found in glycoproteins from various sources.

The occurrence of anti-nutritional proteins of both legumes and cereals, their physical and chemical properties, and their physiological significance in both plants and humans has been reviewed by Gatehouse (1984). Phytohemagglutinin (PHA), the lectin of dry beans, has been described (Bollini and Chrispeels, 1978) as a 6.4S protein with two subunits of molecular weight 34 kd and 36 kd. Estimates of PHA's molecular weight range from a low of 115 kd to a high of 150 kd (Coffey, 1985). Coffey (1985) reviewed seven different studies on the amino acid composition of PHA; and concluded that PHA had a large amount of aspartic acid and serine but had practically no cysteine and methionine. Felsted et al. (1981) investigated the subunit composition of the PHA of kidney bean. These studies revealed that there were five isomers of PHA present. These isomers, in turn, were composed of different combinations of an erythrocyte reactive subunit (E) and a lymphocyte reactive subunit (L): E_4, E_3L_1, E_2L_2, E_1L_3, L_4. SDS-

PAGE showed that E_4 and L_4 were single proteins with molecular weights of 31.7 and 29.9 kd, respectively. Einhoff et al. (1985) have shown that *Leguminosa* lectins will bind both the storage protein and the glycosidase enzymes and that both the lectins and the lectin-bound proteins are found in the protein bodies. They suggested that lectins act during maturation of the plant to contribute to an orderly arrangement of the storage proteins in the protein bodies.

Bollini and Chrispeels (1978) confirmed that vicilin and PHA are reserve proteins for the seedling. Seedling growth over a period of 11 days was accompanied by a decrease in the amount of both proteins. The amount of the original polypeptide decreased while the proportion of smaller molecular weight polypeptides increased. Isolated protein bodies from the beans were found to contain the two subunits of PHA, the three subunits of vicilin, and a major polypeptide with a molecular weight of 60 kd.

Total biological utilization of the legume protein is relatively low. Protein digestibility may be impaired possibly by the presence of numerous anti-nutritional compounds which must be removed or destroyed during processing (Bressani et al., 1982 and Aw and Swanson, 1985). Raw legumes are poorly digested, but adequate heat treatment improves the digestibility significantly (Coffey et al., 1985). However, in many parts of the world, the thermal treatment that can be provided for bean preparation in the home setting is not sufficient to inactivate toxic lectins and is often just sufficient to heat and hydrate the beans. Gomez-Brenes et al. (1975) reported that peak digestibility and Protein Efficiency Ratios (PER) of dry *P. vulgaris* were obtained after soaking for 8 or 16 hours and cooking at 121°C for 10 to 30 minutes. Heating for longer than this resulted in lowered protein quality and decreased available lysine.

Koehler et al. (1987) investigated the protein content and protein digestibility of thirty-six varieties of eight types of dry beans for protein content and protein quality as determined by *Tetrahymena pyriformis* on the raw bean flour. The amount of protein ranged from 17.5% for the Pinto (cultivar NW-590) to 28.7% for the red kidney (cultivar

Royal Red). Except for kidney beans, there was little varietal difference in protein content. Pinto beans had the highest protein quality with six varieties having values of 90 % or more and the rest having values of 80 % or more. Red kidney beans had the lowest protein quality with a mean value of 59. Thus, there can be a great deal of variability among different commercial classes of *Phaseolus vulgaris* and also among cultivars within a class.

Lipids

A low lipid content is characteristic of dry beans, with total fat content (the ether extractable material) ranging from 1.2 to 2.1% (Korytnyk and Metzler, 1963 and Koehler and Burke, 1981). Neutral lipids are the predominant class of lipids present in legume seeds and account for 60% of the total lipid content (Takayama et al., 1965 and Sahasrabudhe et al., 1981). The glycolipids and phospholipids are essential constituents of the cell membrane because of their hydrophilic and hydrophobic properties (Mazliak, 1983). The glycolipids account for up to 10% and the phospholipids make up 24-35% of the total lipid content of legume seeds (Sathe et al., 1984). A comparison of fatty acid composition of several cultivars of legumes show a significant amount of variability. Legume lipids are highly unsaturated, with linolenic acid present in the highest concentration. Linoleic and oleic acids are present in lesser quantities. Palmitic acid is the predominant saturated fatty acid. Unsaturated lipids have high oxidation potential and the end products of this reaction, such as carbonyl compounds, can chemically interact with, for example, the decomposition products of proteins to yield cross-linked end products. Thus, the storage of legumes can result in a loss of quality (off flavors and odors), nutritional value and functionality.

Vitamins

Dry edible beans provide some water soluble vitamins: thiamine, riboflavin, niacin and folic acid, but very little ascorbic acid (Watt and Merril, 1963; Fordham et al., 1975 and Tobin and Carpenter, 1978). Common commercial preparation methods of canned beans cause a significant loss of water soluble vitamins. Therefore, many workers have

studied the retention values of water soluble vitamins in order to optimize the quality of bean products (Augustin et al., 1981 and Carpenter, 1981). There is no evidence in the literature which indicates that dry beans contain appreciable amounts of fat soluble vitamins. Watt and Merrill (1963) and Kay (1979) reported that *Phaseolus vulgaris* provides less than 30 International Units of vitamin A per 100 grams of raw beans. Variability of vitamin content is high. Augustin et al. (1981) suggested that geographic location of growth appeared to have had a significant effect on vitamin content; however, further research is warranted to evaluate the influence of agronomic factors which affect growth rate and subsequent vitamin levels in beans.

Ash and Minerals

The total ash content of Phaseolus vulgaris ranges from 3.5% to 4.1% (Fordham et al., 1975; Tobin and Carpenter, 1978 and Kay, 1979). Beans are generally considered to be a good source of some minerals, such as calcium and iron, but they also contain significant amounts of phosphorus and potassium. Adams (1972) and Patel et al. (1980) observed that navy bean flour had 2 to 17 times as many minerals as wheat flour. The specific mineral content in mature, raw legumes has been reported by several researchers in recent years; however, most values show large variability. Augustin et al. (1981) pointed out that bean class and environmental factors greatly influence this variability.

Ash content decreases after cooking due to leaching, with losses ranging from 10 to 70% (Watt and Merrill, 1963 and Meiners et al., 1976). The wide range of losses could be due to different soaking and cooking methods, and in the case of raw beans, due to factors such as variety, growing location and soil composition.

Dry beans are good sources of zinc, iron and potassium and also contain substantial amounts of calcium, magnesium and phosphorous (Augustin et al., 1981, Fordham et al., 1975, Koehler and Burke, 1981, Meiners et al., 1976, Watt and Merrill, 1963 and Sgarbieri et al., 1979).

The ranges of mineral content (ppm concentration) in mature, raw beans reported by several researchers are: Ca, 595 - 2600; Cu, 5 - 14; Fe, 13 - 135, Mg, 1230 - 2300; Mn, 6 - 232; Na, 17 - 210; P, 2800 - 5700; Zn, 17 - 65 and K, 8202 - 19400 (Watt and Merrill, 1963, Fordham et al., 1975, Meiners et al, 1976, Augustin et al., 1981 and Koehler and Burke, 1981). Reported values from these studies were found to be in agreement with the exception of Mn and Na.

Minerals in cooked legumes were about one-third to one-half of the values in raw beans, with Mg, P and K leaching into the cooking water (Meiners et al., 1976). On the other hand, mineral retention during cooking was found to be 80 to 90% with the exception of sodium and total calcium retentions (Augustin et al., 1981 and Koehler and Burke, 1981). Calcium variabilities were high both between and within classes, although growing area had little effect (Watt and Merrill, 1963, Meiners et al., 1976 and Augustin, et al., 1981). The effect of the growing area on Fe was noted only in navy beans. Sodium variabilities among and within classes were observed to be high and associated with the analysis method (Tittiranonda, 1984). Data agree with the findings of Watt and Merrill (1963) and Augustin et al. (1981), but were lower than those of Meiners et al. (1976). Variation in mineral content may be attributed to differences in bean varieties, growing location, cooking methods and method of analysis.

Relationship between mineral content and proximate composition of 28 varieties of *Phaseolus vulgaris* showed significant correlations between fat content and Ca, Fe and Zn. Significant correlations were also observed between raffinose content and P and K. However, although the relationships were statistically significant, they were not directly proportional since the correlation coefficients were all less than 0.60 (Walker and Hymowitz, 1972). Working on whole navy bean flour and air classified navy bean protein concentrate and starch residue, Patet et al. (1980) found the former fraction to contain the highest ash content and K, Mg, P, Al, B, Cu, Fe, Mn, Zn and Na to be partitioned in this fraction. Ca was concentrated in the starch residue fraction. Similarly, Tecklenburg et al

(1984) found a higher ash value and Zn, Fe, P and Mg partitioned into the protein flours of navy, pinto and black bean flours. For the navy and black bean flours, the hull fraction contained the most Ca, while the fine starch fraction (starch I) of the pinto flours had the highest Ca content. Sodium was the only element studied which was not present in significantly greater amounts in any one fraction than another for any bean type.

Studies indicate that phytic acid (myoinositol hexaphosphoric acid), an anti-nutritional factor, reduce the biological availability of minerals (Sathe and Krishnamurthy, 1953; Roberts and Yudkin, 1960; O'Dell et al., 1972 and Davies and Nightingale, 1975). The study of Tecklenburg et al. (1984) indicate phytic acid to be partitioned with the protein fraction. The high degree of correlation between protein content and Zn, Fe, K and Mg, and between phytic acid and these minerals suggested that these elements are present as metallic phytates.

Tannins and Polyphenols

Vegetable tannins are plant polyphenolic compounds with molecular weights ranging from 500 to 3000. The tannin content of dry beans ranges from 0.4 to 1.0% (Sgarbieri and Garruti, 1986). Only condensed tannins have been identified and quantitated in dry beans. The tannins are localized in the bean seed coat with low or negligible amounts present in the cotyledon (Ma and Bliss, 1978). The hydroxyl groups of the phenol ring enable the tannins to form crosslinks with proteins (Ma and Bliss, 1978 and Haslam 1979) which may be implicated in post-harvest seed hardening or decreased digestibility.

Phenolic acids, esters, and glycosides are widely distributed in various plant tissues, including legume seeds. These compounds contribute to the formation of adverse flavors and colors and to changes in nutritional quality as a result of enzymatic and autolytic reactions during processing (Sosulski, 1979). The coumaric and ferruic acids predominate in navy beans. Research by Huang et al. (1986) suggests that these esterified acids are associated with water-soluble components of the tissue.

Dry Bean Processing

Thermal Processing of Canned Foods

The U.S. Food and Drug Administration requires all commercial processors to file a scheduled process for any low-acid (pH > 4.6 and a_w > 0.85) or acidified foods (acidified to pH $\leq$ 4.6, with $a_w \geq 0.85$) that are hermetically sealed. A scheduled process "means the process selected by the processor as adequate under the conditions of manufacture for a given product to achieve commercial sterility. This process may be in excess of that necessary to ensure destruction of microorganisms of public health significance, and shall be at least equivalent to the process established by a competent processing authority to achieve commercial sterility" (CFR Title 21:113). The scheduled process shall include: the processing method, the type of retort, the minimum initial temperature, time and temperature of processing, the sterilizing value (Fo) or any other equivalent scientific evidence of process adequacy, the critical control factors (minimum head space, consistency, maximum filling or drain weight, a_w and etc.), and the source and date of the establishment of the process for each food and container size. The establishment of the scheduled process needs to be done such that the type, range, and combination of variations encountered in commercial production shall be adequately accounted. Methods used to adequately provide for commercial sterility are to "include, when necessary, but not limited to, microbial thermal death time data, process calculations based on product heat penetration data, and inoculated packs. Calculations shall be performed according to procedures recognized by competent processing authorities (CFR Title 21:113).

Thermal Process Calculation

Various thermal process calculation methods developed include the "General Method" graphically (Bigelow et al., 1920) and numerically (Patashnik, 1953), "Formula Methods" (Ball, 1923 and 1928; Ball and Olson, 1957), "Nomogram Method" (Olson and Stevens, 1939) and "Computer Method" (Sasseen, 1969). The General Method for

process calculations by Bigelow (1920) was later improved by Ball in 1928 to "Improved General Method", by the incorporation of a reference temperature (250°F) to which all processes could be compared, and the lethal rate concept. The improved general method remains the most acceptable procedure in estimating the sterilizing value of a process. This method requires actual processing data (time and temperature and Z value) in order to measure the sterilizing value (F)

The sterilizing value (F) of a heat process is usually the equivalent time (minutes) at reference temperature (121.1°C or 250°F). Two pieces of data must be available before the sterilizing value of a heat process can be determined: 1) a Z value and 2) experimental time-temperature data. If a Z value of 10°C or 18°F (*Clostridium botulinum*) is used, the sterilizing values at 121.1°C or 250°F is designated as the F_c or F_o value.

Heat Penetration Test

The obtainment of accurate data regarding the heating and/or cooling of a food in a container is extremely important to determine product sterilization. The result of a heat-penetration test is experimentally derived heating and cooling curves which depend on the kind of product involved. Bitting and Bitting (1917) were among the first to utilize thermocouples for measuring the temperature profile of the canned product. Improvements were made in thermocouple design by a number of individuals until Ecklund (1949) designed the non-projecting plug-in thermocouple. Holes are punched, usually in the side of the can and the thermocouples are inserted into the container before it is filled with food. The cans are then sealed on commercial seaming equipment with the thermocouple in place. The closed container, with the thermocouple in place, should be shaken to give a uniform product temperature throughout before retort processing and heat penetration tests begin. The retort temperature, product temperature at cold spot and their corresponding times can be recorded by computerized data acquisition with sufficient time interval established.

For a new product with unknown heating characteristics, a cold spot (slowest heating location) must be determined. This should be done with thermocouples located in

different positions and the data should demonstrate that the cold spot has been bracketed between faster heating zones.

For convection heating products, the slowest heating zone in containers processed in a vertical position is about 1/3 of the longitudinal axis length above the bottom of the containers. During heating and cooling, these products are in continuous motion, owing to convection currents set up by temperature differences between product and heating medium. Because of product movement in convection-heating products, temperatures throughout the product are reasonably uniform during heating and cooling. For conduction heating products, the slowest heating zone is in the geometric center of the conduction heating product mass. There are no canned foods that heat exclusively by either conduction or by convection and thus the slowest heating zone will usually be between the geometric center of the can and the slowest point for convection heating for the can size tested. Light-consistency products that exhibit straight-line semi-logarithmic heating curves are referred to as convection-heating products. There are a number of variables which may affect the heating rate. These may include: a) product (kind, size, consistency, ratio of solids to liquid, method of product preparation); b) fill (weight, volume, temperature); c) container (size, head space and orientation) and d) retort system utilized (still or agitated)

Thermal Processing of Dry Beans

Many factors influence bean product quality which include dry bean physico-chemical characteristics (structural and chemical composition) and processing parameters (product formulation including pH, processing time and temperature). Variability in the physico-chemical composition of beans occurs among cultivars with different genetic backgrounds, cultural practices and growing environments (Hosfield and Uebersax, 1980 and Ghaderi et al., 1984). Post-harvest handling and storage conditions further induce changes of physico-chemical properties of dry beans. Under adverse conditions, storage defects such as bin burn, hard-shell and hard-to-cook phenomena may occur, resulting in significant loss of bean quality and its economic value. Improved utilization of dry beans

can be maximized through an understanding of how bean physical and chemical components (primary factors) function and interact during a given process condition (secondary factor) to yield final bean product quality perceived by processors and consumers. This basic understanding will ultimately provide the opportunity for new dry bean cultivars and for innovative product development.

In general, dry beans are cooked, fried, or baked to be used in soups, eaten as a vegetable, or combined with other protein foods to make a main dish. Commercially, they are processed in cans to produce a number of bean-based foods. Research and quality control programs are designed to provide a consistent product of good characteristic flavor, bright color, attractive appearance and possessing good textural properties.

In the developed nations, beans are generally prepared by commercial food processing operations and consumed as canned beans in sauce. Beans to be processed should contain a moisture level of about 12% to 16%, be of uniform size, fully mature and free from foreign materials and seed coat defects. Direct cooking of beans in water is the prevalent means of preparation in lesser developed countries.

Soak/Blanch Processing

Dry beans have been traditionally soaked for 8 to 16 hours at room temperature prior to further processing. Soaking is essential to remove foreign material and facilitate cleaning of beans, aid in can filling through uniform expansion, ensure product tenderness and improve color (Crafts, 1944; Cain, 1950; Hoff and Nelson, 1966 and Deshpande and Cheryan, 1986).

Several studies on the role of microstructural constituents of the seed in water absorption have been carried out using scanning electron microscopy (SEM). Sefa-Dedeh and Stanley (1979) suggested that all three constituents (seed coat, hilum and micropyle) together may form an integral water absorption/removal system. In terms of the relative surface area of the beans, the hilum and micropyle were found to be the most important structural features influencing the initial water uptake of beans (Kyle and Randall, 1963;

Korban et al, 1981 and Desphande and Cheryan, 1986). The seed coats apparently influenced water uptake only after 30 - 60 minutes of soaking. When the primary path for water entry was established within the seed coat layers, water absorption seemed to proceed rapidly (Deshpande and Cheryan, 1986). The raphe was found to be the primary site of water uptake in pinto beans (Korban et al, 1981) and Red Mexican beans and the micropyle the least important (Kyle and Randall, 1963). Using autoradiography, Varriano-Marston and Jackson (1981) showed that for intact black beans, water entered the bean at the hilum, believed to be the rate limiting barrier, and was transported around the periphery of the cotyledon via the spongy parenchyma cells of the testa. Water penetration increased uniformly to the center of the bean as time progressed. The rate of hydration of beans without seed coats was more rapid, indicating that the transport of water across the hilum and into the cotyledon is slower than the rate of diffusion through the cotyledon. Sefa-Dedeh and Stanley (1979 b) observed that during the first three hours of water uptake in cowpeas, approximately 80% of the absorbed water was imbibed, making the seed coat thickness the most important variable; from 3 - 12 hours, the size of the hilum became the most important variable. Hsu et al. (1983) found temperature, solute concentration and initial moisture content to be highly correlated with maximal water absorption in soybeans while protein content, density and bean size had little correlation. Powrie et al. (1960) indicated that for the navy commercial class, water migrated through the seed coat and hydrated the cotyledon during soaking.

The different anatomical structures were used to explain the water uptake of legume seeds. Varieties with relatively thick seed coats such as Black beauty (Phaseolus vulgaris L. biotype) and winged beans showed a slow initial rate of water absorption as compared to the thin seed coat varieties (Deshpande and Cheryan, 1986). They also found the arrangement of the sub-hilar tissue to influence water uptake rate. Varieties with low initial water uptake had a narrow hilar fissure and a very highly developed tracheids system.

However, once the initial resistance to water uptake was overcome, the seed coats because of their relatively large surface area played the dominant role in water uptake of legumes.

Agbo et al. (1987) observed isogenic strains, 'Nep-2' and 'San Fernando' and 'Sanilac' a navy bean cultivar using SEM. The open and heart-shaped micropyle and prominent seed coat pores of 'Sanilac' favor rapid water uptake. 'San Fernando', which hydrated the least, had an occluded micropyle which Agbo (1982) suggested acted as a barrier to water uptake. It also lacked seed coat pores. 'Nep-2', the intermediate one, had a partially opened micropyle and a few prominent seed coat pores. Although water uptake may occur through seed coat pores, this mechanism of water entry appears mainly a feature of white seeded beans of the navy commercial class (Powrie et al., 1960; Adams and Bedford, 1973)

Generally, seed size is negatively correlated with water uptake in legumes. This relationship was established for several soybean lines investigated by Calero et al. (1981). Small seeds also have a higher percentage by weight of the seed coat. Although the hilar fissure seemed to provide the primary path for water entry in the seeds, the entire system of the seed coat, hilum and micropyle might constitute an integrated water absorption process in legume seeds and the influence of other variables in any given variety has to be considered (Deshpande and Cheryan, 1986).

Differences in water uptake in legumes are also related to their composition. Small hard soybeans were observed to also contain high amounts of crude fiber and calcium than normal beans, resulting in their resistance to water absorption (Siao, 1976).

According to Mayer and Poljakoff-Mayber (1985), the main component that imbibes water in seeds is protein. Sefa-Dedeh and Stanley (1979 b) observed that with increasing soaking time and the consequent hydration of the seed coat, seed coat thickness no longer exerted an important influence and that at later stages of soaking, factors such as protein content, hilum size, initial water content, seed coat thickness and seed volume can influence the water absorption of non-homogeneous legume seeds.

SEM examinations of the common bean (Powrie et al. 1960; Sefa-Dedeh and Stanley, 1979b), faba bean (McEwen et al., 1974), and cowpea (Sefa-Dedeh and Stanley, 1979a) all revealed cotyledon cells containing spherical starch granules embedded in a protein matrix. The protein matrix is made up of protein localized in protein bodies (Graham and Gunning, 1970; Varner and Schidlovsky, 1963). SEM revealed structural differences between fresh and stored dry black beans that were accentuated during imbibition. Using SEM, detached plasmalemma membranes from cell walls in aged beans and a looseness among protein bodies and starch were resolved. After 12 to 24 hours of soaking, the percentage of protein in the cotyledon was the most important variable in water uptake (Sefa-Dedeh and Stanley, 1979c). Generalized losses of membrane integrity may be related to increased peroxidation within the cytoplasm during storage (Varriano-Marston and Jackson, 1981).

Reduction in the rate of cotyledonary cell separation due to reduced middle lamella (pectin) solubility and reduction in the ability of the seed to imbibe water were found responsible for increased hardness in beans. Pectin solubility was decreased because phytin breakdown released calcium and magnesium ions which formed cation bridges with the pectinaceous middle lamella, making it less soluble. The ability of the seed to imbibe water is reduced by loss of solids (Jones and Boulter, 1983a; 1983b).

El-Shimi et al (1980) observed the swelling of starch granules and protein bodies in water soaked broad beans, which Siao et al. (1973) observed swelling in the seed coat structured cells (palisade, hourglass and parenchyma cells) of soybeans. Agbo et al. (1987) observed a thicker seed coat palisade cell layer in the genotype 'San Fernando' which had the slowest rate of water uptake implying a slow hydration of the seed coat leading to a slow hydration of seed cotyledonary structures. The starch granule viewed from SEM cross sections of the cotyledons appeared smaller, more densely packed and more tightly enveloped by a tough protein matrix than the starch granules of the other genotypes ('Sanilac and 'Nep-2') characterized as having a faster hydration rate. Starch

granules of the latter were also embeded in a protein matrix, but not to the same extent. Starch granules were mostly globoid, although a few had irregular shapes.

Soaking dry beans before cooking can provide many beneficial attributes to the final cooked product. Soaking serves to remove foreign material, facilitate cleaning of beans, aid in can filling through uniform expansion, ensure product tenderness and improve color (Cain, 1950; Crafts, 1944 and Hoff and Nelson, 1966). Several methods of soaking have been proposed to accelerate water uptake during soaking thus decreasing the cook time required to tenderize the bean. Various soak methods or pre-treatments include: 1) heat treatments (Gloyer, 1921; Dawson et al., 1952; Morris, et al., 1950 and Snyder, 1936); 2) soak water additives (Greenwood, 1935; Morris et al., 1950; Reeve, 1947; Elbert, 1961; Rockland, 1963 and Synder, 1936); 3) vacuumization or sonification (Hoff and Nelson, 1967); 4) scarification of seed coat (Morris et al., 1950); and 5) dipping in concentrated sulfuric acid (Gloyer, 1921). The results of these soak treatments provide a wide range of variability in quality attributes of cooked beans. In addition, many bean physico-chemical factors that contribute to the water absorption rate during soaking include seed coat thickness, availability of possible paths (the hilum, micropyle and raphe) of water entry (Kyle and Randall, 1963; Sefa-Dedeh and Staley, 1979 a, b and Korban et al. 1981), pectic substances, storage temperature and humidity, age of bean, initial moisture content, protein content and seed density and size.

Dry beans have been traditionally soaked for 8 to 16 hours (overnight) at room temperature. To increase the efficiency of water uptake and possibly improve quality aspects of the finished product, a thermal blanch has been suggested to be effective. Junek et al. (1980) found different soak temperatures to have no effect on drained weight of navy beans but kidney and pinto beans had greatest drained weight when soaked at 25°C and 35°C compared to 15°C. Kidney and pinto beans showed increased splitting and decreased firmness when soaked at 35°C. Kon (1979) found that increasing the temperature of soak water yielded elevated rates of water uptake and shorter soak times to

attain maximum imbibition. Hoff and Nelson (1966) while using soak temperatures from 50 to 90°C established the range for maximum uptake from 60 to 80°C. They attribute the rate of water uptake to the trapped or adsorbed gases in interstitial tissues being released from the bean surfaces by steam pressure, vacuum and sonic energy. Other researchers believe that heat is needed to precipitate the Ca and Mg ions to prevent tough pectin metal complex formation (Mattson, 1946). Another hypothesis suggests that heat causes an inactivation of phytase and pectin esterase (Morris and Seifert, 1961). If these enzymes are allowed to act, they could cause a release of divalent ions from phytate and cause tough pectin-metal complexes. More recent work shows that heating effects vegetable texture by causing cell separation and softening from the thermal degradation of intercellular and cohesive materials (Bourne, 1976 and Loh et al., 1982).

During soaking and blanching, water plays an important role in chemical reactions, heat transfer and chemical transformations such as protein denaturation and starch gelatinization. Inadequate water uptake may result in insufficient heat transfer to inactivate anti-nutritional factors and thus, results in poor quality beans. Thermal processing induces the largest alteration in structure and concomitantly various chemical reactions among the chemical constituents in dry beans. Uebersax and Ruengsakulrach (1989) studied the structural changes in soak/blanched beans (30 min. soak at room temperature and 30 min. water blanch at 88°C). They observed the increase in solubility of protein (loss of indigenous spherical structure) and the relatively unchanged starch granules. During this soak/blanch treatment, native protopectin can be depolymerized to yield pectin.

Soaked/blanched beans are thermally processed to meet the sterilization standard and to obtain the desired smooth texture. In order to obtain the desired tenderness, it has been found necessary for beans to be processed longer than the processing time required for sterilization (Adams and Bedford, 1973 and Hosfield and Uebersax, 1980). The micro-structure of canned navy bean under scanning electron microscope (Uebersax and Ruengsakulrach, 1989) indicated that the absorbed water and heating during retort

processing initiated the thermal degradation of intercellular and cohesive materials (middle lamella) and thus allowed cells to separate and soften. It was also noted that, in the uncooked dry bean, fracture occurred across the cell wall while in the cooked sample fracture occurred in the middle lamella portion, leaving the cell intact. Various chemical changes have been significantly induced within the cell inclusions. Protein bodies lose their normal spherical structures due to swelling and denaturation. Starch granules demonstrate the deformation, expansion and loss of birefringence associated with gelatinization, although the presence of intact cell walls impede conformational changes. Hahn et al. (1977) reported that the range of intracellular starch gelatinization in soaked beans is from 76°C to over 95°C. Intracellular starch gelatinization and protein denaturation occurs during moist heating which develops a uniform smooth texture. The characteristics of cooked bean flavors develop through chemical reactions which involve the degradation or interaction of tissue constituents. Environmental factors such as temperature, pH, ionic strength, and the presence of selected food constituents (product sauce formulation) may influence the predominant reactions which affect bean quality performance.

The content of available carbohydrates, total soluble sugars, reducing sugars, and non-reducing sugars in legumes decreases during soaking and cooking. Reducing sugars can participate in non-enzymatic browning reactions and contribute to flavor formation (Maga, 1973). The sugar content of soaked beans is a function of soaking time (Silva and Braga, 1982 and Jood et al., 1986), but not the bean-to-brine ratio (Silva and Braga, 1982). The sucrose, raffinose and stachyose contents of dry beans decreased approximately 20%, 35% and 45%, respectively after soaking (Silva and Braga, 1982 and Jood et al., 1986). Elias et al. (1979) have studied the effects of processing on the protein content of five cultivars of dry beans. On a dry weight basis, the cooked beans had a protein content which was 70 to 86% of that of the raw beans. Similar losses have been observed during the processing of other plant tissues (El-Refai et al., 1987). The loss in

protein is attributed to the extraction of soluble proteins, hydrolysis of protein to free amino acids, and non-enzymatic browning reactions. Heat treatment improves the protein bioavailability and protein quality of dry beans through the inactivation of anti-nutritional factors (Evans and Bandemer, 1967; Elias et al., 1979 and Sgarbieri and Garruti, 1986). However, with excess heat treatment, protein destruction with a subsequent decrease in protein quality occurs (Koehler and Burke, 1981). Almas and Bender (1980) attributed the reduction in the available lysine content and protein quality of legumes during heating to non-enzymatic browning reactions.

CHAPTER 1: QUALITY ASSESSMENT METHODOLOGY OF EARLY GENERATION BEAN BREEDING LINES FOR OPTIMUM CANNING QUALITY

INTRODUCTION

One of the constraints in bean breeding programs is the lack of quality assessment methodology for screening and selecting a superior genetic materials in the early generations after hybridization. The objective of early generation quality testing is to eliminate lines or populations that do not merit consideration for further inbreeding and selection. Early generation testing is necessary to reduce time and cost involved to release a new cultivar. The quality testing methodology should be rapid, reliable, inexpensive and importantly, require a small amount of sample due to limited seed supply in early generations of selection. In addition, quality assessment technology should also simulate a commercial processing operation so that the quality of new cultivars released meet standard criteria of processors.

EXPERIMENTAL PLAN

Part of the research task in Dry bean Research Program at Michigan State University is to develop new cultivars which possess both high crop yield and premium food quality, especially in canned products. The general breeding scheme is illustrated in Figure 2. As previously stated, there is a need of developing the quality testing method for early breeding generations between F_2 and F_4. These seeds are primarily maintained as stock seeds for later propagation and thus, the available amount of a dry bean sample for quality testing is a limiting factor.

Generally, breeding accessions from the F_4 (600 - 900 g/sample) and F_6 generations (50 kg/sample) are evaluated for their canning quality (Figure 2). Dry beans are canned using a method with 303 x 406 can size (16 oz.) (Uebersax and Hosfield, 1985). This canning method has been demonstrated to be an accurate assessment of bean

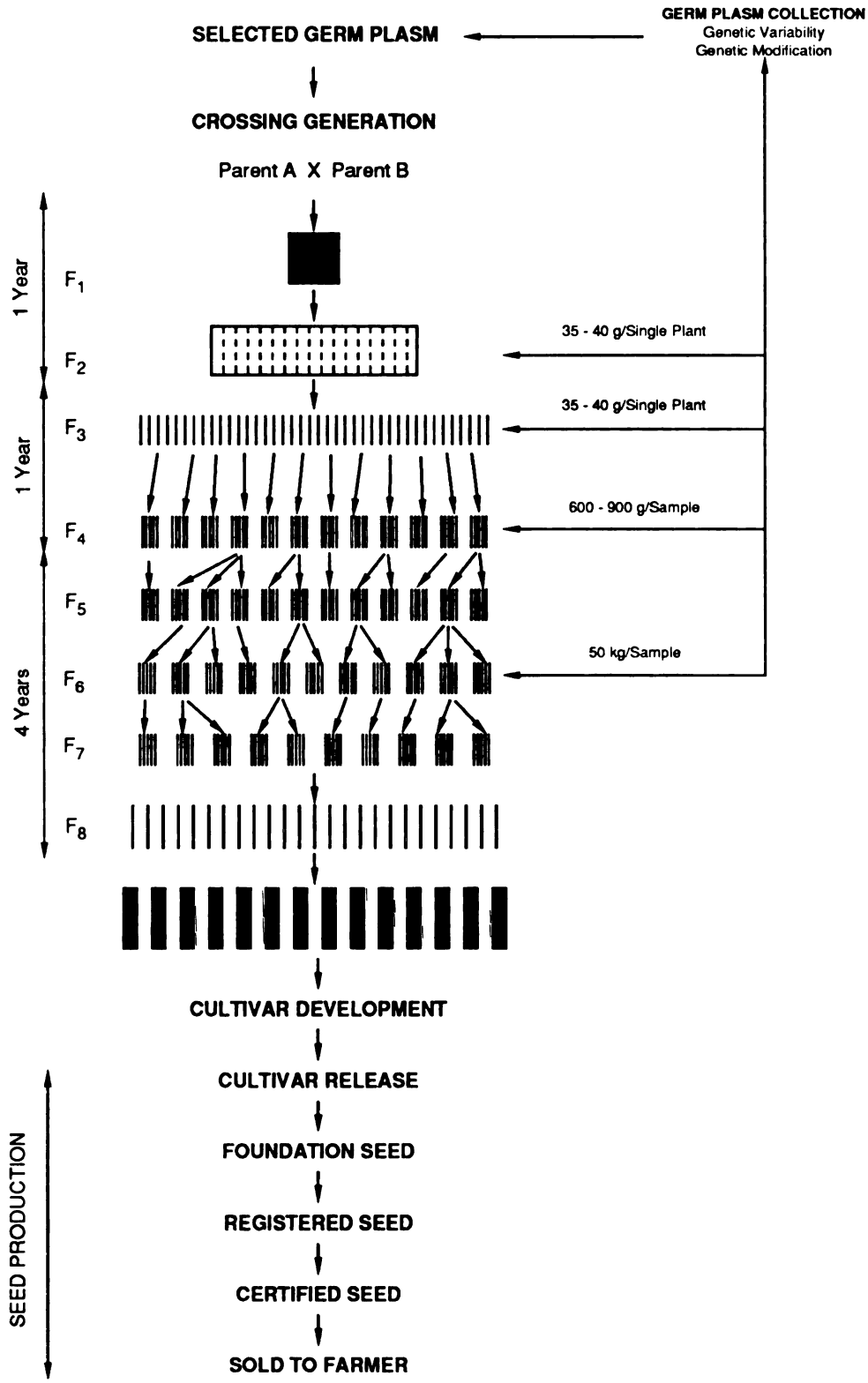

Figure 2. A Breeding Program Illustrating the F Generations and the Quantity of Dry Bean Samples Available for Quality Testing

canning quality; however, it still requires at least 400-500 g dry bean sample for tripicate cans of each breeding line tested (Hosfield and Uebersax, 1980).

The direct canning quality testing using smaller can sizes was proposed to be used as an alternative method in **Study 1**. Dry bean samples are simply prepared by the same soak and/or blanch procedure. However, the retort conditions used, temperature (115.6°C) must be reduced to achieve equivalent thermal process history. This direct scale-down method will enable breeders to assess the canning quality of dry bean with a minimum amount of 100-150 g bean samples (F_4 - F_5).

In order to further reduce the sample size, **Study 2** was designed to evaluate the potential use of whole bean flour pasting characteristic as a criterion in screening breeding lines. This procedure is called an in-direct method because the relationship between bean canning quality and whole bean flour pasting characteristic must first be established. The in-direct method offers an advantage of using a smaller amount of bean sample, enabling the researcher to evaluate the canning quality of Bean Breeding lines in the F_3 generation.

MATERIALS AND METHODS

Study 1. Direct: Scale-down Canning Method

The 202 x 214 (4 oz.) and 211 x 300 (7 oz.) (National Can Co., Chicago, Illinois) cans were selected for this experiment since they are smaller than the 303 x 406 (16 oz.) normally used in canning research (Hosfield and Uebersax, 1980). The first part of this study was to establish processing procedures to ensure an equivalent thermal process history among the 202 x 214; 211 x 300; and 303 x 406 cans used to process beans. Dry beans utilized in this experiment were navy beans var. Seafarer. Either the established 202 x 214 or 211 x 300 method could have been selected as a procedure to be used in screening breeding materials. The selected established methods were tested on various bean cultivars and breeding lines during 1987 and 1988 crop year.

A. Processing Procedures for 202 x 214 and 211 x 300 Canned Beans

Navy beans (Seafarer) obtained from the Cooperative Elevator Company, Pigeon, MI during the 1987 crop year were used throughout this study. Dry beans for use in 202 x 214, 211 x 300 and 303 x 406 cans were prepared by the same soaking/blanching and brining methods. However, the pack ratio (soaked/blanched bean and brine ratio) of 202 x 214 and 211 x 300 cans had to be determined and set equal to the pack ratio of the standard 303 x 406 can. Cans, after filling and exhausting, were hermatically sealed and retorted at 115.6°C for a pre-determined time to obtain the same thermal process history (F_o) which is equivalent to that of established 303 x 406 process.

Pack Specification. The following outlines the MSU laboratory canning procedures for dry beans (Uebersax and Hosfield, 1985) and the comparisons of thermal process history of canned beans processed in studied can sizes.

Dry Bean Sample

One-hundred gram bean solids are the standard requirement for processing in each 303 x 406 can. To determine 100 g of bean solids, the following equation was used:

$$\text{Fresh weight to yield Required Solids (100 g)} = \frac{\text{Solid Required (100 g)}}{100 - \%\ \text{Dry Bean Moisture}} \times 100$$

The initial moisture content of beans was determined by Motomco Moisture meter (Motomco Inc., Clark, N.J.). The bean solids required for each 202 x 214 and 211 x 300 can were subsequently determined according to this procedure after the standard soaked-blanched bean/brine ratio of 303 x 406 was obtained in the following sections.

Soaking and Blanching **(30/30)**

Soaking/Blanching Medium:

100 ppm Ca^{++} Water (Distilled water + Pre-determined Ca^{++})

Ambient Cold Soaking	30 minutes at ambient temperature (25°C)
Water Blanching	30 minutes at 87.8°C. Soaked beans were blanched in an in-direct steam heated temperature controlled blancher maintaining at 87.8°C.

Brining and Exhausting

The soaked bean samples were filled into the 303 x 406 can and covered with hot brine (97.8°C), allowing 2/16" headspace. The brine contained 1.25% NaCl, 1.56% sugar and 100 ppm Ca ions. Total fill volume (headspace 2/16"), soaked-blanched bean/brine ratio (w/v) and approximate dry bean solids for each can size were then calculated. Brining was performed immediately prior to a seven minute thermal exhaust. Exhaust box temperature was maintained at 98.9°C to 100°C.

Sealing and Processing

The cans of beans were hermetically sealed immediately upon removal from the exhaust box. Sealed cans were inverted and transferred to the retort (process schedule: a) 2 min. come-up time, b) process at 115.6°C/45 min. for 303 x 406 can, c) steam off and d) cool with running water for 15 min.). Processed cans were removed from the retort, turned right side up and air dried on a table top. Dried processed cans were stored in designated controlled temperature cabinets at 21°C for two weeks until canned product evaluation.

Heat Penetration Test. Heat penetration tests were conducted using the CNS Needle Type Thermocouples (Ecklund Custom Thermocouples, Cape Coral, FL). The temperatures and times of each thermocouple were recorded at 25 second intervals by an electronic data acquisition system and Hewlett Packard 85 computer.

Cold Spot Determination: An appropriate thermocouple length was selected to measure horizontal center temperature for each can size. The cold spot was determined by varying the vertical position of the thermocouple along the horizontal center of each can size. Four specific locations for each can size were selected and are presented in Table 1.

Table 1. Positions of Thermocouples Along the Horizontal Center of 303 x 406, 211 x 300 and 202 x 214 Cans

303 x 406	211 x 300	202 x 214
5.4[1]	3.6	3.4
4.8	3.2	3.0
4.2	2.8	2.7
3.6	2.4	2.3

[1]cm from Base for Each Can Size.

Thermal Process Calculation. The temperature and time data at cold spots (slowest heating spots) of each can size were used to calculate the lethality and sterilizing value (F_0) of the process using Improved General Method (Lethal Rate = $10^{(T-121.1)/Z}$, Z = 10°C for *Clostridium botulinum*) (Figure 3). The F_0 value is equivalent to the number of min at 121.1°C when Z-value is 10°C. F_0 value is equal to 1 when the cold-point temperature of a canned products is held at 121.1°C for 1 min (Stumbo, 1973 and Toledo, 1980). The F_0 of the 303 x 406 can was used as standard to assess process time of 211 x 300 and 202 x 214 cans.

B. Direct: Scale-down 202 x 214 Method Testing

The established canning procedure for 202 x 214 (4 oz.) was selected because it requires the smallest amount of dry bean sample. Various bean cultivars and breeding lines, obtained from the Michigan Dry Edible Bean Production Research and Advisory Board Agronomist (Gregory Varner) during 1987 and 1988, were processed by the standard processing procedure using the 303 x 406 can and the established procedure using the 202 x 214 can. The canning quality of both can sizes, including soaked weight, drained weight and processed bean color and texture were compared.

Study 2. In-direct Pasting Characteristic of Whole Bean Flour

Navy beans (C-20, Seafarer, Fleetwood and Experimental line 84004) were selected for use in this experiment. Dry bean samples were ground using a Udy Cyclone Mill (Udy Co., Fort Collins, CO) to pass through a 20 mesh screen to produce whole bean flour. Dry bean samples were canned in a 303 x 406 can, according to the method previously described in Study 1. The relationships between the pasting characteristics of whole bean flours and the textural quality of corresponding dry bean samples were determined.

STEP-BY-STEP PROCEDURE FOR IMPROVED GENERAL METHOD

1. Selection of most critical organism(s)

2. Determine by experimental methods or established literature the followings:

   a) Z value
   b) Obtain D_{250}

3. Select appropriate (USDA-FDA) Sterilizing Value (SV)

4. Calculate F_{250} using the following Equation:

$$F_{250}^{Z} = (SV)\, D_{250}$$

5. Determine "Cold-Spot" Temperature (T_c) vs Process Time Profile either experimentally or predicted via Transient Heat Transfer Methods.

6. Arrange T_c vs Time data in tabular form in such a way that the Δ t selected does not produce significant curvature errors.

7. Calculate the Lethality Value "L_i" using

$$L_i = 10^{(T_i - 250)/Z}$$

8. Determine the Sterilizing Value using the following Equation:

$$F_{250}^{Z} = \Sigma L_i \times \Delta t_i$$

9. Determine process time to achieve the pre-selected F_o in Step 4.

Figure 3. Step-by-Step Procedure to Determine Sterilizing Value (F) by Improved General Method

Pasting Characteristic By Brookfield Viscometer

In this study, the whole bean flour pasting characteristic was determined by the method described by Steffe et al. (1989) with some modification. A schematic diagram of the apparatus used is shown in Figure 4. Deionized-distilled water was added to pre-weighed whole bean flour to achieve 6%, w/w concentration. The bean flour slurry was mixed at approximately 800 rpm (Corning PC-351 magnetic stirrer) to ensure full dispersion.

The Brookfield sample chamber was inserted in the heating/cooling jacket and the pre-installed impeller was turned on at 100 rpm, to prevent the sample from settling. Twelve milliliters of this homogeneous bean flour slurry were pipetted into the chamber. Heating of the slurry sample began within 15 seconds, by circulating glycerolglycol from the heating bath through the sample heating/cooling jacket. The time required to reach maximum temperature ($94.0 \pm 1.0^{o}C$) was approximately 8 minutes and the total heating time was set for 23 minutes. Temperature and torque were recorded every 10 seconds by a 16 - bit data acquisition board and accompanying software (ACSE - 16-8 board and Analog Connection WorkBench software, Strawberry Tree Computers, Inc., Sunnyvale CA.) with an Apple Macintosh SE computer (Apple Computer, Inc., Cupertino, CA). The cooling cycle started immediately after heating was completed by circulating the $-6^{o}C$ coolant. This analysis was completed and manually terminated when the temperature of sample slurry reached $10^{o}C$. The cooling time for each sample varied in the range of $\pm$ 1 min.

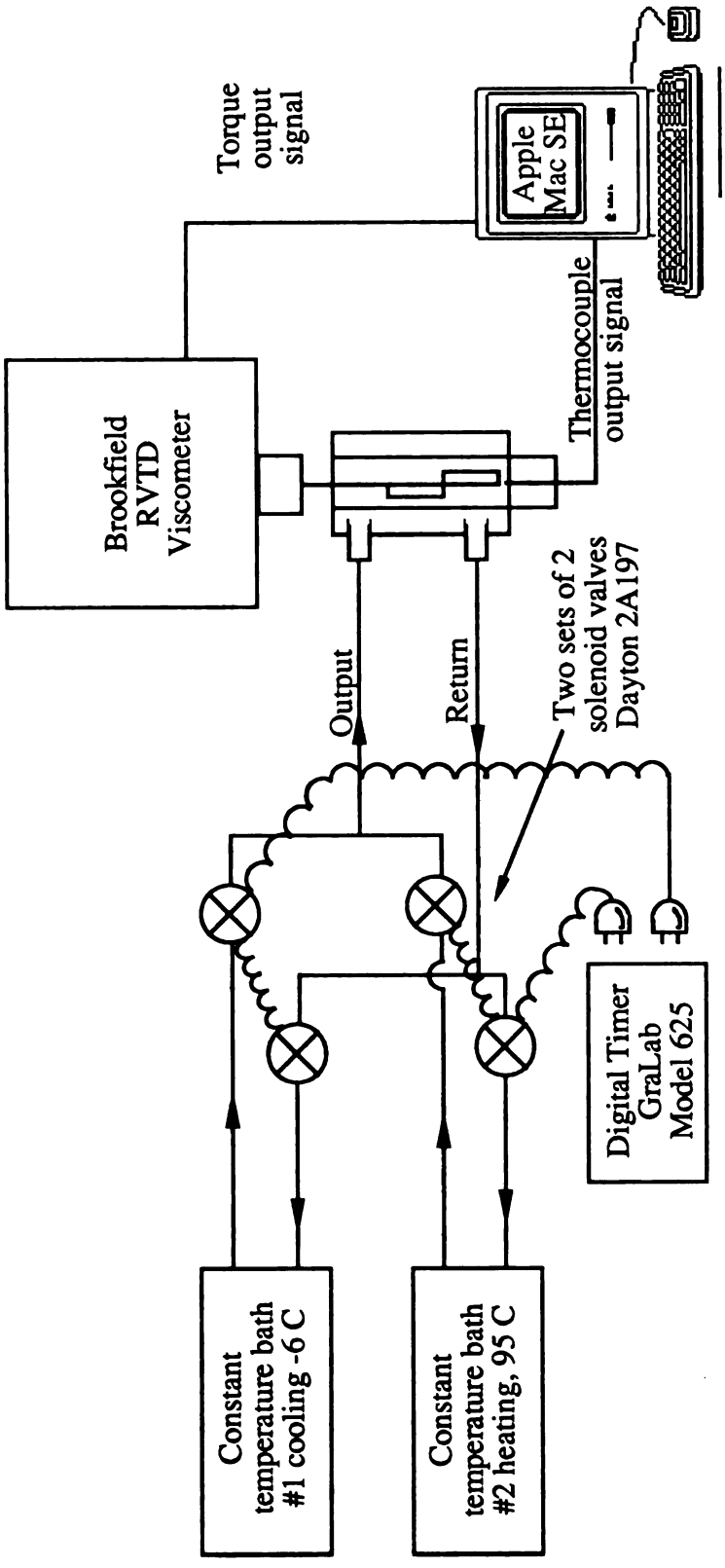

Figure 4. Schematic Diagram Illustrating Brookfield Viscometer Set-up for Pasting Characteristic Analyses of Whole Bean Flour

RESULTS AND DISCUSSION

Study 1. Direct: Scale-down Canning Method

A. Processing Procedures for 202 x 214 and 211 x 300 Canned Beans

It is imperative that various factors involved in heating characteristics of canned products be evaluated prior to establishment of proper thermal processing procedures. For canned beans, these factors include: a) dry bean quality (seed - size, color, moisture and absence of defects), b) brine or sauce formulation, c) container specification (dimension and configuration); and d) pack specification (soaked-blanched bean/brine ratio, headspace and fill weight). Furthermore, the entire processing system and its critical control points must be identified and precisely regulated according to USDA or FDA standards. The process used for commercial sterility of low acid foods such as canned bean should give at least a sterilization value of 10 (Toledo, 1980, Stumbo et al., 1975 and Pflug and Odlaug, 1978).

Pack Specification. In general, dry beans processed in a can should be uniform in size, fully mature, and free from foreign materials and seed coat defects. The moisture content of Seafarer was determined and the weight of fresh beans required to obtain 100 g dry solids was calculated. This procedure was used for each sample processed in a 303 x 406 can. Four major steps in the canning process were: 1) soaking 100 g dry bean solids for 30 min at room temperature; 2) water blanching at 87.8^{o}C for another 30 min; 3) filling and exhausting and 4) retort processing (Hosfield and Uebersax, 1980).

After soaking and blanching, each lot (100 g dry solids) of beans was transferred into 303 x 406 can and subsequently covered with brine containing a predetermined amount of salt, sugar and calcium chloride to a headspace of 2/16". The pack ratio (soaked-blanched bean/brine) of 303 x 406 was determined and is presented in Table 2. Pack ratio of 303 x 406 can (0.72) was then used as a standard for 211 x 300 and 202 x 214 cans. Based on total fill volume and soaked-blanched bean weight, the dry bean solids for 211 x

Table 2. Total Fill Volume (mL, headspace 2/16"), Soaked-blanched Bean/Brine Ratio (w/v) and Approximate Dry Bean Solids for 303 x 406, 211 x 300 and 202 x 214 Cans

Can Size (oz)	Total Fill Volume[1] (mL)	Soaked-Blacnhed Bean and Brine Ratio	Approximate Dry Bean Solids (g)
303 x 406 (16)	480.2	0.72	100.0
211 x 300 (7)	232.0	0.72	48.0
202 x 214 (4)	139.1	0.72	28.5

[1] Determined by filling can with water to obtain the headspace of 2/16", n = 20
[2] n = 30 (3 cultivars x 10 cans each)

300 and 202 x 214 cans were estimated to be 49.5 and 28.5 g (Table 2). These established dry solid requirements were then used in canning procedure throughout this study.

Heat Penetration Test. To calculate thermal process history, product temperature at the cold spot or slowest heating spot must be experimentally determined (Stumbo, 1973 and Holdsworth, 1985). Dry beans processed in brine can be considered as a light consistency product. Based on the literature, the slowest heating spot of beans processed in cans should be on the vertical position, along the horizontal center of the can, between the geometric center point (ideal conduction heating product) and the point about 1/3 of the can height measured from the vertical end (ideal convection heating product) (Stumbo, 1973 and Holdsworth, 1985). With this assumption, the thermocouple positions for each can size were thus selected and are presented in Table 1. One additional criterion was used to ensure that the accuracy of the slowest heating location was obtained. The slowest heating spot should be bracketed between two spots of higher heating rates. The process time and temperature data of beans canned in 303 x 406, 211 x 300 and 202 x 214 cans at selected thermocouple positions were experimentally determined and are reported in Tables 3, 4 and 5, respectively. These results indicated the slowest heating spots during retort processing of the three studied can sizes (Table 6).

Thermal Process Calculation. Thermal process history of canned products can be estimated by their sterilizing value or F_0. The F_0 value can be calculated using Improved General Method and Lethality Concept (Stumbo, 1973). This thermal process calculation method requires the actual process time and temperature data at the slowest heating spot. These process time and temperature data for 303 x 406, 211 x 300 and 202 x 214 cans were determined in three replicates (three retort operations) and are presented in Table 7. Canned beans processed in brine exhibit a straight-line semi-logarithmic heating curve (Figure 5) and is referred to as the convection heating product. The sterilizing value (F_0) of 303 x 406 can was calculated to be 11.6 min (as described in Material and Method Section). This F_0 value (11.6 min) was then used to calculate the process times of 211 x

Table 3. Process Time and Temperature Data Measured at Four Pre-determined Locations in 303 x 406 Can for Navy Beans Processed at 115.6°C for 45 min

Time	Location (cm from base)			
	5.4	4.8	4.2	3.6
0	64.0 ± 3.2	61.2 ± 1.9	62.8 ± 3.6	62.5 ± 3.0
25	91.8 ± 1.6	88.7 ± 1.8	73.1 ± 3.1	88.9 ± 1.8
50	96.5 ± 1.6	94.5 ± 1.2	82.1 ± 3.5	93.7 ± 2.4
75	103.5 ± 1.4	101.5 ± 0.8	91.7 ± 1.0	101.3 ± 1.3
100	108.5 ± 1.6	106.3 ± 0.5	99.4 ± 1.8	105.7 ± 1.3
125	110.3 ± 0.9	109.7 ± 0.3	104.2 ± 1.3	108.4 ± 1.8
150	112.4 ± 0.9	111.5 ± 0.1	107.9 ± 1.7	111.1 ± 3.2
175	113.8 ± 1.1	112.9 ± 0.9	109.9 ± 1.0	112.4 ± 0.9
200	114.6 ± 0.7	113.9 ± 0.7	111.7 ± 1.5	113.3 ± 0.8
225	115.0 ± 0.6	114.5 ± 0.3	112.7 ± 1.5	114.1 ± 0.4
250	115.4 ± 0.3	115.1 ± 0.3	113.5 ± 1.0	114.7 ± 0.4
275	115.6 ± 0.2	115.4 ± 0.2	114.1 ± 0.8	115.1 ± 0.2
300	115.6 ± 0.2	115.6 ± 0.3	114.7 ± 0.7	115.5 ± 0.2
325	115.6 ± 0.0	115.6 ± 0.2	115.2 ± 0.5	115.6 ± 0.2
350	115.6 ± 0.1	115.6 ± 0.2	115.5 ± 0.3	115.6 ± 0.2
375	115.6 ± 0.2	115.6 ± 0.2	115.6 ± 0.3	115.6 ± 0.2
400	115.6 ± 0.1	115.6 ± 0.4	115.6 ± 0.2	115.6 ± 0.2
425	115.6 ± 0.1	115.6 ± 0.2	115.6 ± 0.2	115.6 ± 0.1
450	115.6 ± 0.1	115.6 ± 0.2	115.6 ± 0.1	115.6 ± 0.0
475	115.6 ± 0.1	115.6 ± 0.0	115.6 ± 0.1	115.6 ± 0.0
500	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
2675	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
2700	114.1 ± 1.6	114.9 ± 1.3	115.0 ± 0.5	115.0 ± 1.8
2725	94.8 ± 3.7	95.1 ± 3.4	100.7 ± 0.5	93.9 ± 5.9
2750	84.3 ± 4.7	86.7 ± 4.1	90.1 ± 1.5	87.6 ± 5.3
2775	74.5 ± 2.1	77.0 ± 2.1	78.7 ± 1.1	75.2 ± 2.6
2800	63.5 ± 2.9	66.7 ± 3.8	69.3 ± 1.5	64.4 ± 3.9
2825	58.0 ± 2.8	60.9 ± 4.1	61.7 ± 4.9	58.6 ± 5.1
2850	52.7 ± 0.5	53.3 ± 1.4	55.6 ± 3.1	54.0 ± 4.9
3600	29.0 ± 1.7	28.4 ± 1.4	29.1 ± 0.8	27.4 ± 1.2

Table 4. Process Time and Temperature Data Measured at Four Pre-determined Locations in 211 x 300 Can for Navy Beans Processed at 115.6°C for 45 min

Time	Location (cm from base)			
	3.6	3.2	2.8	2.4
0	61.4 ± 3.0	61.0 ± 4.5	60.4 ± 3.6	62.1 ± 4.7
25	84.8 ± 1.4	85.3 ± 1.1	89.0 ± 1.1	95.8 ± 1.5
50	89.9 ± 1.9	91.5 ± 2.1	96.8 ± 1.8	100.2 ± 0.6
75	97.6 ± 1.4	98.8 ± 1.4	101.9 ± 1.3	106.4 ± 1.0
100	103.2 ± 1.3	104.4 ± 1.2	106.9 ± 1.3	109.7 ± 0.5
125	106.2 ± 0.5	107.8 ± 1.0	110.0 ± 1.2	111.8 ± 0.6
150	108.7 ± 0.1	110.2 ± 1.0	111.3 ± 0.7	113.5 ± 0.6
175	110.8 ± 0.5	112.1 ± 0.2	112.7 ± 0.6	114.3 ± 0.6
200	112.1 ± 0.4	113.0 ± 0.6	113.8 ± 0.9	114.5 ± 0.7
225	112.9 ± 0.5	113.9 ± 0.1	114.4 ± 0.5	115.1 ± 0.6
250	113.7 ± 0.2	114.6 ± 0.1	115.0 ± 0.5	115.6 ± 0.3
275	114.5 ± 0.1	115.0 ± 0.1	115.5 ± 0.4	115.6 ± 0.3
300	115.2 ± 0.2	115.5 ± 0.3	115.6 ± 0.3	115.6 ± 0.2
325	115.4 ± 0.2	115.6 ± 0.3	115.6 ± 0.3	115.6 ± 0.2
350	115.6 ± 0.1	115.6 ± 0.3	115.6 ± 0.4	115.6 ± 0.1
375	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
400	115.6 ± 0.2	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
425	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
450	115.6 ± 0.0	115.6 ± 0.0	115.6 ± 0.1	115.6 ± 0.1
475	115.6 ± 0.0	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
500	115.6 ± 0.0	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
2675	115.6 ± 0.1	115.6 ± 0.0	115.6 ± 0.0	115.6 ± 0.0
2700	115.3 ± 1.2	113.9 ± 0.6	112.0 ± 1.2	109.6 ± 5.9
2725	104.0 ± 1.5	99.5 ± 3.0	99.0 ± 1.6	94.6 ± 2.1
2750	92.1 ± 1.5	88.0 ± 4.5	86.1 ± 3.9	83.3 ± 2.7
2775	81.5 ± 1.1	76.0 ± 4.5	74.4 ± 4.4	73.2 ± 3.0
2800	70.4 ± 2.1	66.6 ± 3.8	66.4 ± 3.0	60.5 ± 5.2
2825	60.0 ± 3.0	56.7 ± 5.3	55.2 ± 5.3	53.5 ± 4.6
2850	48.3 ± 2.1	47.5 ± 1.9	46.2 ± 1.6	44.9 ± 2.3
3600	27.9 ± 2.2	26.8 ± 1.3	26.6 ± 1.1	26.3 ± 0.9

Table 5. Process Time and Temperature Data Measured at Four Pre-determined Locations in 202 x 214 Can for Navy Beans Processed at 115.6°C for 45 min

Time	Location (cm from base)			
	3.4	3.0	2.7	2.3
0	65.8 ± 3.0	63.5 ± 2.8	62.9 ± 4.0	99.7 ± 1.8
25	83.6 ± 0.9	88.8 ± 1.1	95.4 ± 4.0	99.7 ± 1.8
50	95.7 ± 1.7	97.3 ± 2.5	101.3 ± 3.2	104.1 ± 1.1
75	103.6 ± 2.0	104.7 ± 0.4	107.4 ± 1.8	108.4 ± 1.9
100	107.9 ± 1.0	108.5 ± 1.4	110.6 ± 1.4	111.0 ± 2.0
125	112.9 ± 0.8	113.7 ± 0.5	114.2 ± 0.3	114.8 ± 0.6
150	114.3 ± 0.4	114.8 ± 0.5	114.9 ± 0.3	115.2 ± 0.6
175	115.6 ± 0.8	115.6 ± 0.8	115.6 ± 0.5	115.6 ± 0.5
200	115.6 ± 0.5	115.6 ± 0.6	115.3 ± 0.5	115.6 ± 0.5
225	115.6 ± 0.6	115.6 ± 0.8	115.6 ± 0.6	115.6 ± 0.5
250	115.6 ± 0.6	115.6 ± 0.8	115.6 ± 0.6	115.6 ± 0.4
275	115.5 ± 0.4	115.6 ± 0.6	115.6 ± 0.4	115.6 ± 0.3
300	115.6 ± 0.4	115.6 ± 0.6	115.6 ± 0.4	115.6 ± 0.1
325	115.6 ± 0.4	115.6 ± 0.6	115.6 ± 0.3	115.6 ± 0.1
350	115.6 ± 0.4	115.6 ± 0.4	115.6 ± 0.3	115.6 ± 0.0
375	115.6 ± 0.2	115.6 ± 0.2	115.6 ± 0.1	115.6 ± 0.0
400	115.6 ± 0.2	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.0
425	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
450	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1
475	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.0	115.6 ± 0.0
500	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.0
2670	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.0	115.6 ± 0.1
2700	115.6 ± 0.3	114.4 ± 1.1	109.9 ± 2.1	103.2 ± 2.2
2725	106.7 ± 1.4	97.1 ± 1.7	89.8 ± 3.1	87.6 ± 1.8
2750	95.4 ± 2.5	86.6 ± 2.9	79.3 ± 2.9	76.0 ± 3.0
2775	83.1 ± 2.1	71.5 ± 1.3	67.3 ± 2.5	66.5 ± 1.9
2800	73.2 ± 1.9	64.9 ± 1.9	61.0 ± 5.6	57.3 ± 3.2
2825	63.2 ± 1.0	58.1 ± 0.7	53.3 ± 3.8	49.9 ± 2.4
2850	48.0 ± 1.8	44.4 ± 0.8	43.1 ± 0.7	41.8 ± 1.3
3600	26.6 ± 1.1	26.2 ± 0.4	26.2 ± 0.7	25.9 ± 1.1

Table 6. Slowest Heating Spots of 303 x 406, 211 x 300 and 202 x 214 Cans for Navy Beans Measured by Thermocouples Inserted at Various Positions[1] Along the Horizontal Center of the Cans

303 x 406	211 x 300	202 x 214
5.4	3.6*	3.4*
4.8	3.2	3.0
4.2*	2.8	2.7
3.6	2.4	2.3

[1] cm from Base for Each Can Size.
* indicate slowest heating spots

Table 7. Process Time (sec) and Temperature (°C) at Slowest Heating Spots of 303 x 406, 211 x 300 and 202 x 214 Cans for Navy Beans During Retort Processing at 115.6°C for 45 min

Process Time (sec)	Slowest Heating Spot Temperature		
	303 x 406	211 x 300	202 x 214
Heating Cycle			
0	62.2 ± 3.3	57.2 ± 1.1	62.6 ± 3.5
25	76.7 ± 4.2	84.9 ± 2.8	84.8 ± 1.2
50	87.1 ± 7.1	90.7 ± 2.9	96.7 ± 3.1
75	93.1 ± 2.5	98.4 ± 2.6	104.2 ± 0.6
100	100.3 ± 1.8	102.6 ± 3.9	108.5 ± 1.2
125	104.1 ± 0.9	106.2 ± 1.5	113.6 ± 0.8
150	106.5 ± 1.8	109.0 ± 1.4	114.8 ± 0.6
175	108.7 ± 1.8	110.6 ± 2.0	115.6 ± 0.2
200	109.7 ± 1.6	112.0 ± 1.5	115.6 ± 0.1
225	111.4 ± 1.3	112.9 ± 1.0	115.6 ± 0.0
250	112.4 ± 1.2	113.7 ± 0.8	115.6 ± 0.1
275	113.2 ± 0.8	114.4 ± 0.5	115.6 ± 0.1
300	114.1 ± 0.4	115.0 ± 0.4	115.6 ± 0.1
325	114.7 ± 0.5	115.3 ± 0.4	115.6 ± 0.0
350	114.9 ± 0.4	115.4 ± 0.3	115.6 ± 0.0
375	115.2 ± 0.3	115.6 ± 0.0	115.6 ± 0.0
400	115.4 ± 0.2	115.6 ± 0.0	115.7 ± 0.1
425	115.5 ± 0.2	115.6 ± 0.0	115.6 ± 0.1
450	115.6 ± 0.1	115.6 ± 0.0	115.6 ± 0.1
475	115.6 ± 0.0	115.6 ± 0.0	115.6 ± 0.1
500	115.6 ± 0.0	115.6 ± 0.0	115.7 ± 0.1
700	115.6 ± 0.0	115.6 ± 0.0	115.7 ± 0.0
900	115.6 ± 0.0	115.5 ± 0.1	115.7 ± 0.0
1100	115.6 ± 0.0	115.6 ± 0.1	115.7 ± 0.1
1300	115.6 ± 0.0	115.6 ± 0.0	115.7 ± 0.1
1500	115.6 ± 0.1	115.6 ± 0.1	115.7 ± 0.1
1700	115.6 ± 0.1	115.6 ± 0.0	115.7 ± 0.0
1900	115.6 ± 0.0	115.6 ± 0.0	115.7 ± 0.1
2100	115.6 ± 0.0	115.6 ± 0.0	115.6 ± 0.1
2300	115.6 ± 0.1	115.6 ± 0.1	115.7 ± 0.1
2500	115.6 ± 0.0	115.6 ± 0.0	115.7 ± 0.1
2525	115.6 ± 0.1	115.6 ± 0.0	115.6 ± 0.1
2550	115.6 ± 0.0	115.6 ± 0.0	115.7 ± 0.0
2575	115.7 ± 0.1	115.6 ± 0.1	115.7 ± 0.1
2600	115.6 ± 0.0	115.6 ± 0.1	115.6 ± 0.1
2625	115.6 ± 0.0	115.6 ± 0.1	115.7 ± 0.0
2650	115.6 ± 0.1	115.6 ± 0.0	115.7 ± 0.1
2675	115.6 ± 0.1	115.6 ± 0.0	115.6 ± 0.1
2700	115.6 ± 0.1	115.6 ± 0.1	115.6 ± 0.1

(Cont'd ...)

Table 7. (Cont'd ...)

Cooling Cycle			
2725	104.0 ± 10.3	103.3 ± 6.3	104.7 ± 8.0
2750	96.2 ± 14.5	92.1 ± 7.0	90.3 ± 8.9
2775	85.8 ± 13.4	85.0 ± 5.7	82.0 ± 10.2
2800	77.3 ± 12.7	75.8 ± 4.7	70.7 ± 3.4
2825	70.8 ± 12.7	68.2 ± 3.6	63.3 ± 0.9
2850	65.6 ± 11.8	63.0 ± 3.8	57.2 ± 1.0
2875	61.8 ± 11.0	58.4 ± 4.2	52.2 ± 2.6
2900	59.2 ± 10.5	54.9 ± 4.7	48.9 ± 3.3
2925	56.2 ± 9.9	52.0 ± 5.0	46.3 ± 3.7
2950	53.7 ± 9.4	49.6 ± 5.3	44.2 ± 3.9
2975	51.5 ± 9.0	47.6 ± 5.4	42.3 ± 4.0
3000	49.5 ± 8.5	45.9 ± 5.6	40.8 ± 4.1
3025	47.8 ± 8.2	44.3 ± 5.6	38.8 ± 3.2
3050	46.4 ± 7.8	43.0 ± 5.7	38.3 ± 4.3
3075	45.0 ± 7.5	41.9 ± 5.7	37.3 ± 4.3
3100	43.8 ± 7.2	40.8 ± 5.7	36.4 ± 4.3
3125	42.7 ± 6.9	39.9 ± 5.7	35.6 ± 4.4
3150	41.8 ± 6.7	39.1 ± 5.7	34.9 ± 4.3
3175	40.9 ± 6.4	38.3 ± 5.7	34.3 ± 4.3
3200	40.1 ± 6.2	37.6 ± 5.7	33.7 ± 4.3
3225	39.3 ± 5.9	37.0 ± 5.6	33.2 ± 4.3
3250	38.7 ± 5.7	36.5 ± 5.6	32.8 ± 4.3
3275	37.8 ± 5.9	36.0 ± 5.6	32.4 ± 4.2
3300	37.3 ± 5.7	35.5 ± 5.6	32.0 ± 4.2
3325	36.8 ± 5.5	35.1 ± 5.5	31.7 ± 4.2
3350	36.3 ± 5.3	34.7 ± 5.5	31.3 ± 4.1
3375	35.8 ± 5.1	34.3 ± 5.5	31.1 ± 4.1
3400	35.4 ± 5.0	34.0 ± 5.5	30.8 ± 4.1
3425	35.1 ± 4.8	33.7 ± 5.5	30.5 ± 4.1
3450	34.7 ± 4.7	33.5 ± 5.5	30.3 ± 4.1
3475	34.3 ± 4.5	33.2 ± 5.5	30.1 ± 4.1
3500	34.0 ± 4.4	33.0 ± 5.5	29.9 ± 4.1
3525	33.7 ± 4.4	32.7 ± 5.5	29.8 ± 4.1
3550	33.4 ± 4.2	32.5 ± 5.4	29.6 ± 4.1
3575	33.2 ± 4.1	32.3 ± 5.4	29.4 ± 4.0
3600	32.9 ± 4.0	32.1 ± 5.4	29.3 ± 4.0

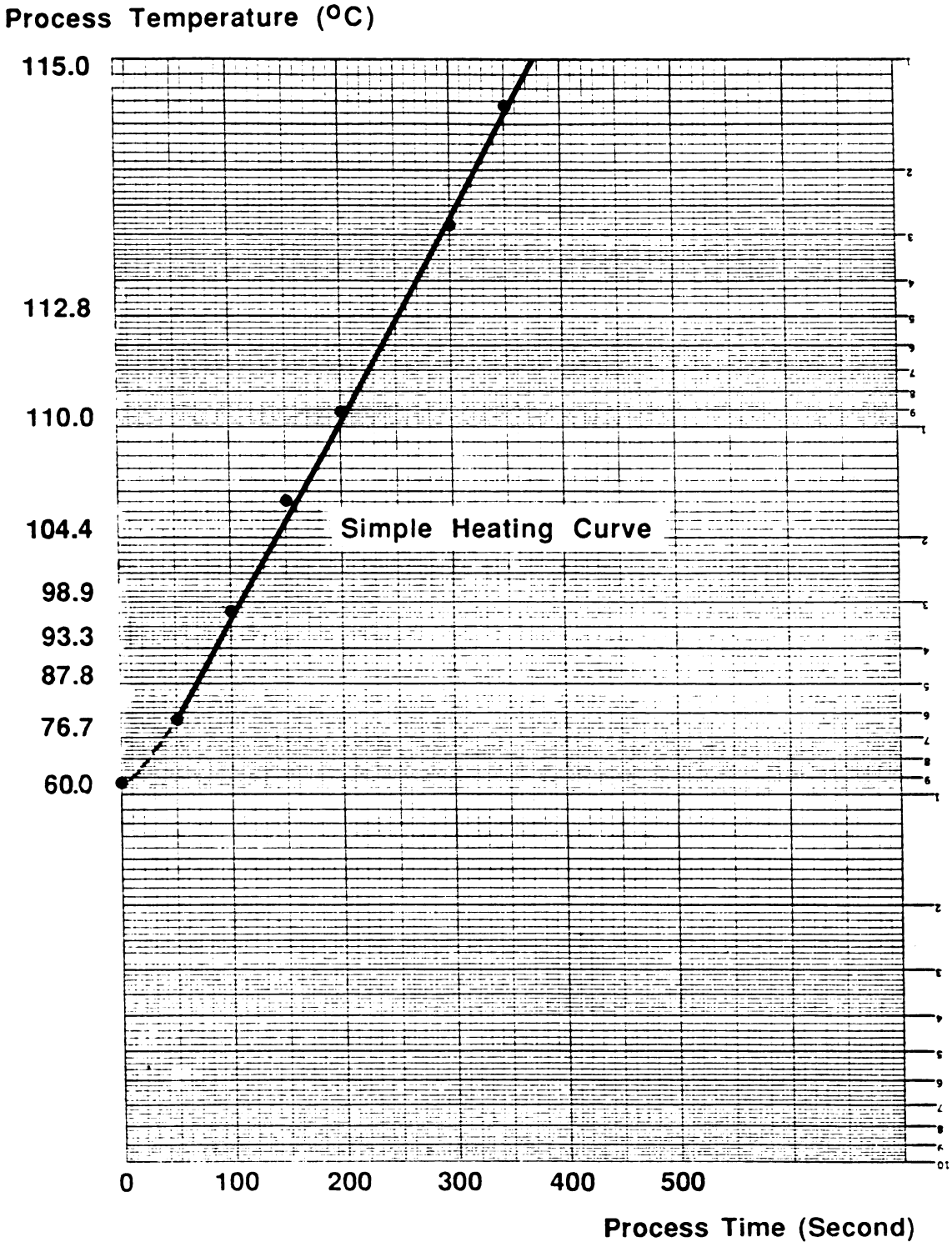

Figure 5. Heat Penetration Characteristic of Navy Beans in Brine Packed in 303 x 406 can and Processed at 115.6°C for 45 min; Product Temperature Measured at Slowest Heating Spot (4.2 cm from the base of can along the horizontal center)

300 and 202 x 214 can required to achieve equivalent F_o (Table 8). The summary of bean thermal processing procedures for the three selected can sizes are presented in Table 9.

B. Direct: Scale-down 202 x 214 Method Testing

The developed method using 202 x 214 can size and 28.5 g dry bean solids was selected for use in screening early generation bean breeding lines for their canning quality because it required a smaller sample size for testing than a 211 x 300 can. Various bean cultivars from 1987 and 1988 crops were canned using the conventional canning process (303 x 406 can, 100 g dry bean solids) and the developed process (202 x 214, 28.5 g dry bean solids).

Three important quality factors of canned beans that processors and consumers usually judge are drained weight (processor yield), color, and texture (Nordstrom and Sistrunk, 1977, Junek et al., 1980 and Hosfield et al., 1984). Therefore, these canning quality characteristics were determined on both can sizes and the respective data are presented in Tables 10 and 11. Drained weight is an indication of total hydration capacity of beans. However, it is difficult to directly compare drained weights of beans obtained from two different can sizes, thus, the drained weight ratio (drained weight/soaked weight) was calculated and used for comparative purposes. The drained weight ratios and canned bean textures (compression and shear) of all bean cultivars and breeding lines processed in both can sizes were similar. However, there were differences in canned bean color L and a_L among some cultivars and breeding lines. Hunter color L values of 1987 crop: Wesland and Midland and 1988 crop: C-20, Fleetwood, Rocket and Stinger processed in 202 x 214 cans were significantly higher ($P<0.05$) than when processed in 303 x 406 cans. In addition, Seafarer, Fleetwood, Wesland, Midland, N 85006 and N 84032 of 1987 crop and C-20, Seafarer, Fleetwood, Bunsi, Rocket and Stinger of 1988 crop processed in 202 x 214 cans exhibited significantly lower a_L value (less intensity of red color) than when processed in 303 x 406 cans. These slight differences in Hunter color L and a_L values may be due to variation in the dry bean seeds themselves.

Table 8. Equivalent Sterilizing Value (Fo) and Process Time at 115.6°C Required to Process Dry Navy Beans in 303 x 406, 211 x 300 and 202 x 214 Cans

	Process Time (min)		
Fo	303 x 406	211 x 300	202 x 214
11.6 ± 0.1	45.0 ± 0.0	44.5 ± 0.3	42.5 ± 0.2

Table 9. Sumarry of Thermal Processing Procedure for Canning Dry Navy Beans Using 303 x 406, 211 x 300 and 202 x 214 Cans

	303 x 406	211 x 300	202 x 214
Dry beans (g dry solids)	100	48.0	28.5
MSU Soak/Blanch Method	<------- 30 min @ 25°C / 30 min @ 87.8°C ------->		
Fill and Brine	<---------- Headspace 2/16" Pack Ratio 7.2 ---------->		
Retort (min @ 115.6oC)	45	44.5	42.5

Table 10. Quality Comparison of Various Bean Cultivars and Breeding Lines (1987 Crop) Canned in 303 x 406 and 202 x 214 Cans[1]

Bean Cultivar	Can Size	Drained Wt. Ratio	Canned Bean Texture		Canned Bean Hunter Color		
			Compression	Shear	L	a	b
Cultivar							
C-20	202 x 214	1.4 ± 0.0a	27.3 ± 0.0a	28.6 ± 0.0a	53.9 ± 0.1a	2.8 ± 0.1a	14.6 ± 0.5a
	303 x 406	1.4 ± 0.0a	28.1 ± 0.8a	29.3 ± 1.0a	52.8 ± 0.1a	3.7 ± 0.4a	14.1 ± 1.1a
Seafarer	202 x 214	1.4 ± 0.0a	27.3 ± 0.0a	28.6 ± 0.0a	52.8 ± 0.9a	2.5 ± 0.1a	14.1 ± 0.4a
	303 x 406	1.4 ± 0.0a	28.0 ± 0.9a	28.9 ± 0.4a	52.2 ± 0.7a	3.7 ± 0.3b	14.0 ± 0.5a
Fleetwood	202 x 214	1.3 ± 0.0a	27.3 ± 1.9a	41.6 ± 2.9a	52.5 ± 0.1a	3.1 ± 0.4a	14.6 ± 0.1a
	303 x 406	1.4 ± 0.0a	26.6 ± 1.0a	41.5 ± 0.8a	52.9 ± 0.2a	4.4 ± 0.0b	14.9 ± 0.1a
84004	202 x 214	1.4 ± 0.0a	19.1 ± 0.0a	21.8 ± 0.0a	53.3 ± 0.7a	2.9 ± 0.3a	14.0 ± 0.2a
	303 x 406	1.4 ± 0.0b	19.1 ± 2.0a	22.5 ± 1.0a	53.3 ± 0.4a	3.7 ± 0.1a	13.5 ± 0.0a
Bunsi	202 x 214	1.3 ± 0.1a	35.5 ± 1.9a	53.2 ± 1.9a	52.8 ± 0.8a	3.3 ± 0.4a	15.5 ± 0.1a
	303 x 406	1.4 ± 0.4a	34.8 ± 1.0a	55.4 ± 0.7a	52.2 ± 0.0a	4.0 ± 0.0a	15.6 ± 0.0a
Wesland	202 x 214	1.3 ± 0.1a	41.6 ± 1.0a	56.9 ± 0.6a	51.8 ± 0.1b	3.6 ± 0.1a	15.6 ± 0.3a
	303 x 406	1.3 ± 0.1a	42.7 ± 1.2a	59.3 ± 1.0a	49.9 ± 0.0a	5.1 ± 0.4b	15.7 ± 0.1a
Midland	202 x 214	1.3 ± 0.0a	42.3 ± 0.0a	63.7 ± 0.6a	52.5 ± 0.0b	3.3 ± 0.4a	15.5 ± 0.4a
	303 x 406	1.3 ± 0.0a	40.9 ± 2.0a	63.2 ± 0.7a	50.2 ± 0.3a	4.6 ± 0.1b	15.4 ± 0.0a
C-15	202 x 214	1.3 ± 0.1a	30.0 ± 2.0a	35.5 ± 1.9a	52.2 ± 0.7a	4.6 ± 0.5a	15.2 ± 0.3a
	303 x 406	1.3 ± 0.0a	30.6 ± 0.8a	35.5 ± 0.0a	51.7 ± 0.3a	5.9 ± 0.2a	15.9 ± 0.1a

(Cont'd ...)

Table 10. (Cont'd)

Breeding Line							
N84024	202 x 214	1.2 ± 0.0a	34.8 ± 1.0a	42.3 ± 0.0a	52.0 ± 0.5a	3.9 ± 0.2a	15.2 ± 0.1a
	303 x 406	1.3 ± 0.1a	35.5 ± 0.0a	41.3 ± 0.5a	50.9 ± 0.4a	4.5 ± 0.1a	15.1 ± 0.3a
N85006	202 x 214	1.2 ± 0.1a	35.4 ± 1.2a	48.4 ± 1.0a	52.0 ± 0.3a	4.5 ± 0.1a	15.0 ± 0.4a
	303 x 406	1.3 ± 0.1a	38.9 ± 0.9a	49.1 ± 2.0a	51.8 ± 0.4a	5.0 ± 0.0b	15.5 ± 0.1a
N85007	202 x 214	1.2 ± 0.0a	38.9 ± 0.9a	51.2 ± 2.9a	53.0 ± 0.9a	3.8 ± 0.4a	15.2 ± 0.4a
	303 x 406	1.3 ± 0.1a	40.2 ± 1.0a	53.3 ± 1.8a	52.6 ± 0.2a	4.6 ± 0.2a	15.3 ± 0.1a
N84032	202 x 214	1.2 ± 0.1a	41.6 ± 1.0a	48.4 ± 1.0a	52.8 ± 0.6a	4.2 ± 0.0a	15.8 ± 0.4a
	303 x 406	1.2 ± 0.0a	42.3 ± 0.0a	50.7 ± 1.6a	51.3 ± 0.1a	5.4 ± 0.1b	16.1 ± 0.1a

[1] n=2, Means in a colum followed by different letters are significantly different ($P<0.05$).

Table 11. Quality Comparison of Various Bean Cultivars and Breeding Lines (1988 Crop) Canned in 303 x 406 and 202 x 214 Cans[1]

Bean Cultivar	Can Size	Drained Wt. Ratio	Canned Bean Texture		Canned Bean Hunter Color		
			Compression	Shear	L	a	b
C-20	202 x 214	1.1 ± 0.0a	27.1 ± 0.2a	28.3 ± 1.0a	46.1 ± 0.0b	3.5 ± 0.4a	14.6 ± 0.3a
	303 x 406	1.1 ± 0.0a	28.8 ± 1.2a	31.0 ± 3.4a	45.5 ± 0.1a	4.5 ± 0.1b	15.3 ± 0.1a
Seafarer	202 x 214	1.2 ± 0.0a	27.3 ± 0.0a	33.8 ± 1.4a	48.4 ± 0.6a	3.5 ± 0.2a	15.6 ± 0.2a
	303 x 406	1.2 ± 0.0a	30.2 ± 0.3b	35.8 ± 0.5a	46.6 ± 0.2a	4.6 ± 0.1b	15.7 ± 0.0a
Fleetwood	202 x 214	1.2 ± 0.0a	22.9 ± 0.5a	40.7 ± 1.2a	48.1 ± 0.1b	3.7 ± 0.0a	15.9 ± 0.1a
	303 x 406	1.2 ± 0.0a	23.9 ± 1.0a	43.0 ± 1.0a	45.6 ± 0.0a	5.3 ± 0.1b	15.9 ± 0.2a
Bunsi	202 x 214	1.2 ± 0.0a	26.8 ± 0.7a	42.4 ± 1.6a	47.0 ± 2.5a	4.1 ± 0.1a	16.0 ± 0.1a
	303 x406	1.2 ± 0.0a	28.7 ± 0.9a	45.0 ± 1.4a	46.5 ± 0.1a	4.6 ± 0.1b	15.8 ± 0.1a
Mayflower	202 x 214	1.1 ± 0.0a	29.3 ± 1.0a	33.4 ± 1.0a	46.1 ± 2.5a	4.5 ± 0.0a	14.6 ± 0.6a
	303 x 406	1.2 ± 0.1a	29.7 ± 0.5a	35.5 ± 3.4a	45.5 ± 0.1a	4.8 ± 0.1a	15.1 ± 0.0a
Midland	202 x 214	1.2 ± 0.0a	30.7 ± 1.0a	43.8 ± 1.2a	46.4 ± 2.4a	4.3 ± 0.1b	15.3 ± 0.5a
	303 x 406	1.2 ± 0.0a	32.7 ± 1.9a	45.7 ± 1.9a	46.4 ± 0.0a	3.9 ± 0.0a	15.0 ± 0.0a
Albion	202 x 214	1.2 ± 0.0a	31.5 ± 0.7a	35.5 ± 1.5a	48.0 ± 2.0a	3.4 ± 0.1a	15.2 ± 0.8a
	303 x 406	1.2 ± 0.0b	33.4 ± 1.0a	38.5 ± 1.4a	46.0 ± 0.0a	3.7 ± 0.0a	15.0 ± 0.1a
Rocket	202 x 214	1.2 ± 0.0a	29.3 ± 1.9a	32.7 ± 0.0a	47.5 ± 0.1b	2.8 ± 0.1a	15.2 ± 0.4a
	303 x 406	1.2 ± 0.0b	29.7 ± 2.4a	33.0 ± 1.8a	44.5 ± 0.0a	4.6 ± 0.2b	15.0 ± 0.1a
Stinger	202 x 214	1.2 ± 0.0a	28.0 ± 1.0a	41.1 ± 0.2a	46.1 ± 0.0b	3.3 ± 0.0a	15.1 ± 0.1a
	303 x 406	1.2 ± 0.0a	28.8 ± 0.7a	44.3 ± 1.0b	45.4 ± 0.1a	4.2 ± 0.1b	15.5 ± 0.0a

[1] n=2,Means in colum followed by different letters are significantly different (P<0.05).

The canning quality of dry beans can be assessed by processing bean samples in 202 x 214 cans. Although this method permits the direct evaluation of canned beans, it requires approximately 32 g dry bean sample for each 202 x 214 can. The 202 x 214 can is appropriate to use for testing the quality of dry beans in early generations (e.g F_3) or wherever dry bean sample size is limited.

Study 2. In-direct: Pasting Characteristics of Whole Bean Flour

Although the canning of dry beans in 202 x 214 cans require 28.5 g bean solids (ca. 32 g sample of dry beans), this method is limited for use in early generations when seed supplies are often limiting. There is a need to develop an alternative quality testing method. Besides the processing conditions, processing performance of beans essentially depends on physical and chemical properties of both seed coat and cotyledon (Hosfield et al., 1984, Nordstrom and Sistrunk, 1979 and Srisuma et al., 1989). Physico-chemical properties can qualitatively and/or quantitatively influence the final product quality. Physical properties include size, shape and microstructure of bean seeds, whereas, chemical properties are mainly the composition and functionality of cell wall polysaccharides, starch, proteins, lipids and minerals (Uebersax and Ruengsakulrach, 1989). Regardless of their physical properties, the functionality of bean chemical constituents may be a useful determination in relation to specific processing quality. Dry beans commonly contain 55 - 60 % carbohydrates and 20 - 25 % protein. Given appropriate access to water and heat, the bean carbohydrates and proteins interact, resulting in certain changes in their viscosity or pasting characteristics that can be determined and subsequently related to physical bean processing quality.

In this experiment, the shear texture of canned beans and the corresponding pasting properties of whole bean flour were studied. Dry bean samples were processed in 303 x 406 cans and the shear texture of canned beans were determined as previously described in Study 1. Pasting properties of bean flour slurries (6%, w/w) were studied using the

Brookfield viscometer (Steffe et al., 1989). Shear texture and viscosity values at 94 ± 1°C, after a 23 min cooking cycle and at 20 ± 1°C, after 2 min cooking cycle are reported in Table 12. Significant differences ($P<0.05$) in torque values at both temperatures indicated similar textural quality trend as observed in the canned bean texture evaluation. These differences were mainly due to the quantitative and/or qualitative differences in bean chemical components. High correlations ($R^2>0.95$) were found between torque values (both heating and cooling) and shear texture value of canned beans. The following regression equations were derived from the data:

Shear force = -18.84 + 10.057 ln (Torque/heating) $R^2 = 0.984$

Shear force = -55.41 + 12.715 ln (Torque/cooling) $R^2 = 0.978$

Viscosity characteristics of whole bean flour in a dilute aqueous system (6%, w/w) dramatically changed with temperature during the Brookfield pasting test. These viscosity changes are primarily due to polymer-type molecules such as starch, protein and fiber in bean flour. The Brookfield viscometer may be used to predict the shear texture of canned beans due to the high correlation coefficient, $R^2 = 0.984$/heating and $R^2 = 0.978$/Cooling.

The advantages of this method include: it is rapid, simple and it requires a small amount of sample (12 mL of 6% whole bean flour slurry, w/w). However, the bean quality estimated by using whole bean flour is entirely based on the functional differences in bean chemical composition and in part, microstructure. Major physical characteristics of dry bean seed such as seed size and color, seed coat thickness, and etc. must be judged separately by the breeder.

Table 12. Canned Bean Shear Texture and Pasting Properties of Corresponding Whole Bean Flours[1]

Bean Cultivar	Texture Shear Force (kg/50g)	Torque x 10^7 (NM) After 23 min of Heating Temp. = 94 ± 1.0°C (T_H)	Torque x 10^7 (NM) After 2 min of Cooling Temp. = 20 ± 1.0°C (T_C)
C-20	29.3 ± 1.0b	111.30 ± 11.14b	888.15 ± 49.55c
Seafarer	28.9 ± 0.4b	104.85 ± 9.59b	689.73 ± 10.53b
Fleetwood	41.5 ± 0.8c	418.14 ± 32.12c	1970.15 ± 63.65d
84004	22.5 ± 1.0a	69.05 ± 9.52a	456.53 ± 42.86a

[1] n = 3, Means in a column followed by different letters are significantly different ($P<0.05$).

SUMMARY AND CONCLUSIONS

The thermal processing procedure of beans using 202 x 214 can (4.5 oz, approx. 28.5 g dry bean solids) has been developed. This method was proven to be appropriate to use in screening F_4 - F_6 bean breeding lines for canning quality. Pasting characteristics of whole bean flour by Brookfield viscometer may also be used to relate to bean canning quality. The advantages of this in-direct method are quick, simple and small amount of samples which are available in the F_3 generation. However, this method must be used in conjunction with other bean physical quality criteria in order to effectively screen breeding lines.

CHAPTER 2: INTERRELATIONSHIPS BETWEEN PHYSICO-CHEMICAL CHARACTERISTICS (SEED MICROSTRUCTURE, PROTEINS, AND MINERALS) AND CANNED PRODUCT QUALITY OF FOUR SELECTED NAVY BEAN CULTIVARS

INTRODUCTION

The physico-chemical characteristics of dry beans are influenced by the genetic background of the cultivars, growing environments, cultural practices, and post-harvest handling conditions. Variability in physico-chemical characteristics results in differential product performance and quality. Increased utilization of dry beans can result from a better understanding of how particular physico-chemical factors under controlled processing conditions govern product qualities, and the ability to modify the key factors and/or processing parameters to achieve a final product which meets the needs of processors and consumers. In addition, key physico-chemical characteristics can be used as criteria in screening breeding materials. Thus, the objective of this research was to identify and establish the inter-relationships of selected dry bean physico-chemical factors (seed microstructure, protein characteristics and mineral profile) contributing to product quality.

EXPERIMENTAL PLAN

Several studies have shown significant variability among bean cultivars and breeding lines for canning quality (Hosfield and Uebersax, 1980; Hosfield et al., 1984 and Ghaderi et al., 1984). In view of the genetic variability that exists in dry beans for canning quality, three cultivars and a breeding line that have been tested for several years were used as the genetic materials. The navy beans, "C-20", "Seafarer", "Fleetwood" and breeding line "MSU 84004" were obtained from the Cooperative Elevator Company, Pigeon, MI during the 1987 crop year. All beans were field-dried, harvested, sorted, packaged, and then transferred to Michigan State University. All dry bean samples were stored in a cooler maintained at 4^{o}C.

The null hypothesis (H_0) for the research was: canned bean quality is not related to its physico-chemical characteristics. The primary bean physical characteristics which may influence the canning quality are seed size, weight and microstructure (seed coat thickness; cell wall thickness; and starch granule size). Major chemical components including polysaccharides, proteins, lipids and minerals may also be important in determining final product quality.

Study 1 was designed to address the canning quality differences of the four cultivars. Study 2 was designed to study the seed physical properties (100 seed weight, density, hilum size, seed coat thickness, cell wall thickness and water absorption ability) and proximate chemical compositions of seed coat and cotyledon flours produced from these cultivars. Study 3 emphasized characterizing bean seed proteins and amino acid patterns. Study 4 determined mineral contents of the model cultivars. Inter-relationships between key canning quality and bean physico-chemical factors was established from the data from all four studies.

MATERIALS AND METHODS

In all studies, dry beans were randomly sampled from the designated storage materials and used directly as whole bean seeds or ground using the Udy Cyclone Mill (Udy Co., Fort Collins, CO) to pass through a 20 mesh screen and produce whole bean flour. In addition, dry beans were infiltrated for 30 minutes with 4°C deionized-distilled water to facilitate seed coat and cotyledon separation prior to manual decortication. The seed coats and cotyledon were frozen in liquid N_2 and subsequently freeze-dried and ground with a Udy Cyclone Mill to pass through a 20 mesh screen to yield seed coat and cotyledon flours. All research samples were kept in tightly capped glass bottles at 4°C throughout the studies to minimize physical and chemical changes. Specific requirements of sample preparation will be outlined individually in each study. All experiments and physico-chemical analyses were replicated three times, unless stated otherwise. Data were

subjected to an analysis of variance to determine significant differences among treatments. Differences between means for the traits were determined using the Tukey test criterion. Correlations were determined by the Least Squares Procedure.

Study 1. Canning Quality Characteristics

Thermal Processing Procedure

Moisture of dry bean samples was determined using a Montomco moisture meter (Motomco, Inc., Clark, NJ). Dry bean samples (equivalent to 100 g dry solids) were placed in nylon mesh bags and soaked in water for 30 minutes at room temperature (21°C). Immediately after this soaking, beans were transferred to an 88°C water bath for an additional 30 min. All soaking was done in distilled water containing 100 ppm calcium as $CaCl_2$. After hot soaking, beans were momentarily cooled under cold tap water, completely drained and weighed. After weighing, beans were filled into 303 x 406 cans and covered with boiling brine (142.0 g of sucrose and 113.4 g of NaCl in 9.1 kg of distilled water containing 100 ppm calcium). Cans were sealed and processed in a still retort for 45 minutes at 115.6°C. After thermal processing , cans were uniformly cooled to 38°C under cold tap water and stored for 2 weeks at room temperature before quality evaluation. The storage period after processing permits canned beans to completely equilibrate with the canning medium.

Canning Quality Evaluation

After the cans were opened, the washed-drained weight of processed beans was determined by decanting the can contents on a number 8 mesh sieve, rinsing them in cold tap water to remove adhering brine, and draining for 2 min on the sieve positioned at a 15° angle. Canned bean color coordinates (L, a_L and b_L) were determined using Hunter Lab Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Texture was determined by using a Kramer Shear Press fitted with a standard multiblade shear compression cell (Food Technology Corp., Reston, VA). A 50-g sample of the washed processed beans

was placed in the compression cell and force was applied until blades passed through the bean sample. The amount of force required, to extrude bean samples were recorded on a force-distance chart. The water content of canned beans (final moisture percentage) was determined from 50 g of the texture samples. These were oven dried at 80°C until the weight remained constant.

Study 2. Physical Characteristics and Proximate Composition of Dry Bean Seeds

Physical Characteristics

One-hundred Seed Weight and Density. One hundred seeds from each entry were randomly sampled and weighed on an analytical balance to obtain a 100-seed weight. The 100-seeds from each sample were placed into a graduate cylinder and subsequently filled with granular salt until completely packed to a known total volume. The salt particles were separated from the bean seeds using a 20 mesh screen, and measured for volume. One-hundred bean volume was calculated by subtracting salt volume from the total volume. Apparent seed density (g/mL) was determined as follows:

$$\text{Apparent seed density} = \frac{\text{100 Seed Weight (g)}}{\text{100 Seed Volume (mL)}}$$

Seed Coat Content. Twenty-five grams of raw beans was soaked in refrigerated (4°C) deionized-distilled water for 2 hr. The seeds were manually decorticated. The seed coats and cotyledons were dried in a vacuum oven at 80°C for 24 hrs, cooled in a desiccator, and then weighed to determine % seed coat (w/w) as follows:

$$\%\ \text{Seed coat} = \frac{\text{Seed Coat wt (g)}}{\text{Seed Coat wt. (g) + Cotyledon wt. (g)}} \times 100\%$$

Hilum Size, Seed Coat Thickness and Cotyledon Parenchyma Cell Wall Thickness. Dry bean seeds of each entry were randomly sampled from the storage lot. Seeds were quickly frozen with liquid N_2 and cracked with a razor blade while frozen, to obtain the appropriate sections to be observed under the Scanning Electron Microscope for hilum size, seed coat thickness and cotyledon cell wall thickness (30

replicate samples for each cultivar). After sectioning the samples, they were immediately freeze-dried in a Virtis Unitrap II model freeze-dryer (Virtis Co., Gardiner, N.Y.) for 12 hours, mounted fracture-side up on circular aluminum stubs with adhesive mounting tab and then coated with a 20 nm layer of gold by "Film Vac" Sputter Coater. The coated samples were examined under a Scanning Electron Microscope at an acceleration voltage of 15 kV (JEOL Model JSM 35CF, Center for Electron Optics, Michigan State University). Hilum size was measured for length and width in millimeters (mm). Seed coat thickness (μ) was measured to obtain three specific layer thickness identified as palisade, hour-glass and parenchyma layers (Figure 6). Total seed coat thickness (μ) was the sum of these three tissue layers. Parenchyma cell walls of the cotyledon tissue was also measured for its thickness (μ) (Figure 7).

Water Uptake Capacity. The water uptake of seeds was determined by soaking 50 g of bean samples in distilled water (1:8, bean and water ratio; w/v) at room temperature (ca 21°C) for 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 8.0, 16.0 and 24.0 hrs, followed by draining, blotting until dry and re-weighing. The increase in weight of soaked beans was taken as due to water absorption. Percent water uptake was calculated from the initial bean weight and weight after soaking.

Seed Coat and Cotyledon Proximate Composition

Moisture. Approximately 4 g of well-mixed seed coat and cotyledon flours were weighed on pre-weighed crucibles and dried to a constant weight at 80°C (ca. 8 hrs) in partial vacuum having pressure equivalent to 25 mm Hg. Percent moisture was determined from the weight loss on the fresh weight basis (AACC Method 44-40):

$$\% \text{ Moisture} = \frac{\text{Loss in Moisture (g)}}{\text{Initial Wt. of Sample (g)}} \times 100\%$$

Ash. Dried flour samples obtained from the above moisture determination were placed in a muffle furnace and incinerated at 525°C for 24 hr. Subsequently, the uniform

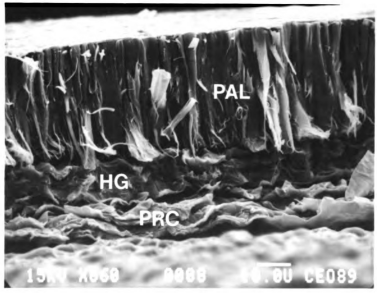

Figure 6. Structural Components of Dry Navy Bean Seed Coat: Cuticle (C) Layer, Palisade (PAL) Cells, Hourglass (HG) Cells and Parenchyma (PRC) Cells (SEM Cross-section)

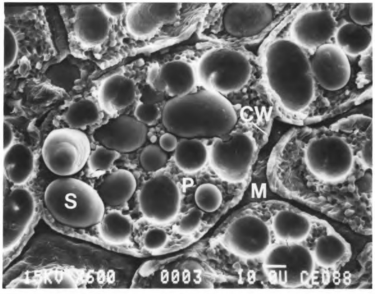

Figure 7. Structural Components of Dry Navy Bean Cotyledonary Cells: Cell Walls (CW), Middle Lamella (M), Protein Bodies (P) and Starch Granules (S) (SEM Cross-section)

grayish white ash was cooled in a desiccator and weighed at room temperature. Percentage of ash was reported on the dry weight basis (AACC Method 08-01):

$$\% \text{ Ash} = \frac{\text{Wt. of Residue (g)}}{\text{Dry Wt. of Sample (g)}} \times 100\%$$

Fat. Approximately 3 g of bean flour was dried in a vacuum oven at 80°C, and extracted with 60 mL petroleum ether in a Goldfish Extractor for 4 hr. The percent crude fat or ether extract was calculated on dry weight basis (AACC Method 30-25):

$$\% \text{ Crude fat or ether extract} = \frac{\text{Wt. of Fat (g)}}{\text{Dry Wt. of Sample (g)}} \times 100\%$$

Protein. The protein content was determined using AOAC method 24.038 (AOAC, 1984). Slight modifications were made as follows. Five milliliters of conc. H_2SO_4 and one catalyst tablet (3.5 g K_2SO_4 + 0.0035 g Selenium, Tecator, England) were added into each digestion tube containing pre-weighed sample (ca 140-150 mg). This tube was then slowly heated to 400°C until digestion was completed (approximately 5 hrs). The protein content was determined on a dry basis by multiplying the percentage nitrogen by a factor of 6.25.

$$\% \text{ Total protein} = \% \text{ Total Nitrogen} \times 6.25$$

Starch. The starch content was quantitatively determined using an enzymatic method. Approximately 150 mg of cotyledon bean flour was accurately weighed into a screw-capped test tube. Two milliliters (2 mL) of dimethylsulfoxide were added. The slurry was mixed thoroughly, then heated for 1 hr. in a boiling water bath to completely solubilize the starch. After heating the sample, it was cooled to approximately room temperature prior to adding 8 mL of 0.1 M acetate-20 mM $CaCl_2$ buffer, pH 4.5. Five milliliters of 5 mg/mL of amyloglucosidase (No. A-7255, Sigma Chemical Co., St. Louis, MO) in 0.1 M acetate containing 20 mM $CaCl_2$ were added, mixed and incubated in a shaking water bath maintained at 55°C for 24 hrs. Following this incubation step, the samples were filtered through Whatman filter paper #4 (Whatman Hilsboro, OR). The diluted filtrate (1:100 v/v) was analyzed for glucose content using a glucose diagnostic kit

(#510, Sigma Chemical Co.). The procedure is based upon the following coupled enzymatic reactions:

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconate} + 2H_2O_2$$

$$H_2O_2 + \underset{\text{(colorless)}}{\text{O - Dianisidine}} \xrightarrow{\text{Peroxidase}} \underset{\text{(Brown)}}{\text{Oxidized O- Dianisidine}}$$

The intensity of the brown color measured at 450 nm is proportional to the original glucose concentration. The total amount of glucose in samples was calculated from a standard curve prepared from known concentrations of glucose. The total starch content in each sample was obtained by multiplying the mg of glucose in each sample by a factor of 0.9 to account for the weight of the water gained during the hydrolysis of starch to glucose.

Total Carbohydrates. Total carbohydrate content was obtained by subtracting percentages (db) of ash, fat and protein from 100.

Study 3. Cotyledonary Protein Characteristics

Protein Isolation and Classification

The protein extraction and fractionation procedure was modified from the method described by Ursula and Lajolo (1981) and is schematically illustrated in Figure 8. Each solvent extraction was performed three times. Condition for centrifugation was 23, 000 g for 30 min.

One gram of cotyledon flour was thoroughly mixed with 10 mL pre-chilled deionized-distilled water (4°C) by vortexing for 1 min. while maintained at 4°C with water-ice bath followed by continuously agitating at 4°C for another 1 hr. Following each water extraction, the slurry sample was centrifuged to obtain a supernatant that was combined and then dialyzed at 4°C for 48 hrs (MW cut off = 3000) against deionized-distilled water. Because of precipitation inside the dialysis tube, precipitates were collected by centrifugation and then combined with the water extracted residue for the next solvent

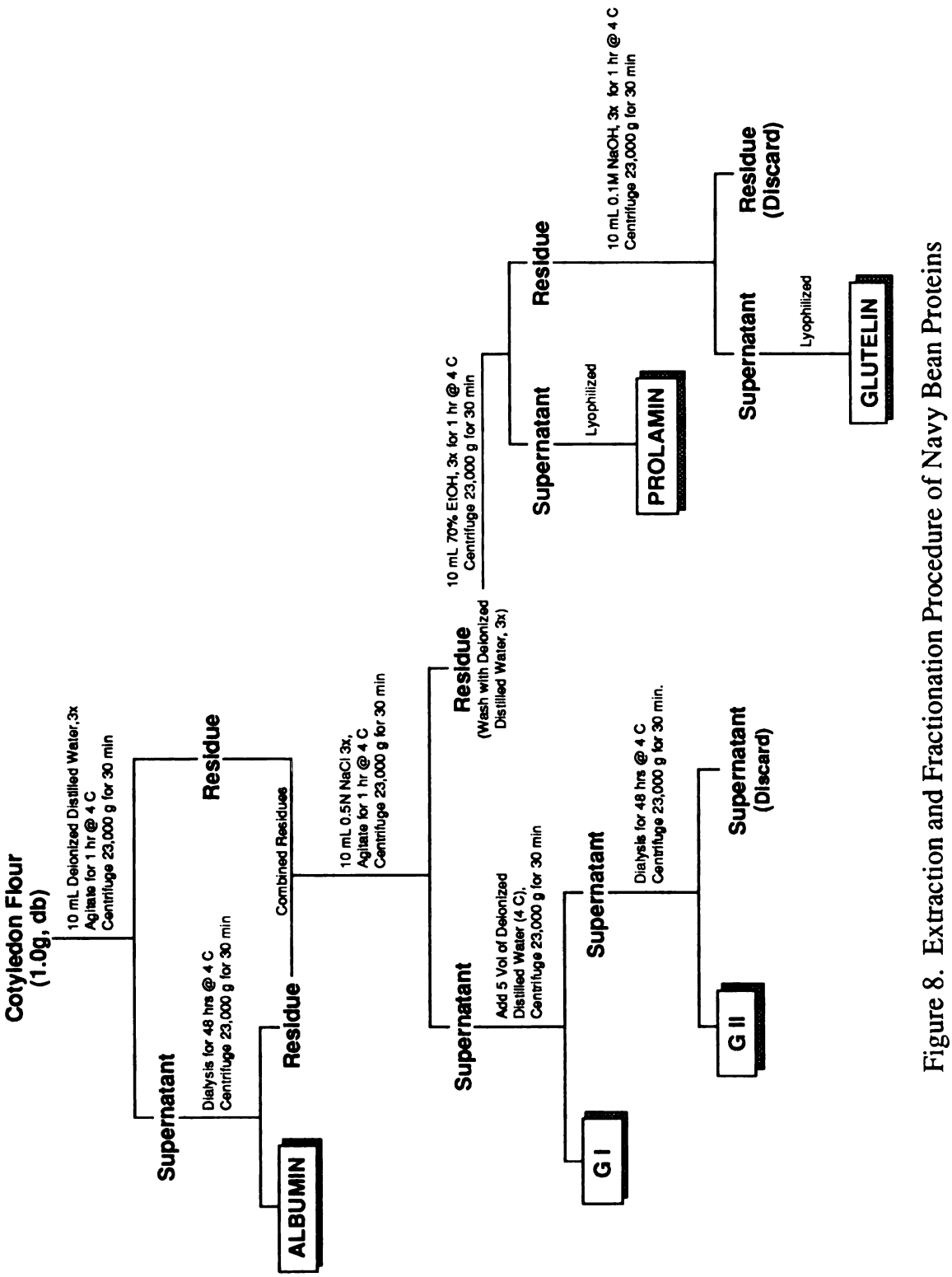

Figure 8. Extraction and Fractionation Procedure of Navy Bean Proteins

extraction. The resulting clear supernatant was then lyophilized to obtain the Albumin water soluble protein.

The combined residue was next extracted with 10 mL 0.5 N NaCl (pH 5.75) by vortex mixing for 1 min. and continuous agitation at 4°C for 1 hr. followed by centrifugation. The supernatants were combined and then five volumes of pre-chilled deionized-distilled water (4°C) was added to precipitate a high-salt soluble protein, Globulin I (GI). The GI was recovered by centrifugation and then lyophilized. The supernatant retaining low salt soluble protein, Globulin II (GII) was then dialyzed against deionized-distilled water at 4°C for 48 hrs to remove small molecular weight species, primarily salt. During dialysis, no precipitation inside the tube occurred. The clear dialyzed protein solution was freeze-dried to obtain GII.

Residue obtained from 0.5 N NaCl extraction step, was washed 3 times with deionized distilled water, prior to 70% EtOH extraction (10 mL) at 4°C for 1 hr followed by centrifugation. The resulting supernatant was lyophilized to obtain alcohol soluble protein, Prolamin.

The alcohol extracted residue was further extracted with 0.1 M NaOH at 4°C for 1 hr. The alkali extract collected by centrifugation was lyophilized to obtain alkali soluble protein, Glutelin. The alkali-extracted residue was washed with deionized-distilled water (3x) before lyophilization . The washed water was combined with the alkali extract.

Each obtained fraction; Albumin, GI, GII, Prolamin, Glutelin and final residue were analyzed for % N content using Kjeldahl. Nitrogen distribution was calculated based on total nitrogen in cotyledonary flour.

SDS Gel Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the bean protein fractions including Albumin, GI and GII was performed on 10% acrylamide running gels with 3% stacking gels using the system of Laemmli (1970). A protein solution of each bean protein was prepared to a final concentration of 10 mg Nitrogen

sample per mL of buffer, heated and mildly vortexed until completely solubilized. A 25 µl of each protein solution sample was applied into the sample well of stacking gel.

Electrophoresis was carried out with a Hoeffer Vertical Electrophoresis unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) using a constant voltage power supply (Fisher Biotech Electrophoresis System, Model FB 458, Pittsburg, PA). A constant current of 30 mA was applied until the proteins migrated into the running gel and then the current was increased to 60 mA until the bromophenol blue tracking dye reached the bottom of the running gel. The gels were removed and stained for 6 hrs. in 0.4% Coomassie Blue in 9/45/45 (v/v/v) acetic acid/methanol/water. The gels were destained using 7.5/25/67.5 (v/v/v) acetic acid/ methanol/water until clear.

Subunit molecular weights of studied proteins were estimated using a mixture of standard molecular weight protein markers (SDS-7 Dalton mark VII - L/Low Molecular Weight Markers and SDS 6H/High Molecular Weight Markers) purchased from Sigma Chemical Corp., St. Louis, Mo. The SDS-7 protein mixture consisted of the following proteins: α-lactalbumin (14.2 kilodalton), soybean trypsin inhibitor (20.1 kd), trypsinogen (24 kd), carbonic anhydrase (29 kd), glyceraldehyde-3-phosphate dehydrogenase (36 kd), egg albumin (45 kd), bovine albumin (66 kd). The SDS-6H protein mixture contained the following proteins: carbonic anhydrase (29 kd), Egg albumin (45 kd), Bovine albumin (66 kd), Phosphorylase B (97.4 kd), β-galactosidase (116 kd) and Myosin (205 kd). The standard protein solutions were prepared according to the method described in Sigma Technical Bulletin No. MWS-877C (Sigma Chemical Corp., St. Louis, MO). The relative mobility (RM) of the protein standards was calculated using the formula:

$$RM = \frac{\text{Distance of Protein Migration (cm)}}{\text{Marker Dye Distance (cm)}}$$

and a plot of relative mobility vs. molecular weight was constructed as a standard curve. The relative mobility of each protein subunit was calculated and the molecular weight was estimated from the standard curve. The protein bands present on the gels were qualitatively

compared using a Shimadzu Dual Wavelength Thin-Layer Chromato Scanner (Model cs-930, Kyoto, Japan). The protein bands were identified by their subunit molecular weights.

Amino Acid Analysis

Amino acid compositions of each major bean protein fraction including Albumin, G I and G II were determined. Isolated bean protein sample (1.0-1.2 mg protein) in a 6 x 50 mm tube was directly solubilized in 500 µl performic acid [formic acid (88%): hydrogen peroxide (30%), 9:1, v/v and held at 22°C for 1 hr]. Following 2 hrs. performic oxidation, this sample was then dried using Speed Vac Concentrators (Savant Instruments Inc., Farmingdale, NY).

Acid hydrolysis and amino acid analysis of the oxidized samples were performed at the Macromolecular Structure Facility in the Biochemistry Department at Michigan State University. Two hundreds µl of constant boiling HCl (Pierce Chemical Co., Rockford, IL) was added to the bottom of the vacuum vial which contained 12 samples per hydrolysis. Three alternate vacuum-nitrogen flushing steps are required to ensure the oxygen-free atmosphere. Vapor phase hydrolysis was progressed at 112 - 116°C for 24 hrs. Following hydrolysis, the samples were re-dried with ethanol: water:triethylamine (2:2:1, v/v), and derivatized. The derivatization reagent consists of a 7:1:1:1 solution of ethanol, triethylamine, water, and phenylisothiocyanate (PITC). After 10 min. derivatization, the samples were vacuum dried and re-dissolved in 500 µl of 5 mM sodium phosphate, pH 7.8. An injection volume of 40 µl was used. A Waters 600 HPLC system (Waters Associates, Milford, MA) was used for the analysis of the derivatized amino acids. Separation was accomplished using a PicoTag reverse phase column (Waters Associates) and a gradient mobile phase system [(A, 15 mM sodium acetate buffer pH 5.9 and 0.05% triethylamine; B, acetonitrile: H_2O (60:40)]. Ultraviolet absorbance at 254 nm was used for detection of the PITC-labelled amino acids.

Study 4. Mineral Content of Dry Bean Seeds

Seed coat and cotyledon flour samples of the four studied cultivars were analyzed for mineral content using an inductively coupled argon plasma (ICP) emission spectrometer (Jarrell-Ash Model 955 Atomcomp) equipped for simultaneous analysis of 19 elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Pb, Se, Tl and Zn) (The Department of Pharmacology and Toxicology, Michigan State University). Detailed sample preparation for mineral analysis are as follows:

All glassware and Tuff-containers were acid washed. All samples were brought to volume using water purified by a Millipore water purification system Class 1 (Millipore Corp., Bedford, MA).

Duplicate samples of freeze-dried bean seed coat and cotyledon flour were combined with 2 mL of concentrated Baker Instra-analyzed nitric acid in 15 mL screw-capped teflon vials (Tuff-Tainer, Pierce Chem. Co., Rockford, IL). The samples were incubated at 70-75^{o}C overnight, cooled and quantitatively transferred to 10 mL Class A volumetric flasks containing 1.0 ml of 100 ppm Yttrium (internal standard). They were then diluted to final volume (10 mL) with Millipore purified water. Along with bean samples, other procedural flasks containing no sample material and a sample of standard material (SRM) were prepared essentially by the same procedure and served as standard. All samples were then rapidly ashed by refluxing the sample in acid solution (HNO_3/H_2SO_4, 5:1, v/v) according to the method of Siemer and Brinkley (1981). All samples were analyzed for their mineral composition and mineral contents reported in ppm on a dry weight basis.

RESULTS AND DISCUSSION

Study 1. Canning Quality Characteristics

Canning characteristics of dry beans, especially navies, largely influence final product acceptability for both processors and consumers. Under identical process conditions, the four navy bean cultivars selected for study exhibited substantial differences in canning quality (Table 13).

Rapid water uptake is an important attribute to dry beans used for human consumption. Soaking dry beans before canning is considered as a necessary step to remove foreign material, facilitate cleaning of beans, aid in can filling, decrease cooking time, increase drained weight and ensure uniform bean expansion in the can during retort processing (Crafts, 1944; Cain, 1950; Hoff and Nelson, 1966; Nordstorm and Sistrunk, 1977 and Quart and da Silva, 1977). The soaking treatment used in this study was a 2-stage procedure that has been shown to maximize differences between genotypes for water uptake, cotyledonary hydration, and the degree of cotyledonary softening during retort processing (Hosfield and Uebersax, 1980). The initial soak was for 30 min in 21^{o}C water to facilitate seed-coat softening and expansion. The second soaking stage, the moderate heat treatment (88^{o}C) was employed to yield elevated rates of water uptake and shorter soak times to attain maximum imbibition. From Table 13, the relative order of soaked weights (g per 100 g of bean solids) ranked from high to low is: C-20 (229.7), the experimental line 84004 (228.4), Seafarer (226.1) and Fleetwood (224.4). Significant differences in soaked weights, shown in Table 13, indicates the variability in seed hydration capability during the soaking treatment. Seed coat characteristics have been suggested to be major factors in controlling seed hydration during soaking (Smith and Nash, 1961 and Quast and da Silva, 1977).

Dry bean physico-chemical constituents undergo various changes during high temperature and pressure cooking in the still retort. The major changes include pectin

Table 13. Canning Characteristics of Four Selected Navy Bean Cultivars[1]

Bean Cultivar	Soaked Weight (g)	Drained Weight (g)	Processed Bean Color			Texture (Kg/50g canned bean)		Dried Solids %, db
			L	a_L	b_L	Compression	Shear	
C-20	229.7 ± 1.2c	313.4 ± 3.9b	52.7 ± 0.0a	3.7 ± 0.4ab	14.1 ± 1.1a	28.1 ± 0.8b	29.3 ± 1.0b	30.9 ± 0.4b
Seafarer	226.1 ± 1.6ab	304.9 ± 4.5a	52.2 ± 0.7a	3.7 ± 0.3ab	14.0 ± 0.5a	28.0 ± 0.9b	28.9 ± 0.4b	30.4 ± 0.3b
Fleetwood	224.4 ± 0.4a	301.7 ± 1.5a	52.8 ± 0.2a	4.4 ± 0.0b	14.9 ± 0.1a	26.6 ± 1.0b	41.5 ± 0.8c	30.9 ± 0.1b
84004	228.4 ± 0.9bc	322.0 ± 0.4c	53.2 ± 0.3a	3.6 ± 0.0a	13.5 ± 0.0a	19.1 ± 1.9a	22.5 ± 1.0a	29.0 ± 0.3a

[1] n = 2, Means in a column followed by different letters are significantly different ($P<0.05$).

depolymerization, starch gelatinization, protein denaturation, cell wall degradation and cell separation. How these changes and other factors and mechanisms regulate canned bean water holding capacity are not entirely understood. After retort processing, cooked beans continue to increase in weight as they equilibrate with water in canning medium until reaching the final moisture of about 65 -70% depending on genotypes (Adams and Bedford, 1973). The processed yield of canned beans was determined by its washed drained weight. It is assumed that intact beans undergo little solid loss during thermal processing. However, excessive bean breakdown during thermal processing will result in the leaching of inter/intra cellular materials (cell wall components, starches etc.) and cotyledon loosened cells purged into canning medium and may lead to graininess of the brine and clumping of beans. Thus, a high water holding capacity of beans with an intact seed coat is one of the most desired product quality characteristics of processors. Differences in drained weight occurred among the cultivars studied (Table 13). The breeding line 84004 possessed higher ($P< 0.05$) drained weight: (322.0 g) as compared to C-20 (313.4 g), Seafarer (304.9 g) and Fleetwood (301.7 g).

Dry navy bean seeds possess a chalky white color and canning the beans in brine results in canned products which are darker in color as indicated by decreased Hunter color L (whiteness) and increase in a_L (redness) & b_L (yellowness) (Wang et al., 1988). Haard (1985) suggested that the Maillard browning reaction might be favored by heat during processing. Melanoidins are the brown products resulting from this non-enzymatic browning reaction and may contribute to the darker color of canned navy beans. In addition, this darker color of canned navy product may be due in part to caramelization of sugars. Minimum variations in color of canned navy beans among the studied cultivars are observed (Table 13). There are no significant differences ($P>0.05$) in L and b_L values among all cultivars. The only significant ($p<0.05$) difference in a_L color is found between Fleetwood (4.4) and 84004 (3.6).

Texture is a primary canning quality character because texture affects the perceived stimulus for chewing and, hence, influences to a large degree a consumer's acceptance of a food product. Textural properties of processed beans must fall within prescribed acceptability limits (Adams and Bedford, 1973). Beans may be unacceptable as they are too firm or too soft after cooking. The textural characteristics of studied canned beans were investigated by examining shear-press tracing of each cultivar (Figure 9). The texture values (Kg/50g processed bean) were reported in Table 13 as compression force and shear force. The curve shapes observed showed that Fleetwood has different textural characteristics from the other three cultivars. Hosfield and Uebersax (1980) examined shear-compression tracings of various bean genotypes. They observed that the textural differences exist among genotypes which can be classified into Type A and Type B (Figure 10). Type A showed a large contribution due to shear force involved in the extrusion of beans between the slots in the sample cut as the head descended and a curve Type B which was characterized by a predominant component due to compression. Based on the work of Hosfield and Uebersax (1980), Fleetwood exhibited Type A curve configuration, whereas C-20, Seafarer and experimental line 84004 demonstrated Type B curve configuration. Within the Type B beans, experimental line 84004 exhibited significantly ($p<0.05$) lower compression force (19.1 Kg/50 g canned bean) and shear force (22.5 Kg/50g canned bean) than C-20 (28.1 and 29.3 Kg/50 g canned bean) and Seafarer (28.0 and 28.9 Kg/50 g canned bean) which were similar. Compared to C-20 and Seafarer, Fleetwood had similar ($P>0.05$) compression force (26.6 Kg/50 g canned bean) but significantly ($P<0.05$) higher shear force (41.5 Kg/50 g canned bean). Curves with large shear force components (Type A) result when a differential component within a product causes an excessive pressure requirement in order to bring the product to a yield point prior to extrusion. Compression type curves (Type B) result when a product is uniformly extruded through the cell compartment. Hosfield and Uebersax (1980) suggested that the Type A curve may have resulted from the highly cohesive bean seed coat that kept individual beans from rupturing

Figure 9. Kramer Texture Curves Characterizing Compression Peaks and Shear Peaks of Selected Navy Bean Samples

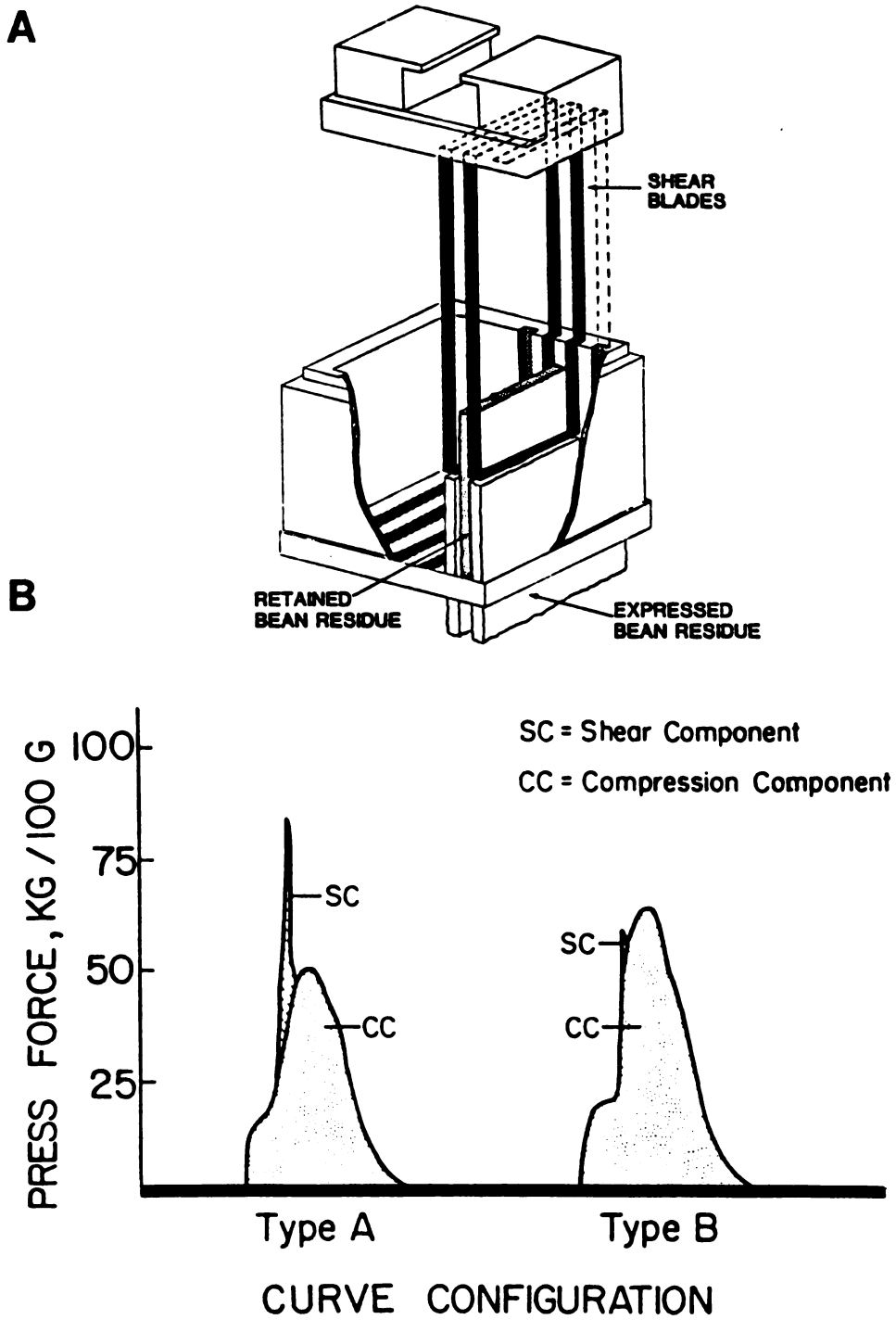

Figure 10. Kramer Compression and Shear Cell (A) and General Curve Configurations (B) Showing Dominant Compression and Shear Textural Characteristics of Canned Bean Products

until a catastrophic failure to shear action resulted. Physico-chemical factors and their mechanisms affecting texture properties have not been clearly established. Canned bean firmness is the result of mechanical deformation of tissues and individual cells and that the force required depends on individual cell strength and the cohesiveness among cells within various tissues.

The amount of bean solids (% w/w) is another quality term used for determining water holding capacity of canned beans. Theoretically, % bean solids and drained weight of canned beans demonstrate an inverse relationship. An analysis of these data (Table 13) showed that % dried solids correlated fairly well with drained weight ($R^2 = 0.601$). The breeding line 84004 exhibited the lowest % dried solids ($P<0.05$) or had the highest water holding capacity of cooked beans of the four beans studied.

The relationship among canning quality characteristics of these four bean cultivars were previously discussed and reported by Srisuma (1989). The relationships are:

Soaked wt.	=	-402.32 + 3.318 (Drained wt.)	$R^2 = 0.65$
Soaked wt.	=	233.85 - 0.220 (Shear value)	$R^2 = 0.55$
Drained wt.	=	340.09 - 0.971 (Shear value)	$R^2 = 0.71$

Study 2. Physical Characteristics and Proximate Composition of Dry Bean Seeds

Physical Characteristics

Seed size of the selected navy bean cultivars was measured and quantitatively reported in Table 14 as: weight in gram of 100 bean seeds (100 seed weight) and volume in mL of 100 bean seed (100 seed volume). The 100 seed weight of Seafarer (24.67 g) was significantly greater ($P<0.05$) than that of C-20 (23.33 g), Fleetwood (22.17 g) and breeding line 84004 (21.83 g). Hundred seed volumes of the studied cultivars which ranged from 15.33 mL (Fleetwood) to 16.67 mL (Seafarer and breeding line 84004), however, are not significantly different ($P>0.05$).

Table 14. Physical Measurements of Four Selected Navy Bean Cultivars[1]

Bean Cultivar	100 Seed Wt. (g)	100 Seed Vol. (mL)	Apparent Density (g/mL)	Seed Coat (% db)
C-20	23.33 ± 0.58b	16.00 ± 0.00a	1.46 ± 0.04b	6.44 ± 0.02a
Seafarer	24.67 ± 0.58c	16.67 ± 1.53a	1.49 ± 0.17b	6.69 ± 0.13a
Fleetwood	22.17 ± 0.76a	15.33 ± 1.53a	1.45 ± 0.09b	7.72 ± 0.36b
84004	21.83 ± 0.29a	16.67 ± 0.58a	1.31 ± 0.05a	6.56 ± 0.08a

[1]n= 3, Means in a column followed by different letters are significantly different (P<0.05).

From the data of 100 seed weight (g) and 100 seed volume (mL), apparent density (g/mL) of seed was calculated to express another quality of dry bean seeds. As shown in Table 14, the breeding line 84004 possessed the least seed density (1.31 g/mL) as compared to the remaining studied cultivars which were similar (1.45 - 1.49 g/mL). These data suggest that the breeding line 84004 seeds may have more intercellular spaces and/or be packed with lesser amounts of the more dense compositional components (i.e. starch). From scanning electron micrographs of cotyledon tissues (Figure 11), the breeding line 84004 did not exhibit any outstanding high intercellular spaces within the cotyledon tissue as compared to the other bean cultivars.

Based on the seed size data (100 seed weight and volume) of the four studied navy bean cultivars, seed size seems to be a poor quality indicator for canned bean products (Table 13). These findings support the previous reports of Hosfield and Uebersax (1980) for *Phaseolus* bean and Bhatty (1984) for lentil. Seed apparent density, however, may be a better means of assessing differentials among seeds. Since seed density accounts not only for seed physical properties but also its chemical composition. Apparent density of studied seeds shows potential correlation with compression force of canned beans.

$$\underset{\text{(g/mL)}}{\text{Apparent Density}} = -52.98 + 55.10 \underset{\text{(Kg/50g canned bean)}}{\text{Compression Force}} \qquad R^2 = 0.98$$

Based on the results of proximate analysis of whole bean flours prepared from the same lot of bean samples (Srisuma, 1989), it is tempting to hypothesize that high density seed is high in starch but low in fat content. Simple correlation with $R^2 = 0.91$ was found between seed apparent density and starch/fat ratio (Appendix A). Further study is needed to develop a better understanding of this relationship.

Seed coats play a significant role in the relationship between structure and quality of legumes (Swanson et al., 1985). Seed coat of studied bean cultivars constituted 6.44% to 7.72% (w/w) of total seed weight (Table 14). Fleetwood cultivar possessed the highest

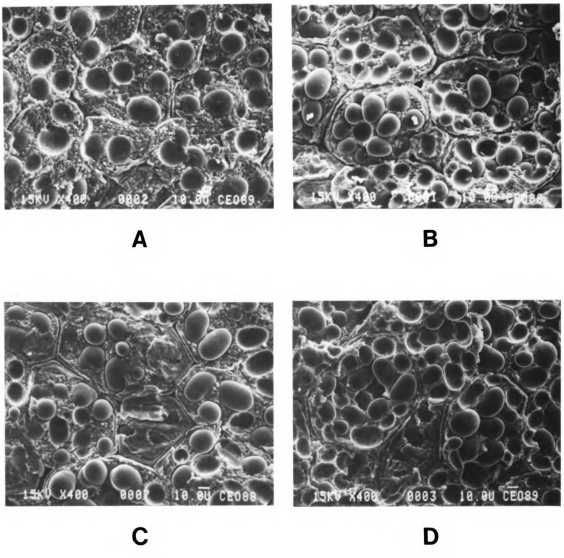

Figure 11. SEM Cross-Sections of Dry Navy Bean Cotyledonary Cells:
A) C-20, B) Seafarer, C) Fleetwood and D) Experimental Line 84004

($P<0.05$) seed coat content (7.72%) while there are no differences ($P>0.05$) in seed coat among C-20 (6.44%), Seafarer (6.69%) and breeding line 84004 (6.56%). Although high seed coat content of Fleetwood may impart its exceptional high shear texture (Table 13), the differences in textural quality of C-20, Seafarer and breeding line 84004 can not be explained by their seed coat contents. Poor relationships between seed coat content and all studied canned bean qualities were obtained with an exception of percentage seed coat and soaked weight:

$$\text{Soaked weight} = 251.06 - 3.491\ (\%\ \text{seed coat}) \qquad R^2 = 0.77$$

Greater seed coat content was associated with lower soaked weight. This relationship confirms that seed coat is a limiting barrier in water imbibition of dry seeds as previously reported by many investigators (Powrie et al., 1960). The relatively poor correlation ($R^2 = 0.77$) may be contributed to the effect of structure and chemical composition of seed coat and cotyledon (Muller, 1967; Sefa-Dedeh and Stanley, 1979; Hsu et al., 1983; Swanson et al., 1985; Deshpande and Cheryan, 1986 and Agbo et al., 1987).

Scanning electron microscopy (SEM) is a valuable tool for studying foods and food products. SEM has been particularly utilized to study the seed microstructure in water imbibition by legumes (Powrie et al., 1960; Opik, 1966; McEwen et al., 1974; Rockland and Jones, 1974; Sefa-Dedeh and Stanley, 1979 a and b; Thorne, 1981; Swanson et al., 1985 and Agbo et al., 1987). The route that water imbibition follows is still controversial (Swanson et al., 1985). In dry beans (Phaseolus vulgaris), four structures have been proposed as possible sites of water entry: the hilum, the micropyle, the raphe and the seed coat. The primary site for water imbibition depends on the cultivars (Adams and Bedford, 1973). Powrie et al. (1960) indicated that for dry beans of the navy commercial class, water migrated through the seed coat and hydrated the cotyledons during soaking. In this study, we employed SEM as a tool to study anatomical structures of the hilum, micropyle, raphe, seed coat characteristics/thickness and cotyledon cell wall thickness.

The studied cultivars exhibited similarity in architecture of hilum, micropyle and raphe. The mean values of hilum size (width and length) of studied bean was presented in Table 15. Similarity in hilum size between Seafarer and Fleetwood; and between the C-20 and 84004 were noticed with the slightly smaller ($P<0.05$) size, in both width and length, of the later pair. The size of hilum cannot explain the differences in canned bean quality characteristics among the studied cultivars (Table 13).

The SEM photographs of the studied bean cultivars, sectioned transversely showed the characteristics structural cells of seed coats (Figure 6) and cotyledon parenchyma (Figure 7). The outermost portion of the legume seed coat is the waxy cuticle layer. The cuticle is composed of a cultivar membrane containing embedded wax, over which is deposited a layer of epicuticular wax. The cuticle is permeable to many polar and nonpolar compounds (Bukovac et al., 1981), but serves as the prime barrier to the penetration of water. Under the cuticle, three distinct cell layers are visible. The uppermost layer of cells are columnar in appearance and consist of palisade cells densely packed without spaces between cells. The mean thickness values of palisade cell layer were 31.5μ for Seafarer, 33.1μ for 84004, 34.3μ for C-20, and 36.0μ for Fleetwood (Table 15). Significant differences in palisade cell layer thickness were observed among cultivars. The layer immediately beneath the palisade layer is referred to as hourglass cells. Hourglass cells are somewhat roughly shaped like an hourglass with spaces between them (Corner, 1951). The average hourglass cell layer thickness (μ) was 10.6, 10.9, 12.4 and 12.5 for Fleetwood, C-20, 84004 and Seafarer, respectively (Table 15). The innermost layer consists of amorphous parenchyma cells. Seed coat parenchyma cell layer of C-20, Fleetwood and 84004 were similar ($P>0.05$), ranged from 11.9μ to 12.9μ (Table 15). Seafarer (14.1μ) had significantly thicker layer of their seed coat parenchyma cells. Total thickness of seed coat was determined by the summation of its three cell layers. The relative order of total seed coat thickness (Table 15) ranked from high to low are Fleetwood (59.4μ), Seafarer (58.1μ), 84004 (57.7μ) and C-20 (57.5μ). The thickness of three seed

Table 15. Hilum Size, Seed Coat Thickness and Cotyledon Cell Wall Thickness of Four Selected Navy Bean Cultivars[1]

Bean Cultivar	Hilum (mm)		Seed Coat Thickness (μ)				Cell Wall Thickness (μ)
	Length	Width	Palisade	Hour Glass	Parenchyma	Total Thickness	
C-20	1.6 ± 0.2a	0.9 ± 0.1a	34.3 ± 1.5c	10.9 ± 1.7a	12.4 ± 2.1a	57.5 ± 2.3a	2.7 ± 0.4b
Seafarer	1.9 ± 0.2c	1.1 ± 0.1b	31.5 ± 1.4a	12.5 ± 2.1b	14.1 ± 2.3b	58.1 ± 2.6ab	2.6 ± 0.4ab
Fleetwood	1.8 ± 0.2c	1.1 ± 0.1b	36.0 ± 1.9d	10.6 ± 1.1a	12.9 ± 2.6a	59.4 ± 3.4b	2.9 ± 0.4c
84004	1.7 ± 0.1b	0.9 ± 0.1a	33.1 ± 2.1b	12.4 ± 2.4b	11.9 ± 2.5a	57.7 ± 3.0a	2.4 ± 0.4a

[1]n= 30, Means in a column followed by different letters are significntly different (P<0.05).

coat cell layer was similar to those reported earlier by Swanson et al. (1985) for Sanilac, San Fernando and Nep-2 beans. Fleetwood's seed coat possessed thickest palisade cell layer, but thinnest hourglass cell layer. An opposite trend was observed from the Seafarer seed coat.

Cotyledon cell wall thickness (μ) of studied bean cultivars was also presented in Table 15. Fleetwood cultivar exhibited the thickest ($P<0.05$) cotyledon cell wall (2.9μ). Breeding line 84004 showed trend of having thinnest cotyledon cell wall (2.4μ), following with Seafarer (2.6μ) and C-20 (2.7μ).

Simple correlation coefficients were calculated between canned product quality (Table 13) and thickness of individual seed coat cell layers, total seed coat thickness and cotyledon cell wall thickness. The relationships with correlation coefficient greater than 0.75 are presented as follows:

Soaked weight (g)	=	376.21 - 2.56 TSCT (μ)	$R^2 = 0.86$
Shear force (Kg/50g canned bean)	=	-453.60 + 8.32 TSCT (μ)	$R^2 = 0.80$
Shear force (Kg/50g canned bean)	=	-66.69 + 36.69 CCWT (μ)	$R^2 = 0.93$

where: TSCT = Total Seed Coat Thickness
CCWT = Cotyledon Cell Wall Thickness

Thick seed coat (total) may impede seed hydration during soaking but not during high temperature/high pressure cooking.

Since a very poor correlation on ($R^2<0.05$) was found between seed coat thickness and drained weight. Greater thickness of total seed coat and cotyledon parenchyma cell wall may cause the high shear texture characteristics of canned bean. Low drained weight and/or high compression force may not associate with the thick cotyledon cell wall or seed coat ($R^2 < 0.60$).

The higher thickness of seed coat and particularly cotyledon cell wall in Fleetwood bean, therefore, may partly explain its high shear textural curve configuration, Type A (Figure 10).

Figure 12 illustrates the percent water uptake of dry beans seeds soaked at room temperature (21°C) for 24 hrs. At each point of measurement time, Fleetwood beans absorbed less amount of water than did other three cultivars, which exhibited similar water uptake pattern. The thickness of both seed coat and cotyledon cell wall may be involved in regulating the movement of water in to the seeds. Agbo et al. (1987) emphasized the importance of palisade cell layer thickness on the rate of seed water uptake of Sanilac, Nep-2 and San Fernando cultivars.

The data obtained from seed coat thickness, cotyledon cell wall thickness and water uptake of seeds in relation to canning quality (Table 13), it seems to provide some evidence for an association among them. In general, beans with a thick seed coat and thick cotyledon cell walls tends to absorb less water during soaking, but not during retort cooking, and exhibit higher shear texture characteristics.

Proximate Composition

Seed Coat. As shown in Table 16, non-starch carbohydrates were a major constituent (83.46% - 87.24%) present in bean seed coats. Protein is the second major constituent, ranging from 10.85% in Fleetwood to 6.99% in C-20. Significant differences in seed coat protein existed among the cultivars, with an exception between Seafarer (9.32%) and breeding line 84004 (9.36%).

Seed coat fat content, ranked from high to low, was Fleetwood (1.14%), Seafarer (1.09%), C-20 (0.36%) and 84004 (0.32%). The differences ($P<0.05$) in seed coat fat content may indicate the variability of seed coat wax content among the studied cultivars, which in turn results in variable water hydration property. High fat content of Fleetwood seed coat may contribute in-part to its low water absorption (Figure 12). However, such a restricted seed hydration effect due primary to high fat content of the seed coat was not

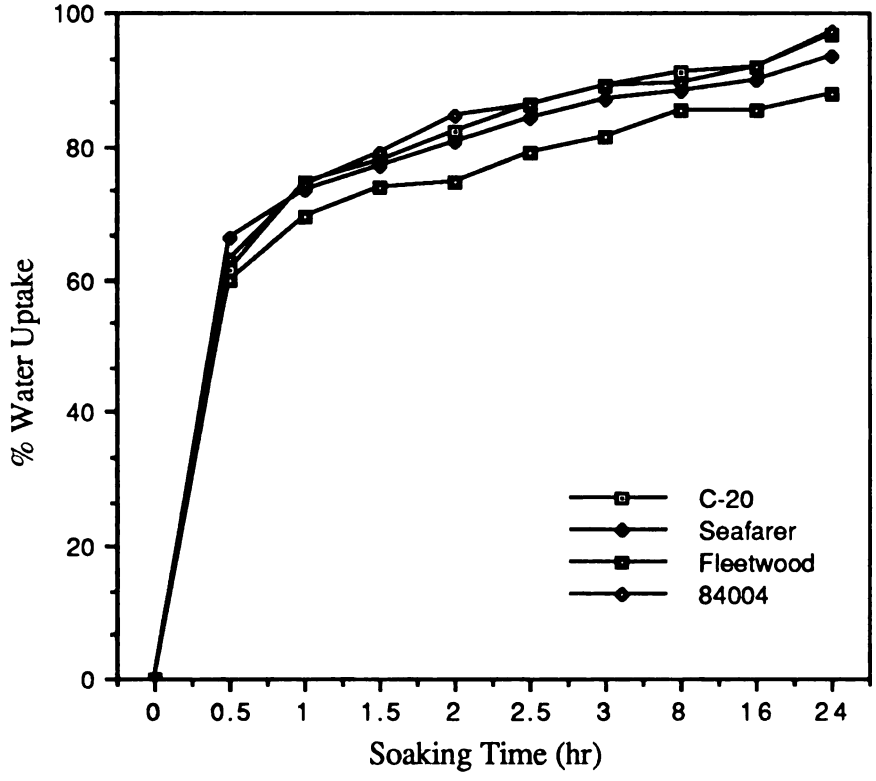

Figure 12. Water Uptake (%) of Studied Navy Bean Seeds Soaked in Deionized-Distilled Water at Room Temperature (25°C) for 24 hrs

Table 16. Proximate Analyses of Seed Coat and Cotyledon Tissues[1]

Bean Component	Bean Cultivar	Ash	Fat	Protein	Carbohydrates	
					Starch	Total[2]
Seed Coat	C-20	5.42 ± 0.08b	0.36 ± 0.01b	6.99 ± 0.05a	ND[3]	87.24 ± 0.11c
	Seafarer	5.88 ± 0.14d	1.09 ± 0.04c	9.32 ± 0.31b	ND	83.71 ± 0.28a
	Fleetwood	4.58 ± 0.06a	1.14 ± 0.02d	10.85 ± 0.27c	ND	83.46 ± 0.30a
	84004	5.64 ± 0.12c	0.32 ± 0.01a	9.36 ± 0.16b	ND	84.69 ± 0.08b
Cotyledon	C-20	3.92 ± 0.02b	1.39 ± 0.0b	30.02 ± 0.07c	42.95 ± 0.62a	64.66 ± 0.09a
	Seafarer	3.79 ± 0.03a	1.37 ± 0.01b	25.77 ± 0.12a	46.72 ± 1.86b	69.07 ± 0.12c
	Fleetwood	3.89 ± 0.05b	1.45 ± 0.05c	28.19 ± 0.04b	45.68 ± 1.73b	66.47 ± 0.13b
	84004	4.07 ± 0.05c	1.05 ± 0.01a	30.45 ± 0.16d	42.61 ± 0.91a	64.43 ± 0.20a

[1] n = 3, Means in a column followed by different letters are significantly different (P<0.05).
[2] % Total Carbohydrates = 100 - (% Ash + % Fat + % Protein).
[3] ND = Not Detectable (Negative Iodine Test).

demonstrated in Seafarer. These current findings further indicate that bean seed hydration is rather complex and may be regulated by multiple factors including structure, composition and post-harvest physiology of the seeds.

Fleetwood seed coat possessed less ($P<0.05$) mineral (ash) content (4.58%) than C-20 (5.42%), 84004 (5.64%) and Seafarer (5.88%). Although no relationship was observed between mineral content and canned bean quality characteristics (Table 13), the details on mineral composition, especially about Na, K, Ca, Mg, and P may further elucidate the minerals' role(s) on canned bean performances (see Study 4).

Cotyledon. As compared to seed coats, cotyledons possessed dissimilarity in major chemical composition. Cotyledons (storage tissues) contained high storage nutrients: starch (42.61% - 46.72%), protein (25.77% - 30.45%) and fat (1.05% - 1.45%), low structural (non-starch) carbohydrates which can be nearly estimated by subtracting starch content from total carbohydrate content (20.79% - 22.35%). Among all cultivars, 84004 (42.61%) and C-20 (42.95%) exhibited similar starch content, and both cultivars have significantly lower ($P<0.05$) starch content than Seafarer (46.72%) and Fleetwood (45.68%). Protein contents exhibited significant differences ($P<0.05$) among cultivars with the relative order of high to low as follows: 84004 (30.45%), C-20 (30.02%), Fleetwood (28.19%) and Seafarer (25.77%). Inverse relationship of high protein and low starch was found in all studied cultivars. Ash content ranged from 3.79% in Seafarer to 4.07% in 84004. There are significant differences ($P<0.05$) in fat content among cultivars with the exception of those between Seafarer (1.37%) and C-20 (1.39%). Cotyledonary fat content is highest ($P<0.05$) in Fleetwood (1.45%) and lowest in 84004 (1.05%).

Srisuma (1989) prepared purified cell walls from the cotyledons of the same lot of studied cultivars and reported results of 16.32%, 16.62% 17.08% and 18.02% for 84004, C-20, Seafarer and Fleetwood, respectively. She further fractionated cell wall components into 6 fractions: Hot water soluble polysaccharides (HWSP), Ammonium oxalate soluble polysaccharides, Hemicellulose A, Hemicellulose B, Cellulose and Lignin. The

outstanding differences of Fleetwood cotyledon cell walls from the other are the lowest in HWSP content and the gelling property of this fraction in 80% Ethanol. Srisuma (1989) also investigated the study on physico-chemical properties of isolated bean starches from the same selected cultivars. She suggested that the difference in starch content appeared to be more prominent on canned bean product quality especially drained weight than the minor variations in the starch characteristics.

Study 3. Cotyledon Protein Characteristics

Protein Isolation and Classification

Srisuma (1989) previously reported that among the same lot of bean samples, Fleetwood exhibited a significantly higher residual protein in dietary fiber (TDF) determined by AOAC method (1985). She suggested that there may be an interaction between cell wall constituents and residual protein which may play an important role in canned product shear texture. In this study, only cotyledon proteins were isolated and classified according to their solubility properties: water soluble protein (Albumin), salt soluble protein (Globulin), alcohol soluble protein (Prolamin), alkali soluble protein (Glutelin) and non-extractable protein. Globulins are further divided into two subgroups: Globulin I (GI) and Globulin II (GII) as proteins soluble in high and low salt concentrations, respectively.

Because of the natural salt presented in the cotyledon when distilled-deionized water was used as an initial solvent to extract Albumin, some Globulins were co-extracted. Therefore, the water extracted protein was subjected to dialysis against deionized distilled water to remove the small molecules of native salts and hence result in precipitation of contaminated Globulins within the dialysis tube. The precipitated Globulins were combined to the water extracted residue which then was extracted with 0.5N NaCl to obtain total Globulins. Pant and Tulsiani (1969), however, called the precipitate protein inside dialysis tube, Globulin A and the salt extracted protein from the water extracted residue,

Globulin B. During protein extractions, the Fleetwood sample behaved quite differently from the remaining samples. For example, Fleetwood cotyledon flour did not readily disperse in water because of a high degree of clumping. The GI precipitate of Fleetwood collected by centrifugation after dilution, possessed a gummy type appearance at the bottom of centrifuge bottle where as GI of the other cultivars did not.

Table 17 shows the nitrogen distribution of cotyledon protein isolated from the studied cultivars. Variations in the nitrogen profiles of cotyledon proteins, were observed among cultivars with an exception of Prolamin. The studied navy beans had Albumin content which ranged from 19.99% in breeding line 84004 to 26.28% in Seafarer. C-20 and Fleetwood exhibited similar ($P>0.05$) Albumin content (22.11% and 22.44%). The Albumin results reported here are slightly higher than those published by Ma and Bliss (11.1% - 19.8%; 1978) for various common beans, but they are much less than the result of Deshpande and Nielsen (37.3%, 1987) for navy bean. However, our results are in very good agreement with those reported by Sathe and Salunkhe (1981). Bhatty (1982) suggested that the discrepancies in the published data are most likely due to cross-contamination of the water and salt soluble proteins. Globulins, salt soluble proteins, are the major storage protein (53.18% - 60.09%) of studied cultivars. These results are in good agreement with many previous investigation (Pant and Tulsiani, 1969; Ma and Bliss, 1978; Sun and Hall, 1974 and Bhatty, 1982). Under the same fractionation conditions, all studied cultivars showed similar ($P>0.05$) percentages of G I (29.86% - 31.54%), but they showed greater variation in percentages of GII (22.88% - 30.04%). Fleetwood possessed higher ($P<0.05$) GII fraction than the other three cultivars. Prolamin is a minor protein fraction (0.56% - 0.69%) in these cultivars. There was no significant differences ($P>0.05$) in percentages of Prolamin among cultivars. C-20 yielded lowest in Glutelin fraction (2.53%), but highest in non-extractable protein (5.89%). Slight variations occurred among Seafarer, Fleetwood and 84004 in the yields of Glutelin (3.06% - 3.21%) and non-extractable protein (5.02% - 5.30%). Non-protein nitrogen (NPN) ranged from 9.38% in

Table 17. Nitrogen Distribution (%) of Studied Navy Bean Cultivars[1]

Bean Cultivar	Albumin	Globulin I	Globulin II	Prolamin	Glutelin	Residue	NPN	% Recovery N
C-20	22.11 ± 0.29b	29.86 ± 1.43a	25.55 ± 1.56b	0.69 ± 0.04a	2.53 ± 0.10a	5.89 ± 0.05c	12.56 ± 0.01c	99.17 ± 3.07ab
Seafarer	26.28 ± 0.47c	30.30 ± 1.35a	22.88 ± 1.10a	0.69 ± 0.04a	3.17 ± 0.07bc	5.02 ± 0.02a	12.36 ± 0.12c	100.65 ± 1.35b
Fleetwood	22.44 ± 0.47b	30.05 ± 1.08a	30.04 ± 1.78c	0.65 ± 0.08a	3.06 ± 0.04b	5.30 ± 0.02b	9.38 ± 0.30a	100.86 ± 0.59b
84004	19.99 ± 0.85a	31.54 ± 0.86a	24.69 ± 0.84ab	0.56 ± 0.06a	3.21 ± 0.08c	5.27 ± 0.01b	11.89 ± 0.21b	97.16 ± 0.78a

[1] n = 3, Means in a column followed by different letters are significantly difference ($P<0.05$).

Fleetwood to 12.56% of C-20. The percentages of prolamin, non-extractable protein and NPN are in conformity with the results of previous authors (Pant and Tulsiani, 1969). The nitrogen distribution of bean proteins does not show any clear relationships with the canned produce quality characteristics (Table 13). The high clumping characteristics of Fleetwood, when its flour dispersed in distilled-deionized water, may relate to its high GII percentage. Because Seafarer possessed lowest percentage of GII, it gave subjectively the least clumping sample when dispersed in distilled-deionized water. Further research is needed to establish their true relationships. Understanding the molecular size/structure and their amino acid composition may provide a better understanding of the protein characteristics and their functional roles in canned bean products.

SDS Slab Gel Electrophoresis

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was employed to study both qualitative and quantitative differences of 3 major proteins: Albumin, GI and GII prepared from the studied bean cotyledons. The SDS-PAGE patterns and their densitometer tracing curves of Albumin, GI and GII are shown in Figures 13-18.

SDS gel patterns of Albumins seem to display cultivar dependent characteristics. Albumin of all studied cultivars consisted of 4 similar major subunits: 68K, 34K, 28K and 16.6K. In general, C-20 and breeding 84004 exhibited similar gel pattern, with an exception of a 34.5K band shown only in 84004 Albumin. This missing subunit of C-20 Albumin are clearly illustrated in densitometer scans-Peak II (Figure 14) when compared to 84004 Albumin. The gel patterns of Seafarer and Fleetwood are essentially the same. The 28.8 K and 30.7K bands of C-20 and 84004 Albumins were absent in Seafarer and Fleetwood. These subunits were illustrated as peak group III on their densitometer scans (Figures 13 and 14). Among 4 cultivars, Fleetwood Albumin tends to have much less 42.3K, and 44.7K subunits shown as peak group I (Figures 13 and 14). The similarity in Albumin gel patterns, especially between C-20 and 84004, Seafarer and Fleetwood, may indicate their close genetic backgrounds.

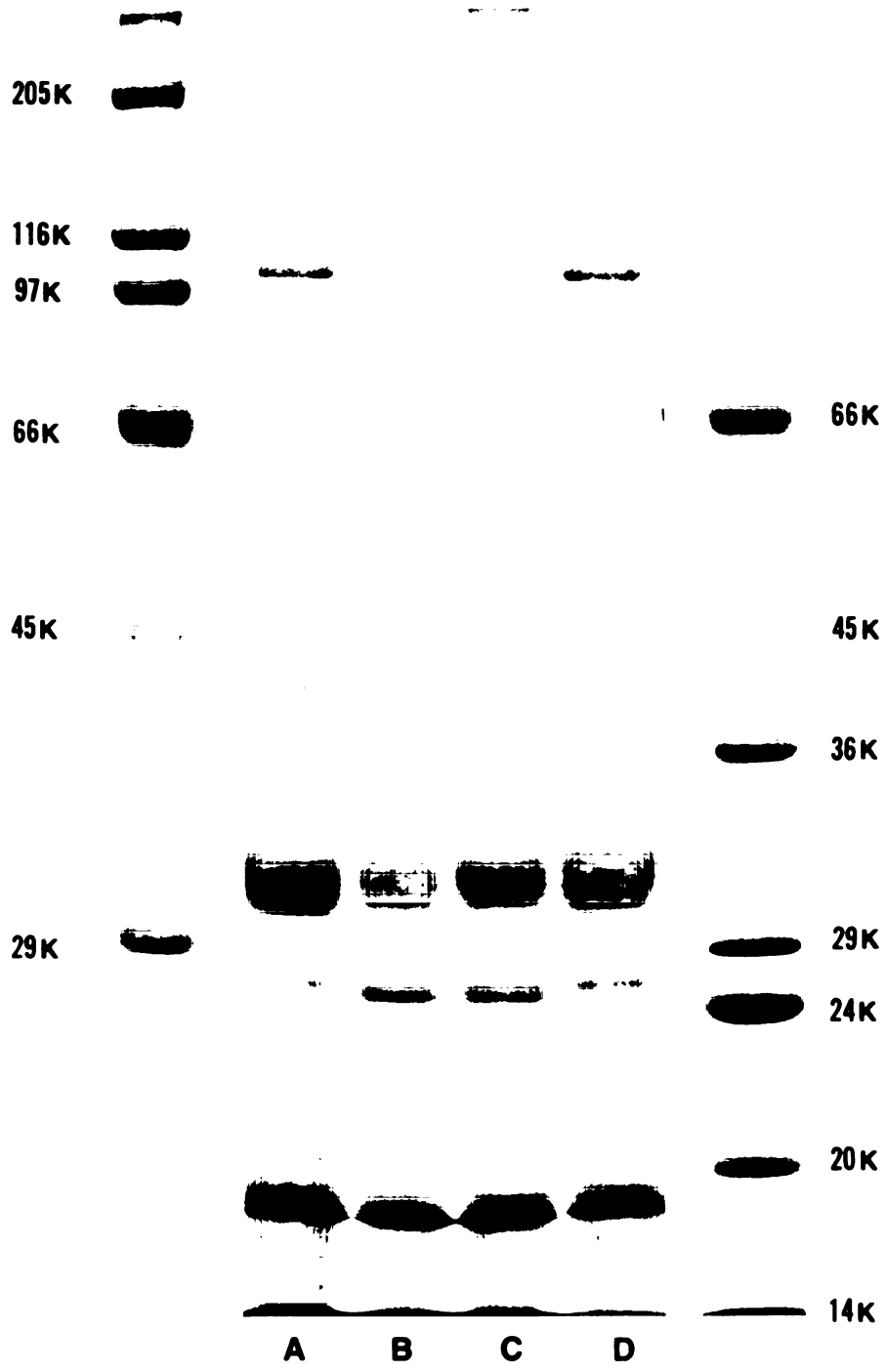

Figure 13. SDS-PAGE Patterns of Albumins Isolated from Dry Navy Beans: A) C-20, B) Seafarer, C) Fleetwood and D) Experimental Line 84004

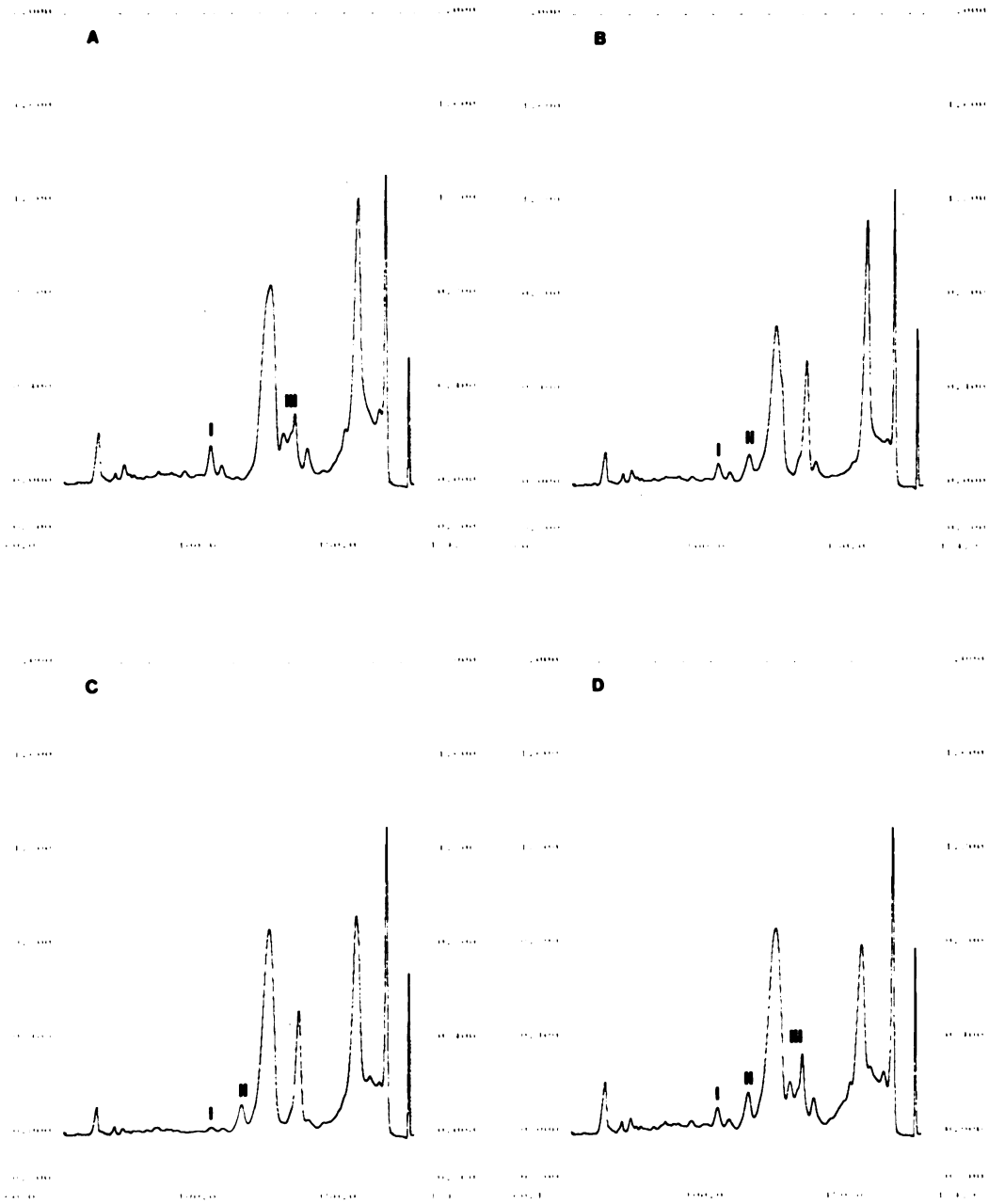

Figure 14. SDS-PAGE Densitometer Scan of Albumins Isolated from Dry Navy Beans: A) C-20, B) Seafarer, C) Fleetwood and D) Experimental Line 84004

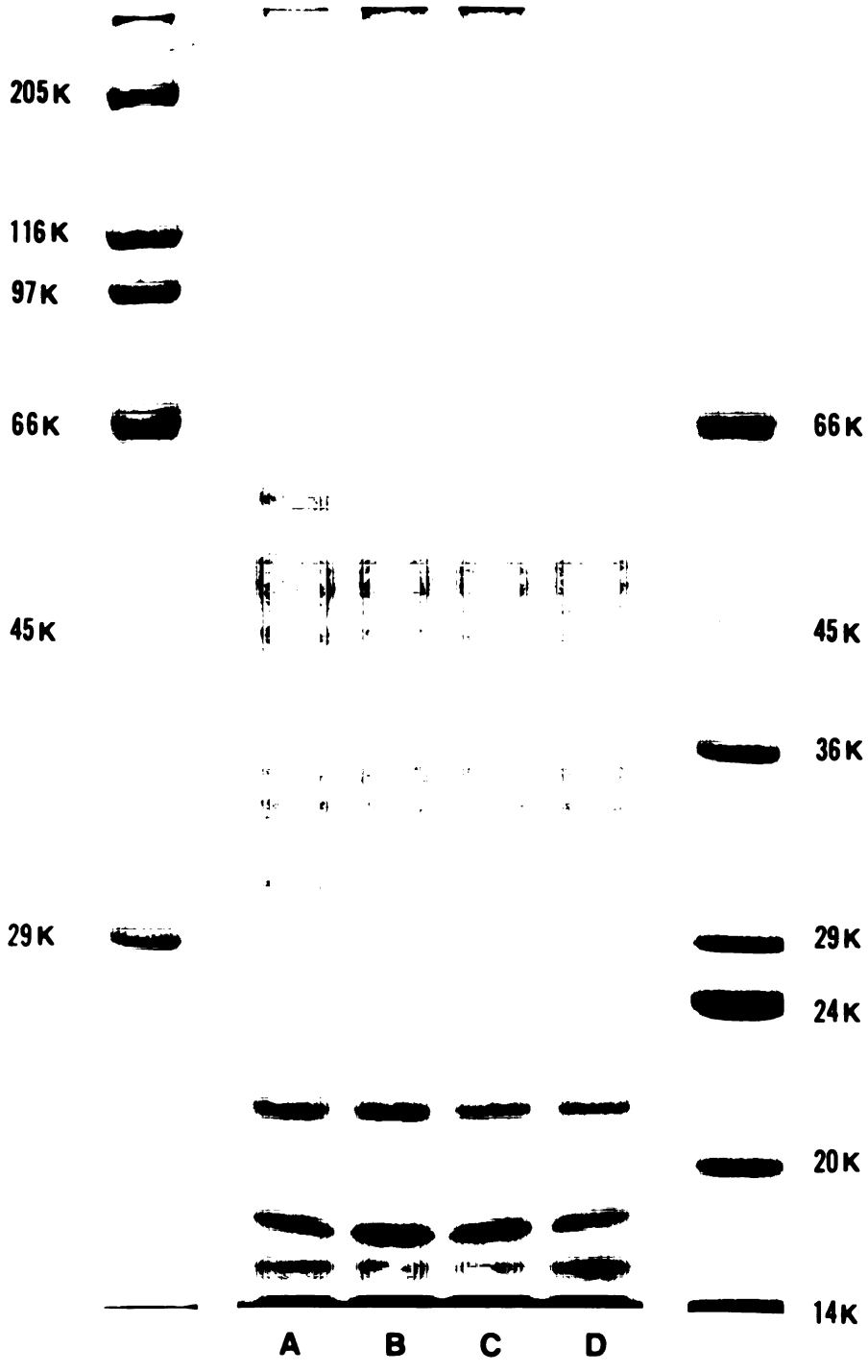

Figure 15. SDS-PAGE Patterns of Globulin I (GI) Isolated from Dry Navy Beans: A) C-20, B) Seafarer, C) Fleetwood and D) Experimental Line 84004

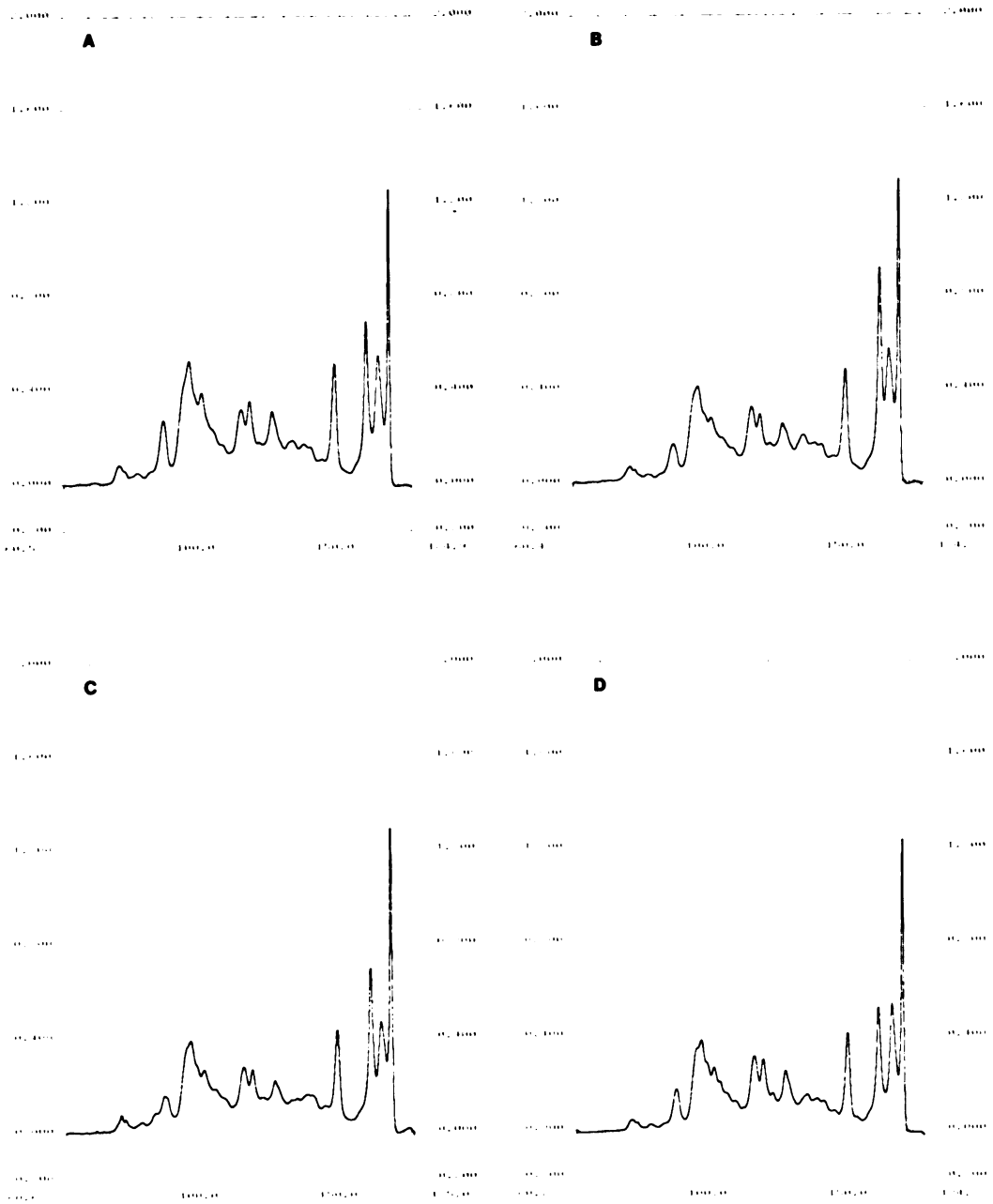

Figure 16. SDS-PAGE Densitometer Scan of Globulin I (GI) Isolated from Dry Navy Beans: A) C-20, B) Seafarer, C) Fleetwood and D) Experimental Line 84004

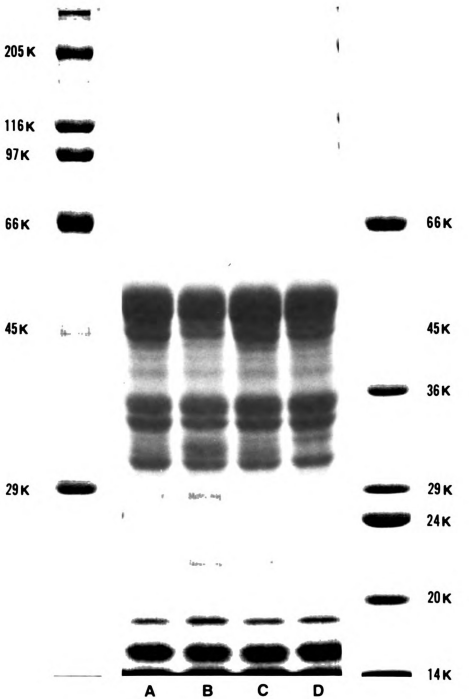

Figure 17. SDS-PAGE Patterns of Globulin II (GII) Isolated from Dry Navy Beans: A) C-20, B) Seafarer, C) Fleetwood and D) Experimental Line 84004

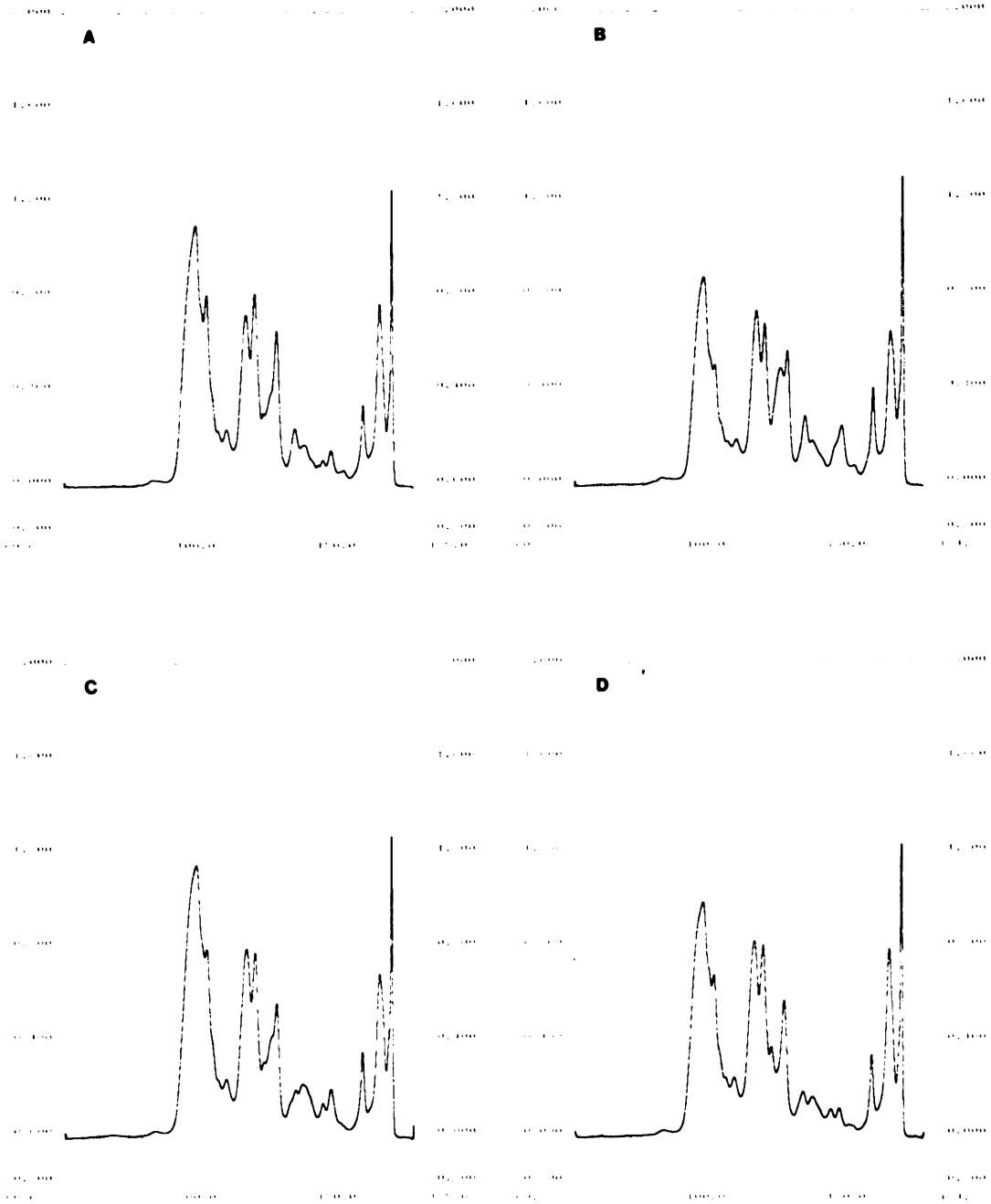

Figure 18. SDS-PAGE Densitometer Scan of Globulin II (GII) Isolated from Dry Navy Beans: A) C-20, B) Seafarer, C) Fleetwood and D) Experimental Line 84004

SDS-PAGE patterns and the corresponding densitometer scans of the GI (Figures 15 and 16) and GII (Figures 17 and 18) are more complex. The similarity in some bands (i.e. 49-40K, 36-33K) of GI and GII indicates high possibility of cross-contamination. The five volume water added to precipitate GI and GII may not be sufficient. To achieve better separation between GI and GII, more water may be needed to completely precipitate GI; however, the large volume of diluted GII will not be very practical for quantitative analysis. Since the diluted GII required dialysis against distilled-deionized water prior to freeze drying. The GI of all studied cultivars exhibited quite similar gel patterns (Figure 15). There are some differences in the subunit concentration as shown by differences in peak height of densitometer tracing curves. More detailed quantitative determination can be done using ultracentrifugation or chromatographic separation. The gel patterns of GII fraction are similar among cultivars with potential differences in their subunit concentrations (Figure 17). The known concentration distribution of each subunit of purified protein may provide better information to explain the differences among cultivars and their canning characteristics. In addition, the possibility of differences in structure and shape through inter/intramolecular bondings could play an important role. Although the observed gummy character of Fleetwood GI was distinctly different from the others, the differences in SDS-PAGE gel pattern are not clearly demonstrated. Other than the subunit concentration distribution, a suggestion of disulfide bonding effects is warranted since β-mercaptoethanol was added during SDS-PAGE sample preparation, it can cleave the disulfide bonds which may have significant affects on physical properties.

Amino Acid Analysis

The amino acid composition of major bean proteins (Albumin, GI and GII), as expressed on a mole percent of total amino acid content basis, is presented in Tables 18 to 21 for C-20, Seafarer, Fleetwood and breeding line 84004, respectively. Differences in amino acid profiles were observed primarily among protein fractions. Aliphatic hydrocarbon amino acids were the predominant amino acid class found in all major protein

Table 18. Amino Acid Composition (% Molar) of Various Protein Fractions Isolated from C-20 Cultivar[1]

Classification	Amino Acid	Protein Fractions		
		Albumin	Globulin I	Globulin II
Aliphatic Hydrocarbon	Glycine	7.5 ± 0.7a	7.8 ± 0.7a	7.0 ± 1.0a
	Alanine	9.2 ± 0.3b	6.9 ± 0.4a	6.1 ± 0.2a
	Valine	6.8 ± 0.3a	8.0 ± 0.1b	6.7 ± 0.1a
	Leucine	7.2 ± 0.7a	10.1 ± 2.1a	10.0 ± 1.2a
	Isoleucine	4.8 ± 0.3a	6.0 ± 1.1a	5.7 ± 0.3a
	Total	**35.6 ± 0.2a**	**38.7 ± 3.0a**	**35.6 ± 0.4a**
Alcohol	Serine	7.3 ± 0.7a	7.1 ± 0.0a	7.8 ± 0.2a
	Threonine	6.9 ± 0.3b	4.5 ± 0.0a	4.1 ± 0.0a
	Total	**14.3 ± 0.9b**	**11.6 ± 0.0a**	**11.8 ± 0.2a**
Acid	Aspartic Acid	11.5 ± 0.2a	9.8 ± 1.8a	13.0 ± 0.1a
	Glutamic Acid	10.7 ± 0.3a	13.4 ± 0.7b	14.4 ± 0.5b
	Total	**22.2 ± 0.5a**	**23.2 ± 2.5ab**	**27.5 ± 0.4b**
Basic	Arginine	4.6 ± 0.0ab	5.0 ± 0.2b	4.5 ± 0.0a
	Histidine	2.3 ± 0.8a	2.6 ± 0.4a	2.5 ± 0.3a
	Lysine	7.2 ± 0.7a	6.4 ± 0.8a	5.9 ± 0.3a
	Total	**14.0 ± 1.5a**	**13.9 ± 1.4a**	**12.9 ± 0.7a**
Sulfur	Cysteic Acid	4.5 ± 0.6b	2.0 ± 0.9a	0.4 ± 0.2a
	Methionine	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	Total	**4.5 ± 0.6b**	**2.0 ± 0.9a**	**0.4 ± 0.2a**
Aromatic	Phenylaline	4.1 ± 0.2a	5.1 ± 0.9a	5.7 ± 0.6a
	Tyrosine	0.3 ± 0.5a	0.0 ± 0.0a	1.4 ± 0.3b
	Total	**4.4 ± 0.7a**	**5.1 ± 0.9ab**	**7.1 ± 0.3b**
Heterocyclic	Proline	5.0 ± 0.2a	6.0 ± 0.1b	4.8 ± 0.0a

[1]n= 2, Means in a row followed by different letters are significantly different ($P<0.05$).

Table 19. Amino Acid Composition (% Molar) of Various Protein Fractions Isolated from Seafarer Cultivar[1]

Classification	Amino Acid	Protein Fractions		
		Albumin	Globulin I	Globulin II
Aliphatic Hydrocarbon	Glycine	7.5 ± 0.8a	7.6 ± 0.3a	7.1 ± 0.3a
	Alanine	8.7 ± 0.2b	6.9 ± 0.4a	6.1 ± 0.1a
	Valine	7.1 ± 0.3a	7.7 ± 0.7a	7.1 ± 0.1a
	Leucine	7.7 ± 0.8a	10.7 ± 1.7a	10.0 ± 0.9a
	Isoleucine	5.4 ± 0.5a	6.4 ± 0.6a	5.9 ± 0.2a
	Total	**36.5 ± 0.5a**	**39.3 ± 3.2a**	**36.2 ± 0.8a**
Alcohol	Serine	8.0 ± 0.4b	6.8 ± 0.4a	8.1 ± 0.2b
	Threonine	7.4 ± 0.1b	4.5 ± 0.0a	4.5 ± 0.0a
	Total	**15.5 ± 0.5c**	**11.3 ± 0.4a**	**12.6 ± 0.2b**
Acid	Aspartic Acid	11.6 ± 0.0ab	9.2 ± 1.6a	12.7 ± 0.4b
	Glutamic Acid	9.7 ± 0.1a	12.6 ± 0.0b	13.2 ± 0.8b
	Total	**21.3 ± 0.1a**	**21.8 ± 1.6a**	**25.9 ± 1.2b**
Basic	Arginine	4.4 ± 0.1ab	4.9 ± 0.2b	4.2 ± 0.1a
	Histidine	2.0 ± 0.7a	2.5 ± 0.3a	2.4 ± 0.3a
	Lysine	6.6 ± 0.1a	6.6 ± 1.4a	6.4 ± 0.4a
	Total	**13.0 ± 0.6a**	**14.0 ± 1.9a**	**13.0 ± 0.2a**
Sulfur	Cysteic Acid	3.7 ± 0.8b	2.3 ± 0.8ab	1.0 ± 0.4a
	Methionine	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	Total	**3.7 ± 0.8b**	**2.3 ± 0.8ab**	**1.0 ± 0.4a**
Aromatic	Phenylaline	4.4 ± 0.4a	5.5 ± 0.5a	5.6 ± 0.5a
	Tyrosine	0.7 ± 0.2ab	0.2 ± 0.2a	0.9 ± 0.1b
	Total	**5.1 ± 0.6a**	**5.7 ± 0.3ab**	**6.6 ± 0.4b**
Heterocyclic	Proline	5.0 ± 0.2a	5.7 ± 0.3b	4.7 ± 0.0a

[1]n= 2, Means in a row followed by different letters are significantly different ($P<0.05$).

Table 20. Amino Acid Composition (% Molar) of Various Protein Fractions Isolated from Fleetwood Cultivar[1]

Classification	Amino Acid	Protein Fractions		
		Albumin	Globulin I	Globulin II
Aliphatic Hydrocarbon	Glycine	8.0 ± 1.5a	7.7 ± 0.6a	6.8 ± 0.9a
	Alanine	9.3 ± 0.6b	6.9 ± 0.2a	5.8 ± 0.1a
	Valine	6.9 ± 0.5a	7.4 ± 0.5a	6.7 ± 0.2a
	Leucine	7.7 ± 1.0a	10.1 ± 1.2a	9.9 ± 1.3a
	Isoleucine	5.4 ± 0.7a	6.2 ± 0.6a	5.8 ± 0.5a
	Total	**37.3 ± 0.1a**	**38.3 ± 1.8a**	**35.1 ± 1.0a**
Alcohol	Serine	8.2 ± 0.4bc	7.0 ± 0.5a	8.0 ± 0.1ab
	Threonine	7.7 ± 0.2b	4.5 ± 0.1a	4.1 ± 0.2a
	Total	**15.9 ± 0.2b**	**11.5 ± 0.4a**	**12.1 ± 0.3a**
Acid	Aspartic Acid	12.0 ± 0.0ab	9.5 ± 1.4a	12.9 ± 0.1b
	Glutamic Acid	10.0 ± 0.1a	12.6 ± 0.3b	14.2 ± 0.8c
	Total	**22.0 ± 0.1a**	**22.1 ± 1.7a**	**27.1 ± 0.9b**
Basic	Arginine	3.8 ± 0.1a	5.0 ± 0.1c	4.4 ± 0.0b
	Histidine	2.0 ± 0.8a	2.4 ± 0.4a	2.5 ± 0.3a
	Lysine	6.0 ± 0.6a	7.7 ± 0.1b	6.3 ± 0.0a
	Total	**11.8 ± 0.3a**	**15.0 ± 0.5c**	**13.2 ± 0.4b**
Sulfur	Cysteic Acid	2.9 ± 0.0c	2.2 ± 0.5bc	0.5 ± 0.1a
	Methionine	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.2a
	Total	**2.9 ± 0.0c**	**2.2 ± 0.5bc**	**0.6 ± 0.3a**
Aromatic	Phenylaline	4.3 ± 0.4a	5.3 ± 0.6a	5.8 ± 0.9a
	Tyrosine	0.5 ± 0.1a	0.0 ± 0.0a	1.4 ± 0.4b
	Total	**4.8 ± 0.5a**	**5.3 ± 0.6a**	**7.2 ± 0.5b**
Heterocyclic	Proline	5.2 ± 0.6a	5.6 ± 0.1a	4.7 ± 0.2a

[1]n= 2, Means in a row followed by different letters are significantly different ($P<0.05$).

Table 21. Amino Acid Composition (% Molar) of Various Protein Fractions Isolated from Experimental Line 84004[1]

Classification	Amino Acid	Protein Fractions		
		Albumin	Globulin I	Globulin II
Aliphatic Hydrocarbon	Glycine	7.8 ± 0.6a	7.5 ± 0.9a	6.8 ± 0.4a
	Alanine	9.5 ± 0.6b	6.6 ±+0.1a	5.8 ± 0.1a
	Valine	6.8 ± 0.5a	7.2 ± 0.1a	6.5 ± 0.1a
	Leucine	7.6 ± 0.6a	9.9 ± 1.3a	10.0 ± 0.8a
	Isoleucine	5.1 ± 0.3a	6.0 ± 0.5a	5.8 ± 0.1a
	Total	**36.6 ± 0.7ab**	**37.2 ± 0.9b**	**34.8 ± 0.4a**
Alcohol	Serine	7.8 ± 0.8a	7.1 ± 0.1a	7.8 ± 0.2a
	Threonine	7.2 ± 0.6b	4.1 ± 0.3a	3.7 ± 0.1a
	Total	**15.0 ± 1.5b**	**11.2 ± 0.2a**	**11.5 ± 0.1a**
Acid	Aspartic Acid	10.8 ± 0.5a	10.5 ± 0.8a	12.9 ± 0.3b
	Glutamic Acid	10.2 ± 0.3a	13.7 ± 0.1b	15.1 ± 0.6c
	Total	**21.0 ± 0.9a**	**24.2 ± 0.9b**	**28.0 ± 0.9c**
Basic	Arginine	4.1 ± 0.1a	4.9 ± 0.1b	4.6 ± 0.2b
	Histidine	1.9 ± 0.3a	2.5 ± 0.4a	2.6 ± 0.2a
	Lysine	7.2 ± 0.6a	7.2 ± 0.6a	6.1 ± 0.1a
	Total	**13.2 ± 1.0a**	**14.6 ± 0.1a**	**13.2 ± 0.2a**
Sulfur	Cysteic Acid	3.1 ± 0.2c	2.2 ± 0.3b	0.7 ± 0.4a
	Methionine	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
	Total	**3.1 ± 0.2c**	**2.2 ± 0.3b**	**0.7 ± 0.4a**
Aromatic	Phenylaline	4.3 ± 0.2a	5.1 ± 0.7a	5.9 ± 0.8a
	Tyrosine	0.5 ± 0.2ab	0.0 ± 0.0a	1.4 ± 0.6b
	Total	**4.8 ± 0.1a**	**5.1 ± 0.7a**	**7.2 ± 0.2b**
Heterocyclic	Proline	5.0 ± 0.0a	5.5 ± 0.2b	4.7 ± 0.1a

[1]n= 2, Means in a row followed by different letters are significantly different (P<0.05).

fractions of studied beans. These amino acids were found slightly greater content in GI (37.2%-39.3%0 than in Albumin (36.5%-37.3%) and G II (34.8%-36.2%). Albumin possessed significantly higher ($P<0.05$) alanine content than GI and GII. The amino acids with acidic or amide side chains are the second higher class of bean proteins. They ranged from 21.0% to 22.2% for albumin, 21.8% to 24.2% for G I and 25.9% to 28.0% for GII. The percentage of glutamic acid/glutimine content (13.2%-15.1%) in GII was significantly greater ($P<0.05$) than that of GI (12.6%-13.7%) and Albumin (9.7%-10.7%). Hydroxy amino acids accounted for 14.3 to 15.9% of the total amino acid content in Albumin, 11.2 to 11.6% in GI and 11.5 to 12.6% in GII. Albumin had higher ($P<0.05$) threonine content (6.9%-7.7%) than GI (4.1%-4.5%) and GII (3.7%-4.5%). Basic and aromatic amino acids of analyzed proteins of all cultivars represented 11.8 to 15.0% and 4.4 to 7.2% of total amino acids, respectively. Lysine and phenylalanine were the major amino acids of their respective classes. All studied protein fractions are a good source of lysine ranging from 5.9% to 7.2% of total amino acids. Very small amount of tyrosine (0.00% - 1.4%) was found in all protein fractions, especially in G I (0.0% - 0.2%). Sulfur amino acids found in studied bean proteins were mostly cysteine/cystine. Methionine content was so low that it was undetected in all samples, except in Fleetwood GII (0.1%). Ma and Bliss (1978) reported that methionine is more concentrated in the alkali soluble fraction, Glutelin than in Albumin, Globulins (GI and GII), Prolamin and residue. Tryptophan is labile to the acid hydrolysis conditions and was not quantified in this study. The results of amino acid compositions are in good agreement with the previous published data (Pant and Tulsiani, 1969 and Marquez and Lajolo, 1981).

When comparison among cultivars was made for each protein fraction, there was no distinct difference observed. The variation in physical properties of GI proteins prepared from Fleetwood may not be explained by the amino acid composition. More work needs to be done on the inter/intra molecular bondings of bean proteins to understand their complex roles in regulation of canned bean product quality.

Study 4. Mineral Content of Dry Bean Seeds

The contents of minerals and trace minerals of studied bean seed coats and cotyledons are shown in Tables 22 and 23, respectively. As compared to cotyledons, seed coats were richer in Na, Ca, Mg, Al, B and Ba; but poorer in P, K and Mn. Distributions of Fe, Zn and Cu in seed coat/cotyledon, however, were varied depending on cultivars. In all bean samples, relatively similar concentration of Se (ppm) was found in seed coats (20.27-21.00) and cotyledons (20.00-20.42). Seafarer, both seed coats and cotyledons, possessed distinctively higher content of Na, Mn and Ba when compared to the other bean samples. The calcium concentration in Fleetwood seed coat is approximately half of those found in the seed coats of C-20, Seafarer and 84004.

The relationships between cooking quality and/or texture quality of bean with its mineral content and/or ratio were previously reported (Mattson et al., 1950; Muller, 1967, Chong et al., 1983 and Bhatty, 1984). These investigators explained the proposed relationships based on phytic acid complexing divalent metal ions (Ca^{2+}, Mg^{2+}), resulting in reduction of insoluble Ca^{2+} and Mg^{2+} pectate formation and hence softening the bean texture. In this study, least square regression equations relating the canned product quality (soaked weight, drained weight, compression force and shear force) with the mineral contents or ratios [$Ca^{2+} + Mg^{2+}$, $(Ca^{2+} + Mg^{2+})/P$, P, $Na^{+} + K^{+}$, $(Na^{+} + K^{+})/P$ and $(Ca^{2+} + Mg^{2+})/(Na^{+} + K^{+})$ as suggested by Bhatty, 1984] were calculated. Relationship of $R^2>0.75$ was arbitrarily selected as a minimum correlation coefficient which would be meaningful. Equations meetings this criteria are:

Soaked weight (g) = $221.9 + 0.23\ (Ca^{2+} + Mg^{2+})_{SC}/P_{SC}$ (1)
$R^2 = 0.983$

Soaked weight (g) = $234.62 - 0.01\ P_{sc,\ ppm}$ (2)
$R^2 = 0.998$

Shear force (Kg/50g canned bean) = $69.72 - 0.002\ (Ca^{2+} + Mg^{2+})_{sc,\ ppm}$ (3)
$R^2 = 0.873$

Table 22. Mineral and Trace Mineral Content (ppm) of Studied Bean Seed Coats[1]

Bean Cultivar	P	K	Na	Ca	Mg	Fe
Seedcoat						
C-20	687.95 ± 0.78a	3773.25 ± 136.83a	38.35 ± 1.36a	19706.70 ± 71.98b	4038.10 ± 21.64c	172.10 ± 0.00d
Seafarer	1208.57 ± 37.32c	6486.17 ± 82.10c	623.25 ± 3.75c	19632.65 ± 149.27b	4121.80 ± 7.50d	77.79 ± 0.45b
Fleetwood	1455.85 ± 14.92d	6968.05 ± 37.26d	38.35 ± 0.82a	10813.35 ± 74.60a	3729.30 ± 22.34b	59.55 ± 0.49a
84004	906.35 ± 25.53b	5348.30 ± 87.40b	46.53 ± 3.13b	20661.60 ± 72.83c	3477.95 ± 7.28a	119.03 ± 5.10c

[1]n = 2, Means in a column followed by different letters are significantly different (P<0.05).

Table 22. (Cont'd) Mineral and Trace Mineral Content (ppm) of Studied Bean Seed Coats[1]

Bean Cultivar	Zn	Cu	Se	Mn	Al	B	Ba
Seedcoat							
C-20	16.70 ± 0.00a	3.84 ± 0.04a	20.27 ± 0.00a	3.79 ± 0.00a	81.83 ± 0.07c	21.89 ± 0.00b	4.95 ± 0.05b
Seafarer	35.15 ± 0.49d	11.08 ± 0.44d	21.00 ± 0.00d	7.83 ± 0.06d	38.32 ± 0.15a	22.59 ± 0.15b	24.53 ± 0.22d
Fleetwood	32.05 ± 1.48c	7.89 ± 0.32c	20.99 ± 0.00e	5.43 ± 0.09c	43.04 ± 0.90b	19.46 ± 0.82a	2.80 ± 0.01a
84004	21.55 ± 0.35b	6.41 ± 0.19b	20.61 ± 0.00b	5.16 ± 0.08b	95.01 ± 2.33d	23.19 ± 0.00bc	7.47 ± 0.01c

[1]n = 2, Means in a column followed by different letters are significantly different ($P<0.05$).

Table 23. Mineral and Trace Mineral Content (ppm) of Studied Bean Cotyledons[1]

Bean Cultivar	P	K	Na	Ca	Mg	Fe
Cotyledon						
C-20	6001.65 ± 21.57c	14635.60 ± 0.00a	9.20 ± 2.79a	305.41 ± 2.16a	1529.60 ± 7.21d	78.92 ± 1.51b
Seafarer	6085.80 ± 0.00d	14624.40 ± 72.55a	57.95 ± 2.19b	331.00 ± 0.71b	1447.00 ± 0.00a	76.76 ± 0.29b
Fleetwood	5428.35 ± 7.14a	15014.57 ± 71.01b	13.36 ± 3.13a	303.30 ± 4.24a	1476.30 ± 0.00b	70.85 ± 1.07a
84004	5683.40 ± 21.35b	15879.40 ± 0.00c	12.46 ± 2.98a	360.80 ± 8.49c	1492.45 ± 7.14c	84.47 ± 0.93c

[1]n = 2, Means in a column followed by different letters are significantly different (P<0.05).

Table 23. (Cont'd) Mineral and Trace Mineral Content (ppm) of Studied Bean Cotyledons[1]

Bean Cultivar	Zn	Cu	Se	Mn	Al	B	Ba
Cotyledon							
C-20	34.56 ± 0.15c	9.27 ± 0.04a	20.23 ± 0.00b	13.92 ± 0.00b	5.07 ± 0.00c	11.03 ± 0.79a	0.51 ± 0.00ac
Seafarer	30.14 ± 0.23a	10.26 ± 0.00c	20.42 ± 0.00c	22.68 ± 0.00d	5.12 ± 0.00b	9.66 ± 0.17a	0.82 ± 0.02b
Fleetwood	32.54 ± 0.57b	9.78 ± 0.21b	20.04 ± 0.07a	12.35 ± 0.00a	5.01 ± 0.01a	9.73 ± 0.59a	0.50 ± 0.00a
84004	43.10 ± 0.42d	11.16 ± 0.00d	20.00 ± 0.00a	15.48 ± 0.00c	5.01 ± 0.01a	12.56 ± 0.14b	0.50 ± 0.00a

[1]n = 2, Means in a column followed by different letters are significantly different (P<0.05).

Shear force (Kg/50g canned bean)	=	$119.02 + 15.95\ (Na^{+} + K^{+})_{SC}/P_{SC}$ $R^2 = 0.910$	(4)
Soaked weight (g)	=	$221.85 + 1.31\ (Ca^{2+} + Mg^{2+})_{SC}/(Na^{+} + K^{+})_{SC}$ $R^2 = 0.963$	(5)
Drained weight (g)	=	$-104.68 - 0.23\ (Ca^{2+} + Mg^{2+})_{COT}$ $R^2 = 0.934$	(6)
Compression force (Kg/50g canned bean)	=	$135.76 - 0.007\ (Na^{+} + K^{+})_{COT}$ $R^2 = 0.980$	(7)

Where: SC = Seed Coat
COT = Cotyledon

The positive correlation with a very high correlation coefficient ($R^2 = 0.9847$) between the contents of total P and phytic acid was previously reported by Lolas and Markakis (1975). According to these investigators, phytic acid is the principal form of P in dry beans and represent about 70% of total P.

There are several correlation equations with $R^2 > 0.75$ (equations 1 - 7), derived from the canned bean quality characteristics and the mineral data. A reaction of phytic acid with divalent cations; however, is not a appropriate explanation for the established correlations. The effects of insoluble Ca/Mg pectate formation in middle lamella on cell strength, hydration and expansion is well known, but it does not appear to clarify the relationships in equations 3, 5, and 6. However, the positive correlations of cotyledon, monovalent ion ($Na^{+} + K^{+}$) concentration and compression force can be simply explained by the enhanced level of soluble Na/K pectates, and hence loosening the intercellular strength. The same principle can also apply to the relationship of compression force and $(Ca^{2+} + Mg^{2+})cot/(Na^{+} + K^{+})_{cot}$ ratio as illustrated in Figure 19. Simple correlation coefficients were calculated again between cotyledon major mineral contents (K^{+}, Mg^{+}, Ca^{+} and Na^{+}, Table 23) and compression force (Table 13). The only meaningful

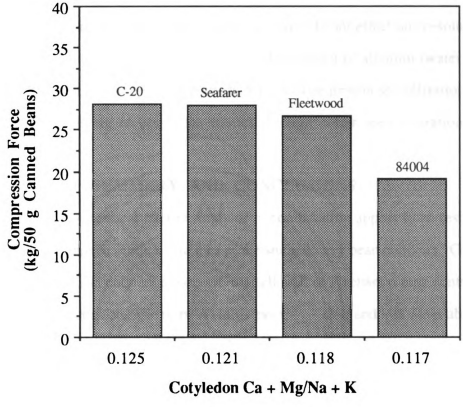

Figure 19. Relationships between Canned Bean Texture: Compression Force and $(Ca^{2+}+Mg^{2+})_{Cot}/(Na^{+}+K^{+})_{Cot}$ Ratio of Studied Navy Beans

correlation with R^2>0.75 is with cotyledon K content (compression force = 133.61 - 0.007 $K^+_{cot,\ ppm}$, R^2 = 0.977). Among the four studied cultivars, the differences in cotyledon K content may be one of the key factors regulating bean texture as indicated by compression force.

From Study 3, native salts in dry beans have a significant effect on co-solubilizing globulins (salt soluble proteins) into the water soluble extract of albumin (water soluble proteins). The influence of minerals (especially Na^+, K^+) on protein solubilization may in part account for softening of cotyledon tissues through better seed hydration during processing.

SUMMARY AND CONCLUSIONS

Many physico-chemical factors singly or in combination appear to be responsible for the variability in canned product qualities of the studied navy bean cultivars. Greater in thickness of seed coat and cotyledon parenchyma cell wall of Fleetwood may contribute to the high shear texture characteristic (type A) of its canned product and less water absorption during soaking and blanching. Water holding capacity during high temperature-high pressure cooking and texture: compression force of the canned beans, however, are more related to their chemical composition, primary starch, protein (GI and GII), cell wall components and minerals (P, Na, K, Ca and Mg). Additional work on protein inter/intra molecular bondings (especially of GI and GII fractions) in relation to their functional characteristics is warranted. The established correlations between cotyledon mineral (Na, K, Ca and Mg) and canned bean quality (drained weight and compression force) need to be tested by a larger sample pool.

CHAPTER 3: IN-VITRO PROTEIN DIGESTIBILITY OF SELECTED NAVY BEANS: CANNED BEANS, COTYLEDON FLOURS AND ISOLATED PROTEIN FRACTIONS

INTRODUCTION

The nutritional quality of a protein is determined by its quantity, availability and proportions of essential and non-essential amino acids. Although legumes are recognized as important dietary sources of protein, their proteins are generally less digestible than other grain and animal proteins (FAO/UN, 1970; Tobin and Carpenter, 1978; Wolzak et al., 1981 and Wolzak et al., 1981). The reasons for the low digestibility of bean protein are not well understood; however, research has shown that this low digestibility may be due to a combination of factors, such as size of food particles in the digestive track, conformation of bean protein (native and/or induced), hindrance effect by starch and fiber components, protease-amylase inhibitor, and lectin. Today consumers are becoming more educated about nutrition and more sophisticated in choosing foods that are wholesome and nutritious. Understanding the digestibility behaviors of different bean protein fractions (raw and cooked) as well as the whole beans could provide the plant breeder the necessary information in selection of superior breeding lines.

EXPERIMENTAL PLAN

The following research was organized into two studies which are partially interrelated. Study 1 was focused on the effect of cultivar/experimental line differences on in-vitro protein digestibility of canned beans. This serves as a preliminary screening for cultivars and breeding lines which may possess high protein digestibility. Study 2 was designed to elaborate some of the factors which might influence navy bean protein digestibility. Two navy bean cultivars: C-20 and Seafarer were selected for this study. Since Albumin, GI and GII were the major protein fractions found in these two bean

cultivars, they were isolated and studied for their digestibility behavior including the effect of moist heat (autoclaved).

MATERIALS AND METHODS

Raw Materials

Various dry bean cultivars were obtained from the Cooperative Elevator Company, (Pigeon, MI) and Michigan Dry Edible Bean Research (grown at Gratiot county) during 1987 crop year. Dry bean samples and their growing sources are provided in Table 24.

Sample Preparations

Study 1: various dry bean samples were canned according to the method described in the Material and Method Section, Chapter 2. After a 4-week canned bean equilibration period, washed canned beans, obtained by rinsing off the residual brine with tap water for 2 min. and draining for 2 min., were lyophilized for 72 hours and then ground through 20 mesh screen to produce flour for in-vitro protein digestibility determination.

Study 2: dry navy bean C-20 and Seafarer cultivars were selected for this study since their chemical composition was comprehensively investigated by Srisuma (1989) and in Chapter 2 of this dissertation. Dry bean samples were decorticated to obtain the cotyledonary sections, and subsequently ground using a UDY Cyclone Mill with 20 mesh screen to yield cotyledonary flour. Cotyledonary proteins of these two cultivars were fractionated by the method described in Material and Method Section, Chapter 2. The three major protein fractions for the digestibility study include Albumin, GI and GII.

In-vitro Protein Digestibility

All samples for in-vitro protein digestibility were determined for their nitrogen content using AOAC method 24.038, previously described in Chapter 2 (AOAC, 1984). The digestibility of the proteins was determined by measuring the extent to which the pH of the protein suspension dropped when treated with a multi-enzyme system, based on AOAC

Table 24. Sources of Dry Bean Samples (1987 Crop Year) Utilized in the Protein Digestibility Studies

Sources	Cultivar/Experimental Line
The Cooperative Elevator Company Pigon, MI	C-20
	Seafarer
	Fleetwood
	84004
Michigan Dry Edible Bean Research Out-State Trials County: Gratiot	C-20
	Seafarer
	Mayflower
	Bunsi
	N85027
	N87007
	N85006
	N84032
	N85606

Method 43.265: In-vitro Digestibility Method for C-PER (AOAC, 1984) using casein as a standard. The enzymes used in this determination include: porcine pancreatic trypsin (Type IX), porcine intestinal peptidase (Grade I), bovine pancreatic α-chymotrypsin (Type II) and bacterial protease (Pronase E). All enzymes were obtained from the Sigma Chemical Co., St. Louis, MO. Standard casein was purchased from the Sigma Chemical Co., St. Louis, MO. In addition, the moist heating effect on protein digestibility was carried out by autoclaving (121°C) the cotyledon flour, albumin, GI and GII of C-20 and Seafarer for 20 min. in aqueous suspensions.

RESULTS AND DISCUSSION

Study 1. In-vitro Protein Digestibility of Selected Canned Navy Beans

In-vitro assay for the measurement of protein digestibility was used in this experiment because it is less expensive, less time consuming and required only a small amount of sample. Protein digestibility indicates the availability of the amino acids contained in foods. The nitrogen contents and in-vitro protein digestibility of canned products from various cultivars and experimental lines are presented in Table 25. The nitrogen contents of studied bean samples varied from 4.25 to 4.73 % (db), probably due to their genetic background, growing environments, cultural practices, and post-harvest handling conditions. There was no significant difference ($P>0.05$) in in-vitro protein digestibility of canned products of dry bean samples obtained from Cooperative Elevator Co. However, protein digestibility values of canned C20 and Seafarer beans from Cooperative Elevator Co. were significantly higher ($P<0.05$) than those from Michigan Dry Edible Bean Research. Growing locations can exert a large influence on processing quality of navy beans (Hosfield et. al., 1984) and, in this case, had a greater effect on canned bean protein digestibility than did cultivar influence.

In general, canning process is applied to cook beans to obtain a palatable product, using substantial amount of heat which excess the requirement for microbiological safety.

Table 25. Nitrogen Content (%, db) and In-vitro Protein Digestibility of Canned Navy Beans from Various Cultivars and Breeding Lines

Bean Cultivar	% N	% Protein Digestibility
Cooperative Elevator Co.		
C-20	4.65 ± 0.03	86.28 ± 0.48
Seafarer	4.51 ± 0.04	86.29 ± 0.16
Fleetwood	4.42 ± 0.01	86.40 ± 0.32
84004	4.73 ± 0.04	86.85 ± 0.64
Michigan Dry Edible Bean Research		
C-20	4.68 ± 0.45	83.91 ± 0.64
Seafarer	4.24 ± 0.12	83.01 ± 1.28
Mayflower	4.53 ± 0.39	82.90 ± 0.16
Bunsi	4.37 ± 0.18	84.72 ± 0.83
N85027	4.44 ± 0.00	83.80 ± 2.07
N87007	4.58 ± 0.04	84.14 ± 0.00
N85006	4.38 ± 0.02	85.15 ± 1.12
N84032	4.43 ± 0.00	85.83 ± 0.16
N85606	4.24 ± 0.03	85.38 ± 0.16
$LSD_{(0.05)}$	0.38	1.81

Appropriate heat treatments were also reported to improve the digestibility of protein due to inactivation of anti-nutrients such as phytohemagglutinin and heat-labile protease inhibitors (Liner and Thompson, 1980 and Deshpande and Nielsen, 1987).

At present, there are limited research findings in protein digestibility of canned navy beans, thus, we are unable to compare the obtained digestibility results with previous research. Two other factors which may cause difficulty in result comparison are the differences in methods for sample preparation and in-vitro protein digestibility determination. The protein digestibility (%) of these canned bean samples are higher than those values found in most literature (70 - 77 %) (FAO/UN, 1970; Tobin and Carpenter, 1978; Marquez and Lajolo, 1981; Wolzak et al., 1981 and Wolzak et al., 1981). These higher digestibility values may be due to the method of sample preparation used in this study, washed canned beans were freeze-dried and ground through 20 mesh screen before in-vitro protein digestibility determination. This preparation treatment, by reducing bean particle size, should significantly improve the protein digestibility of canned bean samples. However, the protein digestibility of canned beans is still lower than that of casein (90% - 92%), thus, suggesting that other factors limit digestibility.

Study 2. In-vitro Protein Digestibility of Cotyledon Flours and Isolated Protein Fractions

In this study, the cotyledon portions of C-20 and Seafarer beans were separately ground through 20 mesh screen to obtain cotyledon flour samples. These flour samples were subsequently extracted to yield the following protein fractions: Albumin, GI and GII. There were significant differences ($P<0.05$) in nitrogen content among these flours and protein fractions within each cultivar, in an ascending order as follows: cotyledon flour, albumin, GI and GII (Table 26). Among isolated protein fractions, the GII fractions possess the highest purity as demonstrated by their highest nitrogen content.

The AOAC in-vitro protein digestibility method requires 10 mg of Nitrogen in 10 mL distilled-deionized water. The characteristics of these sample solutions of C-20 and

Table 26. Nitrogen Content[1] (%, db) of Cotyledonary Flours and Isolated Bean Protein Fractions

Flour/Protein Fraction	Bean Cultivar	
	C-20	Seafarer
Cotyledonary Flour	4.80 ± 0.02a	4.12 ± 0.03a
Albumin	9.86 ± 0.35b	9.90 ± 0.04b
Globulin I	14.04 ± 0.18c	13.76 ± 0.22c
Globulin II	15.71 ± 0.21d	15.50 ± 0.82d

[1] n = 3, Means in a column followed by different letters are significantly difference ($P<0.05$).

Seafarer are illustrated in Figures 20 and 21. Casein (standard) and albumin were completely solubilized in aqueous solution where as GI, GII and cotyledon flour formed an aqueous suspension. However, upon autoclaving at 121°C for 20 min, albumin and GI fractions formed precipitates at the bottom of test-tube (Figures 22 and 23). When adjusting pH to 8.0 $\pm$ 0.03, GII fractions of both C-20 and Seafarer were completely solubilized whereas the other samples were partially solubilized. The protein digestibility of all samples, both uncooked and autoclaved, are presented in Table 27. Without heat treatment, GI of both C-20 and Seafarer were the most digestible (88.5 -91.2%) fractions, following with GII (83.35 and 85.38%), cotyledon flour (72.75 and 73.09%) and albumin (69.82 and 71.74%). Phytohemagglutinin (PHA) has been found in GII fraction and thus, may contribute to low protein digestibility of this fraction. The least digestible fractions, albumins, from C-20 (69.8%) and Seafarer (71.7%) may be due to the natural protease inhibitor activity such as trypsin inhibitor. Marquez and Lajolo (1981) have published that large amount (73% of total) trypsin inhibitor activity is associated with the albumin fraction isolated from Brazilian beans (*Phaseolus vulgaris*). However, they reported that albumin was more digestible than GI and GII in a raw state using three separate enzyme systems: trypsin, pancreatin and pepsin-pancreatin. The use of multi-enzyme system (trypsin, peptidase, chymotrypsin and protease) in this study may improve the digestion of GI and GII over albumin. The applicability of multi-enzyme digestion is preferable since it is similar to the human complex proteolytic enzyme system.

The protein digestibility values of cooked (autoclaved) samples are summarized in Table 27. Comparison between the protein digestibility of raw and cooked samples were illustrated in Figures 24 and 25 for C-20 and Seafarer, respectively. Heat treatment by autoclaving at 121°C for 20 min greatly improved the protein digestibility of cotyledon flour and GII fraction. Slight increases in protein digestibility of albumins were observed after heat treatment. In contrast, the lowering of digestibility of bean albumin when heat treated was claimed by Marquez and Lajolo (1981). They suggested that it was due to the

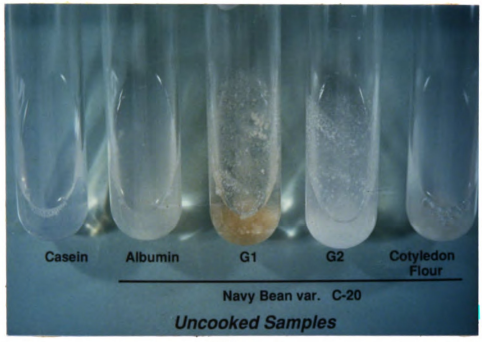

Figure 20. Characteristics of Uncooked Flours/Protein Fractions from Navy Bean "C-20" and Casein in Deionized-Distilled Water During In-vitro Protein Digestibility Assay

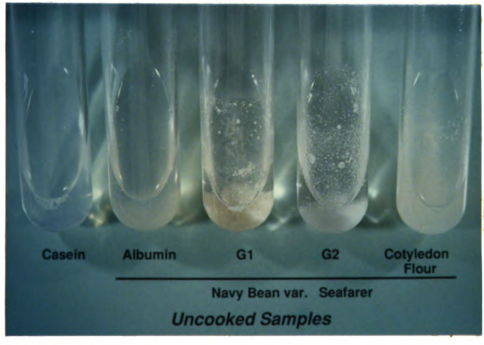

Figure 21. Characteristics of Uncooked Flours/Protein Fractions from Navy Bean "Seafarer" and Casein in Deionized-Distilled Water During In-vitro Protein Digestibility Assay

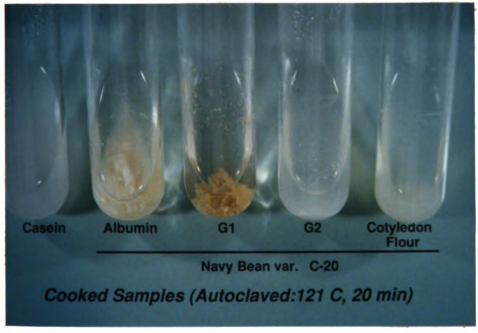

Figure 22. Characteristics of Cooked (Autoclaved) Flours/Protein Fractions from Navy Bean "C-20" and Casein in Deionized-Distilled Water During In-vitro Protein Digestibility Assay

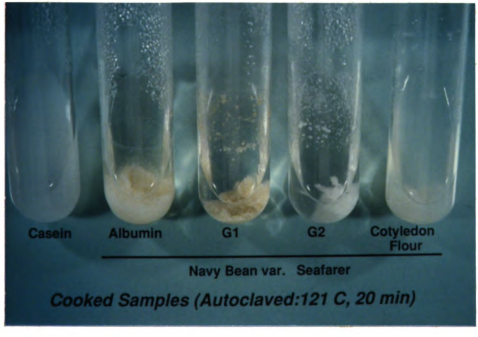

Figure 23. Characteristics of Cooked (Autoclaved) Flours/Protein Fractions from Navy Bean "Seafarer" and Casein in Deionized-Distilled Water During In-vitro Protein Digestibility Assay

Table 27. The Effect of Heating (Autoclave, 121°C for 20 min) on In-vitro Protein Digestibility of Cotyledon Flours/Isolated Protein Fractions from C-20 and Seafarer

Heating Effect/ Protein Fraction	% Protein Digestibility[1]	
	C-20	Seafarer
Raw		
Cotyledonary Flour	72.75 + 0.16b	73.09 + 0.32a
Albumin	69.82 ± 0.80a	71.74 ± 0.98a
GI	88.54 ± 0.16d	91.21 ± 0.11c
GII	83.35 ± 0.79c	85.38 ± 0.48b
Autoclaved		
Cotyledonary Flour	80.98 + 0.96b	81.66 + 0.32b
Albumin	73.20 ± 0.48a	73.54 ± 0.00a
GI	91.59 ± 1.59c	91.58 ± 0.00c
GII	92.60 ± 0.48c	92.94 ± 0.32d

[1] n = 3, Means in a column followed by different letters are significantly difference ($P<0.05$).

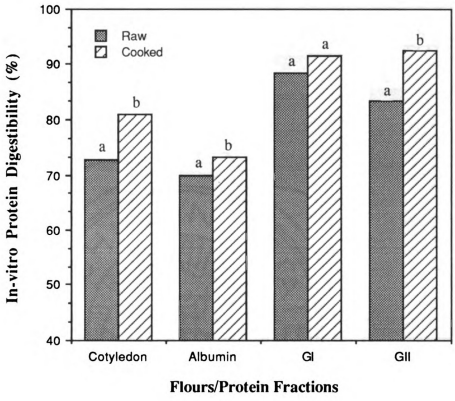

Figure 24. Effect of Heating (Autoclaved, 121°C for 20 min) on In-vitro Protein Digestibility of Cotyledon Flours/Isolated Protein Fractions from Navy Bean "C-20"

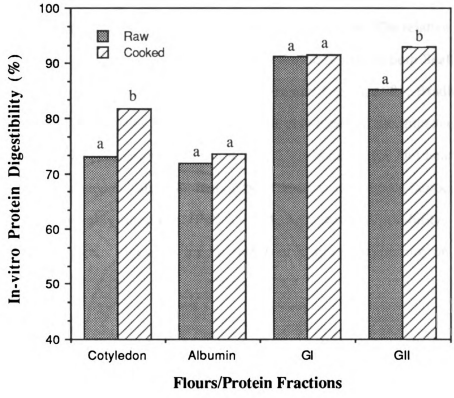

Figure 25. Effect of Heating (Autoclaved, 121°C for 20 min) on In-vitro Protein Digestibility of Cotyledon Flours/Isolated Protein Fractions from Navy Bean "Seafarer"

aggregation of autoclaved albumins and the presence of heat stable trypsin inhibitor. In this study, all samples (raw and cooked) were vortex-mixed for 1 min prior to enzyme digestion, since all solid foods were usually chewed before passing to the stomach and further mixed inside the stomach and along the digestive tract. The partial breakdown of heated albumin aggregates may facilitate enzyme-substrate interaction. The relative order of % digestibility ranked from high to low is GII, GI, cotyledon flours for both Seafarer and C-20 cultivars. Many previous investigators (Marquez and Lajolo, 1981; Bradbear and Boulter, 1984 and Deshpande and Nielsen, 1987) have also reported the improvement of protein digestibility by heat treatments. The ANOVA analysis of raw and heated bean protein samples is presented in Table 28. Bean protein fraction and heating (autoclaving at 121^{o}C, 20 min) have a highly significant ($P<0.001$) effect on protein digestibility. Heating improves the bean protein digestion but to different degrees depending on protein fraction/form.

SUMMARY AND CONCLUSIONS

Protein digestibility assessment of beans comprised of different cultivars, crop locations and/or conditions resulted in significant differences. Greater differences were demonstrated between growing locations than between cultivars. The agronomic environmental influence on protein digestibility warrants additional research.

Fractionation and evaluation of specific cotyledonary proteins demonstrated that the Globulin I (GI) possesses the highest percent digestibility in contrast to albumin and Globulin II (GII) which had lower digestibility.

Heat treatment (autoclave: 121^{o}C for 20 min) of the primary protein fractions resulted in enhanced digestibility of GII fraction to that equivalent of GI and casein. Heat induced improvement was not substantially demonstrated for albumin, suggesting the presence of a heat stable protein coagulation structure and/or an active protease inhibitor.

Table 28. Analysis of Variance for the Effect of Isolated Bean Protein Fractions and Heat Treatment on Protein Digestibility

Treatment	df	Mean Square	
		C-20	Seafarer
Bean Protein Fraction	3	315.41***	329.84***
Cooking Effect (Autoclave)	1	142.97***	83.48***
Bean Protein Fraction x Cooking Effect	3	10.37***	16.83***
Error	8		

SUMMARY AND CONCLUSIONS

This research was conducted to improve dry navy bean quality through a) the development of quality assessment methodology and b) a greater understanding of fundamental compositional relationships within the seed.

Canning quality of navy beans in F_4 - F_6 generations can be assessed by processing these bean samples using a 202 x 214 can. This method requires a smaller sample size (28.5 g dry bean solids) as compared to the conventional 303 x 406 (100 g dry bean solids) method. Whole flour pasting characteristics (torque values) of navy beans had high correlation ($R^2 = 0.97$) with textural quality of corresponding canned beans and yet required smaller sample size (< 2 g, db), thus providing an alternative quality evaluation in early generation breeding lines (F_2 - F_3).

Physico-chemical composition of dry beans varies widely among cultivars and undergoes various changes during processing resulting in differential product quality. In this study, selected physico-chemical characteristics of C-20, Seafarer, Fleetwood and Experimental line 84004 were correlated to their canning quality, especially drained weight and texture. Distinct differences in canned bean texture (compression/shear) were found among these cultivars as follows: Fleetwood, 26.6/41.5; C-20, 28.1/29.3; Seafarer, 28.0/28.9 and 84004, 19.1/22.5 kg force/50 g canned beans. Apparent seed density was highly correlated ($R^2 = 0.98$) with compression texture of canned beans. Fleetwood exhibited textural characteristic of Type A configuration, whereas C-20, Seafarer and experimental line 84004 demonstrated Type B curve configuration. Highest seed coat and cotyledonary parenchyma cell wall thickness of Fleetwood may contribute to its highest shear texture. Cotyledonary proteins of these navy beans were isolated and fractionated into six fractions, quantitatively ranked from high to low as: globulins (G I + G II), albumin, non-extractable protein, glutelin and prolamin based on their solubility properties. The three major proteins: albumin (20 - 26%), G I (29 - 31%) and G II (22 - 30%) were

further characterized for SDS-PAGE peptide pattern and amino acid composition. Nitrogen distribution of cotyledon proteins varied among cultivars. Albumin gel patterns demonstrated similarity of the following pairs: C-20 and 84004, and Seafarer and Fleetwood. Greater complexity of GI and G II gel patterns with differential concentration in protein sub-units was observed. Larger differences in amino acid composition were found among protein fractions than among cultivars. The contents in P, Na^+, K^+, Ca^{2+}, Mg^{2+}, $(Na^+ + K^+)/P$, $(Ca^{2+} + Mg^{2+})/P$ and $(Ca^{2+} + Mg^{2+})/(Na^+ + K^+)$ of seed coats and cotyledons were analyzed in relation to the canned bean quality. Significant correlations were obtained between compression texture and selected cotyledon minerals: a) K^+, b) $(Na^+ + K^+)$ and c) $(Ca^{2+} + Mg^{2+})/(Na^+ + K^+)$.

In-vitro protein digestibility assessment of beans comprised of different cultivars and crop growing conditions resulted in significant differences. Greater differences were demonstrated between growing conditions than those obtained among cultivars. Fractionation and evaluation of specific cotyledonary proteins demonstrated that the globulin I (G I) possesses the highest percent digestibility in contrast to albumin and globulin II (G II) which had lower digestibility. Heat treatment (autoclave: 121°C for 20 min) of the primary protein fractions resulted in enhancing the digestibility of G II fraction to that of G I and casein. Heat induced improvement was not substantially demonstrated for albumin, suggesting the presence of a heat stable protein coagulation structure and/or an active protease inhibitor.

APPENDIX A

APPENDIX A

Correlation between Seed Apparent Density and Starch/Fat Ratio

$(\%\ \text{Starch}/\%\ \text{Fat})_{Cot}$ = 99.95 - 48.21 Seed Apparent Density (g/mL)

$R^2 = 0.91$

where Cot = Cotyledon Tissue

APPENDIX B

Table 29. Analysis of Variance for Quality Comparison of Various Bean Cultivars and Breeding Lines (1987 Crop) Canned in 303 x 406 and 202 x 214 Cans

Source of Variation	df	Mean Square					
		Drained Wt. Ratio	Canned Bean Texture		Canned Bean Hunter Color		
			Compression	Shear	L	a	b
C-20							
Can Size	1	0.0001	0.56	0.49	1.21**	0.90	0.20
Error	2	0.0001	0.30	0.49	0.005	0.09	0.76
Seafarer							
Can Size	1	0.0002	0.42	0.06	0.39	1.44*	0.02
Error	2	0.0001	0.42	0.06	0.64	0.05	0.21
Fleetwood							
Can Size	1	0.0004	0.42	0.01	0.16	1.82*	0.06
Error	2	0.0003	2.31	4.51	0.03	0.06	0.01
84004							
Can Size	1	0.0001	0.00	0.49	0.0025	0.56	0.20
Error	2	0.00	1.96	0.49	0.31	0.04	0.02
Bunsi							
Can Size	1	0.01	0.42	5.06	0.30	0.56	0.01
Error	2	0.01	2.31	2.07	0.30	0.06	0.01

Cont'd

Table 29 (Cont'd)

Wesland							
Can Size	1	0.00	1.10	5.76	3.61**	2.10*	0.0025
Error	2	0.01	1.21	0.65	0.01	0.07	0.04
Midland							
Can Size	1	0.0004	1.96	0.20	5.29**	1.69*	0.0025
Error	2	0.0009	1.96	0.45	0.04	0.06	0.06
N84024							
Can Size	1	0.00302	0.49	1.10	1.10	0.42	0.01
Error	2	0.00272	0.49	0.12	0.21	0.03	0.05
N85006							
Can Size	1	0.0016	12.25	0.49	0.04	0.25*	0.20
Error	2	0.01	1.15	2.45	0.13	0.01	0.09
N85007							
Can Size	1	0.0016	1.82	4.41	0.20	0.64	0.01
Error	2	0.0029	0.91	5.77	0.38	0.09	0.10
N84032							
Can Size	1	0.00123	0.49	5.06	2.25	1.32**	0.09
Error	2	0.00123	0.49	1.81	0.17	0.003	0.07
C-15							
Can Size	1	0.000625	0.30	0.0025	0.25	1.69	0.42
Error	2	0.000302	2.26	1.82	0.29	0.15	0.04

Table 30. Analysis of Variance for Quality Comparison of Various Bean Cultivars and Breeding Lines (1988 Crop) Canned in 303 x 406 and 202 x 214 Cans

Treatment	df	Drained Wt. Ratio	Canned Bean Texture		Canned Bean Hunter Color		
			Compression	Shear	L	a	b
C-20							
Can Size	1	0.0036	2.91	7.45	0.36*	1.10	0.42
Error	2	0.0004	0.76	6.16	0.01	0.07	0.04
Seafarer							
Can Size	1	0.0009	8.38**	4.28	3.24	1.21*	0.02
Error	2	0.0004	0.03	1.14	0.22	0.02	0.02
Fleetwood							
Can Size	1	0.00063	1.02	4.88	6.00***	2.33**	0.00
Error	2	0.00043	0.58	1.18	0.0025	0.00312	0.02
Bunsi							
Can Size	1	0.0004	3.55	6.89	0.25	0.25*	0.04
Error	2	0.00085	0.71	2.31	3.07	0.005	0.005
Mayflower							
Can Size	1	0.0009	0.11	4.18	0.25	0.09	0.30
Error	2	0.00016	0.57	6.15	3.06	0.01	0.20

Cont'd

Table 30 (Cont'd)

Midland							
Can Size	1	0.0003	4.18	3.63	0.00	0.12*	0.06
Error	2	0.00063	2.33	2.53	2.89	0.0025	0.12
Albion							
Can Size	1	0.0020*	3.61	9.33	4.00	0.09	0.04
Error	2	0.0025	0.70	2.11	1.96	0.01	0.30
Rocket							
Can Size	1	0.0020*	0.12	0.05	9.00**	3.06*	0.04
Error	2	0.0025	4.77	1.56	0.01	0.03	0.07
Stinger							
Can Size	1	0.0012	0.72	10.50*	0.56**	0.72**	0.16
Error	2	0.00063	0.73	0.49	0.0025	0.0025	0.01

Table 31. Analysis of Variance for Pasting Properties of 6% (w/w) Whole Bean Flour Solutions from Four Bean Cultivars

Source of Variation	Degrees of Freedom	T_H	T_C
		MEAN SQUARES	
Cultivar	3	79317.278***	1345313.645***
Error	8	334.586	2113.486

Table 32. Analysis of Variance for Canned Bean Quality Attributes of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Soaked Weight	Drained Weight	Processed Color			Texture Shear Force	Bean Solids
				L	a_L	b_L		
				MEAN SQUARES				
Bean Cultivar	3	10.985*	166.307***	0.375	0.258	0.631	125.375***	1.613**
Error	4	1.191	9.325	0.171	0.064	0.383	0.673	0.090

Table 33. Analysis of Variance for One-hundred Seed Weight and Volume

Source of Variation	df	Mean Square	
		100 Seed Weight	100 Seed Volume
Bean Cultivar	3	4.94**	10.75
Error	2	0.33	3.50

Table 34. Analysis of Variance for Hilum Size, Seed Coat Thickness and Cotyledon Cell Wall Thickness of Four Selected Navy Bean Samples

Source of Variation	df	Hilum (L)	Hilum (W)	Seedcoat Thickness (Palisade)	Seedcoat Thickness (Hour Glass)	Seedcoat Thickness (Parenchyma)	Cell Wall Thickness
Bean Cultivar	3	0.41***	0.19***	108.90***	36.82***	27.53**	1.37***
Error	116	0.02	0.01	3.00	3.55	5.56	0.15

Table 35. Analysis of Variance for Proximate Chemical Analyses of Seed Coat Tissues from Four Bean Cultivars

Source of Variation	Degrees of Freedom	Ash	Fat	Protein	Total Carbohydrates
			MEAN SQUARES		
Cultivar	3	0.954***	0.613***	6.565***	8.332***
Error	8	0.011	4.533E-4	0.046	0.041

Table 36. Analysis of Variance for Proximate Chemical Analyses of Cotyledon Tissues from Four Bean Cultivars

Source of Variation	Degrees of Freedom	Ash	Fat	Protein	Carbohydrates	
					Starch	Total
			MEAN SQUARES			
Cultivar	3	0.041***	0.095***	13.605***	12.284***	13.809***
Error	8	0.002	0.001	0.012	1.922	0.020

Table 37. Analysis of Variance for Nitrogen Distribution (%) of Studied Navy Bean Cultivars

Source of Variation	df	Mean Square							
		Albumin	G I	G II	Prolamin	Glutelin	Residue	NPN	% Recovery
Bean Cultivar	3	20.54***	1.73	27.79**	0.01	0.30***	0.41***	6.49***	8.78
Error	8	0.31	1.44	1.88	0.01	0.01	0.00	0.04	3.05

Table 38. Analysis of Variance for Mineral and Trace Mineral Content (ppm) of Studied Bean Seed Coats

Source of Variation	df	Mean Square					
		Ca	Mg	K	P	Fe	Al
Bean Cultivar	3	42639301.71***	174651.17***	4033007.45***	227141.46***	4991.33***	1584.77***
Error	4	9583.21	269.17	8622.25	566.92	6.61	1.57

Table 38 (Cont'd)

Source of Variation	df	Mean Square					
		Na	B	Zn	Ba	Cu	Mn
Bean Cultivar	3	169494.56***	5.35**	150.73***	196.61***	18.29***	5.64***
Error	4	6.6	0.17	0.64	0.01	0.08	0.004

Table 39. Analysis of Variance for Mineral and Trace Mineral Content (ppm) of Studied Bean Cotyledons

Source of Variation	df	Mean Square					
		Ca	Mg	K	P	Fe	Al
Bean Cultivar	3	1448.02***	2371.46***	694328.61***	182708.99***	63.40***	0.01***
Error	4	23.79	25.76	2576.62	243.04	1.09	0.00002

Table 39 (Cont'd)

Source of Variation	df	Mean Square					
		Na	B	Zn	Ba	Cu	Mn
Bean Cultivar	3	1077.06***	3.73*	63.67***	0.05***	1.30***	41.66
Error	4	7.82	0.26	0.14	0.0001	0.01	0.00

Table 40. Analysis of Variance for Nitrogen Content (%, db) and In-vitro Protein Digestibility of Canned Navy Beans from Various Cultivars and Breeding Lines

Source of Variation	df	Mean Square	
		% N	% Protein Digestion
Protein Fraction	12	0.05	3.56**
Error	13	0.03	0.70

Table 41. Analysis of Variance for Nitrogen Content (%, db) of Cotyledon Flours and Isolated Bean Protein Fractions

Source of Variation	df	Mean Square	
		C-20	Seafarer
Protein Fraction	3	47.36***	50.87***
Error	4	0.05	0.01

Table 42. Analysis of Variance for the Effect of Heating (Autoclave) on In-vitro Protein Digestibility of Cotyledon Flours/Isolated Protein Fractions from C-20 and Seafarer

Source of Variation	df	Mean Square	
		C-20	Seafarer
Raw			
Protein Fraction	3	155.22***	180.10***
Error	4	0.33	0.32
Autoclaved			
Protein Fraction	3	170.57***	165.84***
Error	4	0.98	0.05

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