INFLUENCE OF NAVY BEAN CHEMICAL COMPOSITION ON CANNING QUALITY: COMPLEX CARBOHYDRATES, CELL WALL HYDROXYPROLINE AND PHENOLIC COMPOUNDS

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By

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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

LIST OF PLATES

INTRODUCTION

Among pulse crops, Phaseolus beans are the most important in terms of world-wide distribution. More than thirty Phaseolus species, the common bean (*Phaseolus vulgaris*) is the most advanced species of the genus in terms of domestication and cultivation (Hidalgo, 1988). Eleven different market classes of common beans are recognized in the U.S.A. Although common beans are similar botanically, they vary widely among classess for color, size, shape and flavor characteristics. World production of major grain legumes exceeds 150 million metric tons per annum (Figure 1). The United States production distribution of major commercial classes of common beans is illustrated in Figure 2. Seven different classes of dry beans grown in Michigan account for nearly one-third of U.S. production annually. These are navy beans, cranberry beans, dark red kidney beans, light red kidney beans, pinto beans, black turtle soup beans and yellow eye beans. Navy beans 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, weighing in the range of 17 g to 19 g per 100 seeds. Approximately 40% of all dry beans exported from the U.S. are shipped from Michigan during an average marketing year. Michigan produces about 70% of the navy beans grown in the U.S. while pinto beans are grown extensively in the western states: North Dakota, Idaho, Colorado, and Wyoming (Conklin and Peacock 1985).

Food legumes contribute a good source of several important nutrients. They provide variety to the human diet and more importantly, an economical source of supplementary protein for many populations lacking animal proteins, especially in underdeveloped or developing countries. In general, dry beans are rich in lysine but limiting in methionine content, thereby complementing the amino acid pattern found in

cereal. Dry beans are also recognized as as a major source of complex carbohydrates including dietary fiber which is thought to have an effect of lowering cholesterol in blood serum.)

However, several factors are found to limit dry bean utilization. These limitations include low levels of the sulfur amino acids (Bressani, 1975), 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), various flatulence factors (Fleming, 1981 a & b), and hard shell and hard-to-cook defects which may develop during dry bean storage (Gloyer, 1921; Bourne, 1967; Stanley and Aguilera, 1985 and Srisuma et al., 1989).

Dry beans are not a staple in the U.S.A. and the consumption of dry beans in U.S. 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 household structure and numbers of women in labor force have adversely affected bean consumption. Most consumer preferences are shifting in favor of convenience foods and commodities which require less preparation. Dry bean products in today's food market unfortunately do not lend themselves to these emerging consumer changes and thus require a significant development of innovative technology for 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 consumer awareness is eating less saturated fat, cholesterol, sugar and salt and 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 the consumer attention.

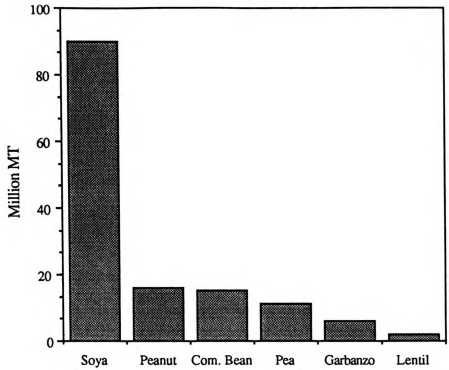

Figure 1. World Production of Major Grain Legumes

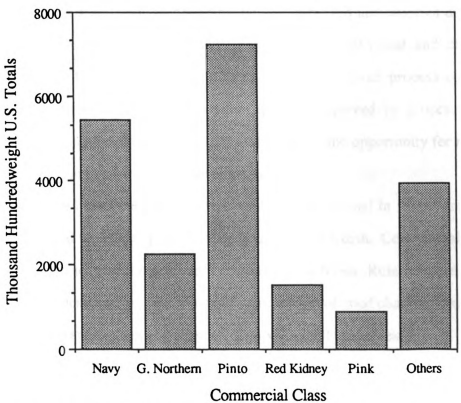

Figure 2. U.S. Commercial Dry Bean Production (5 Year Average: 1982-1986)

REVIEW OF LITERATURE

Dry Bean Processing

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 practises 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 factors) 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.

Physico-Chemical Composition of Dry Navy Beans

The physical and chemical properties of dry beans are primary key factors in determining processing parameters and subsequent final product quality. Dry bean seed structure is comprised of seed coat, cotyledon and embryonic tissue. Structurally, seed coat, cell wall, middle lamella and other cellular membranes immensely influence the dry bean product quality. Furthermore, the dry bean chemical constituents such as carbohydrates, proteins, phytate, polyphenols and lignin also have profound effects on bean product quality.

Structural Characteristics

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. Seed coat consists of approximately 7-8 % of the total dry weight in the mature bean (*Phaseolus vulgaris*) with a protein content of 5% (db) (Powrie et al., 1960 and Ott and Ball, 1943). The major components in the seed coat structure of legumes include waxy cuticle layer, palisade cell layer, hourglass cells and thick cell-walled parenchyma cells. The waxy cuticle layer is the outermost portion of the seedcoat 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) found that seed coat thickness, seed volume, and hilum size along with protein content 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 leaf tissue 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 and thus, forming intercellular polyelectrolyte gel which contribute significantly to the textural quality (Dull and Leeper, 1975 and Van Buren, 1979 & 1980).

Cell Wall Constituents

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) and a significant proportion of the crude fiber (80-93%) is localized in the seed coat (Reddy et al., 1984 and Salunkhe et al., 1985).

Plant cell wall varies 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 of fibrous structure (partially crystalline) and provide basic structural strength of the cell wall. The cellulose fibers are embedded with 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, pectin 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 ionic strength of the medium. This aggregation effect will result in overestimating molecular weights using viscometry, ultracentrifugation, light scattering or osmometry.

The extent of pectin methyl esterificaion vary 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 should occur randomly. A random distribution of methyl groups was also found in lemon pectin (DeVries et al., 1984).

Cell Wall Isolation and Fractionation

In recent years, cell wall material 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 established 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 works on bean cell wall were conducted using different preparation procedures which cause the difficulty in result comparisons (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 would be lost with the cooking water, eventhough the bean was intact and 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 respectively 67, 73 and 42 % pectic polysaccharides associated with 16, 12 and 10% cellulose (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). From their work, raw phaseolus legumes, pinto and white beans, contain respectively 3.73 and 4.14% cellulose and 1.58 and 1.04% lignin. In addition, they observed the higher total dietary fiber in cooked beans than in 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 lingkages). 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. 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 found 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 dry weight basis. Researchers of dry beans (*Phaseolus vulgaris*) have reported the protein content ranging from 18.8 to 29.3% (Meiners et al., 1976; Varriano-Marston and DeOmana, 1979 and Hosfiled and Uebersax, 1980). Total utilization of the legume protein is relatively low. Protein digestibility may be impaired possibly by the presence of numerous antinutritional compounds which must be removed or destroyed during processing (Bressani et al., 1982 and Aw and Swanson, 1985).

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). Hall et al. (1979) reported that French bean seed contain 60% globulin (salt soluble), 20% albumin (water soluble), 10% glutelin (alkali soluble) and 3% prolamine (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-1 (G1: high salt concentration for solubility) and globulin-2 (G2: low salt concentration for solubility). The ratio of G1 and G2 is about 6 to 1. The G1 fraction is also reported to be vicilin which is 6.9S proteins and can aggregate to form 18S tetramer at pH 4.5. Phytohemagglutinin (PHA) is identified as G2 with a characteristic of 6.4S protein. This PHA has ability to agglutinate the red blood cell and considered as antinutritional factor that limits the use of dry bean.

Bressani (1975) and Kay (1979) reported that the predominant class of protein present in Phaseolus beans is salt soluble globulins of which three distinct proteins have been identified: phaseolin, phaselin and conphaseolin. 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 hydrophoic 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.

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 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.

Lipids

A low lipid content is characteristic of dry beans and the total fat content (the ether extractable material) of dry beans ranges 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 crosslinked 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 methods of preparation 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 this content.

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.

Tannins

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 seed coat of bean 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).

Polyphenol

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.

Flavor Factors

Low molecular weight alcohols and aldehydes, aromatic hydrocarbons, chlorinated compounds and other very volatile constituents were identified by a direct transfer gas chromatographic method to be present in raw bean. The presence of aliphatic and aromatic hydrocarbons in plant tissues has been attributed to the uptake of these compound from the soil. Murray et al. (1976) suggested that these compounds result from plant decomposition, adsorption of petroleum hydrocarbons, and degradation of plant carotenoids in the soil.

Changes During Post-Harvest and Thermal Processing

During Post-Harvest

The handling and storage of beans in the field critically affect the ultimate quality of beans and bean products. The moisture content is the most important consideration during harvest and storage. Seeds with a moisture content above 18% are subjected to excessive damage during storage and in the processing line, due to physical susceptibility to

mechanical forces and microbial spoilage from mold (Weston and Morris, 1954). On the other hand, seeds with a moisture content below 15% are sensitive to impact damage.

During storage, seed deterioration can be retarded by providing proper storage conditions. The two major controlling factors are moisture content and temperature.

Moisture Content

Beans stored at too low moisture exhibit clumping and splitting due to seed coat and cotyledon rupture, while storage at high initial moisture encourages discoloration, off-flavor development, loss of water uptake capacity and mold growth. Morris and Wood (1956) reported that beans with moisture content above 13% deteriorated significantly in both flavor and texture after six months at 77°F and became unpalatable within 12 months. Burr et al. (1968) and Bedford (1972) reported that beans stored at high moisture showed a significant increase in their required cooking time while low moisture did not lose their cooking quality.

Temperature

Quality degradation is faster at high temperature than at low temperature. Beans stored at high temperature become darker in color and require longer cooking times. The deteriorative effect of high moisture on bean quality is increased by high temperature. Long cooking time of beans from high temperature storage was observed by Burr et al. (1968). Uebersax (1972) reported that deterioration rate both in discoloration and mold growth was minimized in beans stored at 55°F under relative humidities ranging from 75% to 86%. The influence of increased storage temperature became greater at high relative humidity.

Vongsarnpigoon et al. (1981) suggested that optimum bean quality was obtained from dry beans stored at 14% moisture at 70°F. Hard-to-cook phenomenon can develop due to improper storage conditions (Morris and Wood, 1956 and Muneta, 1964). Recommendations to prevent storage loss of dry bean from hardening (Mejia, 1980) include: 1) beans should be stored at the lowest possible moisture content and 2) beans

should be stored in a dry and cool environmet. Aeration with the proper flow rate, relative humidity and temperature improve the stored bean quality. Aeration, which is the practice of moving air at low flow rates to cool all beans in a bin, prevents moisture migration and also reduces mold growth and development of musty odors and off flavors (Maddex, 1978). Vongsampigoon et al. (1981) observed that organic acid treatment provided limited mold inhibition and resulted in beans with brown discoloration and firm texture, whereas $NaHSO_3$ treatment provided limited color stability without adversely influencing processing characteristics. In addition, they reported that vacuum and CO_2 storage did not significantly improve bean quality.

Hard Shell and Hard-to-Cook

The textural defects of cooked bean seeds can be divided into two categories: hard shell and hard-to-cook (HTC) (Stanley and Aguilera, 1985). Legumes that will not soften sufficiently because of a seed failure to imbibe water during soaking are termed "hard shell". HTC legumes, however, absorb adequate water during soaking (based on soaked weight) but will not soften sufficiently during a reasonable cooking time. HTC defect has been demonstrated in various cultivars of Phaseolus and Vigna as well as other legumes (Sefa-Dedeh et al., 1979 and Stanley and Aguilera, 1985).

The most noticeable structural changes caused by HTC defect is a failure of cotyledon cells to separate during cooking. This has been observed by various researchers (Sefa-Dedeh et al., 1979 and Varriano-Marston and Jackson, 1981). Varriano-Marston and Jackson (1981) using transmission electron microscope, described disintegrations of cytoplasmic organelles, and inclusions and loosening of attachments between the cell wall and the plasmalemma in HTC beans. They considered loss of plasmalemma integrity to be responsible for increases in electrolyte leakage observed in HTC beans as previously reported by Ching and Schoolcraft (1968).

At present, the HTC mechanism is not fully elucidated. Mattson (1946) emphasized the role of phytate in bean hardening. This author demonstrated that hard-to-cook peas

contained much less (over 50%) phytate than normal and that removal of this compound by soaking or enzymatic action induced the textural defect. Several other researchers observed the same phenomena (Jones and Boulter, 1983 a & b and Kon and Sanshuck, 1981). However, Crean and Haisman (1963) argued that since the available phytate can only, at maximum, complex less than 50% of the divalent cations in beans during cooking, free calcium and magnesium ions are always available to cross-link middle lamella pectins and, hence, the influence of phytate on texture should be limited.

Polyphenols have been known to contribute adverse effect on color, flavor and nutritional quality in cereals and legumes. Tannins are the polyphenols that seem to be of most interest in legumes (Hulse, 1980 and Salunkhe et al., 1982). These tannins consist of water soluble phenolic compounds which have molecular weights between 500 to 3,000 daltons and possess the ability to precipitate alkaloids and gelatin and other proteins (Gupta and Haslam, 1980). Rolston (1978) observed that the impermeability of legume seed coats is associated with higher levels of phenolic compounds in the seed coats and with their level of oxidation involving polyphenolases. The browning or tanning reaction, found to be correlated with impermeability, is considered to be a consequence of quinone formation via the action of catechol oxidase on the diphenols resulting from polyphenolase activity. It seems possible that the enzymatic oxidation of polyphenols is also involved in the hard-to-cook phenomenon.

Lignin is also thought to be capable of influence the bean texture. Lignin has a three dimensional structure consisting of short linear chains crosslinked by a variety of interchain covalent bonds. It is insoluble and exists covalently bound to the hemicellulose components of the cell walls and middle lamella (Blouin et al., 1982). The function of lignin is to decrease the permeation of water across cell walls, impart rigidity and bond cells, thus creating a structure resistant to impact and compression (Sarkanen and Ludwig, 1971). The role of lignin influencing hard-to-cook phenomenon is not fully known.

During Thermal Processing

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.

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 pretreatments 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 variablity 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 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 heat 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^{o}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 opinion lies with heat causing 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 degredation 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 antinutritional factors and thus, results in poor quality beans. Thermal processing induces the largest alteration in structure and concomitantly various chemical reactions among 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 otain 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 but 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 lossess 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 antinutritional 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 STORAGE INDUCED CHANGES OF PHENOLIC ACIDS AND THE DEVELOPMENT OF HARD-TO-COOK IN DRY BEANS (*Phaseolus vulgaris*, var. Seafarer)

Introduction

Storage of legume seeds at high temperature and high humidity typically results in a "hard-to-cook" (HTC) phenomenon. This defect is characterized by extended cooking time for cotyledon softening (a failure of cells to separate). HTC beans present: 1) an energy problem during preparation, 2) inferior nutritional qualities, and 3) poor acceptance by consumers (Stanley and Aguilera, 1985).

Several hypotheses have been proposed to explain the cause of bean hardening - 1) lipid oxidation and/or polymerization (Morris and Wood, 1956; Muneta, 1964; and Takayama et al., 1965); 2) phytin catabolism, and pectin demethylation with subsequent formation of insoluble pectate (Mattson, 1946; Jones and Boulter, 1983 a & b; Moscoso et al., 1984; and Vindiola et al., 1986); 3) autolysis of cytoplasmic organelles, weakening plasmalemma integrity and lignification of middle lamella (Varriano-Marston and Jackson, 1981); and 4) interactions of proteins and polyphenols and polymerization of polyphenolic compounds (Rozo, 1982). Recently, Hincks and Stanley (1986) proposed a multiple mechanism of bean hardening which included phytate loss as a minor contributor during initial storage and phenol metabolism as a major contributor during extended storage.

Phenolic acids and their derivatives are widely distributed in plants (Krygier et al., 1982). These acids impose significant influences on both plant physiology and ecology, for example, plant growth regulators, germination inhibitors, antimicrobial agents, feeding deterrents and pigments (Harborne, 1980). Phenolic compounds in plant tissues can be present in either free (extractable) and/or bound (both extractable and non-extractable) forms. Analysis of changes in total phenolic content of plant tissues must involve determining the content of all forms of phenolics.

The objective of this study was to determine whether changes in specific phenolic components (both free and bound forms) and lignin in the seed coat and cotyledon of navy beans held under various storage conditions correlate with development of the HTC phenomenon.

Experimental Plan

The experiment was conducted using a completely randomized design with two replications. The schematic plan to study the effect of storage conditions on changes of phenolic constituents and lignin content in navy bean is illustrated in Figure 3.

Materials and Methods

Materials

Navy beans (*Phaseolus vulgaris*, var. Seafarer) were provided by the Dept. of Crop & Soil Science, Michigan State University. The beans were field dried to final moisture content of 13%.

Enzymes used for preparing starch and protein free cell wall fractions were papain (30,000 FCC, No. 3658), fungal protease (60,000, No. F6886E2) from Miles Laboratories, Inc., Elkhart, IN and amyloglucosidase (A-7255) from Sigma Chemical Co., St. Louis, MO.

Standard phenolic acids (Sigma Chemical Co.) used for qualitative and quantitative TLC were ferulic, p-hydroxybenzoic, caffeic, chlorogenic, cinnamic, p-coumaric, sinapic, syringic, and vanillic acids.

Thioglycolic acid (Sigma Chemical Co.) was used for lignin determination.

Methods

Storage Conditions

Dry beans were stored for 9 months under 3 conditions to induce different degrees of the HTC phenomenon as follows: 5°C/40% RH (Control), 20°C/73% RH (Partially Hard) and 35°C/80% RH (Hard) (Figure 3). Selected saturated salt saturated salt

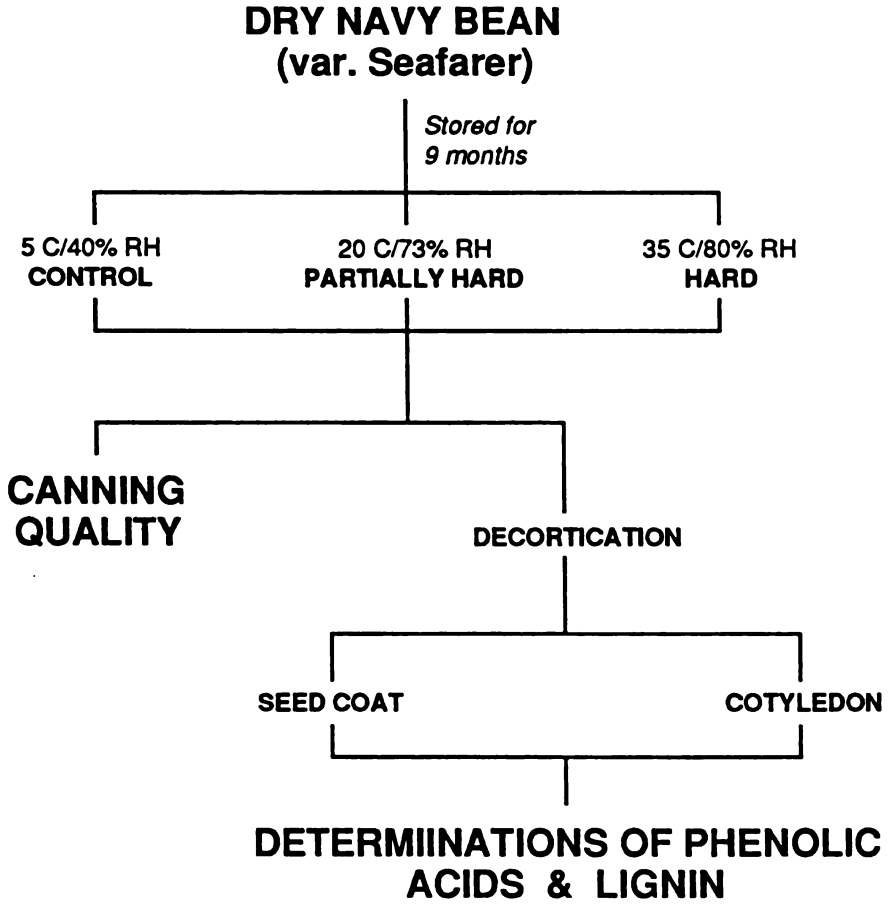

Figure 3. Schematic Plan for the Study on Storage Induced Changes of Phenolic Acids and the Hard-to-cook Development

solutions: K_2CO_3, NaCl and $[NH_4]_2SO_4$ was prepared to produce 40%, 73% and 80% RH, respectively. A standard large size desiccator (250mm inner diameter) was the container of control for storing the beans. After 9 month storage, the moisture content of control, partially hard and hard beans were determined using a Motomco moisture meter (Motomco, Inc., Clark, NJ) to be 10, 14 and 18% respectively.

Canning and Evaluation

After a 9 month storage period, 100g (dry basis, db) beans were soaked in distilled water, filled into 303x406 cans, covered with boiling brine (brine formula: distilled water 9.1 kg + sucrose 142.0 g + salt 113.4 g) and thermally processed at 116°C for 45 min. After thermal processing, cans were uniformly cooled to 38°C under cold tap water and stored for 3 weeks at room temperature (21°C) before evaluation. The canned beans were tested for drained weight, color (Hunter Lab Colorimeter, Hunter Associates, Fairfax, VA.), texture and percentage of dry solids. Firmness was measured by using a Kramer Shear Press (Food Technology Corp., Reston, VA.) and expressed as peak force (kg) per 100 g sample. The details of canning and evaluation procedure were previously described by Hosfield and Uebersax (1980).

Sample Preparation

Sample seeds were soaked in cold (4°C) distilled water for 45 min. prior to manual decortication. Subsequently, the seed coats and cotyledons were freeze-dried in the Unitrap Model II (VirTis Co., NY) freeze drier. The samples were ground before the following phenolic acid extraction and purification procedure.

Extraction and Purification

Isolation procedure for phenolic acids was modified from the methods described by Krygier et al. (1982) and Fry (1983). The detailed extraction and purification procedure for phenolic acids and lignin is shown in Figure 4. One gram of seed coat or 3 g of cotyledon was extracted 3 times with 25 mL hexane. Since phenolic acid esters of lipid are known to be present in some plant tissues (Harborne, 1980), the hexane extract was

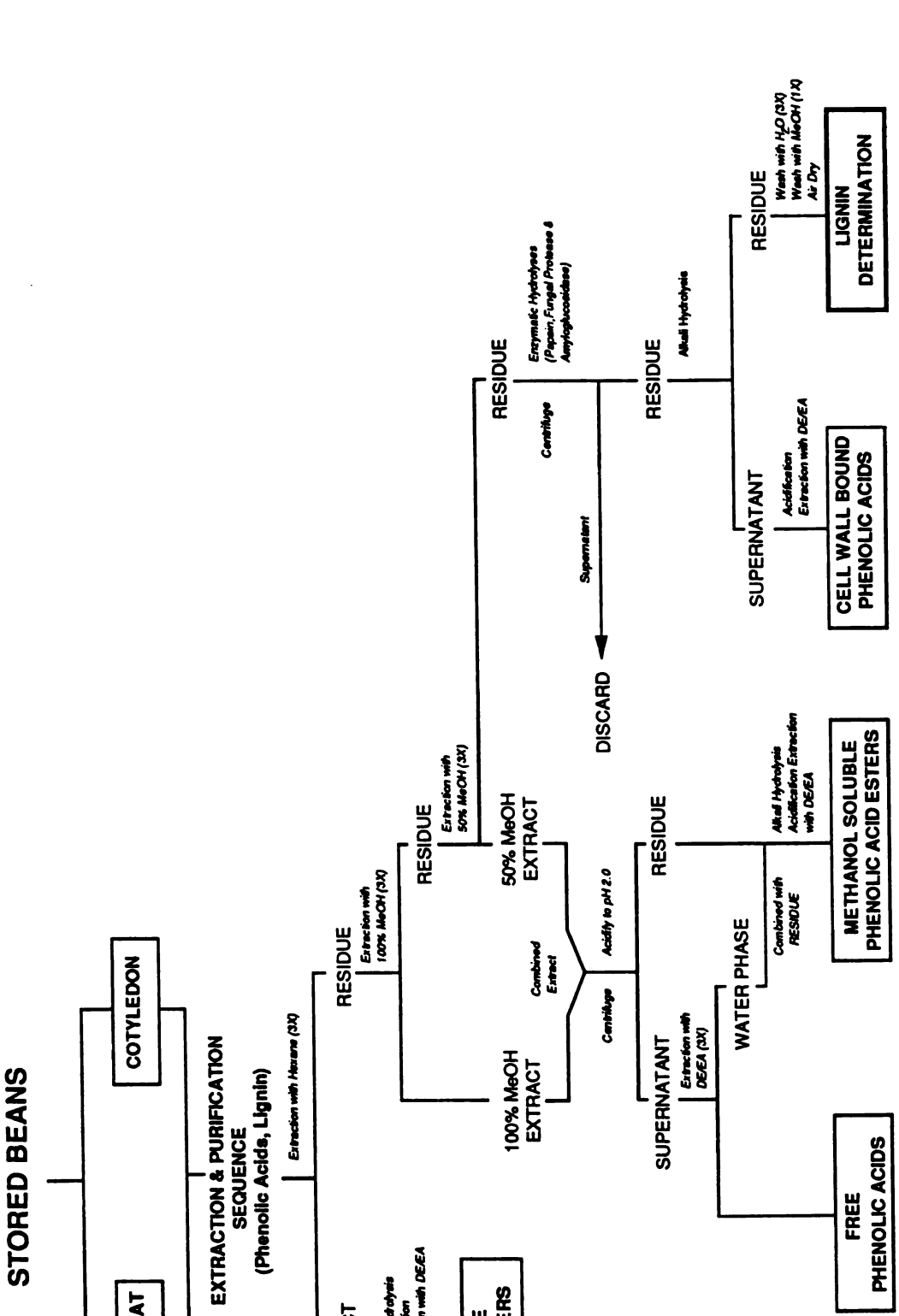

Figure 4. Extraction and Purification for Phenolic Acid and Lignin Analyses

evaporated and hydrolyzed with 20 mL 0.5 N NaOH (degassed) at 20°C for 16 hr under N_2 in the dark. After alkaline hydrolysis, the pH was adjusted to 2.0 with HCl and extracted (3X) with diethyl ether/ethyl acetate (DE/EA, 1:1, v/v) at a solvent to H_2O phase ratio of 1:1 (v/v). The DE/EA extracts were dehydrated with anhydrous sodium sulfate, filtered, and evaporated to dryness and redissolved in 1 mL methanol. This fraction represents phenolic acid esters of hexane soluble compounds. The hexane-extracted seed coat or cotyledon residues were then extracted with 25 mL absolute methanol (3X) followed by 25 mL 50% methanol (3X). The methanolic and aqueous methanolic extracts were combined, evaporated under vacuum at 45°C to approximately 20 mL and then transferred to a 50 mL graduated conical centrifuge tube. The 20 mL aqueous suspension was adjusted to pH 2.0 and centrifuged (1,000 g, 15 min) to seperate a cloudy precipitate. The supernatant obtained from this step was extracted (3X) with hexane (1:1, v/v) to remove free fatty acids and other lipid contaminants not previously extracted. The aqueous phase was then extracted (3X) with DE/EA (1:1), dehydrated, filtered, evaporated, and redissolved as described earlier to yield the free phenolic acid fraction. The cloudy precipitate was combined with the remaining aqueous phase, hydrolysed with 0.5 N NaOH and extracted with DE/EA (1:1, 3X), dehydrated, filtered, evaporated and redissolved as described above. This solution represented the phenolic acids liberated from methanol-soluble phenolic acid esters (presumably esters of sugars or other polar compounds). The residue from methanol extraction was used to prepare cell walls free from starch and soluble proteins using a series of enzymatic hydrolyses. Proteolytic hydrolysis was done with an enzyme-to-protein ratio of 1:10 (w/w) with papain (pH 4.5, 70°C) for 21 hr followed by fungal protease (pH 6.5, 70°C) for 21 hr. The protein content of the tissue was determined by a micro-Kjeldhal method of AACC (1983). Starch was hydrolyzed by amyloglucosidase (22,400 units) for 24 hr at pH 4.5, 55°C. After enzymatic hydrolysis, the indigestible residues collected by centrifugation were washed with distilled water and methanol to obtain the cell wall free from starch and protein. Cell wall bound phenolic acid

fraction was prepared by hydrolyzing the starch-and protein-free cell wall in 0.5N NaOH for 16 hr., acidification, and extraction with DE/EA (1:1). The residue from alkaline hydrolysis of cell wall was washed with distilled water and methanol, then air dried for lignin analysis.

Thin Layer Chromatography

Two types of TLC absorbents: 1) silica gel and 2) cellulose, were used in this study. The solvent systems employed for separation of phenolic acids were: 1) $CHCl_3$:CH_3COOH:H_2O (4:1:1), lower phase; 2) Toluene:CH_3COOH (9:1); and 3) 5% CH_3COOH. Phenolic acids were located by fluorescence under 254 and 366 nm UV light before and after fuming with NH_3 vapor, by staining with I_2 vapor, or by their staining with Folin and Ciocalteu's phenol reagent followed by NH_3 vapor. The specific phenolic acids were quantitated from the phenol reagent sprayed chromatograms by densitometry (SHIMADZU Dual-Wavelength Thin layer Chromatoscanner, Model CS-930, Kyoto, Japan). Ferulic, p-coumaric and sinapic acids were identified by comparison of Rf values, fluorescence properties, and ultraviolet spectra with known standards (Table 1)

Lignin Determination

Relative lignin content was determined by the thioglycolic acid procedure (Hammerschmidt, 1984) and expressed as absorbance at 280 nm. Tissues previously extracted with hexane, absolute methanol, 50% methanol and 0.5N NaOH were ground. Approximately 200 mg (db) seed coat or 500 mg (db) cotyledon were derivatized with 10% thioglycolic acid in 2N HCl. After derivatization, the residue was collected by contrifugation and washed with distilled water. The produced ligninthioglycolate was solubilized in 2.5 mL 0.5N NaOH. The soluble material was precipitated with 8 mL conc. HCl and then collected by centrifugation. The final solution was centifuged to remove any insoluble materials prior to measuring the absorbance at 280 nm.

Table 1. TLC Characteristics of Standard Phenolic Acids Used in Qualitative and Quantitative Analyses

Phenolic Acid	Rf Value			Visual Characteristics			
				UV Light		Iodine	Phenol Reagent
	$C_6H_5CH_3$ CH_3COOH	$CHCl_3$ CH_3COOH H_2O	5% CH_3COOH	254nm	366nm	Vapor	NH_3 Vapor
Ferulic	0.39	0.68	0.40	Blue Bright	Blue	Dark Brown	Purple
p-Hydroxy benzoic	0.27	0.50	0.68	Dark Purple	Invisible	Light Brown	Purple
Caffeic	0.10	0.29	0.35	Brown	Brown	Dark Brown	Purple
Chlorogenic	0.00	0.00	0.61	Greenish-Blue	Greenish-Blue	Dark Brown	Purple
Cinnamic	0.52	0.82	0.65	Purple	Invisible	PaleYellow	Invisible
p-Coumaric	0.26	0.53	0.46	Purple	Brownish	Green	Purple
Sinapic	0.29	0.68	0.34	Greenish-Blue	Greenish-Blue	Dark Brown	Purple
Syringic	0.32	0.70	0.61	Purple	Invisible	Red Purple	Purple
Vanillic	0.42	0.69	0.67	Purple	Invisible	DarkBrown	Purple

Statistical Analysis

Bean canning quality was performed on duplicate cans (n=2). Phenolic acid and lignin contents of dry beans were determined from samples with two determinations for each replicate (n=2). All data were analyzed using analysis of variance and Tukey mean separation (MSTAT, 1986).

Results and Discussion

Canning Quality of HTC Beans

Changes in canning quality of beans stored to produce different degrees of HTC are presented in Table 2. The soaked weight of partially hard bean is significantly higher than those of control and hard beans, but these slight differences are unlikely ascribed to HTC defect. Varriano-Marston and Jackson (1981) using autoradiography to follow the mode of water penetration into beans also found similar water absorption for aged and fresh black beans. After thermal processing, however, the hardened seeds showed a decrease ($P<0.05$) in drained weight which was confirmed by an increase ($P<0.05$) in percentage of dry solid. Increasing degree of hardness resulted in undesirable darker color of canned products, as shown by the decreases of L (whiteness) and b_L (yellowness) values but increase of a_L (redness) value. Burr et al. (1968) and Garruti and Bourne (1985) attributed the darker color in beans stored at high temperature and humidity to polymerization of phenolic compounds.

The most noticeable undesirable quality of canned hard-to-cook beans was the textural defect. As shown in Table 2, the hard beans required approximately 4.5 times the shear force compared to the control beans. In addition, we observed a larger texture difference (shear values) between partially hard and hard-to-cook beans than between control and partially hard beans. These findings are similar to previous works of Stanley and Aguilera (1985), Hinck and Stanley (1986) and Hohlberg and Stanley (1987). For example, Hohlberg and Stanley (1987) stored black beans (*P. vulgaris*) for 10 months

Table 2. Canned Bean Quality Attributes of Navy Beans Stored under Selected Conditions for 9 Months[1]

Storage Condition (°C/% RH)	Bean Category	Soaked weight (g)	Color			Drained weight (g)	Shear force (kg/100 g)	% Dried solid
			L	a_L	b_L			
5/40	Control	211.0a	52.4c	-4.9a	15.2b	301.0c	51.0a	29.4a
20/73	Partially Hard	218.5b	51.8b	-4.8a	15.1b	285.4b	58.5b	29.6a
35/80	Hard	212.6a	34.0a	0.01b	13.4a	234.2a	237.9c	36.5b

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

under 3 environmental conditions: high temperature/ humidity (HTHH:30°C, 85% RH); medium temperature/ humidity (MTMH:25°C, 65% RH) and low temperature/humidity (LTLH:15°C, 35% RH). They reported the cooked bean texture (hardness value) as follows: 20, 25, and 52 kg/30g for LTLH, MTMH and HTHH, respectively. At present, there is no single theory to fully explain this complex HTC phenomenon. Based on light microscopy and scanning electron microscopy this textural defect was proposed to be due to a failure of cotyledon cell separation during heating (Rockland and Jones, 1974; Varriano-Marston and Jackson, 1981 and Jones and Boulter, 1983 a). The presence of intact middle lamella may restrict further water absorption during canning and hence, produce a lower drained weight. Hard-to-cook beans stored at 35°C/80% RH produced the most undesirable canned bean characteristics, while the partially hard (20°C/73% RH) gave only slight significant ($P<0.05$) changes in overall bean quality (firmness, drained weight and color) compared to control beans (5°C/40% RH).

Phenolic Acid and Lignin Analyses of Stored Beans

Many authors (Morris et al., 1950; Bourne, 1967; Variano-Marston and Jackson, 1981; Jones and Boulter, 1983 a & b and Hincks and Stanley, 1986) attribute the hard-to-cook property to cotyledonary defects; however, the seed coat can play an important role as well. The reported shear values (Table 2.) are the composite textural values of 100 gm processed whole beans. The effect of bean seed coats on the textural profile of cooked beans (especially shear force component) has been previously reported by Binder and Rockland (1964). Thus, changes in hydroxycinnamic acid contents of seed coats and cotyledons were independently evaluated in this study. After 9 month storage, stored beans were soaked in 4°C distilled water for 45 minutes prior to manual decortication. Most bean seed coats were easily removed, especially the hard and partially hard beans. The soak water was not analyzed for phenolics, since phenolic acids are only slightly soluble in cold water. In addition, all stored beans were treated under the same condition. Thus, the relative differences or relationships would be similar. Phenolic acid contents of

control and storage-induced HTC bean seed coats and cotyledons are presented in Table 3. The hexane-soluble fraction of seed coat and cotyledon contained relatively small amounts of compounds that yielded ferulic acid upon alkaline hydrolysis. The concentration of hexane-soluble ferulic acid esters in seed coats was higher ($P<0.05$) for hard beans than for partially hard beans. No hexane-soluble ferulic acid esters were found in the control beans. Hexane-soluble ferulic acid esters in cotyledons were detected only in the hard beans at low levels. The release of ferulic acid from hexane-extracted compounds upon alkali hydrolysis suggested that ferulic acid was conjugated with lipids and/or waxes. Lipid-soluble phenols have been reported in the coating material of plant leaves (Harborne, 1980). Also, Takata and Scheuer (1976) reported the presence of monoglyceride esters of the common hydroxycinnamic acid, caffeic acid, in oat seeds.

The distribution of seed coat and cotyledon hydroxycinnamic acids esters obtained from the methanol soluble fraction is also presented in Table 3. By employing separation with two dimensional Thin Layer Chromatography (TLC:Cellulose plate, toluene: CH_3COOH-solvent first direction, 5% CH_3COOH-solvent second direction), many different phenolic acids were detected. However, since ferulic, sinapic and p-coumaric acids were quantitatively important, only these phenolic compounds were measured. Phenolic acid ester contents of seed coats were much greater ($P<0.05$) in hard beans than in partially hard or control beans. The ferulic acid content of seed coats from hard beans was double that of the partially hard and more than 17 times that of the control. However, the opposite trend was observed in the cotyledon portion of the same fraction (approximately 50% decrease in phenolic acid esters of hard beans compared to that of control). Partially hard seed; however, showed a non-significant decrease in these phenolic compounds to control beans. The differences in changes of phenolic content in this fraction of seed coat and cotyledon may be due to the different function of these two tissues. Seed coats are recognized as protective tissues whereas cotyledons are storage tissues.

Table 3. Phenolic Aicd Content (μg/g, db) in Control, Partially Hard and Hard Bean Components[1]

Fraction	Bean Category	Seed Coat			Cotyledon		
		Ferulic	Sinapic	p-Coumaric	Ferulic	Sinapic	p-Coumaric
Hexane Soluble Phenolic Acid Esters	Control	ND[2]a	NDa	NDa	NDa	NDa	NDa
	Partially Hard	1.3 ± 0.3b	NDa	NDa	NDa	NDa	NDa
	Hard	2.3 ± 0.2c	NDa	NDa	0.9 ± 0.1b	NDa	NDa
Methanol Soluble Phenolic Acid Esters	Control	25.9 ± 5.7a	5.8 ± 2.3a	NDa	299.3 ± 5.1b	137.7 ± 4.2c	34.2 ± 1.0b
	Partially Hard	242.0 ± 20.1b	32.4 ± 5.8b	30.8 ± 3.6b	289.0 ± 5.7b	104.1 ± 4.7b	25.5 ± 2.8ab
	Hard	458.6 ± 22.3c	173.8 ± 3.4c	106.5 ± 3.8c	147.4 ± 6.8a	53.8 ± 2.7a	19.6 ± 1.8a
Free Phenolic Acids	Control	NDa	NDa	NDa	8.4 ± 0.7a	5.3 ± 0.5a	5.7 ± 1.4a
	Partially Hard	23.1 ± 2.2b	7.5 ± 1.2b	22.8 ± 2.2b	46.6 ± 3.0b	8.5 ± 1.4ab	9.4 ± 1.5a
	Hard	86.3 ± 2.0c	28.0 ± 6.1c	28.9 ± 2.9b	64.8 ± 3.0c	14.4 ± 2.5b	26.5 ± 1.4b
Cell Wall Bound Phenolic Acids	Control	36.2 ± 1.7a	0.7 ± 0.2a	NDa	15.9 ± 2.1a	1.2 ± 0.6a	NDa
	Partially Hard	65.2 ± 3.2b	5.7 ± 0.6c	NDa	18.0 ± 3.6a	1.1 ± 0.3a	NDa
	Hard	68.2 ± 7.2b	3.2 ± 0.3b	NDa	18.3 ± 2.1a	1.6 ± 0.2a	NDa

[1] Means in a column within a fraction followed by different letters are significantly different ($p<0.05$).
[2] ND = Not detectable

The higher degree of hardness was related with increases ($P<0.05$) in free phenolic acid content (Table 3) of both seed coat and cotyledon. Phenolic compounds are normally present in bound or conjugated forms in living plant tissue (Harbourne, 1980). Increasing the concentration of free phenolic acids in hard beans appears to be induced by the adverse storage conditions. Phenolic acids could be liberated form bound phenolic derivatives (eg. methanol soluble phenolic acid esters) and/or synthesized de novo. Aromatic amino acids such as phenylalanine and tyrosine are known as immediate precursors of hydroxycinnamic acids (C6-C3 molecules) biosynthesis via phenylalanine and tyrosine ammonia lyases (Koukol and Conn, 1961). Hohlberg and Stanley (1987) observed significant increases in low molecular weight protein and free aromatic amino acid in hard-to-cook beans.

Free ferulic acid is known to inhibit seed germination in sugar beets and cereal seeds (Van Sumere et al., 1972). This may explain, in part, the loss of seed viability in beans stored at high temperatures and humidity (Villiers, 1972; Hallam et al., 1973 and Varriano-Marston and Jackson, 1981). In addition, since free phenolic acids have high affinity for interacting with protein (Harbourne, 1980), protein-phenol interaction may be related to the loss of granularity of protein bodies found in cotyledon tissue of aged bean (Varriano-Marston and Jackson, 1981). This protein-phenol interaction may increase protein hydrophobicity through cross-linking, resulting in the subsequent decreased seed hydration during cooking, thus aggravating the HTC defect.

The amounts of hydroxycinnamic acids released from cell walls by alkaline hydrolysis are given in Table 3. The two major phenolic acids found in this fraction are ferulic and sinapic acids. Ferulic acid content was similar in partially hard and hard bean seed coats, but was greater ($P<0.05$) in both than in the control beans. In the cotyledon, the ferulic acid contents of partially hard and hard beans were similar, and not significantly higher than that of control beans. Ferulic and sinapic acids are thought to be esterified to cell wall polysaccharides because of their release from cell wall on treatment with alkali. Fry (1983) presented evidence that ferulic acid was esterified to the non-reducing terminus

of unbranded arabinose chains. Feruloyl pectins may have a possible role in regulation of cell expansion, in disease resistance, and in the initiation of lignification (Fry, 1983). It was suggested that the peroxidase-catalyzed coupling of pectin-bound phenols (e.g. diferulic acid formation) would increase the tendency of the pectins to bind tightly in the cell wall (Fry 1982 & 1986). This may explain the work of Jones & Boulter (1983 a) who sequentially extracted pectins from hard and soft (control) beans with different solvents: cold water, hot water, 0.5% ammonium oxalate (chelating solvent) and 0.05 N NaOH solution. They observed a decrease in hot water and ammonium oxalate soluble pectins in hard beans with a concomitant increase in insoluble pectin and NaOH soluble pectin. An extensive increase in NaOH soluble pectin indicated that phenolic compounds are esterified to pectins, with possible phenolic cross-linking between the pectins (Fry 1986). Formation of insoluble pectin, however, may also be due to a complex physical entanglement of pectin with extensin, a type of hydroxyproline-rich glycoprotein, in plant cell walls. Increased cross-linking of cell wall components may also occur via the peroxidase coupling of tyrosine units in extensin molecules to form a diphenyl-ether bridge, isodityrosine, which is resistant to alkaline hydrolysis (Fry, 1986). The decrease in pectin solubility of HTC beans is correlated with a decrease in cell separation which caused textural defects (Rockland and Jones, 1974; Jones and Boulter, 1983 a & b and Moscoso et al., 1984).

Greater changes in phenolic acid constituents occurred in seed coats than in cotyledons. This is probably due to the direct exposure of the protective type of tissues (seed coat) in adverse storage environments. In addition, seed coats and pericarps of many plant species contain higher levels of extensin (Cassab et al., 1985) which is presumably capable of being oxidatively coupled with polysaccharide bound phenols via tyrosine residues (Neukom and Markwalder, 1978).

The comparison of relative lignification of bean components (seed coat and cotyledon) is shown in Figure 5. Lignin content of both seed coat and cotyledon remained unchanged, even though beans were stored at an extremely adverse condition (35°C/80%

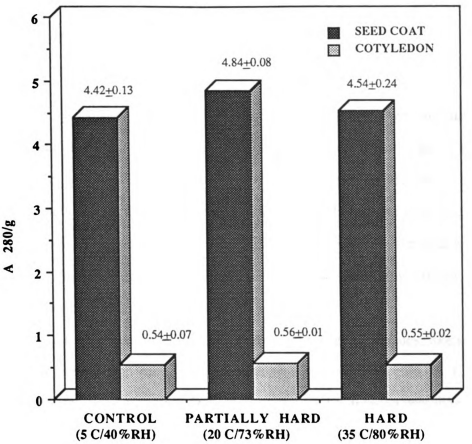

Figure 5. Comparison of Relative Lignification of Bean Components (Seed Coat and Cotyledon)

RH for 9 mo.) and developed the HTC phenomenon. As expected, seed coat lignin content is much greater (8X, $P<0.05$) than cotyledonary lignin. These data suggest that enhanced lignification is not a major cause of HTC beans as proposed by Muller, 1967; Varriano-Marston and Jackson, 1981 and Hinck and Stanley, 1986.

Summary and Conclusions

The major hydroxycinnamic acids found in stored navy beans were ferulic, sinapic and p-coumaric acid. Storage for 9 months under high temperatures and humidities resulted in an increase in phenolic acid content (both free and bound forms) with the exception of the methanol soluble phenolic ester and cell wall bound phenolic acid contents of cotyledon fraction. The development of HTC defects was best associated with large increases in free hydroxycinnamic acids. No significant changes in lignin content of either seed coat or cotyledon were detected.

Increases in the hydroxycinnamic acid content of protective tissues, like seed coat, leads to discoloration of bean seed coat and increased seed coat toughness. Liberation and/or synthesis of free phenolic acids in both seed coat and cotyledon provides phenolic compounds for cross-linking to pectin in middle lamella, and/or proteins, which could result in bean textural defects as well as decreased seed viability.

CHAPTER 2 INTERRELATIONSHIPS BETWEEN PHYSICO-CHEMICAL CHARACTERISTICS AND CANNED BEAN 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 bean can result from a better understanding of how key physico-chemical factors under defined processing condition govern product qualities and the ability to modify these key factors and/or processing parameters to achieve a final product which processor and consumer need. Further, key physico-chemical characteristics can be used as criteria in screening breeding materials. Thus, the primary objectives of this research were to identify and establish the interrelationships of selected dry bean chemical factors (starch, cell wall components and phenolic acid constituents) and their functional properties contributing to product quality.

Experimental Plan

The Michigan State University Dry Bean Breeding Program (CSS & FSHN) and the Michigan Dry Edible Bean Research Advisory Board agronomist (Gregory V. Varner, Director) have shown significant variability among bean cultivars and breeding lines for canning quality. Four navy bean cultivars: C-20 (commercial standard), Seafarer (commercial standard), Fleetwood (firm) and Experimental line 84004 (soft) were selected as model cultivars for the following studies due to their differences in textural characteristics under identical production sites and practices. Selected bean cultivars were obtained from the Cooperative Elevator Company during 1987 crop year. 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°C. The focus of this research was stated as a null hypothesis (H_o): canned bean quality is not related to physico-chemical characteristics. The experimental design is outlined in Figure 6. Study I was designed to address the canning quality differences of the four model cultivars. Study II was designed to study the physical properties and proximate chemical compositions of whole bean flours produced from these cultivars. Study III emphasized bean starch characteristics and Study IV determined cell wall constituents and phenolic acid content of the model cultivars. Inter-relationships between key canning quality and bean physico-chemical factors would be established from the data from all four studies.

Materials and Methods

In Study I, II, and III, 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) with 20 mesh screen to 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 obtained seed coat and cotyledon were frozen in liquid N_2 and subsequently freeze-dried and ground with a Udy Cyclone Mill with 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 explained in each study. All experiments and physico-chemical analyses were replicated three times, unless stated otherwise. When appropriate, data were analyzed using analysis of variance, Tukey mean separation and Least square regression correlation (StatView II, 1984).

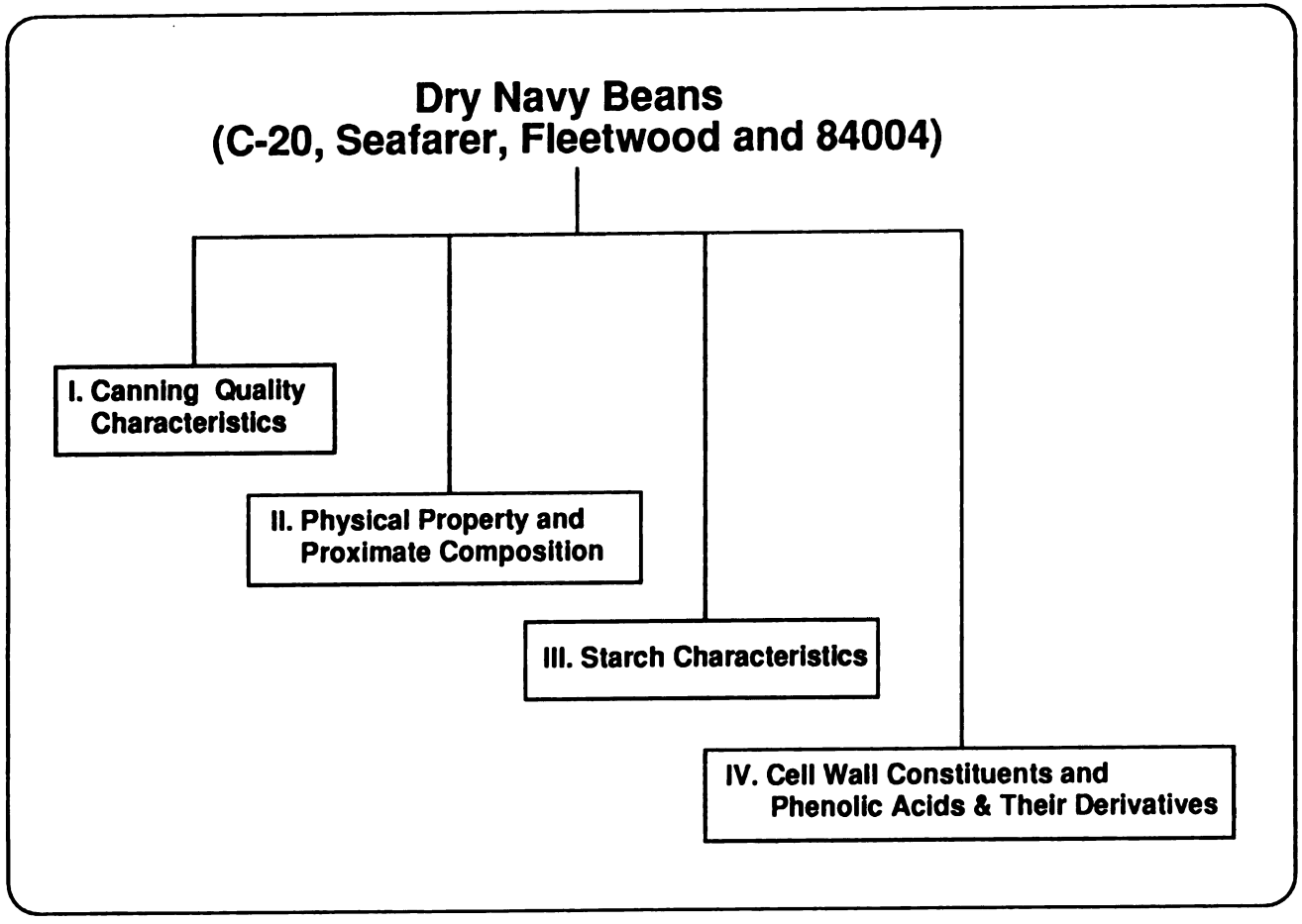

Figure 6. Schematic Plan for Chapter II: Interrelationship between Physico-Chemical Characteristics and Canning Quality of Four Seclected Navy Bean Cultivars

Study I: 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 a 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 303x406 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 116°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. 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 water content of canned beans (final moisture percentage) was determined from the 50 g texture samples. These were oven dried at 80°C until the weight remained constant.

Study II: Physical Property and Proximate Composition

Physical Properties

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 hr, cooled in a desiccator, and then weighed to determine % seed coat (w/w) as follows:

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

Pasting characteristics of whole bean flour. Pasting characteristics of the whole flour made from these studied bean cultivars were evaluated using the method described by Steffe et al. (1989). A schematic diagram of the apparatus used is illustrated in Figure 7. 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 fully dispersed.

Twelve milliliters of this homogeneous bean flour slurry were pipetted into the Brookfield sample chamber and immediately placed inside the heating/cooling jacket. As the sample chamber was being inserted in the jacket, the pre-installed impeller was introduced into the sample slurry and subsequently turned on at 100 rpm to prevent sample setting. Heating of the slurry sample began within 15 seconds by circulating glycerolglycol from the heating bath through the sample heating/cooling jacket. Time to reach maximum temperature (94.0 $\pm$ 1.0°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°C coolant. This

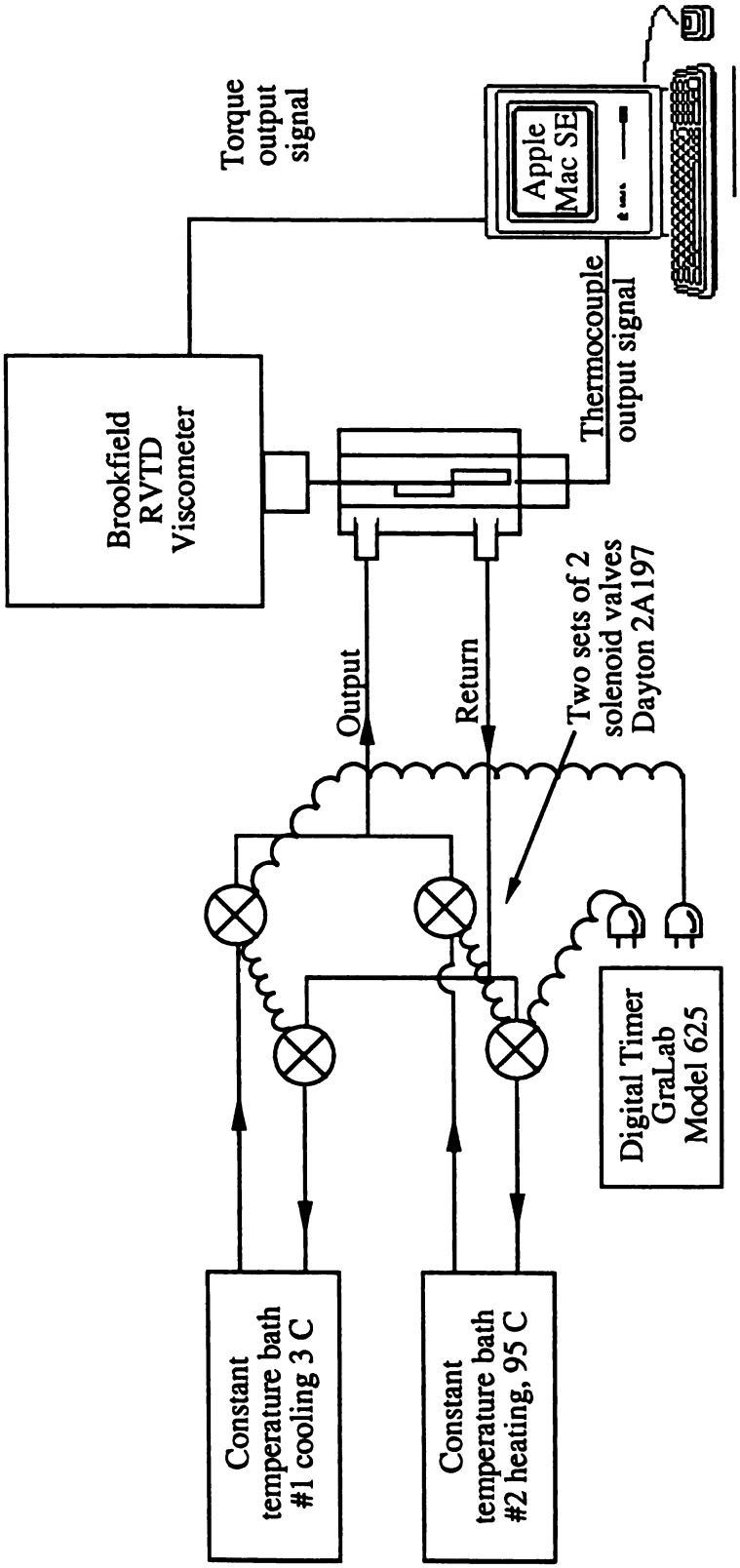

Figure 7. Schematic Diagram Illustrating Brookfield Vicosmeter Set-up for Pasting Characteristic Analyses of Whole Bean Flour and Starch

analysis was completed and manually terminated when the temperature of sample slurry reached 10°C. The cooling time for each sample varied in the range of ± 1 min.

Gel rheological properties of whole bean flour. The gel rheological characteristics of whole flour were studied and compared among selected bean cultivars. Ten grams of 12% (w/w) flour slurry including 10 ppm Thimerosal (Bacteriocide, Sigma Chemical Co.) were prepared in 14 mm, (I.D.) screw-cap test tubes. These slurries were mixed well, heated in boiling water bath and intermittently mixed (30 seconds interval, 3x) using a vortex mixer at medium high speed. After 15 min heating, the samples were cooled in a 20°C water bath for 2 minutes. The cooked flour gel was then incubated at room temperature for 7 days prior to gel rheological analysis. Rheological characteristics of bean flour gel including an apparent viscosity and an apparent elasticity, were determined using back extrusion technique (Hickson et al., 1982). A cylindrical plunger (8.51 mm diameter) is equipped to the load cell of the Instron Universal Testing Machine. As the testing began, this plunger was set to travel through the gel at a constant speed of 100 mm/min. The force required to drive the plunger is registered by a load cell connected to a Hewlett Packard data acquisition and a computer system. The detail procedure and calculation was essentially the same as described by Hickson et al. (1982).

Proximate Composition

Moisture. Approximately 4g of well-mixed whole bean flour was accurately weighed into pre-weighed crucibles and dried to a constant weight at 80°C (ca 8 hr) in partial vacuum having pressure equivalent to 25 mm Hg. Percentage of moisture was determined from the weight loss on the fresh weight basis (AACC Method 44-40):

$$\% \text{ Moisture} = \text{loss in moisture (g)/initial wt. of sample (g)} \times 100\%$$

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 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):

% Ash = wt. of residue (g)/dry wt. of sample (g) x 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 the digestion was completed (approximately 5 hr). The protein content was determined on a dry basis using a nitrogen conversion factor of 6.25.

% Total protein = % Total Nitrogen x 6.25

Fat. Approximately 3g of bean flour were dried in vacuum over 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 then calculated on dry weight basis (AACC Method 30-25):

% Crude fat or ether extract = wt. of fat (g)/dry wt. of sample (g) x 100%

Total starch. The starch content was quantitatively determined using an enzymatic method. Approximately 150 mg of whole 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 hour in a boiling water bath to completely solubilize the starch. After heating, the sample 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 shaking water bath maintaining at 55°C for 24 hr. 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 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 dietary fiber (TDF). The TDF content was determined by the method of Prosky et al. (1984) and JAOAC (1985) using total dietary fiber assay kit (TDFAB-1, Sigma Chemical Co.). Because of the low fat content in the whole bean flours, these flour samples can be directly used to analyze for the dry bean TDF contents.

Approximately 1 g of whole bean flour was accurately weighed into 400 mL beaker and 50 mL of phosphate buffer (0.05 M, pH 6.0) was added. After mixing the bean slurry thoroughly, 0.2 mL of heat stable α-amylase solution (No. A-0164) was added to hydrolyse the starch for 30 minutes at 95°C. After this starch hydrolysis, the treated flour slurry was cooled to room temperature and the pH was adjusted to 7.5 ± 0.1 using 0.171 N NaOH with the aid of a pH meter. Protein hydrolysis was carried out by adding 1 mL of protease (No. P-3910) solution (5 mg/mL phosphate buffer) and incubation at 60°C for 30 minutes with continuous agitation. Following protein hydrolysis, the treated whole bean flour slurry was cooled to room temperature and the pH was readjusted to 4.5 with 0.205 M H_3PO_4. Then, 0.3 mL of amyloglucosidase (No. A-9913) was added to further hydrolyse starch at 60°C for 30 min. The fiber residue precipitated with 4 volumes of room temperature ethanol and collected by filtering through pre-weighed crucibles containing celite. The fiber residue was then washed with 78% ethanol (3x), 95% ethanol (2x) and acetone (2x). Crucibles containing the residue were dried overnight in 70°C

vacuum oven, cooled to room temperature in a desiccator and weighed to determine total weight. The sample residue weight is obtained by subtracting the original celite and crucible weight from the total weight. Along with whole bean flour sample, 2 blanks (without flour sample) were also performed throughout the entire TDF analysis procedure and any recovered blank residue weight will be used as a correction factor. TDF analysis for each whole bean flour sample and blank was replicated four times. Two replicates were used to analyze for protein using Kjeldahl method (AOAC 24.038) with 6.25 as the conversion factor. The other two replicate samples were analyzed for ash content (525°C, 5 hr). The calculation for TDF percentage is shown as follows.

$$\text{BLANK} = \text{BR (mg)} - \left[\frac{(\%\ \text{Protein in BR} + \%\ \text{Ash in BR})}{100} \times \text{BR (mg)}\right]$$

$$\%\ \text{TDF} = 100 \times \frac{\text{SR (mg)} - \left[\frac{(\%\ \text{Protein in SR} + \%\ \text{Ash in SR})}{100} \times \text{SR (mg)}\right] - \text{BR (mg)}}{\text{Initial Dry Whole Bean Flour Sample Weight (mg)}}$$

Where : BR = Blank Residue SR = Sample Residue

Study III: Starch Characteristics

Starch Isolation

Bean starches were isolated from the selected navy bean cultivars by the method of Naivikul and D'Appolonia (1979) with some minor modifications. The whole bean flour (500g) was extracted with 1.5 liter of 0.016N NaOH by mixing in a Waring Blender for 2 min. Water-soluble material was removed by centrifugation (2000 x g for 20 min). The obtained precipitate was sieved through 60 mesh screen retaining the non-starch components (mainly celluloses), thus yielding crude starch. The crude starch was then washed with deionized-distilled water (3x), 80% ethanol (2x) and deionized-distilled water (2x). The supernatant and floating sludge were removed and discarded after each washing

step. The prime starch was air dried at 40°C for two days and sieved through a 60 mesh screen to obtain a bean starch sample. Commercial corn starch (AGRO R Pure Corn Starch, CPC International Inc. Englewood Cliffs, NJ) was used as a standard for comparison.

Bean Starch Chemical Composition

Proximate analyses. Moisture, ash and protein contents were determined using the previously described standard methods (AACC, 1983 and AOAC, 1984).

Amylose content. The amylose content was determined by the method modified from the improved colorimetric procedure of Morrison and Laignelet (1983). Approximately 80 mg of bean starch sample were accurately weighed into a screw cap test tube. Ten milliliters of urea-dimethylsulphoxide (UDMSO: prepared by mixing 9 volume of dimethylsulphoxide with 1 volume of 6 M urea), were added. This sample tube was then capped, and immediately mixed vigorously with a vortex mixer. After this mixing, the sample tube was placed in a boiling water bath for 90 min with intermittent mixing, until the bean starch-UDMSO solution was transparent and homogeneous (without any gel particulate). The prepared starch-UDMSO solution was cooled to room temperature and a 1 mL aliquot of this solution was weighed into a 100 ml volumetric flask. Three to four portions from 95 mL deionized-distilled water was added into the sample flask with concomitantly well-mixing at each addition. Subsequently, 2 mL I_2-KI solution (2 mg I_2, 20 mg KI/mL) was added and mixed immediately with the content in sample flask. The time required for dilution and color development was always less than 60 seconds. The final sample volume was adjusted to 100 mL. At 15 minutes after I_2-KI addition, the absorbance of sample solution was measured with spectrophotometer at 635 nm using UDMSO-I_2-KI solution as a blank.

Blue Value is defined as the absorbance/cm at 635 nm of 10 mg anhydrous starch in 100 mL dilute I_2-KI solution at 20°C. Blue value was converted into amylose content using the following equation:

Amylose (%) = (28.414 x Blue Value) - 6.218

Bean Starch Physical Properties

Starch swelling power and solubility. Swelling power and solubility of bean starch were carried out in duplication for the temperature range from 70 to 90°C, at 5°C intervals, by using the method of Leach et al. (1959).

Differential scanning calorimetry. Phase transition temperatures of bean starches were studied using Differential Scanning Calorimetry (DSC). Starch with known moisture content was mixed with the appropriate amount of deionized-distilled water to obtain 10% (w/w), and allowed to equilibrate for 1 hr at room temperature before heating. Approximately 10 ± 2 mg of starch solution was transferred to DSC sample pan which was then hermatically sealed. A sample pan was placed on a 910 DCS cell base equipped with a Du Pont Model 990 Thermal Analyzer. An empty sealed pan was used as a reference. Samples were heated from 25° to 120°C at a constant rate of 5°C/min. The instrumental sensitivity was set at 0.02 m cal/s/in and the analysis was performed in a nitrogen atmosphere (50cc/min). The cell calibration coefficient was determined from the standard indium with known enthalpy of fusion. The phase transition temperatures were obtained from the enthalpy (heat flow)-temperature plot. Heat of transition or gelatinization enthalpy (ΔH) were calculated using the following equation:

$$\Delta H = \frac{A \times 60 \times B \times E \times qs}{M \times C}$$

where

A	=	peak area during phase transition
M	=	sample mass (mg)
C	=	sample concentration (%, w/w)
B	=	time base setting (min/in)
E	=	cell calibration coefficient
qs	=	Y - axis range (mcal/sec/in)

Each reported ΔH value is the average from 4 analyses of bean starch solution samples. The gelatinization characteristics of starch are illustrated in the DSC thermogram

(Figure 8) and can be represented by various temperatures: the onset temperature (To); the peak temperature (Tp) and the melting point (Tm) of the most perfect crystallites. The temperatures and the areas taken for calculating ΔH are also shown in Figure 8.

Pasting properties. The method utilized to study the pasting characteristics of bean starch 6% (w/w) was essentially the same as the method previously described in the pasting properties of whole bean flour. Pasting curves of corn and isolated bean (var. Seafarer) starches are shown in Figure 9 and the viscosities are reported for 2 selected time-temperature histories: after 23 min heating, Temp. = 94 $\pm$ 1.0°C and after 2 min cooling, Temp. = 20 $\pm$ 1.0°C.

Degree of syneresis and gel rheological properties. Starch gels (8%, w/w) were prepared according to the method previously described in preparation of whole bean flour gel except that there was no addition of Thimerasol to the gel. The prepared starch gel was incubated at room temperature for 7 and 10 days prior to further analyses. Degree of syneresis (DS) of bean starch was determined by measuring the liquid exuded after a specified storage period. Centrifugation at 2,000 g for 20 minutes was employed to separate the exudate from the starch gel. DS is expressed as the percentage weight loss of the gel after discarding the exudate. After weighing, the gel was then subjected to gel rheological study using the method previously described in gel rheological properties of whole bean flour gel. In addition, Seafarer bean starch was selected to study the starch concentration effect on gel rheological properties. These gels were prepared at 4%, 6%, 8% and 10% (w/w) concentrations and incubated at room temperature for 7 days before further analyses.

Scanning Electron Microscopy

Raw starch. The size and shape of each bean starch was studied using the scanning electron microscope (SEM). Starch samples was dried overnight at 40°C in a vacuum oven, mounted on circular aluminium stubs with adhesive mounting tab and then coated with 20 nm of gold by "Film Vac" Sputter Coated and examined under a Scanning

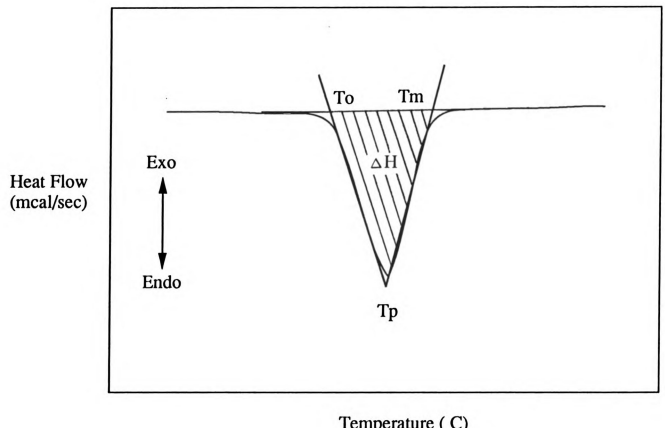

Figure 8. Typical DSC Thermogram for Bean Starch at 10% (w/w) Concentration
(To = Onset Temperature, Tp = Peak Temperature, Tm = Melting Temperature)

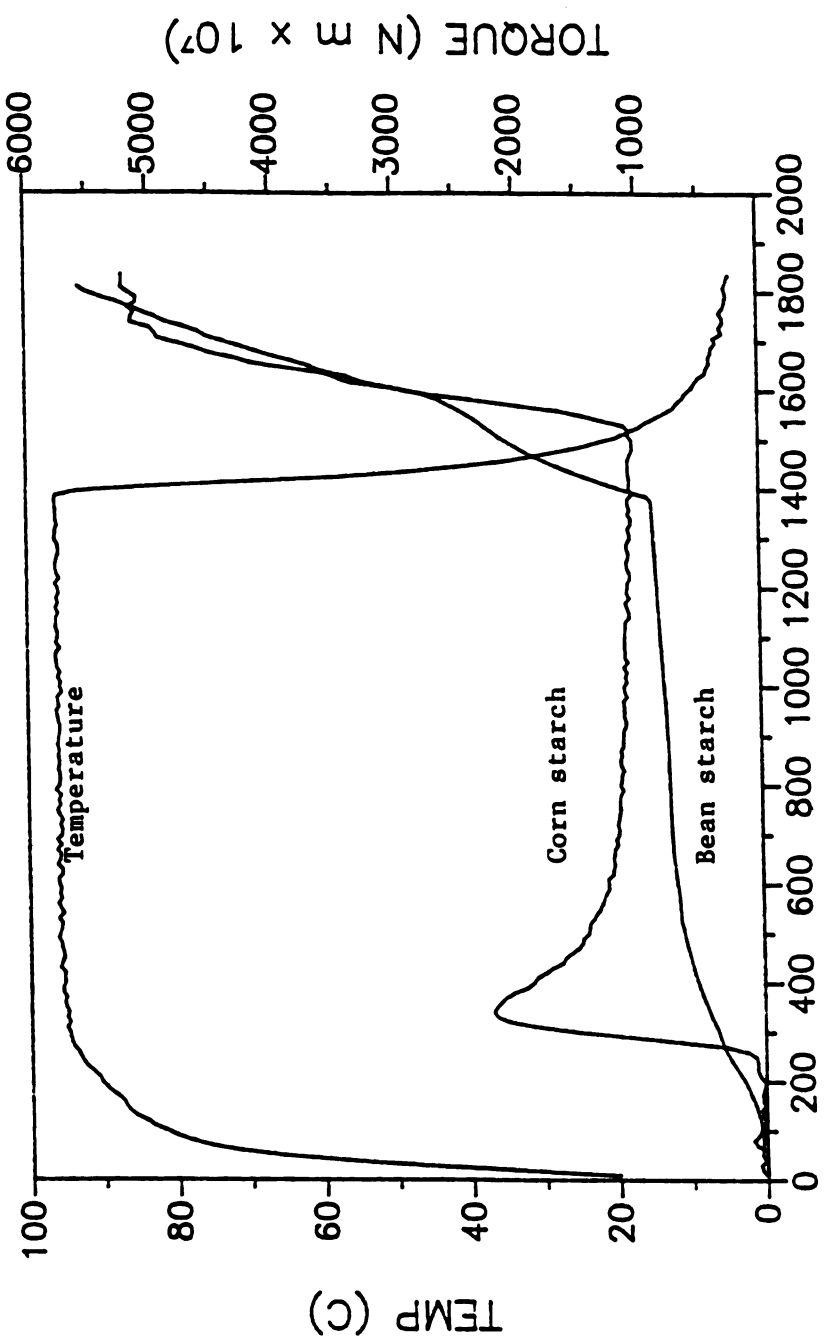

Figure 9. Pasting Curves of Bean (var. Seafarer) and Corn Starches at 6% (w/w) Concentration

Electron Microscope (JEOL Model JSM 35CF, Center for Electron Optics, Michigan State University).

Cooked starch. The changes of starch morphology when it was cooked at 70°, 80°, and 90°C were observed under SEM. Starch slurry (10%, w/w) was prepared in a screw cap test tube, then heated in a temperature-controlled water bath with gentle agitation for 1 hr. After heating, the starch paste from the bottom of the tube was prepared for SEM examination using the method modified from techniques by Pearse (1960 a & b), Bancroft (1975 a, b & c) and Varriano-Marston et al. (1985). This preparation method includes a) rapidly dropping the cooked starch paste in -70°C n-propanol bath (temperature maintained by solidified acetone and liquid N_2), b) transferring the frozen starch droplet into liquid N_2 bath and c) freeze-drying the starch droplet. The dried samples were prepared for viewing under SEM as previously described.

Study IV: Cell Wall Constituents and Phenolic Acids & Their Derivatives

The materials used in the cell wall and phenolic acid study included seed coat and cotyledon flours. Sample preparation was previously described in the Materials and Methods section. Figure 10 presents the protocol for the Study of Cell Walls. Figure 11 illustrates the schematic procedure for cell wall preparation.

Seed Coat and Cotyledon Proximate Composition

The prepared seed coat and cotyledon flour samples were analyzed for moisture, ash, fat, protein and starch using standard methods (AACC, 1983 and AOAC, 1984). The defatted flour samples after fat analysis were collected for further cell wall preparation.

Cell Wall Constituents

Cell Wall Preparation

Seed coat cell wall. The defatted seed coat (1.0g) sample was extracted (3x) with 80% ethanol at 80°C (in water bath) for 30 min. During each extraction, the sample was centrifuged at 17,300g for 20 minutes to obtain the precipitate and the supernatant was

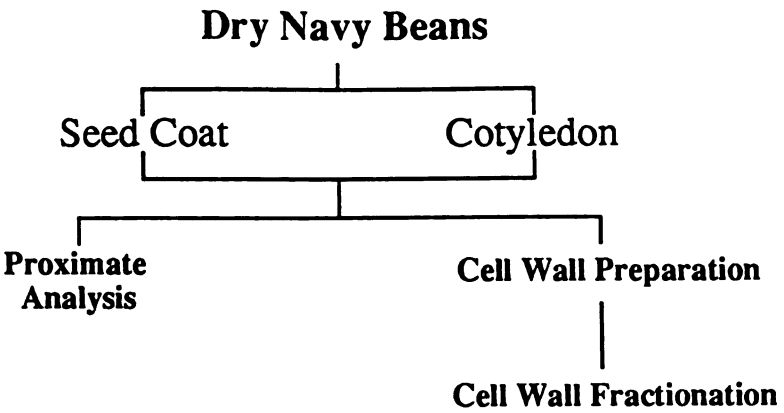

Figure 10. Protocol for Cell Wall Study

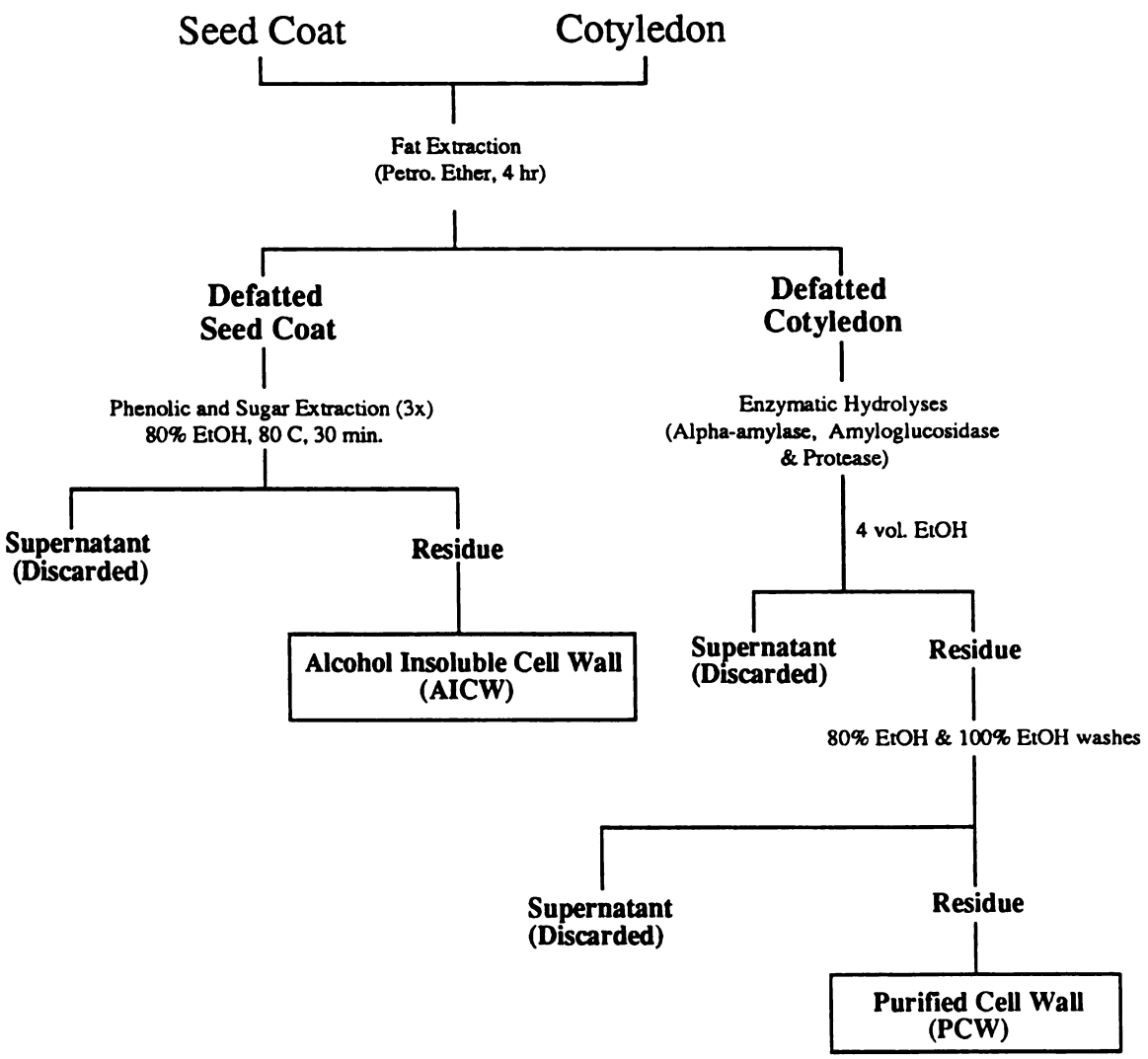

Figure 11. Schematic Procedure for Cell Wall Preparation

discarded. After three ethanol extractions, the recovered precipitate or residue was dried at 70°C in a vacuum oven for 6 hr. This residue is designated as alcohol insoluble cell wall (AICW) and kept for the following cell wall fractionation.

Cotyledon cell wall. Cell wall preparation of bean cotyledon tissues requires additional steps to remove their storage nutrients such as starch and protein. The enzyme hydrolysis used in total dietary fiber (JAOAC 1985) were applied with extended incubation periods as follows: amylase-2 hr; protease-8 hr; and amyloglucosidase-16 hr. Four volumes of ethanol were used to precipitate soluble polysaccharides, and the precipitate recovered by centrifugation at 17,300g for 20 minutes was designated as purified cell wall (PCW). PCW was further washed with 80% ethanol (1x) and absolute ethanol (2x), then dried at 70°C in a vacuum oven for 6 hr. A Blank was also analyzed throughout the entire procedure along with samples and used as a correction factor for any precipitate contributed by reagents. Parts of PCW were analyzed for ash (AACC, 1983) and protein (AOAC 1984) contents to assess its purity, the rest of the PCW was used for the fractionation study. For each flour sample, a total of 7 replicates were performed so that 4 replicates was used for purity tests (n=2, ash and protein analyses) and 3 replicates was used for subsequent cell wall fractionation.

Cell Wall Fractionation

Prepared cell wall materials (AICW and PCW) were fractionated using various salt solutions. The fractionation method of Monte and Maga (1980) was utilized with some modifications applied from the methods of Ring and Selvendran (1980) and Salimath and Tharanathan (1982). The outline of the fractionation method used in this study is illustrated in Figure 12. Cell wall fractionation started with the isolated cell wall from one gram tissues (seed coat or cotyledon). Centrifugation at 23,000 g for 20 min was used throughout the procedure to separate the extracts from the extracted cell wall residues. The extracted cell wall polymers were recovered by precipitating the combined extracts with 4 volumes ethanol. Water was added to the precipitate to increase freezing rate before

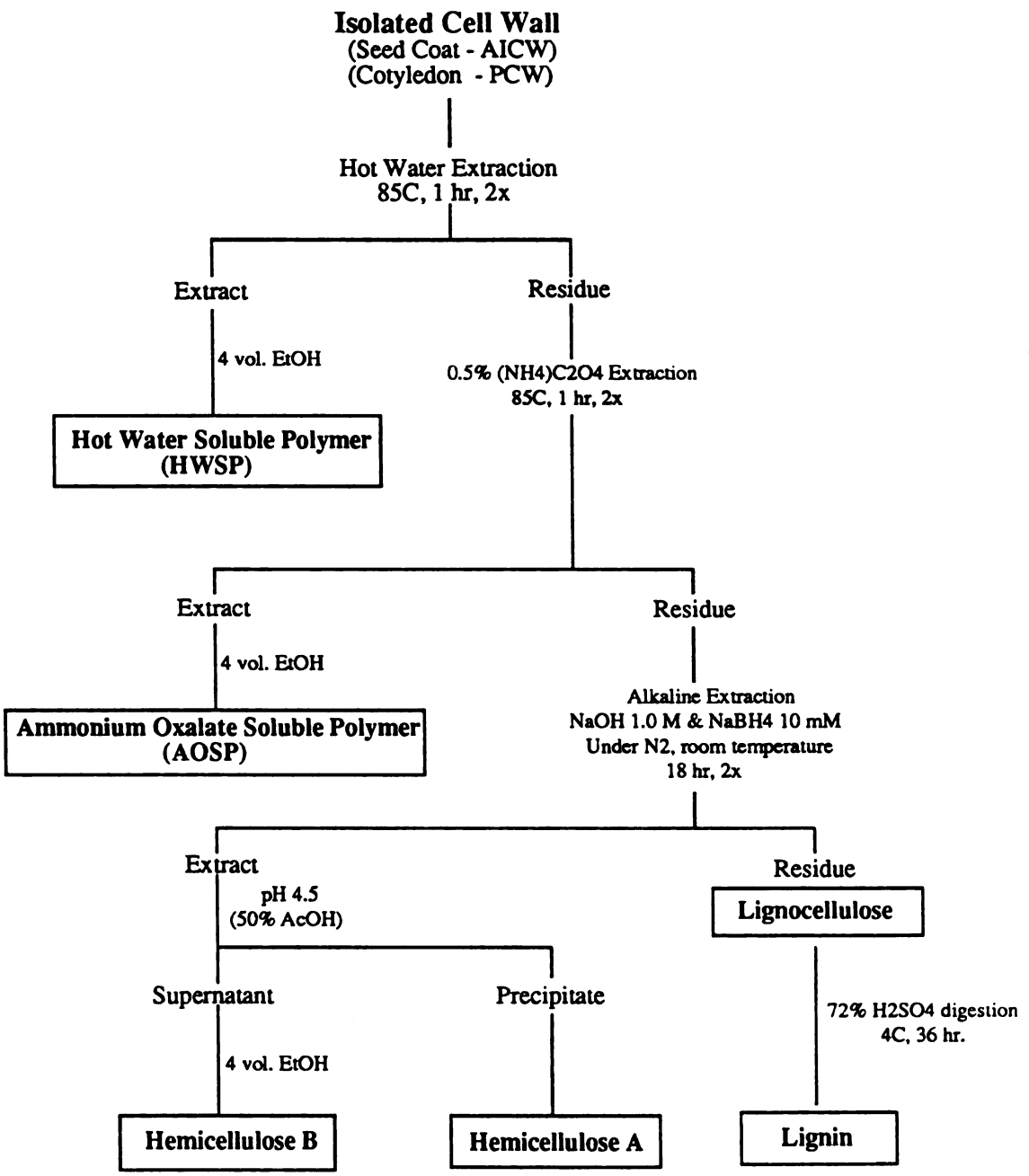

Figure 12. Procedure for Fractionation of Cell Walls

lyophilization. This technique was also used throughout whenever an ethanol precipitate was to be lyophilized. The lyophilized cell wall polymers were weighed and expressed as % of isolated cell wall (AICW or PCW). The followings are the sequential fractionation steps:

a) Extraction of hot water soluble polymers (WSP). The isolated cell wall material was extracted twice with hot deionized-distilled water for 1hr in 85°C shaking water bath.

b) Extraction of ammonium oxalate soluble polymers (AOSP). The water extracted cell wall material was treated twice with 20ml of 0.5% ammonium oxalate for 1 hr at 85°C. The oxalate extracted cell wall was recovered by centrifugation and washed with 15 mL, hot deionized-distilled water before subjection to the next step. The two oxalate extracts and the water wash were combined before ethanol precipitation.

c) Extraction of hemicellulose. The oxalate extracted cell wall material was extracted twice under nitrogen with 1 M sodium hydroxide containing 10 mM sodium borohydride for 18 hr at room temperature. After each centrifugation, the supernatant was cooled to 4°C and the pH adjusted to 4.5 using 50% acetic acid. To prevent foaming a few drops of octanol-1 were added. The combined acidified supernatant were allowed to stand overnight at 4°C and any precipitate (Hemicellulose A) formed was collected by centrifugation. Addition of 4 volume ethanol to the supernatant yield hemicellulose B.

d) Determination of cellulose. The alkali insoluble residue left after alkali extraction represented lignocellulosic fraction. The remaining alkali was removed from lignocellulose by washing with water and filtering through a pre-weighed coarse filter glass crucible. The washing was then continued with 80% ethanol, absolute ethanol and acetone. The washed lignocellulose was further dried in 40°C vacuum oven overnight, cooled and weighed. Part of lignocellulose was analyzed for lignin content. Cellulose content was obtained by

subtracting lignin content (Step e below) from the lignocellulose content.

e) Determination of lignin. Lignocellulose (approx. 100 mg) was hydrolysed with a cold 72% H_2SO_4 solution in a Pyrex beaker. The mixture was kept at 0-4°C for 36 hr and was occasionally stirred with a glass rod. After hydrolysis, 30 mL cold water were added, mixed and filtered through the pre-weighed coarse filter glass crucible. The residue left on the filter crucible was washed with deionized-distilled water until no acid was detectable. The washing was then continued with 80% EtOH, 100% EtOH and acetone. It was then dried in 100°C oven, cooled in a desiccator, and weighed.

Cell Wall Hydroxyproline

To prepare cell walls, one gram of seed coat or cotyledon flour was sequentially extracted (2x, each) with the following solvents: 0.5 M NaCl, deionized-distilled H_2O, 90% aqueous dimethylsulfoxide, deionized-distilled H_2O, 80% ethanol, absolute ethanol and acetone. The final residue, which consisted mainly of cell walls, was freeze-dried and stored over a desiccant until analyzed.

Cell walls were then hydrolysed to determine the hydroxyproline content. Ten milligrams of cell wall material were hydrolysed in 1 mL of 5.5 M HCl at 110°C for 18 hr. After hydrolysis, the sample was blown dry under a stream of nitrogen gas and re-suspended in 1 mL deionized-distilled H_2O. The mixture was mixed well then centrifuged to remove solids. The supernatant was taken for the colorimetric determination of hydroxyproline by the method of Lamport and Miller (1971). The assay consisted of combining 0.5 mL sample aliquot with 1 mL dilute sodium hypobromite (NaOBr) solution (20 mL stock NaOBr + 94 mL cold 5% NaOH; stock NaOBr: 6.4 mL bromine + 1 Liter of cold 5% NaOH). After 5 min at room temperature, 0.5 mL 6N HCL and 1.0 mL PDMAB solution (5 g p-dimethylaminobenzaldehyde in 100 mL n-propanol) were added consecutively. The mixed solution was then incubated 15 min in 70°C water bath and cooled to room temperature. A blank containing 0.5 mL deionized-distilled H_2O was run

through the entire assay along with the samples. The intensity of pink color measured at 560 nm is proportional to the original hydroxyproline concentration. With a standard curve prepared from known concentrations of hydroxyproline, the amount of hydroxyproline in cell wall sample was calculated.

Phenolic Acids and Their Derivatives

Total Extractable Phenol

One gram of seed coat or cotyledon flour was successively extracted three times with 20mL absolute methanol. The combined extracts were determined for total phenol concentration using Folin-Ciocalteu's phenol reagent (Bray and Thorpe, 1954). The procedure consists of combining 0.1 ml extract with 6 mL 2% Na_2CO_3. After 2 min, 0.1 mL of 50% Folin-Ciocalteu's phenol reagent (Sigma chemical Co.) was added, then the mixture was incubated 30 minutes at room temperature prior to measuring the absorbance at 750 nm. Blank containing 0.1 mL methanol, 6 mL 2% Na_2CO_3 and 0.1 mL 50% Folin-Ciocalteu's phenol reagent was used. Relative total extractable phenol content was expressed as absorbance at 750 nm.

Phenolic Fractionation

Phenolic acids were isolated, fractionated and determined according to the method described in Chapter I. Slight modification was done on the step of starch/protein free cell wall preparation for cell wall bound phenolic analysis. A series of enzymatic hydrolysis using α-amylase, protease and amyloglucosidase previously reported in cell wall preparation of Study IV was employed. Silica gel TLC plate with 2 solvent systems {1) $CHCl_3$:CH_3COOH:H_2O [4:1:1], lower phase, and 2) Toluene: CH_3COOH (9:1)} were employed to separate phenolic acids. Phenolic acids were located by their staining with Folin and Ciocalteu's phenol reagent followed by NH_3 vapor. The specific phenolic acids were quantitated from the phenol reagent sprayed chromatogram by densitometry. Standard phenolic acids used for comparison and quantitation were ferulic and p-coumaric acids.

Results and Discussion

Study I: Canning Quality Characteristics

Canned Bean Quality

Under identical process conditions, the four navy bean cultivars selected for study exhibited canning quality differences (Table 4). Soaking dry beans before canning is considered as a necessary step to decrease cooking time, increase drained weight and ensure uniform bean expansion in the can during processing (Nordstorm and Sistrunk, 1977 and Quast and da Silva, 1977). Soaked weight indicates the seed hydration ability during soaking treatment. From Table 4, the relative order of soaked weights (g) 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 are shown in Table 4. Seed coat characteristics were suggested to be major factors in controlling seed hydration during soaking (Smith and Nash, 1961 and Quast and da Silva, 1977).

The processed yield of canned beans was determined by its washed drained weight. 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 4). The breeding line 84004 and C-20 cultivar possessed high drained weights: 322.0 and 313.4 g, respectively ($P<0.05$). Dry bean physico-chemical constituents undergo various changes during high temperature and pressure cooking in the still retort. The major changes include pectin 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.

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

Table 4. Canned Bean Quality Attributes[1] of Four Bean Cultivars

Bean Cultivar	Soaked Weight (g)	Drained Weight (g)	Processed Color			Texture Shear Force (kg/50g)	Bean Solids % db
			L	a_L	b_L		
C-20	229.7 ± 1.2c	313.4 ± 3.9b	52.7 ± 0.0a	3.7 ± 0.4ab	14.1 ± 1.1a	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	28.9 ± 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	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	22.5 ± 1.0a	29.0 ± 0.3a

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

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 4). There are no significant differences ($P<0.05$) in L and b_L values among all cultivars. The only significant difference in a_L color is found between Fleetwood (4.4) and 84004 (3.6).

The genetic background of cultivars has a large impact on the shear texture of canned bean product. For example, Fleetwood, the most firm bean cultivar (41.5 kg/50 g canned bean) studied had a shear value twice that of the softest bean, 84004 (22.5 kg/50 g canned beans). The reported shear values of C-20 and Seafarer are 29.3 and 28.9 kg/50 g canned beans, respectively. Physico-chemical factors and their mechanisms affecting texture properties have not been clearly established.

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 demonstrated an inverse relationship. An analysis of these data (Table 4) showed that % dried solids correlated fairly well with drained weight ($R^2 = 0.601$). The breeding line 84004 exhibited the lowest % dried solids ($P<0.05$) and had the highest water holding capacity of cooked beans of the four bean strains studied.

Relationships Among Canning Quality Characteristics

Soaked weight, drained weight and shear texture of canned beans are primary quality characteristics influencing processor and consumer acceptabilities. The mechanisms governing these product performance traits are confounded and thus, the relationships among these characteristics are of interest. Least square regression equations relating these primary product qualities were calculated using the data in Table 4 and presented as follows:

Soaked wt. = -402.32 + 3.318 (Drained wt.), R^2 = 0.650

Soaked wt. = 233.85 - 0.220 (Shear value), R^2 = 0.553

Drained wt. = 340.09 - 0.971 (Shear value), R^2 = 0.711

Greater soaked weight is associated with greater drained weight (R^2=0.65). Van Buren et al. (1986) found the same relationship in kidney beans with R^2 = 0.60. However, this relationship may not be true when dry beans were subjected to adverse post-harvest handling and storage conditions resulting in quality defects such as hard-to-cook (HTC). Previous works by Variano-Marston and Jackson (1981) and Srisuma et al. (1989) have demonstrated that HTC beans exhibited normal water absorption during soaking while the drained weight after thermal processing was significantly lower. Soaked weight and shear texture demonstrated a poor relationship (R^2=0.553). Similar findings was previously reported by Silva et al. (1981) and Van Buren et al. (1986). An increase in drained weight of canned bean is associated with a decrease in shear texture (R^2= 0.711). This relationship was previously suggested by Nordstrom and Sistrunk (1977), Hosfield and Uebersax (1980) and Van Buren et al (1986).

Study II: Physical Property and Proximate Composition

Physical Properties

Seed Coat Content

The canning quality of dry beans can be greatly influenced by their structural components especially seed coat. The seed coat is known as a major barrier to water absorption by legume seeds. Youssef et al. (1982) studied the cookability of faba beans using a penetrometer and found that seed coat content of the whole bean had a highly significant correlation (P<0.001) with faba bean cookability index.

Seed coat contents (%w/w of total seed weight) of the studied dry beans are presented in Table 5. These data indicate that the Fleetwood cultivar possessed the highest

Table 5. Percentage of Seed Coat of Four Bean Cultivars[1]

Bean Cultivar	Seed Coat % db
C-20	6.44 ± 0.02a
Seafarer	6.69 ± 0.13a
Fleetwood	7.72 ± 0.36b
84004	6.56 ± 0.08a

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

($P<0.05$) seed coat content (7.72%) while there are no differences ($P>0.05$) in seed coat content among C-20 (6.44%), Seafarer (6.69%) and 84004 (6.56%). Although high seed coat content of Fleetwood may impart its exceptional high shear texture, the relative differences in textural quality of C-20, Seafarer and 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 % seed coat and soaked weight: soaked wt. = 251.06 - 3.491 (% seed coat), R^2 = 0.765. These poor correlations may be contributed to the effect of both structures and compositions of seed coat and cotyldon (Hsu et al., 1983; Muller, 1967 and Sefa-Dedeh and Stanley, 1979).

Pasting Characteristics and Gel Strength of Whole Bean Flour

Two general factors contributing to canning quality are bean physical structure and bean chemical compositions. Physical quality tests utilizing whole bean flour will eliminate effects due to physical structure and therefore, any differences obtained among cultivars should result mainly from the differences in bean chemical composition and microstructure. Pasting properties and gel strength were utilized to evaluate the effect of bean chemical compositions contributing to their canning quality.

Pasting properties of bean flour slurries (6%, w/w) were studied using the Brookfield viscometer. Viscosity values at 94 $\pm$ 1°C, after a 23 min cooking cycle and at 20 $\pm$ 1°C, after 2 min cooking cycle were reported in Table 6. Significant differences ($P<0.05$) in torque values at both temperatures indicate similar textural quality trend as observed in the canned bean 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 bean (Table 4). The following regression equations were derived from the data:

$$\text{Shear force} = -18.84 + 10.057 \ln (\text{Torque/heating}), \quad R^2 = 0.984$$

$$\text{Shear force} = -55.41 + 12.715 \ln (\text{Torque/cooling}), \quad R^2 = 0.978$$

Table 6. Pasting Properties of 6% (w/w) Solution of Whole Bean Flour at Selected Time-Temperature History[1]

Bean Cultivar	Torque x 10^7 (NM)	
	After 23 min of Heating Temp. = 94 ± 1.0°C (TH)	After 2 min of Cooling Temp. = 20 ± 1.0°C (TC)
C-20	111.30 ± 11.14b	888.15 ± 49.55c
Seafarer	104.85 ± 9.59b	689.73 ± 10.53b
Fleetwood	418.14 ± 32.12c	1970.15 ± 63.65d
84004	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).

Table 7. Rheological Properties of 12% (w/w) Whole Bean Flour Gels from Four Bean Cultivars[1]

Bean Cultivar	Apparent Viscosity (poise)	Apparent Elasticity (N/cm^2)
C-20	2168.96 ± 138.76ab	2.84 ± 1.44b
Seafarer	2064.18 ± 82.31a	2.40 ± 1.73ab
Fleetwood	2972.50 ± 132.56c	0.79 ± 0.30a
84004	2305.99 ± 38.85b	3.55 ± 0.76b

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

Viscosity characteristic of whole bean flour in dilute 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 are quick, simple and small amount of sample required.

The results obtained from the study of whole bean flour gel (12%, w/w) after 7 days storage at room temperature (Table 7) showed that the gel of Fleetwood was firmer ($P<0.05$) with relatively less elastic character compared to the other cultivars. The low mechanical shear force was applied during gel preparation in order to ensure homogeneity of the gel and yet provide limited disruption to the microsturcture of whole bean flour. Whole bean received minimum disruption in the can during retort processing. Seven day storage time of whole flour gel further allowed any molecular arrangements which yield the similar effect of equilibration period before canning evaluation. There was no conclusive relationship between gel strength (apparent viscosity) and canning quality. This is due to the similarity in gel characteristics of C-20, Seafarer, and 84004. High variations found among replicates of each cultivar may obscure any effects due to cultivars. Furthermore, the relatively high concentration gel system (12%, w/w) along the low mechanical shear force used for dispersion may not have allowed homogeneous preparation and causing high variations within cultivar.

Proximate Composition

Proximate analyses of raw whole navy beans on dry basis are presented in Table 8. Fat (ether soluble extract) content is in the range of 1.14% (84004) to 1.57% (Seafarer). There are significant differences ($P<0.05$) in fat content among cultivars with the exception of those between C-20 (1.53%) and Seafarer (1.57%). Ash contents of the raw whole beans (4.03%-4.16%), however, showed no significant difference ($P>0.05$) among the cultivars. Protein contents determined by the Kjeldhal method (conversion factor = 6.25)

Table 8. Proximate Chemical Compositions (%, db) of Four Navy Bean Cultivars[1]

Bean Cultivar	Moisture (%)	Fat	Ash	Protein	Starch	TDF
C-20	10.94 ± 0.11a	1.53 ± 0.04c	4.16 ± 0.02a	28.10 ± 0.20c	43.27 ± 0.58b	18.74 ± 0.29b
Seafarer	12.96 ± 0.07c	1.57 ± 0.01c	4.03 ± 0.01a	24.39 ± 0.26a	44.85 ± 0.73c	19.96 ± 0.62c
Fleetwood	15.98 ± 0.10d	1.42 ± 0.01b	4.06 ± 0.18a	27.11 ± 0.31b	45.15 ± 0.32c	19.49 ± 0.38bc
84004	12.24 ± 0.04b	1.14 ± 0.02a	4.12 ± 0.02a	29.10 ± 0.22d	41.83 ± 0.42a	17.50 ± 0.85a

[1] n = 3 except for TDF, n = 4
Means in a column followed by different letters are significantly different ($P<0.05$)

exhibited significant differences ($P<0.05$) among cultivars with the relative order of high to low as follows: 84004 (29.10%), C-20 (28.10%), Fleetwood (27.11%) and Seafarer (24.39%). Starch is the major component found in navy beans (41.83%-45.15%). Inverse relationship of high protein and low starch was found in all cultivars, except Seafarer. Among all cultivars, 84004 contained the lowest starch content of 41.83% ($P<0.05$). Similar starch contents ($P>0.05$) were found in Fleetwood (45.15%) and Seafarer (44.85%), and both cultivars have significant higher starch content than that in C-20 (43.27%).

In the physical property study, pasting characteristics of Fleetwood exhibited a much more viscous (3-4 times) paste than the Seafarer cultivar. Because of their similar starch contents, this higher viscosity effect of Fleetwood should not be due to the quantity difference in starch. However, the characteristics of starch (Study III) and cell wall polysaccharides (Study IV) may play important roles in this complex phenomenon.

Total dietary fiber (TDF) study revealed that 84004 possessed the lowest TDF content (17.50%) ($P<0.05$). Fleetwood TDF content (19.49%) is not significantly different ($P>0.05$) from Seafarer's but both TDF contents are significant higher than that of C-20.

Plate 1 illustrates the overnight precipitates of 80% ethanol fiber during the TDF determinations of the studied cultivars. The Fleetwood precipitate has fibrous-like aggregates suspended in the solution, where as the fibers from C-20, Seafarer and 84004 exhibited more particulate precipitations. Another noticeable characteristic of the completely precipitated fibers from C-20, Seafarer and 84004 is the granular appearance. Finer grains of precipitated fiber was observed for 84004 compared to C-20 or Seafarer.

Table 9 presents the detailed data from TDF analysis. The 80% alcohol precipitates, as shown in Plate 1, were recovered to determine the percent yield expressed as percentage of fiber residue (fiber + ash + protein). The fiber residue content is highest ($P<0.05$) in Fleetwood cultivar (27.64%), and lowest ($P<0.05$) in 84004 cultivar

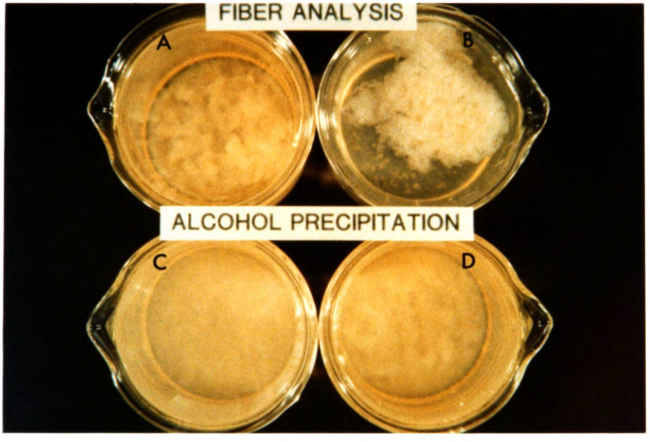

Plate 1. Precipitation of Fiber Residues in 80% Ethanol during TDF Determinations of Studied Cultivars: A) C-20, B) Fleetwood, C) 84004 and D) Seafarer

Table 9. Total Dietary Fiber Analysis[1]

Bean Cultivar	Fiber Residue[2]	Residual Ash[3]	Residual Protein[3]	Retained Protein[4]	TDF[2]
C-20	25.89 ± 0.40b	8.41 ± 1.89a	18.80 ± 0.18b	17.41 ± 0.25b	18.74 ± 0.29b
Seafarer	25.82 ± 0.80b	9.46 ± 0.37a	13.07 ± 0.27a	14.14 ± 0.15a	19.96 ± 0.62c
Fleetwood	27.64 ± 0.53c	7.33 ± 1.13a	22.84 ± 0.15c	22.62 ± 1.24c	19.49 ± 0.38bc
84004	24.14 ± 1.16a	8.30 ± 0.23a	17.98 ± 0.77b	15.78 ± 0.93ab	17.50 ± 0.85a

1 Means in a column followed by different letters are significantly different ($P<0.05$)
2 Percentage in whole bean flour, with blank correction, n = 4
3 Percentage in fiber residue, with blank correction, n = 2
4 Percentage in whole bean flour protein, n = 2

(24.14%). Fiber residue content of C-20 (25.89%) is similar to that of Seafarer (25.82%) ($P>0.05$). The obtained fiber residue was analyzed for its residual ash and protein contents. Among the studied cultivars, there is no significant difference ($P>0.05$) in residual ash content. Fiber residual protein, however, demonstrated significant differences ($P<0.05$) among cultivars, except between C-20 (18.80%) and 84004 (17.98%). The fiber residue of Fleetwood contained the highest residual protein content (22.84%) where as that of seafarer is the lowest (13.07%). Fleetwood protein is more resistant ($P<0.05$) to protease hydrolysis during TDF determination as shown by high % retained protein when protein remnants of fiber residue were calculated as percentages in the whole bean flour protein. The susceptibility of protein in Seafarer and 84004 are similar ($P>0.05$), but there is a significant difference between that in Seafarer and C-20. TDF content, derived from the fiber free ash and free protein, was previously compared among cultivars. Saunders and Betschart (1980) argued that dietary fiber should not be considered as polysaccharide free of protein because the evidence for cell wall protein has been well established. In addition, cell wall protein, a hydroxyproline rich glycoprotein may be covalently bound to polysaccharides and therefore it may not completely removed by simple solvents or protease enzymes (Fry, 1986). Because of the amino acid pattern of cell wall protein, some investigators (Brillouet and Carre, 1983 and Champ et al., 1986) use a factor of 5.80 instead of 6.25 to convert nitrogen determined by the Kjeldahl procedure into crude protein content.

Relationships Between Chemical Composition and Canned Bean Quality

Least square regression analysis was employed to determine the relationships of proximate chemical analyses (Tables 8 and 9) and canning quality (Table 4). Since only four cultivars were utilized in this study, relationships are meaningful only if the regression equation has a high coefficient of correlation (R^2). Relationship of $R^2 > 0.75$ were arbitrarily selected as a minimum coefficient correlation which would be meaningful. Equations meeting this criteria are:

Drained wt.	=	569.68-5.922 (% Starch),	R^2 = 0.996
Drained wt.	=	462.10-8.014 (%TDF),	R^2 = 0.890
ln (Shear force)	=	-1.13+.175 (%Fiber residue),	R^2 = 0.990

Greater starch content in navy bean may result in lower drained wt. Compared to corn and potato starches, this relationship may be explained by the bean starch characteristics of high amylose content which contributes to high retrogradation potential and high degree of syneresis (Halbrook and Kurtzman, 1975). Higher total dietary fiber content was associated with lower drained weight. This probably related to the biological function of cell wall (fiber) as a cell expansion regulator during water absorption (Goodwin and Mercer, 1983). A high correlation between % fiber residue and shear force value was obscured, the correlation between TDF and shear force was not high. Since the ash content of the fiber residue did not vary, but the protein content of the fiber residue did vary, the relationship between fiber residue and shear force must be in part due to protein interaction with cell wall fiber and/or characteristics of the residue protein. Since significant variation ($P<0.05$) in residual protein but not in residual ash was found in fiber residues.

Summary and Conclusions

The genotype of a navy bean cultivar affected its canned bean quality, especially shear texture value. Among 4 studied cultivars, the firmness of canned bean is highest in Fleetwood (41.45 kg/50 g Processed bean) and lowest in 84004 (22.5 kg/50 processed bean). Seafarer and C-20 produce similar shear texture which is relatively higher ($P<0.05$) than for 84004.

Bean physico-chemical properties seem to influence its canning quality. From least squares regression analysis, the content of starch ($R^2 = 0.996$), and TDF ($R^2 = 0.890$) are inversely associated with drained weight. Characteristics of 80% ethanol precipitates and residual protein content found during TDF analysis suggested the influences of protein and fiber characteristics. Fleetwood protein had more ($P<0.05$) protease resistant protein associated with the fiber residue. Further, very high correlation ($R^2 = 0.990$) between

shear force and fiber residue content, but poor correlation between shear force and TDF confirm the effects of protein and/or protein and fiber interaction. Seed coat content may partly regulate seed water absorption during soaking, but less during thermally processing. The results from the study of pasting and gel properties indicated that the differences in canning quality of the studied beans are influenced by their chemical components primarily starch, protein and fiber. The differences could be due to quantitative and/or qualitative basis. Further studies on characteristics of starch and fiber could further screen the factors affecting canning quality.

Study III: Starch Characteristics

Study II demonstrated that starch, the major component (41.83% - 45.15%) of navy beans, is a major factor in determining drained weight of canned beans. In this study, physico-chemical characteristics of isolated bean starch were determined in an effort to further explain relationships between their canned bean quality attributes and bean starch properties.

Starch Isolation

Starches were isolated from the whole flours of 4 model bean cultivars (C-20, Seafarer, Fleetwood and 84004) using the method of Naivikul and D'Appolonia (1979) with some modifications to improve the starch purity. Extractant solvents used in the isolation and purification process include 0.016 N NaOH, water and 80% ethanol. Dilute NaOH has been used by many investigators and is suggested as a good medium to dissolve protein without causing starch gelatinization (Schoch and Maywald, 1968). In addition, bean fibers are also partially dissolved by NaOH and dilute alkali is able to saponify the existent glycerides which are then removed by the following water washing step. Water was used to remove alkali and to wash off various water soluble fibers, monosaccharides and other water soluble constituents. Ethanol was used to extract sugars, phenolic acids and non-sponifiable lipids. An additional step using 60 mesh sieve to strain the non-starch

fine particles (mainly cellulosic fiber) from crude starch further improved the starch purification.

Physico-Chemical Analyses of Bean Starch

Proximate Composition and Microstructure

Corn starch was selected as a standard since its physico-chemical properties are well established. Purity determination of the isolated bean starches and corn starch was assessed on the basis of chemical composition (Table 10) and microscopic structure by scanning electron microscopy. Isolated bean starch appears to be pure and no adhering protein was observed (Plate 2). Ash contents of all starches, except 84004 starch, are in the range of 0.07% to 0.09%. The ash content (0.16%) of 84004 bean starch is approximately 2 times higher ($p<0.05$) than those in other studied cultivars. The protein contents of bean starches (0.20%-0.27%) are similar ($p>0.05$) in all cultivars and significantly lower ($p<0.05$) than that of corn starch (0.39%). The isolated navy bean starch prepared by Naivikul and D'Appolonia (1979) contained slightly higher protein content (0.38%) and comparable ash content (0.14%).

Amylose Content

Amylose contents of studied starches were determined using the improved "Blue Value" method (Morrison and Laignilet, 1983). For bean starches, the amylose content of C-20 (35.26%) was significantly higher ($p<0.05$) than that of Seafarer (34.13%) but there was no significant difference among amylose content of 84004 (34.39%), Fleetwood (34.88%) and Seafarer or C-20. Naivikul and D'Appolonia (1979) reported a much lower amylose content (22.10%). The current results from this study agree closely with latter investigators: 36.0%, Biliaderis et al. (1980); 32.1%, Hoover and Sosulski (1985); and 34.24% Srisuma et al. (1988). Corn starch possesses a much lower ($p<0.05$) amylose content (20.43%) than any other bean starches. Biliaderis et al. (1980) previously reported the amylose content of commercial corn starch to be 22.6%.

Table 10. Chemical Characteristics of Corn and Isolated Bean Starches[1]

Starch Source	Moisture (%)	Ash (%, db)	Protein (%, db)	Amylose in Starch (%)
Corn	9.33 ± 0.05a	0.09 ± 0.02b	0.39 ± 0.02b	20.43 ± 0.39a
C-20	12.09 ± 0.07b	0.09 ± 0.01ab	0.22 ± 0.06a	35.26 ± 0.04c
Seafarer	20.36 ± 0.75c	0.07 ± 0.00ab	0.27 ± 0.04a	34.13 ± 0.42b
Fleetwood	16.65 ± 0.05d	0.07 ± 0.00a	0.24 ± 0.03a	34.88 ± 0.60bc
84004	14.53 ± 0.03c	0.16 ± 0.02c	0.20 ± 0.02a	34.39 ± 0.42bc

[1] n = 3, Means in a column followed by different letters are significantly different (P<.005).

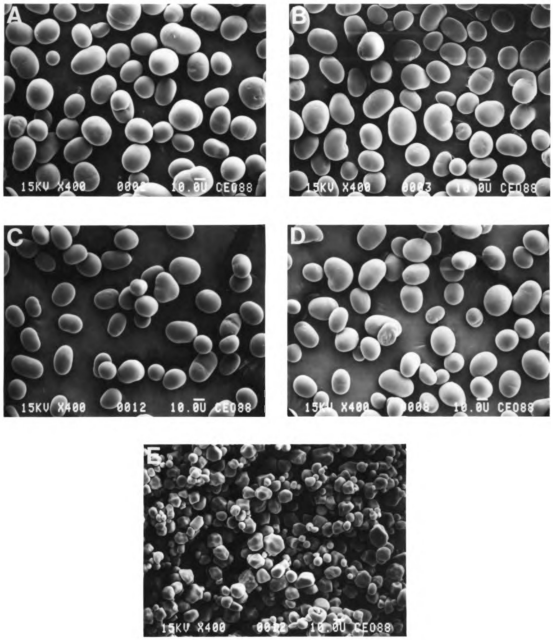

Plate 2. Scanning Electron Micrographs of Raw Starches:
A) C-20, B) Seafarer, C) Fleetwood, D) 84004 and E) Corn

Swelling Power and Solubility

The swelling power and solubility patterns recorded at different temperatures are presented in Figure 13 and 14, respectively (See the values in Appendix B). All bean starches exhibited single stage, restricted swelling patterns with continuous increases in swelling power throughout the testing temperature range (70-90°C). During 70 to 80°C, the swelling power of Seafarer and Fleetwood was significantly ($p<0.05$) higher than that of C-20 and 84004. At higher temperature range (80-90°C), Seafarer starch maintained its higher ($p<0.05$) swelling power potential compared to Fleetwood starch. However, there was no significant ($p>0.05$) difference in swelling power among all bean starches at the end of the test (90°C). Similar phenomenon was found in the bean starch solubility study.

Corn starch exhibited two-stage swelling power (70-80°C/slow rate and 80-90°C/fast rate) and solubility patterns. Corn starch granules possess much higher degree of swelling than bean starches throughout the testing temperature profile. Its solubility, however, was higher than bean starches only at the temperature range of 60°C to 75°C and at 90°C.

Swelling power and solubility pattern of starches have been used to provide evidence for associative binding within the granules (Leach et al., 1959 and Lorenz, 1979). Bean starches exhibited the restricted single stage swelling and solubility patterns which indicate the existence of strong bonding forces. Bonding forces within granules allow bean starches to relax over the entire temperature range (70 - 90°C). Linear molecules of amylose preferentially leach out of the starch granules at or slightly above temperatures in the gelatinization range while the branched molecules of amylopectin retain in the swollen granules (Greenwood and Thompson, 1962). Consequently, the higher solubility of bean starches at 80 - 85°C may be explained by their higher amylose contents and relatively higher gelatinization temperatures, compared to corn stareh.

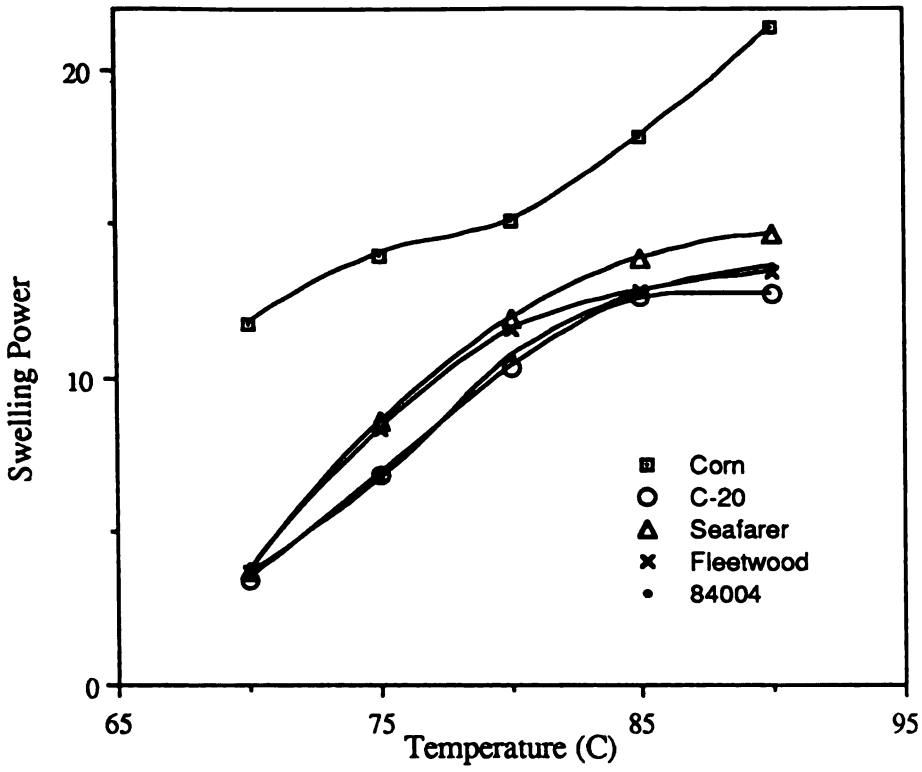

Figure 13. Swelling Power Patterns of Corn and Isolated Bean Starches

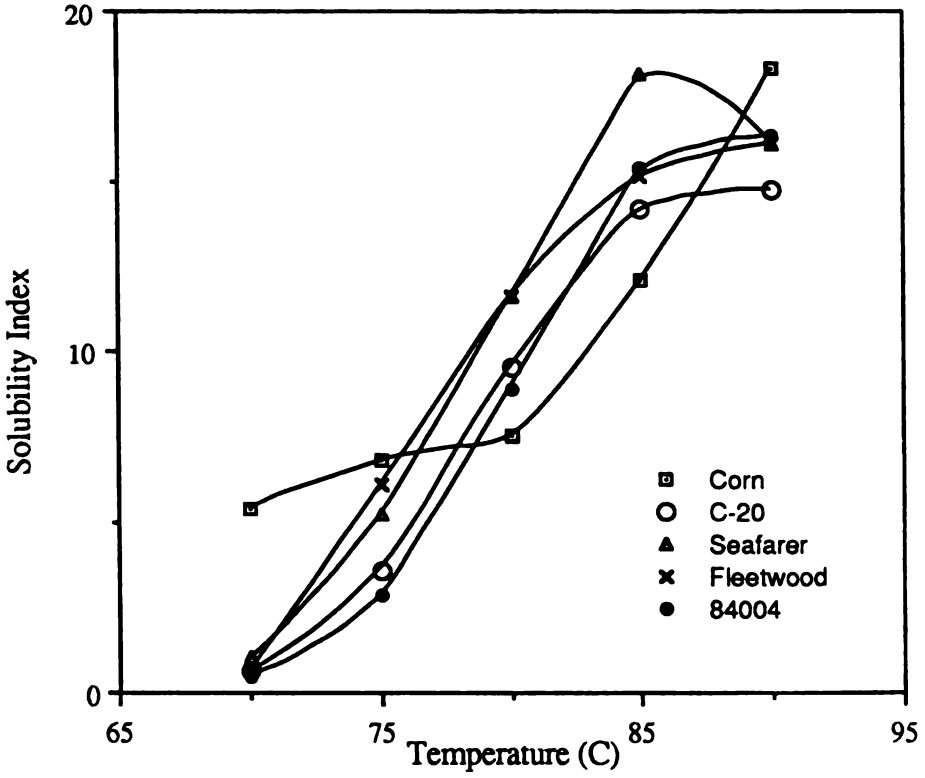

Figure 14. Solubility Patterns of Corn and Isolated Bean Starches

Granule Size

As observed by scanning electron microscopy (SEM), the shapes and sizes of isolated bean starches are shown in Plate 2. Bean starches vary widely by size which includes a mixture of large (elliptical to oval), intermediate (oval) and small (oval to round) granules. The granule sizes characterized by their widths and lengths are presented in Table 11. Ranked by average granule sizes, C-20 and Fleetwood have the largest and smallest granules, respectively. Starch granules from Seafarer and 84004 starch are fairly similar and are categorized as intermediate sized granules. C-20 starch showed a broader range in granule size than the starches of Seafarer, 84004 and Fleetwood, which were similar. These results on granule size and shape were comparable to previous reported values by Hoover and Sosulski (1985) and Sathe and Salunkhe (1981).

DSC Characteristics

DSC was used to study the gelatinization process of corn and isolated bean starches. At 10% w/w concentration, all starches exhibited the single endothermic transition type characterized by their single endothermic peaks with very narrow temperature range (Tm-To) Excess water during heating in DSC resulted in extensive hydration/swelling of the amorphous starch and thus facilitated the melting process of the starch crystallites. Table 12 presents the data obtained from DSC thermograms including gelatinization or phase transition temperatures (To-onset temperature, Tp-peak temperature and Tm-melting temperature), and gelatinization enthalpy (ΔH). Between corn and bean starches, corn starch has much lower ($p<0.05$) transition temperatures (To, Tp and Tm), and smaller ($p<0.05$) gelatinization enthalpy (ΔH). Among bean starches, small variations were observed in their To and Tp, but not in Tm. The Tm values ranked from high to low are as follows: 84004, C-20, Fleetwood and Seafarer. These findings are somewhat correlated to the previous results that Seafarer starch exhibited highest in swelling power and solubility.

Table 11. Size of Isolated Bean Starches[1]

Starch Source	Width (μm) mean	Width (μm) range	Length (μm) mean	Length (μm) range
C-20	21.22	12.5 - 35.0	26.21	12.5 - 42.5
Seafarer	20.26	12.5 - 27.5	25.74	16.3 -35.0
Fleetwood	18.65	12.5 - 27.5	23.21	15.0 - 35.0
84004	20.04	12.5 - 27.5	25.73	15.0 - 35.0

[1] n = 100

Table 12. DSC Characteristics of Corn and Isolated Bean Starches at 10% (w/w) Concentration[1]

Starch Source	Endothermic Transition (°C)			Tm - To	Enthalpy of Gelatinization
	To	Tp	Tm		(ΔH, Cal/g)
Corn	64.84 ± 0.24a	69.26 ± 0.61a	73.01 ± 0.46a	8.17 ± 0.30a	3.38 ± 0.04a
C-20	70.35 ± 0.72bc	77.15 ± 0.24c	84.43 ± 0.65d	14.08 ± 1.36d	4.44 ± 0.08d
Seafarer	71.80 ± 0.14d	76.58 ± 0.17b	81.70 ± 0.37b	9.90 ± 0.37b	4.18 ± 0.23c
Fleetwood	70.98 ± 0.67cd	76.08 ± 0.24b	82.93 ± 0.86c	11.95 ± 1.47c	3.89 ± 0.20b
84004	69.80 ± 0.76b	77.45 ± 0.41c	85.75 ± 0.66c	15.95 ± 0.83c	4.77 ± 0.13c

[1] n = 4, Means in a column followed by different letters are significantly different (P<0.05).

The heat (energy) required to disintegrate granular organization is proportional to the bonding strength in crystalline regions within starch granules (Biliaderis et al., 1980 and Hoover and Sosulski, 1985). Differences in Tm indicate variations in structural characteristics of amylopectin, the principle component of the starch crystallites. Increased degree of branching of the amylopectin is detrimental to crystallization and, therefore lowers Tm (Biliaderis et al., 1980). From the above, one would conclude that Seafarer starch is less resistant to gelatinizaion because its amylopectin molecules have a higher degree of branching. Enthalpy of gelatinization (ΔH) represents the net amount of thermal energy per gram of dry starch required to rupture hydrogen bonds (exothermic process) and to form new bonds between molecules (endothermic process involving water). The result of this primary endothermal reaction is a less ordered structure with increased entropy (Stevens and Elton, 1971). The relative order of ΔH obtained from this study is similar to that of Tm, except Seafarer and Fleetwood are reversed. Donovan (1979) suggested that transition enthalpy of starch in dilute system represents the enthalpy of granule swelling, crystallite melting and extensive hydration of starch molecules. From the results of swelling power and solubility studies, Seafarer starch exhibited the most granule swelling with highest solubility. This may provide an explanation of slightly higher ΔH in Seafarer starch than in Fleetwood starch. DSC characteristics of corn starch are similar to that described by Sweat et al. (1984). Results of navy bean starches, however, are slightly higher than those reported by Biliaderis et al. (1980). Differences may reflect variations in starch source, starch concentration and heating rate (Sweat et al., 1984).

Pasting Characteristics

Brookfield viscographs of corn starch and navy bean starch (var. Seafarer) are illustrated in Figure 9. These two types of starch produce very different viscosity patterns. During the heating cycle, corn starch showed a moderately high peak viscosity. This effect obviously reflects fragility of the swollen granules, which first swell and then break down under the continuous mechanical shear. The much higher swelling power of corn starch

may contribute to this viscosity characteristic. All bean starches gave similar viscograph patterns described by their restricted-swelling power and no pasting peak with gradual increase in viscosity throughout the heating cycle. Viscosity developed during heating the starch solution is due to the entanglements of swollen granules, exudates (from granules) and granule remnants. From Table 13, at the same starch concentration (6%, w/w), the relative order of increasing viscosities at the end of heating (T= 94 $\pm$ 1.0°C) cycle were corn>Seafarer>84004>C-20>Fleetwood. The swelling power and solubility characteristics of C-20, Fleetwood and 84004 starches cannot be used to explain their relative order of hot paste viscosity because constant mechanical shear forces were applied during these viscosity measurements. Hoover and Sosulski (1985) studied the granule morphological changes of black bean starch (viscous hot paste/high set-back) and pinto bean starch (thin hot paste/low set-back) during viscosity measurement using Brabender Viscoamylograph. Retention of starch granule integrity at 95°C for both beans and after 30min holding at 95°C for pinto beans were regarded as evidence for covalent bonding(s), providing intermolecular forces to stabilize granule integrity and hence resulting in the absence of peak viscostiy. Hoover and Sosulski (1985) also suggested that the higher viscosity shown by black bean starch during the heating cycle could be due to less extensive hydrogen and covalent bondings, higher swelling power and higher amylose leaching.

Among bean starches, the positive set-back trend (Tc-TH) was not significantly different ($P< 0.05$). Compared to corn starch, the set-back magnitude of bean starches was much lower. Corn starch was also reported previously to exhibit higher set-back viscosity (Brabender units) than legume starches isolated from two varieties of *Phaseolus vulgaris* (negro gueretaro and bayoeel) (Paredes-Lopez et al., 1988).

Positive set-back trend indicates a molecular reassociation after withdrawing heat energy. This characteristic is closely associated with amylose content and its chain length (Hoover and Sosulski, 1985). Similarity in set-back trend and amylose content of bean starches suggest somewhat identical amylose characteristics. However, the above

Table 13. Pasting Properties of Corn and Isolated Bean Starches at 6% (w/w) Concentration[1]

Starch Source	Torque x 10^7 (NM)		
	After 23 min of Heating Temp. = 94 ± 1.0°C (T_H)	After 2 min of Cooling Temp. = 20 ± 1.0°C (T_C)	Set Back Trend (T_C - T_H)
Corn	1045.61 ± 10.54d	4343.89 ± 90.48c	3298.28 ± 100.48b
C-20	638.21 ± 46.73b	1854.06 ± 209.74a	1215.85 ± 163.07a
Seafarer	886.98 ± 9.39c	2103.85 ± 19.91b	1216.87 ± 12.66a
Fleetwood	569.41 ± 44.54a	1703.83 ± 81.00a	1134.42 ± 36.97a
84004	647.21 ± 19.71b	1890.07 ± 22.57a	1242.86 ± 25.07a

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

hypothesis can not be used to explain the relationships of high set-back trend and low amylose content (Table 10) of corn starch. This may be due to differences in degree of granule dissociation during heating, and/or molecular structure of both amylose and amylopectin.

Gel Properties

The degree of syneresis of starch gels stored at room temperature for a period of 7 and 10 days is presented in Table 14. Among bean starches, the percentage of exudate from the gels after 7 days ranged from 17.60% for seafarer to 21.06% for C-20. Corn starch gels possessed an excellent ability to hold liquid, therefore squeezed out very little exudate (0.12% - 7 days; 0.16% - 10 days). This wide range in syneresis among the starch gels were directly correlated to amylose content (R^2 = 0.999, 7 days and R^2 = 0.997, 10 days; see equations in Appendix C). Extending storage time to 10 days caused all starches to have an increase in degree of syneresis.

Gel rheological properties (apparent viscosity and apparent elasticity) of starches stored at room temperature for 7 and 10 days are reported in Table 14. Relatively high variation in these results, especially for bean starch samples, may be caused by their low liquid holding capacity and consequently gel shrinkage. In both storage periods, Seafarer gel exhibited higher apparent viscosity ($p<0.05$) and were firmer than Fleetwood gel. The firmness of corn starch gel was significant lower ($p<0.05$) than the gels prepared from bean starches and stored for 7 and 10 days. It should be emphasized that a mild mechanical shear force was applied during gel preparation to disperse starch granules. Thus, the results can be explained as follows. Starch gel is a composite material (Eliasson and Bohlin, 1982) and constitutes a phase of more or less swollen starch granules dispersed in a continous phase composed of polysaccharides materials (mostly amylose and some amylopectin) that leach from swollen granules. Previous work on starch gelation by Ring (1985) has demonstrated two main factors affecting gel rigidity: 1) strength and stability of matrix amylose gel and 2) granule deformability. Therefore, the softer corn starch gel, in

Table 14. Degree of Syneresis and Rheological Properties of 8% (w/w) Starch Gels after 7 & 10 Day Storage at Room Temperature[1]

Starch Source	7 Day Storage			10 Day Storage		
	DS (%)	App. Viscosity (poise)	App. Elasticity (N/cm^2)	DS (%)	App. Viscosity (poise)	App. Elasticity (N/cm^2)
Corn	0.12 ± 0.06a	1217.08 ± 162.26a	0.33 ± 0.07a	0.16 ± 0.05a	1472.49 ± 169.92a	0.47 ± 0.05a
C-20	21.06 ± 1.08d	4395.00 ± 1014.70e	0.59 ± 0.16ab	24.60 ± 0.80d	4383.15 ± 1009.32bc	0.43 ± 0.17a
Seafarer	17.60 ± 0.16b	4632.96 ± 708.89c	0.76 ± 0.21b	19.43 ± 1.21b	5461.56 ± 555.57c	0.88 ± 0.33b
Fleetwood	18.40 ± 0.20bc	3156.45 ± 589.12b	0.59 ± 0.24ab	22.49 ± 0.43c	4030.45 ± 701.03b	0.55 ± 0.28ab
84004	19.52 ± 1.23c	3178.46 ± 154.09b	0.44 ± 0.07a	21.42 ± 0.59c	5071.88 ± 54.17bc	0.52 ± 0.04ab

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

comparison to bean starch, can be explained by its low amylose content and high swelling power of starch granule. Due to their linear structure, amylose can reassociate or retrograde through the intermolecular hydrogen bondings and this reassociation partially contributes to the firmer gel characteristic of bean starches. Furthermore, the highly swollen granules of corn starch tend to be more susceptible to deformability, tend to be more fragile than the restricted swollen granules from bean, and therefore poorly reinforce gel matrix (Ring, 1985).

Seafarer and Fleetwood starches have similar amylose contents ($P>0.05$) which can not be used to explain why Fleetwood starch gel was softer than Seafarer's. Neither does lower swelling power of Fleetwood starch explain its softer texture. During aging of starch gel (7 and 10 day storage), amylose as well as amylopectin have the ability to crystallize. The crystallization process might occur both inside the starch granules and/or outside in the continuous phase. Both cases will result in an increased gel firmness either due to the stiffer granule remnants or to an increasing number of "junction zones" present in the crystallizing network (Graessley, 1974). Bohlin et al. (1986) reported that crystallization of amylose was more or less complete within a day whereas the crystallization of amylopectin continued for weeks (Miles et al., 1985). The firmer starch gel from Seafarer, even though it has a higher swelling power, may be due to more crystallization of amylopectin within the granules which will produce stronger particles to reinforce the amylose matrix gel.

Starch Concentration and Gel Rheological Properties

The effect of starch concentrations on Seafarer gel properties is presented in Table 15. Greater starch concentrations result in lower degree of gel syneresis, stronger storage gel (apparent viscosity) and more rubbery-like texture (apparent elasticity). As starch concentration increases, the starch granules must increasingly compete for water. The swelling of individual granules was reduced and gel strength and elasticity concomitantly increased. Although, the dissolution of amylose from the granules was limited, the

Table 15. Effect of Starch Concentrations on Gel Rheological Properties[1]

Starch Concentration (%, w/w)	DS (%)	App. Viscosity (poise)	App. Elasticity (N/cm^2)
4	39.08 ± 1.94d	359.63 ± 49.35a	0.06 ± 0.02a
6	26.17 ± 1.39c	896.57 ± 115.01a	0.08 ± 0.01a
8	19.52 ± 1.23b	3178.46 ± 154.09b	0.44 ± 0.07a
10	12.94 ± 0.79a	15989.56 ± 1568.16c	2.81 ± 0.99b

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

amylose concentration in the continuous phase tended to increase due to the reduced available water in continuous phase. As a result, higher starch concentration promotes greater restriction of molecular movements and so causes a faster rate of amylose matrix formation and less degree of syneresis. As illustrated in Figures 15, 16 and 17, starch concentrations exhibit the exponential correlation with degree of syneresis, apparent viscosity and apparent elasticity.

Structural Changes in Cooked Starch Granules

Microscopic structural changes of corn and bean starches cooked at 70, 80 and 90°C for 1 hr are illustrated in Plates 3, 4 and 5, respectively. The starch solutions (10%, w/w) were cooked at designated temperatures with minimum applied shear force designed to provide adequate homogeneous dispersion with minimum granule disruption. The first step in SEM sample preparation of cooked starches requires the rapid freezing of samples to preserve the morphological structural changes resulting from cooking. Liquid nitrogen was previously recommended by Varriano-Marston et al. (1985) to rapidly freeze starch paste sample before the following freeze drying, mounting and coating processes. The disadvantage of using liquid N_2 is when hot sample pastes are submerged in liquid N_2, nitrogen gas is produced which can form pockets around starch pastes resulting in slow heat transfer (or freezing) rate. In addition, the slow freezing rate induces formation of large ice crystals which may disrupt the fine morphological structure of starch paste. Liquid nitrogen/acetone cooled isopropanol (-70°C) was used in this experiment to rapidly freeze starch paste samples. Isopropanol was placed in a bath cooled with liquid N_2/acetone (-70°C). Isopropanol turns viscous at this temperature and allows quick-freezing the samples without forming gas. The frozen samples were then transferred to liquid N_2, freeze-dried, followed by general mounting and coating steps prior to viewing with SEM.

The granule size of corn starches, both raw and cooked, was smaller than those of bean starches (Plates 2-5). The structural changes of corn starch granules during cooking

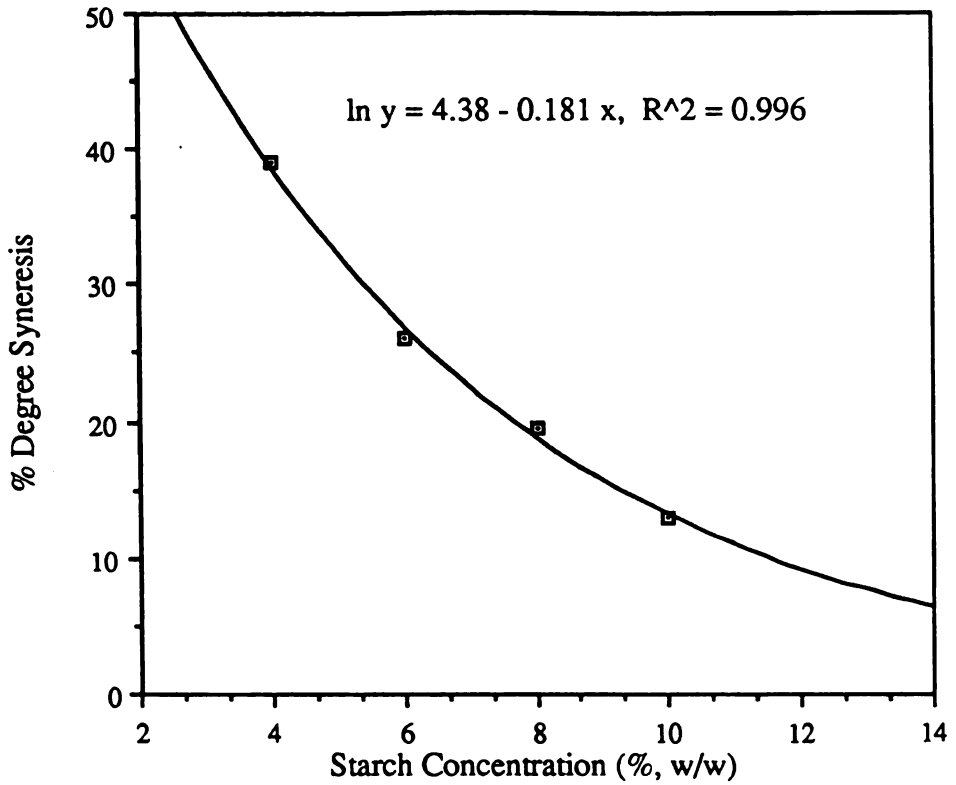

Figure 15. Effect of Starch Concentrations on Gel Degree Syneresis

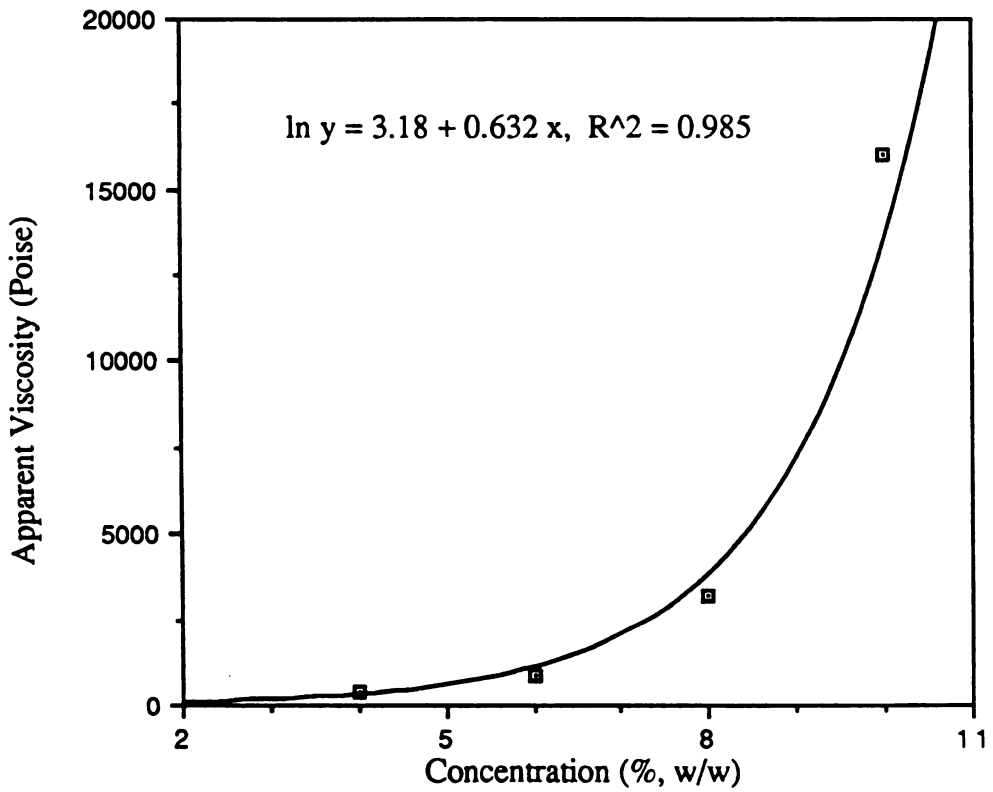

Figure 16. Effect of Starch Concentrations on Gel Apparent Viscosity

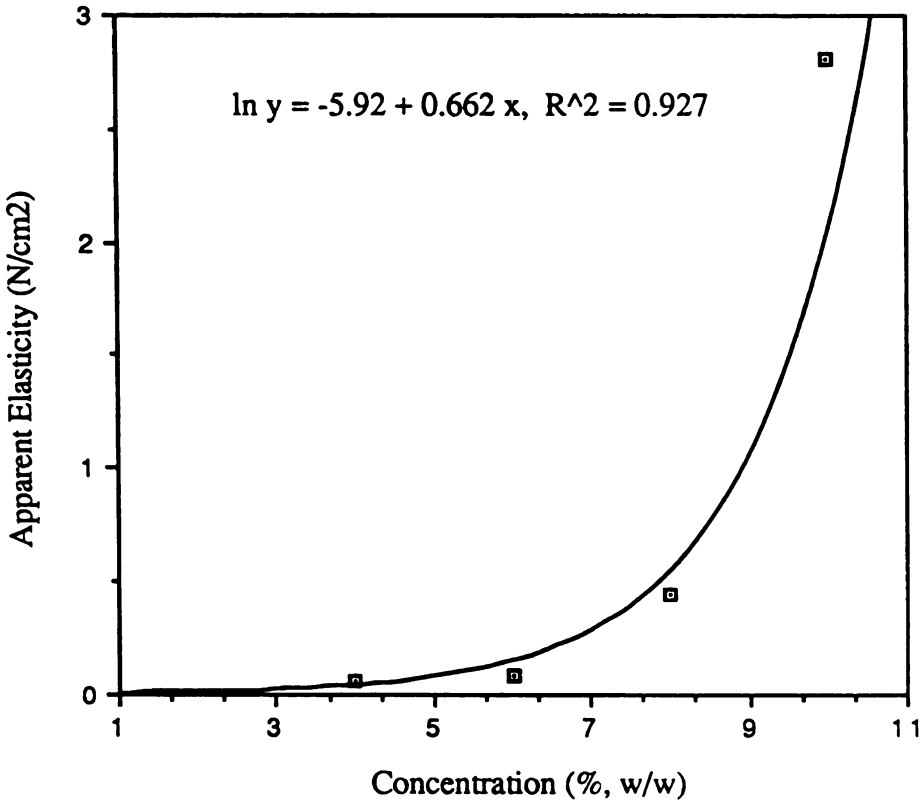

Figure 17. Effect of Starch Concentrations on Gel Apparent Elasticity

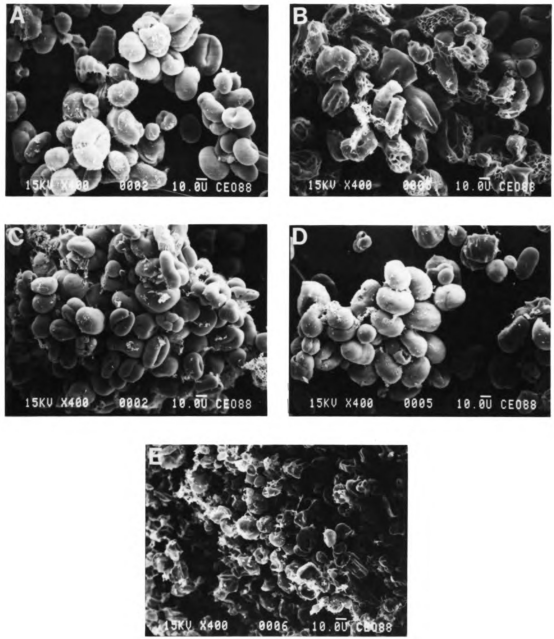

Plate 3. Scanning Electron Micrographs of Starches Cooked in Water at 70ºC for 1 hr: A) C-20, B) Seafarer, C) Fleetwood, D) 84004 and E) Corn

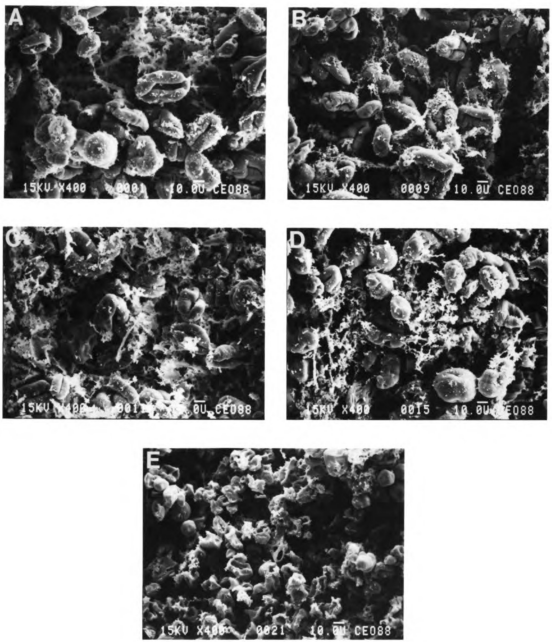

Plate 4. Scanning Electron Micrographs of Starches Cooked in Water at 80°C for 1 hr: A) C-20, B) Seafarer, C) Fleetwood, D) 84004 and E) Corn

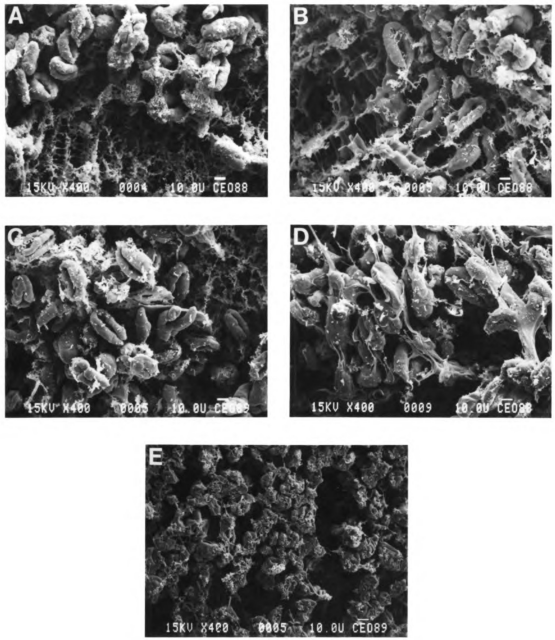

Plate 5. Scanning Electron Micrographs of Starches Cooked in Water at 90°C for 1 hr: A) C-20, B) Seafarer, C) Fleetwood, D) 84004 and E) Corn

were unlike the changes that occur with bean starch granules. At 70°C, corn starch granules had been heated to the midpoint of the gelatinization range (Table 12), where most of granules had collapsed and melted as a result of amylose solubilization. All bean starch granules, except Seafarer's, retained swollen granule shape with noticeable signs of shrinkage at the center of the starch granules, indicating a weak bonding region. Mixed characteristics of Seafarer starch granules included swollen granules with ridges and other granules with smooth surface and central shrinkage. At 80°C, fibrous-like exudates (amylose) increasingly leached out of granules, leaving major portions of amylopectin (as a granule's major structural component) inside the remnant granules. The melting of remnant granules is more obvious in corn starch. Among bean starches, a slightly more severe collapse of granules were observed for the Seafarer starch. Greater amount of fibrous materials leaching from bean starch granules evidently coincide with their higher amylose contents. Severe granule disintegration and matrix formations from leached materials and the melting granules were found in starch cooked at 90°C, for 1hr. Interestingly, there was no evidence of the matrix formation in the Fleetwood starch. This phenomenon may explain its low viscosity (Table 13) and soft gel characteristics (Table 14). Fleetwood starch granule size and its structural changes at various temperatures seem to correlate with its physical properties, especially swelling power and solubility and pasting characteristics.

Relationships between Starch Characteristics and Canned Bean Quality

Variation in bean starch characteristics among studied bean cultivars (C-20, Seafarer, Fleetwood and 84004) are relatively minor in comparison to the differences found between corn starch and bean starches. Slight variations in starch properties among bean cultivars do not provide any meaningful correlations to their canning quality. Starch content, however, is highly correlated with drained weight ($R^2 = 0.990$) and possibly to shear texture ($R^2 = 0.650$). These parameters must be influenced by 1) the large quantity (41.83% to 45.15%) of starch; 2) exceptional high amylose content; 3) strong bondings within starch granules and 4) firm gel formation with a high degree of syneresis. It is

difficult to establish relationships between starch properties and canned bean quality due to the fact that chemical interactions among dry bean components and their structural/compositional changes during thermal processing are complex phenomena. In-vitro study of each component may help explain processing effects.

However, some meaningful conclusions can be obtained when combining the findings on bean starch properties (Study III) and bean flour properties (Study II) which were determined under similar procedures. Two evidences in pasting property studies of bean flours and bean starches indicate the influences of macro-molecules, other than starch, on viscosity development of flours during heat treatment. First, comparing Seafarer and Fleetwood cultivars, the much higher viscosity of Fleetwood bean flour cannot be explained by the starch content and/or starch characteristics since both bean cultivars contain similar ($p>0.05$) starch content. At the same concentration, Seafarer starch produced more viscous paste than Fleetwood starch. Secondly, comparing C-20 and Seafarer cultivars, C-20 contains lower ($p<0.05$) starch content and yet the flour viscosity values were similar. In addition, the starch paste of C-20 was less viscous than that of Seafarer at the same conditions. These lines of interpretation further substantiate significant influences of other macro-molecules in beans. The possible macromolecules involved in viscosity development during heat treatment include proteins and/or fiber materials. The least viscous flour paste of 84004 may be partly due to its lowest starch content, but not starch characteristics since 84004 starch behaves similarly to C-20 starch.

From the studies on gel properties prepared from bean flours (Study II) and bean starches (Study III), the obvious characteristic difference between the whole flour and starch gels from the same bean cultivar is their water holding capacity. Opposite to starch gels, flour gels exhibit trace amount of exudates during storage. This indicates that other components in whole bean flour interfere with starch retrogradation. Furthermore, flour gels at 12% (w/w) concentration demonstrate weaker bondings (lower apparent viscosity) than starch gel at 8% (w/w) concentration. This means that the bonds between starch and

other components in beans are less crystalline than between starch molecules, and hence are able to hold more liquid in the gel matrix. The high swelling power and solubility, and weaker granule integrity of Seafarer starch may allow more interactions between starch and other bean components; therefore, among bean cultivars, Seafarer flour gel is the softest (Table 7). However, Seafarer starch gels are the firmest among bean starch gels prepared under the same conditions.

Summary and Conclusions

Compared to corn starch, overall characteristics of isolated bean starches are: high amylose content (34.13% - 35.26%), restricted swelling power, temperature dependent solubility, high gelatinization temperature range (69.8°C - 85.75°C) & high enthalpy (3.89 - 4.77 Cal/g), strong granule integrity and firmer gel formation with high degree of syneresis.

Although, the properties of isolated starches isolated from selected bean cultivars do not show a close relationship to canned bean quality, the starch content in beans seems to correlate well with drained weight ($R^2 = 0.990$) and to a lower degree with shear texture ($R^2 = 0.650$). The findings from physico-chemical analyses of bean flours and their corresponding starches further implicate the possible roles of other macromolecules in bean such as protein and/or fibers, in determining canning quality. The soft texture of 84004 bean cultivar may be partly related to its lowest starch content.

Study IV: Cell Wall Phenolic Acids and Their Derivatives

In this section, seed coat and cotyledon tissues of studied bean cultvars were independently studied for their cell wall components and phenolic acid constituents since these tissues differ vastly in their biological functions. Seed coats are recognized as protective tissues, whereas cotyledons are storage tissues.

Seed Coat and Cotyledon Proximate Composition

Study II demonstrated that pasting characteristics and gel strength of whole bean flours are significantly influenced by the chemical composition of dry beans which in turn can significantly influence bean functional quality. Therefore, chemical characterization of bean seed coats and cotyledons may improve our understanding of factors relating to canning quality. Although, seed coat is of obvious concern in determining seed water absorption, the role of its components on water hydration is not clearly eatablished. In a study of 13 varieties of legumes, Muller (1967) concluded that the thickness of palisade layer as well as lignin and cellulose content of seed coat were important determinates for bean water hydration and cooking quality.

Results from proximate chemical analyses of studied beans presented in Table 16 further indicate the dissimilarity in major chemical composition between seed coat and cotyledonary tissues. By comparison, seed coats contain more non-starch carbohydrates (mainly fibers) and minerals (presented as ash content) but less fat and protein content. Fleetwood seed coat has highest fat (1.14%) and protein (10.85%) contents, but lowest in ash (4.58%) and carbohydrate (83.46%) contents. C-20 seed coat possessed the lowest protein content (6.99%) commensurated with the highest carbohydrate (87.24%) content. Another noticeable observation from seed coat chemical analyses is that the fat content of Seafarer (1.09%) and Fleetwood (1.14%) are approximately three times (3x) higher than those of C-20 (0.36%) and 84004 (0.32%). High fat content of seed coat indicates a greater hydrophobic property which could impede seed hydration during soaking. However, there is no high correlation between chemical composition of seed coats and bean canning quality. The effect of seed coats on canning quality may be masked by the cotyledon component due to its greater proportion of the bean seeds (92.28-93.56 %, w/w) (Table 5). Chemical composition of cotyledon flours (Table 16) are quite similar in their contents and parallelled that of its whole bean flour which was previously discussed in Study II.

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).

Cell Wall Polysaccharides

Cell Wall Preparation

Because of their dissimilarities in chemical composition, seed coat and cotyledon tissues require different methods for their cell wall preparation. Since seed coat tissues are comprised mostly of non-starch carbohydrates (83.46% - 87.24%), their cell walls can be obtained by simple successive alcoholic extractions of defatted seed coat flour to further remove small and relatively polar molecules (i.e. sugars, phenolic acids etc.). The yields of alcohol insoluble cell walls (AICW) are reported in Table 17. C-20 seed coat contained more AICW (95.33%) than 84004 (93.52%), Fleetwood (92.24%) and Seafarer (91.96%).

Cotyledon tissues, however, require several additional steps of enzymatic hydrolyses to remove the storage starch and protein (Figure 11). The general method of Prosky et al. (1984) was used except the hydrolysis periods were extended: α-amylase-2 hr; protease-8 hr and amyloglucosidase-16 hr. The yield of cotyledon purified cell wall (PCW) and their residual contaminants (protein and ash) are also presented in Table 17. C-20 (16.62%), Seafarer (17.68%) and 84004 (16.32%) demonstrated similar ($p>0.05$) PCW contents, while Fleetwood (18.62%) had a significantly ($p<0.05$) higher PCW content than those of C-20 and 84004. The cotyledon PCWs contained 11.36 - 14.27% protein , 6.94 - 8.47% ash; and no residual starch (negative I_2 test). The relative order of residual proteins in PCW, from high to low, is Fleetwood > C-20 > 84004 > Seafarer. Significant differences ($p<0.05$) in PCW residual protein contents were found between Seafarer and C-20 or Fleetwood. The residual ash content of PCW were similar ($p>0.05$) among all cultivars. In a previous study (Study II, Table 9), similar trends were observed for total dietary fiber in whole bean flours.

Cell Wall Fractionation

Fractionation of the cell wall materials (AICW and PCW) was performed by sequential solvent extractions (Figure 12). The isolated fractions are classified according to

Table 17. Data (%, db) from the Cell Wall Preparations of Seed Coat and Cotyledon Tissues[1]

Bean	Seed Coat	Cotyledon		
Cultivar	AICW[2]	PCW[2]	Residual Protein[3]	Residual Ash[3]
C-20	95.33 ± 0.10c	16.62 ± 0.47a	14.24 ± 0.32b	8.47 ± 0.91a
Seafarer	91.96 ± 0.27a	17.08 ± 0.60ab	11.36 ± 1.25a	7.07 ± 0.02a
Fleetwood	92.24 ± 0.03a	18.02 ± 0.55b	14.27 ± 0.19b	6.94 ± 1.46a
84004	93.52 ± 0.09b	16.32 ± 0.93a	13.55 ± 0.88ab	8.09 ± 0.57a

[1] Means in a column followed by different letters are significantly different ($P<0.05$).
[2] The yield of alcohol insoluble cell wall (AICW) and purified cell wall (PCW) were reported as %, db of seed coat (n = 3) and cotyldeon tissues (n = 7), respectively
[3] Residues are expressed as %, db in purified cell wall (PCW), n = 2

their solubility: 1) hot water soluble polymer (HWSP), 2) ammonium oxalate soluble polymer (AOSP), 3) hemicellulose A (H_A), 4) hemicellulose B (H_B), 5) cellulose, and 6) lignin. During hot water extraction, cell wall polysaccharides which are loosely bound primarily through hydrogen bonds, were solubilized. Hot aqueous ammonium oxalate (calcium chelating solvent) further provides disruption of calcium-stabilized ionic bondings of polysaccharide chain aggregates. It is expected that most of the pectins would be extracted by hot water (highly esterified pectins) and ammonium oxalate (less esterified pectins) treatments (Stevens and Selvendran, 1980). Hemicelluloses are solubilized in alkali medium indicating their covalent linkages to other cell wall materials. The alkali extracted polysaccharides, hemicelluloses (H) can be further differentiated into H_A and H_B. H_B is soluble in dilute salt solution at pH 4.5, whereas H_A requires 1 N NaOH for solubilization. Earlier works on the hemicelluloses (H_A and H_B) of various plant cell walls (Brillouet and Mercier, 1981; Salimath and Tharanathan, 1982 and Wen et al., 1988) demonstrated the differences in sugar composition of the two hemicellulose classes. All of these investigators found a higher ratio of arabinose to xylose in H_B than in H_A. The pectins and the hemicelluloses are generally recognized as matrix cell wall polysaccharides. They exist in the non-crystalline forms stabilized by their multibranched molecules with several species of sugar monomers. Unlike pectins and hemicellulose, cellulose is a microfibrillar polysaccharide with repeating molecules of glucose linked by β-1,4 glycosidic bonds. The long, unbranched molecules of cellulose are orderly arranged to form microfibrils and then macrofibrils, respectively. This characteristic of cellulose results in its crystalline structure. Therefore, cellulose is not easily solubilized in most solvents. Lignin, a complex, highly ramified polymer of phenylpropane (C_6 - C_3) residues, is also present in cell wall and serves to add rigidity to the wall.

The amount of each cell wall fraction in seed coats and cotyledons were calculated as percentages (w/w) of AICW and PCW and reported in Table 18. The sum of cell wall fractions are less than 100% due to the impurities (residual protein and ash) of the prepared

Table 18. Data on Yield (%, db) of Cell Wall Fractions[1] in Seed Coats and Cotyledons[2]

Bean Component	Bean Cultivar	HWSP	AOSP	Hemicellulose (H)			Cellulose	Lignin	Sum of Fractions
				Total	H_A	H_B			
Seed Coat	C-20	2.42 ± 0.38a	9.85 ± 0.24ab	18.68 ± 0.52a	10.53 ± 0.55a	8.14 ± 0.03b	60.70 ± 0.83c	1.68 ± 0.25bc	93.32 ± 1.01a
	Seafarer	3.79 ± 0.09b	12.13 ± 0.77c	19.66 ± 0.33ab	10.11 ± 0.27a	9.55 ± 0.14c	58.43 ± 0.58b	1.37 ± 0.05ab	95.38 ± 0.98b
	Fleetwood	3.57 ± 0.16b	8.90 ± 0.07a	20.80 ± 0.98b	13.48 ± 1.23b	7.32 ± 0.25a	58.65 ± 1.15b	1.38 ± 0.06ab	93.29 ± 0.31a
	84004	3.55 ± 0.10b	9.87 ± 0.62b	24.40 ± 0.55c	16.17 ± 0.32c	8.23 ± 0.31b	56.42 ± 0.51a	1.86 ± 0.22c	96.10 ± 0.85b
Cotyledon	C-20	23.70 ± 1.86b	9.05 ± 0.34a	22.01 ± 1.15c	4.50 ± 0.51d	17.52 ± 0.87b	27.12 ± 0.91a	0.39 ± 0.10a	82.15 ± 1.91b
	Seafarer	25.23 ± 1.79b	12.81 ± 0.30c	20.52 ± 0.79b	1.30 ± 0.14a	19.22 ± 0.74c	30.91 ± 0.57b	0.56 ± 0.02a	89.84 ± 1.91c
	Fleetwood	19.35 ± 0.49a	12.40 ± 0.25c	17.18 ± 0.43a	2.58 ± 0.05b	14.60 ± 0.38a	25.98 ± 0.29a	0.45 ± 0.03a	75.21 ± 1.06a
	84004	28.41 ± 1.25c	11.42 ± 0.37b	20.64 ± 0.21bc	3.78 ± 0.29c	16.86 ± 0.37b	26.80 ± 1.73a	0.39 ± 0.09a	87.53 ± 0.30c

[1] All cell wall fractions: Hot water soluble polymer (HWSP), Ammonium oxalate soluble polymer (AOSP), Hemicellulose (H), Cellulose and Lignin, were reported as % of AICW and PCW in Seed Coat and Cotyledon, respectively.

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

cell wall materials, especially cotyledon PCW. In order to make meaningful comparisons among bean cultivars, the percentages of each cell wall fraction were recalculated based on the sum of all cell wall fractions (Table 19).

Seed coat cell walls. The major cell wall fraction in studied bean seed coats is cellulose. Significant differences ($p<0.05$) in cellulose contents were found among cultivars, with the highest in C-20 (65.04%) and the lowest in 84004 (58.71%).

Hemicellulose is the next major cell wall fraction which contains more H_A (10.60 - 16.83%) than H_B (7.85 - 10.01%). The H_A contents of C-20 (11.29%) and Seafarer (10.60%) are similar ($p>0.05$), but both are significantly lower ($p<0.05$) than those of Fleetwood (14.45%) and 84004 (16.83%). The H_B (salt soluble hemicellulose) contents are highest and lowest in Seafarer (10.01%) and Fleetwood (7.85%), respectively.

The cation bound pectin (AOSP) contents are in the range of 9.54 - 12.71% among these studied bean seed coats. These AOSP structures exhibited gel-like characteristics in 80% ethanol. Precipitation of this material by centrifugation at 23,000 g for 15 min produced a transparent, gel-like material indicating good liquid holding capability (Plate 6).

Seed coat cell walls contain small amounts of HWSP (2.59 - 3.98%). There is no significant difference ($p>0.05$) in the HWSP content among cultivars, with the exception of C-20 which contained the lowest amount (2.59%). Lignin contents in seed coat cell walls (1.44 - 1.94%) are comparable to earlier findings (Champ et al., 1986), with the highest content in 84004 (1.94%) and the lowest content in Seafarer (1.44%).

Cotyledon cell walls. In contrast to the seed coat cell walls, the matrix fractions: HWSP, AOSP, H_A and H_B are the predominant polysaccharides in cotyledon cell walls. Cellulose makes up roughly one-third (30.61 - 34.55%) of the bean cotyledon cell walls whereas the cellulose constitutes nearly two-thirds of the seed coats (58.71 - 65.04%). Although the total content of hemicelluloses in cell walls of seed coats (20.02 - 25.39%) and cotyledons (22.84 - 26.79%) are comparable, the distributions of H_A and H_B are very different. H_B is the predominant form in the cotyledon cell walls while H_A is

Table 19. Percentage Distribution of Cell Wall Fractions[1] in Seed Coats and Cotyledons[2]

Bean Component	Bean Cultivar	HWSP	AOSP	Hemicellulose (H)			Cellulose	Lignin	Sum of Fractions
				Total	H_A	H_B			
Seed Coat	C-20	2.59 ± 0.37a	10.55 ± 0.15b	20.02 ± 0.59a	11.29 ± 0.60a	8.73 ± 0.09b	65.04 ± 0.67d	1.80 ± 0.28bc	100.00 ± 0.00
	Seafarer	3.98 ± 0.06b	12.71 ± 0.69c	20.61 ± 0.42a	10.60 ± 0.36a	10.01 ± 0.10c	61.26 ± 0.70b	1.44 ± 0.05a	100.00 ± 0.00
	Fleetwood	3.82 ± 0.18b	9.54 ± 0.04a	22.30 ± 1.07b	14.45 ± 1.33b	7.85 ± 0.26a	62.86 ± 1.16c	1.48 ± 0.06ab	100.00 ± 0.00
	84004	3.69 ± 0.07b	10.27 ± 0.58ab	25.39 ± 0.62c	16.83 ± 0.30c	8.56 ± 0.36b	58.71 ± 0.22a	1.94 ± 0.25c	100.00 ± 0.00
Cotyledon	C-20	28.85 ± 2.06b	11.03 ± 0.63a	26.79 ± 1.21b	5.46 ± 0.51d	21.33 ± 1.13b	33.01 ± 0.40b	0.48 ± 0.10a	100.00 ± 0.00
	Seafarer	28.06 ± 1.43ab	14.26 ± 0.18c	22.85 ± 1.22a	1.45 ± 0.19a	21.41 ± 1.08b	34.41 ± 0.12b	0.63 ± 0.03a	100.00 ± 0.00
	Fleetwood	25.73 ± 0.30a	16.49 ± 0.17d	22.84 ± 0.36a	3.43 ± 0.04b	19.41 ± 0.32a	34.55 ± 0.75b	0.59 ± 0.03a	100.00 ± 0.00
	84004	32.47 ± 1.48c	13.05 ± 0.43b	23.58 ± 0.19a	4.32 ± 0.34c	19.27 ± 0.36a	30.61 ± 1.91a	0.44 ± 0.10a	100.00 ± 0.00

[1] All cell wall fractions: Hot water soluble polymer (HWSP), Ammonium oxalate soluble polymer (AOSP), Hemicellulose (H), Cellulose and Lignin, were reported as % of AICW and PCW in Seed Coat and Cotyledon, respectively.

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

Plate 6. Ethanol Precipitates of Ammonium Oxalate Soluble Polysaccharides Extracted from Bean Seed Coat Cell Walls after Centrifugation at 23,000 g for 15 minutes

the predominant form in the seed coat. AOSP contents are slightly greater in cotyledon cell walls (11.03 - 16.49%) than in seed coat cell walls (9.54 - 12.71%). The HWSP fractions of cotyledon cell walls (25.73 - 32.47%) was dramatically higher than those observed in the seed coat. Further, approximately 2.5 to 3.0 times lower amount of lignin were found in cotyledons (0.44 - 0.63%). All of these cell wall component characteristics in cotyledons suggest a more flexible or less rigid nature of cotyledon tissues. Among these cultivars, the cotyledon cell wall of 84004 exhibited the highest HWSP content (32.47%) and the lowest cellulose content (30.61%). Conversely, the cotyleodn cell wall of Fleetwood is lowest in HWSP content (25.73%) and highest in cellulose content (34.55%). However, the HWSP of Fleetwood is not significantly different from that of Seafarer (28.06%). Further, the percentage of cellulose in Fleetwood is rather similar ($p>0.05$) to that found in Seafarer (34.41%) and C-20 (33.01%).

During the recovery of extracted HWSP by 80% ethanol, the Fleetwood HWSP exhibited a unique gel-like, fibrous characteristic. This material aggregated and stayed in suspension in 80% ethanol, whereas the HWSP from other beans showed complete precipitation. The mechanism of recovery (precipitation) cell wall polymers by adding 4 volume ethanol is based on ethanol competition for water molecules resulting in limited free water to fully hydrate the solubilized polymers. Upon centrifugation (23,000 g, 15 min), the Fleetwood HWSP precipitate retained its gel-like, transparent structure which is characteristic of materials with a high capacity for liquid entrapment (Plate 7). The ability to form polysaccharide matrix entrapping liquid suggests the "junction zones" formation (Graessley, 1974) probably through bondings between linear polymer molecules and/or linear sections of branched molecules (analogy to amylose and amylopectin, respectively). Furthermore, the observed characteristics of Fleetwood HWSP were similar to the characteristics of the AOSPs' in both Fleetwood seed coat and cotyledon. H_B contents in C-20 (21.33%) and Seafarer (21.41%) are significantly higher than those in Fleetwood (19.41%) and 84004 (19.27%). Significant differences ($p<0.05$) in H_A and AOSP

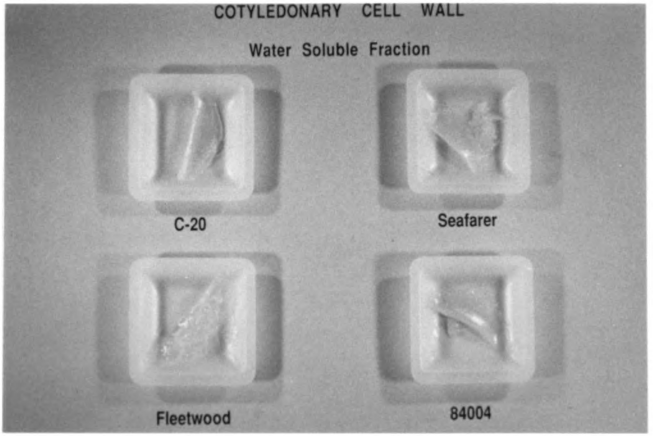

Plate 7. Ethanol Precipitates of Hot Water Soluble Polysaccharides Extracted from Cotyledon Cell Walls of Studied Beans after Centrifugation at 23,000 g for 15 minutes

contents were observed among all cultivars suggesting genetic dependent effects. H_A and AOSP contents are ranked from high to low as follows: C-20 > 84004 > Fleetwood > Seafarer and Fleetwood > Seafarer > 84004 > C-20, respectively. There is no significant difference in lignin content among cultivars. The results from cell wall fractionations (both seed coat and cotyledon) agree fairly well with the earlier findings on the basis of per gram starting tissues (Monte and Maga, 1980; Champ et al., 1986 and Anderson and Bridges, 1988). Minor variations could be due to different methodology for cell wall preparation and/or cell wall fractionation.

Relationships between Cell Wall Polysaccharides and Canned Bean Quality

Fractionation and quantitative analysis of bean cell walls, both in seed coats and cotyledons, provides data which can help explain differences in canned bean characteristics, especially texture. The soft bean, 84004 possesses significantly less ($p<0.05$) cellulose in both seed coat and cotyledon cell walls. Cellulose provides rigid structural characteristics to cell walls and has strong intermolecular bonding as evidenced by the difficulty in extracting this material. On the other hand, the HWSP fraction is less rigid and is otained by simple extraction of prepared cell walls with hot water, which indicates weak bonds between HWSP and other cell wall materials. At the same time, 84004 contained the highest cotyledon HWSP. The relatively low cellulose and high HWSP may partially explain its soft canned bean texture. Fleetwood (firm bean), however, showed the opposite trends having the lowest HWSP and the highest cellulose content. The standard culivars: C-20 and Seafarer, which possess similar shear texture, also contain similar cotyledon HWSP contents. C-20 cotyledon cell wall shows a slight trend of more matrix polysaccharides of H_A ($p<0.05$) and HWSP ($p>0.05$). In term of seed coat cell wall, C-20 demonstrates the stronger wall characteristics due to its higher ($P<0.05$) celluose and lower ($p<0.05$) contents in all matrix cell wall components except H_A. The lignin and AOSP contents of seed coat and cotyledon cell walls did not appear to be associated with canned bean texture.

From all results of this study, no mathematic relationship can be derived with correlation coefficient (R^2) higher than 0.75. However, the cotyledon HWSP appears to the best potential indicator of canned bean shear texture (increase HWSP content, decrease shear texture value). Fleetwood cotyledon HWSP is of interest for further physico-chemical analyses due to its distinct characteritstics. In-depth study on this HWSP fraction may provide further insight regarding textural qualities of canned beans. Further, some chemical modifications of cell wall constituents can occur during thermal processing of dry beans. Monte and Maga (1980) studied the cell wall constituents in pinto beans and indicated that the hemicellulose B became more water soluble after two hour boiling in water. They also suggested that the water soluble fractions would be lost with the cooking water, even though the bean was intact. High temperature and high pressure cooking in the retort can dramatically alter the cell wall physico-chemical properties, especially the matrix polysaccharides. Therefore, thermal history as well as the bean cell wall physico-chemical composition are necessary information in order to predict the texture of cooked beans.

Cell Wall Hydroxyproline

A significant amount of protein (11.36 - 14.27%) remained in the purified cotyledon cell wall (Table 17). The resistance of these proteins to extensive protease treatment could be due to the existence of unrecognized bonding(s) of protein. Lamport (1967 and 1969) demonstrated the presence of hydroxyproline rich glycoprotein in higher plant cell wall. He proposed that this protein may exert a possible role in cell wall extention which led to name of this glycoprotein extensin. More recent works (Epstein and Lamport, 1984; Fry, 1983 and Fry, 1986) have shown that the extensin can be cross-linked through isodityrosine bridges. This may be why the protein is not soluble and to some extent, resistant to proteolysis (arab-gal on protein resistant to proteolysis). The levels of cell wall bound extensin in the bean seed coats and cotyledons were estimated by determining the amount of hydroxyproline remaining in the cell walls. Because of the insoluble nature of extensin (Fry, 1986), extensive extractions with various solvents: 0.5 N NaCl (2x),

distilled water (2x), DMSO (2x), 80% alcohol (2x) to remove storage, enzymatic and ionically bound cell wall proteins and starch were used to prepare cell walls for hydroxyproline analysis with an intention to avoid any enzymatic treatments. Table 20 presents the hydroxyproline content of cell walls in seed coats and cotyledons. Seed coat cell walls contain more hydroxyproline (1.00 - 1.50 mg/g cell wall) than cotyledon cell walls (0.15 - 0.34 mg/g cell wall). The four times higher content of extensin in seed coat cell wall may be associated with its biological function as a protective tissue. Previous works (Hammerschmidt et al., 1984 and Stermer and Hammerschmidt, 1987) have shown that extensin accumulation is a rapid response to pathogenic infection and to heat shock and this is thought to be involved in defense process. Among the cultivars, the seed coat hydroxyproline contents of Seafarer and Fleetwood (1.05 and 1.00 mg/g cell wall, respectively) are significantly lower ($p<0.05$) than C-20 (1.23 mg/g cell wall) and 84004 (1.50 mg/g cell wall). In cotyledon, all beans exhibited significantly different hydroxyproline contents and are ranked as follows: C-20 > Seafarer > 84004 > Fleetwood. The differences in hydroxyproline content of the studied bean cell walls (both of seed coats and cotyledons) indicated their differences in genetic background. Based on the assumption that hydroxyproline content represents the level of extensin, there is no correlation found between the extensin content and the canned bean quality attributes.

Phenolic Acids and Their Derivatives

From Chapter I, the development of undesirable firm texture characteristic in hard-to-cook beans was proposed to be associated with changes in the phenolic acid distribution (free, ester and cell wall bound forms), primarily ferulic acid. In this section of Chapter II, the seed coats and cotyledons of model beans with different textural quality were also studied for their phenolic acid patterns as well as the total extractable phenol contents.

Total Extractable Phenol

The relative comparision of total extractable phenol in bean seed coat and cotyledon are illustrated in Figure 18. The total extractable phenols of C-20 and 84004 seed coats are

Table 20. Hydroxyproline Content in Seed Coat and Cotyledon Cell Walls[1]

Bean Cultivar	Hydroxyproline (mg/g cell wall)	
	Seed Coat	Cotyledon
C-20	1.23 ± 0.02b	0.34 ± 0.01d
Seafarer	1.05 ± 0.12a	0.25 ± 0.01c
Fleetwood	1.00 ± 0.03a	0.15 ± 0.01a
84004	1.50 ± 0.14c	0.22 ± 0.02b

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

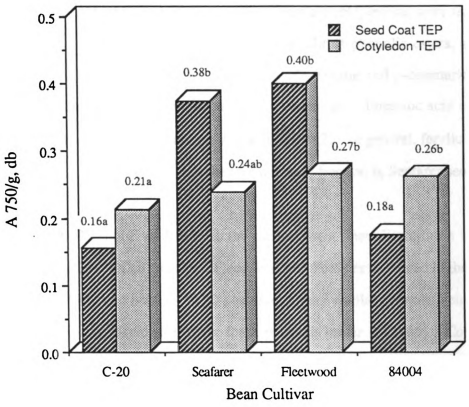

Figure 18. Comparison of Relative Total Extractable Phenol (TEP) from Seed Coat and Cotyledon Tissues

similar ($p>0.05$) and are approximately half of those of Fleetwood or Seafarer. In cotyledons, however, all beans demonstrated fairly similar extractable phenol contents, with the lowest in C-20.

Phenolic Acid Fractionation

Phenolic constituents in model bean seed coats and cotyledons were fractionated into 3 fractions: 1) free phenolic acids, 2) methanol soluble phenolic esters, and 3) cell wall bound phenolic acids. Two major phenolic acids (ferulic and p-coumaric acids) in navy beans were seperated and quantitated as μg/g tissue (db). Phenolic acid contents of the bean seed coats and cotyledons are presented in Table 21. In general, ferulic acid is the predominant form in all fractions except the free phenolic fraction in Seafarer seed coat and C-20 cotyledon.

Seed Coat. Phenolic acid ester is the predominant phenolic fraction in the seed coat of the bean studied. Of all fractions, Fleetwood and Seafarer exhibited higher phenolic acid contents than C-20 and 84004. C-20 possesses a very similar phenolic acid pattern to 84004 except the p-coumaric acid ester form is much lower ($P<0.05$). Compared to Seafarer, Fleetwood contains more ($p<0.05$) free ferulic acid but less ($P<0.05$) esters of both acids.

Cotyledon. Similar to seed coat tissues, the major phenolic fraction in cotyledons is the ester form. In general, when compared with the seed coats, the cotyledons of the model beans has less free phenolic acids, greater phenolic acid esters and less cell wall bound ferulic acids. Minor exceptions were noted for C-20 and Seafarer. Among the cultivars, the phenolic acid distribution are fairly similar.

The results from the study of phenolic acid fractionation confirm the previous works on the total extractable phenol. The distribution of phenolic acids in seed coats and cotyledons seems to be cultivar dependent, and they show no relationships to texture characteristics.

Table 21. Phenolic Acid Contents (μg/g, db) in Seed Coat and Cotyledon Tissues[1]

Phenolic Acid Fraction	Bean Cultivar	Seed Coat		Cotyledon	
		p-Coumaric	Ferulic	p-Coumaric	Ferulic
Free Phenolic Acids	C-20	5.20 ± 1.14a	10.74 ± 0.70a	15.81 ± 1.13b	7.11 ± 2.23a
	Seafarer	21.48 ± 0.06b	16.96 ± 1.03b	6.67 ± 0.14a	10.71 ± 1.95ab
	Fleetwood	22.44 ± 0.05b	23.84 ± 2.26c	6.52 ± 0.38a	13.74 ± 1.57b
	84004	4.74 ± 1.04a	8.86 ± 0.89a	4.96 ± 1.15a	7.32 ± 2.01a
Phenolic Esters	C-20	6.17 ± 0.65a	91.81 ± 10.29a	32.74 ± 0.93b	209.49 ± 31.28ab
	Seafarer	65.74 ± 4.25d	222.25 ± 16.01b	24.21 ± 7.10ab	212.34 ± 22.53ab
	Fleetwood	46.21 ± 0.29c	121.32 ± 5.03a	20.12 ± 3.80a	176.17 ± 5.20a
	84004	25.50 ± 0.62b	117.72 ± 21.81a	17.48 ± 3.74a	247.56 ± 2.19b
Cell Wall Bound Phenolic Acids	C-20	ND	7.21 ± 1.35a	ND	10.46 ± 3.53a
	Seafarer	ND	29.26 ± 5.71b	ND	10.47 ± 1.32a
	Fleetwood	ND	20.40 ± 4.59b	ND	10.74 ± 3.45a
	84004	ND	9.65 ± 1.77a	ND	7.19 ± 1.27a

[1] n = 4, Means in a column within a fraction followed by different letters are significantly different ($P<0.05$).

Summary and Conclusions

Cell wall components in seed coat and cotyledon tissues were isolated into six fractions based on their solubility: HWSP, AOSP, H_A, H_B, cellulose and lignin. Seed coat cell wall is mainly composed of microfibrillar polysaccharide, cellulose. Its predominant matrix polysaccharides are the group of hemicelluloses with slightly more H_A than H_B. The major pectins are present in the AOSP fraction rather than in the HWSP fraction. Lignin content in seed coat is low. Cotyledon cell wall, however, contains mostly matrix polysaccharides, especially HWSP. The predominant hemicellulose is H_B. Very little lignin is in the cotyledon tissues. More extensin was found in cell walls of seed coats than in cotyledon.

Higher contents of cellulose, lignin and cell wall extensin in seed coat cell wall support the concept that biological function of seed coat is for protection. Some possible relationships were found between cell wall composition and canned bean texture characteristics, especially the HWSP of cotyledon cell wall (high HWSP content - lower shear texture value). The distinct different characteristics of Fleetwood HWSP in 80% alcohol suggests dissimilarities in its molecular structure and/or physico-chemical properties which may contribute to its high shear textural quality.

The contents of extensin, total extractable phenol, free phenolic acids, phenolic acid esters and cell wall bound phenolic acids in the model bean components (both seed coat and cotyledon) appear to be cultivar dependent and are not associated with their canning quality.

Summary and Conclusions

Four selected navy bean cultivars with significant textural differences (Fleetwood, 41.5; C-20, 29.3; Seafarer, 28.9 and Experimental line 84004, 22.5 kg force/50 g canned beans) were studied for their proximate chemical composition. Starch, cell wall components and phenolic acid constituents of these bean cultivars were also isolated, purified and studied for their physico-chemical properties. Subsequently, the relationships between physico-chemical characteristics of bean constiuents and their corresponding canning qualites were hypothesized. The following are some significant findings which may explain the differences in canned bean qualities of the studied beans.

Fleetwood. Canned product of this bean cultivar exhibited the lowest drained weight and the highest shear texture value. Fleetwood's highest content of starch, fiber residue and seed coat may contribute to these canning qualities. Although high starch content in this bean is partially responsible for its firm product texture, the starch characteristics do not appear to explain its firmness quality. Fleetwood bean flour developed unsual high viscosity upon heating/cooling during Brookfield viscosity test. Since the starch content of Fleetwood is slightly greater than that of Seafarer, the four times higher viscosity of Fleetwood bean flour must have resulted from other macro-polymers such as protein and soluble fibers. Another supporting evidence is that Seafarer starch also exhibited the higher viscosity than Fleetwood starch.

Highest residual proteins were found in Fleetwood fibers during TDF analysis and cell wall preparation. However, there was no meaningful correlation between either residual protein content or TDF (protein free fiber) content and shear texture of model cultivars. The interaction between cell wall constituents and residual protein suggests unique bonding characteristics which may play an important role in canned product texture. The lowest amount of HWSP in cotyledon cell wall and its distinct characteristics in 80% alcohol may also contribute to its high shear value.

Experimental Line 84004. Distinct canning quality of 84004 including the highest drained weight and the softest shear texture is of researchers' interest. This high drained weight and soft texture of canned 84004 may be due to its lowest contents of starch and fiber (TDF and cellulose). Furthermore, 84004 seed coat and cotyledon cell walls also contain the highest content of matrix polysaccharides, especially the cotyledon HWSP fraction. Other studied physico-chemical characteristics were found to be fairly similar to those of C-20 and Seafarer cultivars.

C-20 and Seafarer. These two cultivars exhibited similar shear textural quality of canned products. The physico-chemical properties of C-20 and Seafarer were relatively comparable. The lower drained weight of canned Seafarer may be related to its higher contents in starch and fiber (TDF). Among the studied cultivars, Seafarer protein seems to be most susceptible to protease hydrolysis resulting in lowest residual protein contents in prepared fibers. Matrix polysaccharides in seed coat cell wall of Seafarer was greater than that of C-20; however, both beans contained very similar amount of matrix cotyledon cell wall, expecially the HWSP fraction.

No relationship was found between canning quality attributes and the contents of total extractable phenol, various fractions (free, ester and cell wall bound) of major phenolic acids (ferulic and p-coumaric acids) and extensin estimated by the level of hydroxyproline in extracted cell wall.

APPENDIX A

APPENDIX A

Selected Multiple Linear Regression Equations for Bean Chemical Composition and Canned Bean Quality

Drained Weight	=	522.75 - 3.888 (% Starch) - 2.230 (% TDF)
		$R^2 = 0.889$
Shear Force	=	-148.45 + 3.606 (% Starch) + 1.161 (% Residual Protein)
		$R^2 = 0.922$

APPENDIX B

APPENDIX B

Swelling Power and Solubility Index of Studied Starches

Temperature (°C)	Starch Source	Swelling Power	Solubility Index
70	Corn	11.77 ± 0.05b	5.44 ± 0.12c
	C-20	3.46 ± 0.17a	0.62 ± 0.06a
	Seafarer	3.70 ± 0.16a	1.01 ± 0.24b
	Fleetwood	3.68 ± 0.14a	0.65 ± 0.15a
	84004	3.65 ± 0.15a	0.47 ± 0.01a
75	Corn	13.97 ± 0.18c	6.82 ± 0.14c
	C-20	6.86 ± 0.71a	3.62 ± 0.89a
	Seafarer	8.55 ± 0.31b	5.23 ± 0.42b
	Fleetwood	8.32 ± 0.08b	6.10 ± 0.11bc
	84004	6.70 ± 0.68a	2.84 ± 0.68a
80	Corn	15.06 ± 0.17c	7.54 ± 0.11a
	C-20	10.37 ± 0.10a	9.56 ± 0.36b
	Seafarer	11.89 ± 0.03b	11.61 ± 0.52c
	Fleetwood	11.56 ± 0.35b	11.67 ± 1.00c
	84004	10.67 ± 0.35a	8.96 ± 0.49b
85	Corn	17.83 ± 0.22c	12.15 ± 0.90a
	C-20	12.59 ± 0.29a	14.17 ± 1.01a
	Seafarer	13.86 ± 0.23b	18.16 ± 0.68c
	Fleetwood	12.82 ± 0.17a	15.10 ± 1.87ab
	84004	12.73 ± 0.07a	15.35 ± 1.27ab
90	Corn	21.39 ± 0.78d	18.35 ± 2.41ba
	C-20	12.73 ± 0.12a	14.77 ± 0.72a
	Seafarer	14.66 ± 0.10c	16.12 ± 1.03ab
	Fleetwood	13.39 ± 0.43ab	16.08 ± 0.95ab
	84004	13.57 ± 0.36b	16.35 ± 1.44ab

APPENDIX C

APPENDIX C

Correlations Between Amylose Content (%) and Degree Syneresis (%DS) of Starch Gels Stored at 7 and 10 Days

7 Day Storage

$\ln \%\ DS = -8.87 + 0.345\ \%\ Amylose, \quad R^2 = 0.999$

10 Day Storage

$\ln \%\ DS = -9.36 + 0.355\ \%\ Amylose, \quad R^2 = 0.997$

APPENDIX D

Table 22. Analysis of Variance for Canned Bean Quality Attributes of Navy Beans Stored Under Selected Conditions for 9 Months

Source of Variation	Degrees of Freedom	Soaked Weight	Drained Weight	Processed Color			Texture Shear Force	Bean Solids
				L	a_L	b_L		
				MEAN SQUARES				
Storage Condition	2	31.207	2442.347**	218.587***	15.784***	2.047**	22385.012***	32.687**
Error	3	11.340	23.633	0.033	1.667E-5	0.020	42.242	0.780
Total	5	19.287	991.119	87.455	6.314	0.831	8979.350	13.543

Table 23. Analysis of Variance for p-Coumaric Acid Content in Control, Partially Hard and Hard Bean Components

Source of Variation	Degrees of Freedom	Seed Coat		Cotyledon	
		Methanol Soluble Ester	Free Acid	Methanol Soluble Ester	Free Acid
		MEAN SQUARES			
Bean Category	2	6006.229***	463.415**	108.206*	247.996**
Error	3	18.312	8.72	6.543	3.924
Total	5	2413.479	190.598	47.208	101.553

Table 24. Analysis of Variance for Ferulic Acid Content in Control, Partially Hard and Hard Bean Components

Source of Variation	Degrees of Freedom	Seed Coat				Cotyledon			
		Hexane Soluble Ester	Methanol Soluble Ester	Free Acid	Cell Wall Bound Acid	Hexane Soluble Ester	Methanol Soluble Ester	Free Acid	Cell Wall Bound Acid
					MEAN SQUARES				
Bean Category	2	2.590**	93625.488**	3995.701***	623.095**	0.505***	15360.175***	1652.802**	3.300
Error	3	0.056	1009.369	3.560	21.488	0.001	57.313	12.375	0.856
Total	5	1.070	38055.817	1600.416	262.131	0.202	6178.458	668.546	1.833

Table 25. Analysis of Variance for Sinapic Acid Content in Control, Partially Hard and Hard Bean Components

Source of Variation	Degrees of Freedom	Seed Coat			Cotyledon		
		Methanol Soluble Ester	Free Acid	Cell Wall Bound Acid	Methanol Soluble Ester	Free Acid	Cell Wall Bound Acid
		MEAN SQUARES					
Bean Category	2	16301.288***	420.869**	11.2200***	3566.926**	42.359*	0.126
Error	3	33.504	25.493	0.069	23.098	4.270	0.275
Total	5	6540.618	183.644	4.529	1440.629	19.505	0.216

Table 26. 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				
Storage Condition	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
Total	7	5.388	76.603	0.258	0.147	0.489	54.116	0.743

Table 27. Analysis of Variance for Seed Coat Contents of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Mean Squares
Cultivar	3	1.025***
Error	8	0.037
Total	11	0.307

Table 28. 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
Total	11	21875.320	368440.802

Table 29. Analysis of Variance for Rheological Properties of 12% (w/w) Whole Bean Flour Gels from Four Bean Cultivars

Source of Variation	Degrees of Freedom	Apparent Viscosity	Apparent Elasticity
		MEAN SQUARES	
Cultivar	3	667717.515***	5.454*
Error	12	11277.780	1.432
Total	15	142565.727	2.236

Table 30. Analysis of Variance for Proximate Chemical Composition of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Moisture	Fat	Ash	Protein	Starch
			MEAN SQUARES			
Cultivar	3	13.656***	0.074***	0.010	12.359***	6.683***
Error	8	0.007	0.001	0.009	0.063	0.253
Total	11	3.729	0.021	0.009	3.417	2.007

Table 31. Analysis of Variance for Total Dietary Fiber Analysis (TDF and Fiber Residue) of Four Bean Cultivars

Source of Variation	Degrees of Freedom	TDF	Fiber Residue
		MEAN SQUARES	
Cultivar	3	4.621***	8.182***
Error	12	0.335	0.603
Total	15	1.192	2.119

Table 32. Analysis of Variance for Total Dietary Fiber Analysis (Residual Components) of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Residual Ash	Residual Protein	Retained Protein
			MEAN SQUARES	
Cultivar	3	1.526	32.187***	26.968***
Error	4	1.257	0.178	0.617
Total	7	1.372	13.896	11.911

Table 33. Analysis of Variance for Chemical Characteristics of Corn and Isolated Bean Starches

Source of Variation	Degrees of Freedom	Moisture	Ash	Protein	Amylose in Starch
			MEAN SQUARES		
Starch Source	4	46.146***	0.004***	0.012**	81.460***
Error	9	0.128	1.235E-4	0.001	0.173
Total	13	14.287	0.001	0.005	25.184

Table 34. Analysis of Variance for Swelling Power of Corn and Isolated Bean Starches at Various Temperatures

Source of Variation	Degrees of Freedom	Swelling Power 70°C	75°C	80°C	85°C	90°C
				MEAN SQUARES		
Starch Source	4	39.857***	26.384***	10.459***	14.753***	38.020***
Error	10	0.020	0.221	0.057	0.044	0.188
Total	14	11.402	7.696	3.029	4.247	10.997

Table 35. Analysis of Variance for Solubility Index of Corn and Isolated Bean Starches at Various Temperatures

Source of Variation	Degrees of Freedom	Solubility Index				
		70°C	75°C	80°C	85°C	90°C
		MEAN SQUARES				
Starch Source	4	13.696***	8.337***	9.482***	14.190**	4.974
Error	10	0.019	0.292	0.331	1.485	2.075
Total	14	3.927	2.590	2.946	5.115	2.904

Table 36. Analysis of Variance for DSC Characteristics of Corn and Isolated Bean Starches at 10% (w/w) Concentration

Source of Variation	Degrees of Freedom	To	Tp	Tm	Tm - To	ΔH
		MEAN SQUARES				
Starch Source	4	24.134***	37.178***	81.503***	35.111***	0.976***
Error	14	0.344	0.120	0.396	1.049	0.025
Total	18	5.630	8.355	18.420	8.619	0.236

Table 37. Analysis of Variance for Pasting Properties of Corn and Isolated Bean Starches at 6% (w/w) Concentration

Source of Variation	Degrees of Freedom	T_H	T_C	$T_C - T_H$
			MEAN SQUARES	
Starch Source	4	121159.700***	3680221.660***	2640354.298***
Error	10	951.141	11928.411	7768.631
Total	14	35296.443	1060012.196	759935.964

Table 38. Analysis of Variance for Degree of Syneresis and Rheological Properties of 8% (w/w) Starch Gels after 7 Day Storage at Room Temperature

Source of Variation	Degrees of Freedom	Degree of Syneresis	Apparent Viscosity	Apparent Elasticity
			MEAN SQUARES	
Starch Source	4	222.200***	5511346.656***	0.081
Error	10	0.548	385853.902	0.028
Total	14	63.877	1850280.403	0.043

Table 39. Analysis of Variance for Degree of Syneresis and Rheological Properties of 8% (w/w) Starch Gels after 10 Day Storage at Room Temperature

Source of Variation	Degrees of Freedom	Degree of Syneresis	Apparent Viscosity	Apparent Elasticity
			MEAN SQUARES	
Starch Source	4	296.147***	7339441.359***	0.095
Error	10	0.528	370128.112	0.044
Total	14	84.991	2361360.468	0.059

Table 40. Analysis of Variance for Properties of Starch Gels Prepared at Various Concentrations

Source of Variation	Degrees of Freedom	Degree of Syneresis	Apparent Viscosity	Apparent Elasticity
			MEAN SQUARES	
Starch Source	3	325.757***	162414643.296***	5.236***
Error	8	2.146	624635.748	0.247
Total	11	90.404	44749183.250	1.608

Table 41. 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
Total	11	0.268	0.167	1.824	2.302

Table 42. 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
Total	11	0.012	0.026	3.719	4.748	3.781

Table 43. Analysis of Variance for Preparation of Seed Coat Alcohol Insoluble Cell Walls (AICW) of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Mean Squares
Cultivar	3	7.080***
Error	8	0.023
Total	11	1.947

Table 44. Analysis of Variance for Preparation of Cotyledon Purified Cell Walls (PCW) of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Mean Squares
Cultivar	3	2.208*
Error	12	0.441
Total	15	0.794

Table 45. Analysis of Variance for Residual Components in PCW of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Residual Ash	Residual Protein
		MEAN SQUARES	
Cultivar	3	0.854	3.756*
Error	4	0.821	0.601
Total	7	0.835	1.953

Table 46. Analysis of Variance for Fractionation of Seed Coat Cell Walls (AICW) from the Four Bean Cultivars

Source of Variation	Degrees of Freedom	HWSP	AOSP	Hemicellulose			Cellulose	Lignin	Sum of Fractions
				Total	H_A	H_B			
				MEAN SQUARES					
Cultivar	3	1.143***	5.651***	18.740***	23.984***	2.536***	9.162**	0.171*	6.184**
Error	8	0.046	0.261	0.410	0.495	0.044	0.650	0.029	0.705
Total	11	0.345	1.731	5.409	6.901	0.724	2.971	0.068	2.199

Table 47. Analysis of Variance for Fractionation of Cotyledon Cell Walls (PCW) from the Four Bean Cultivars

Source of Variation	Degrees of Freedom	HWSP	AOSP	Hemicellulose			Cellulose	Lignin	Sum of Fractions
				Total	H_A	H_B			
				MEAN SQUARES					
Cultivar	3	42.559***	8.498***	12.651***	5.910***	10.965***	14.422**	0.012	126.792***
Error	8	2.112	0.100	0.544	0.092	0.394	1.052	0.005	2.133
Total	11	13.143	2.390	3.846	1.679	3.277	4.699	0.005	36.131

Table 48. Analysis of Variance for Percentage Distribution of Cell Wall Fractions in Seed Coats

Source of Variation	Degrees of Freedom	HWSP	AOSP	Hemicellulose			Cellulose	Lignin
				Total	H_A	H_B		
				MEAN SQUARES				
Cultivar	3	1.190***	5.591***	17.433***	25.083***	2.423***	21.346***	0.178*
Error	8	0.045	0.207	0.511	0.581	0.054	0.586	0.036
Total	11	0.357	1.675	5.126	7.263	0.700	6.248	0.075

Table 49. Analysis of Variance for Percentage Distribution of Cell Wall Fractions in Cotyledons

Source of Variation	Degrees of Freedom	HWSP	AOSP	Hemicellulose			Cellulose	Lignin
				Total	H_A	H_B		
				MEAN SQUARES				
Cultivar	3	23.417**	15.642***	10.640**	8.637***	4.130*	10.010**	0.015
Error	8	2.138	0.160	0.774	0.103	0.665	1.092	0.006
Total	11	7.941	4.382	3.465	2.430	1.610	3.524	0.006

Table 50. Analysis of Variance for Hydroxyproline Content in Seed Coat and Cotyledon Cell Walls of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Seed Coat	Cotyledon
		MEAN SQUARES	
Cultivar	3	0.150**	0.018***
Error	8	0.008	8.283E-5
Total	11	0.047	0.005

Table 51. Analysis of Variance for Total Extractable Phenol from Seed Coat and Cotyledon Tissues of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Seed Coat	Cotyledon
		MEAN SQUARES	
Cultivar	3	0.033**	0.001
Error	4	1.423E-4	2.901E-4
Total	7	0.014	7.143E-4

Table 52. Analysis of Variance for p-Coumaric Acid Content in Seed Coat and Cotyledon Tissues of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Seed Coat		Cotyledon	
		Free	Ester	Free	Ester
		MEAN SQUARES			
Cultivar	3	192.775***	1325.865***	48.821***	88.976
Error	4	0.599	4.734	0.687	19.914
Total	7	82.960	570.933	21.316	49.512

Table 53. Analysis of Variance for Ferulic Acid Content in Seed Coat and Cotyledon Tissues of Four Bean Cultivars

Source of Variation	Degrees of Freedom	Seed Coat			Cotyledon		
		Free	Ester	Cell Wall Bound	Free	Ester	Cell Wall Bound
		MEAN SQUARES					
Cultivar	3	91.887**	6613.471**	207.491*	19.804*	1702.367	5.690
Error	4	1.861	215.875	14.637	2.829	379.485	6.929
Total	7	40.444	2957.702	97.289	10.104	946.435	6.398

LIST OF REFERENCES

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AACC. 1983. "Approved Methods," 8th ed. American Association of Cereal Chemists, St. Paul, MN.

Adams, M.W. 1972. On the quest for quality in the field bean. In "Nutritional Improvement of Food Legumes by Breeding," Protein Advisory group of the United Nations System, United Nations, NY.

Adams, M.W. and Bedford, C.L. 1973. Breeding food legumes for improved processing and consumer acceptance properties. In "Nutritional Improvement of Food Legumes by Breeding," Proceedings of a symposium sponsored by PAG, Rome, July 1972. M. Milner (Ed.), p. 299. Protein Advisory Group (PAG), United Nations, New York, NY.

Akpapunam, M.A. and Markakis, P. 1979. Oligosaccharides of 13 American cultivars of cowpeas (*Vigna sinensis*). J. Food Sci. 44:1317.

Almas, K. and Bender, A.E. 1980. Effect of heat treatment of legumes on available lysine. J. Sci. Food Agric. 31:448.

Anderson, J.W. and Bridges, S.R. 1988. Dietary fiber content of selected foods. Am J. Clin. Nutr. 47:440.

AOAC. 1984. "Official Methods of Analysis", 14th ed. Association of Official Analytical Chemists, Washington, D.C.

Augustin, J., Beck, C.B., Kalbfleish, G., and Kagel, L.C. 1981. Variation in the vitamin and mineral contents of raw and cooked commercial *Phaseolus vulgaris* classes. J. Food Sci. 46:1701.

Aw, T.L. and Swanson, B.G. 1985. Influence of tannins on *Phaseolus vulgaris* protein digestibility and quality. J. Food Sci. 50:67.

Bancroft, J.D. 1975 a. Cryostats. In "Histochemical Techniques," Butterworths and Co. Ltd. (2nd Edition), p. 30 - 47, London and Boston.

Bancroft, J.D. 1975 b. Fixation. In "Histochemical Techniques," Butterworths and Co. Ltd. (2nd Edition), p. 5 - 15, London and Boston.

Bancroft, J.D. 1975. Freeze Drying and Freeze Substitution. In "Histochemical Techniques," p. 48 - 67, Butterworths and Co. Ltd. (2nd Edition), London and Boston.

Becker, R., Olson, A.C.,Frederick, D.P., Kon, S., Gumbmann, M.R. and Wagner, J.R. 1974. Condition for the autolysis of alpha-galactosides and phytic acid in california small white beans. J. Food Sci. 39:766.

Bedford, C.L. 1972. Bean storage and processing. In "Proceedings of the International Dry Bean Symposium," August 22-24. Michigan State University, East Lansing, MI.

Biliaderis, C.G., Maurice, T.J. and Vose, J.R. 1980. Starch gelatinization phenomena studied by differential scanning calorimetry. J. Food Sci. 45:1669.

Binder, L. and Rockland, L. 1964. Use of the automatic recording shear press in cooking studies of large dry lima beans (*Phaseolus lumatus*). Food Technol. 18:127.

Blouin, F.A., Zarins, Z.M., and Cherry, J.P. 1982. Discoloration of proteins by binding with phenolic compounds. In "Food Protein Deterioration," J.P. Cherry (Ed.), p. 67-91, American Chemical Society, Washington, D.C.

Bohlin, L., Eliasson, A.-C., and Mita, T. 1986. Shear stress relaxation of native and modified potato starch gels. Starch 38:120.

Bourne, M.C. 1967. Size, density and hardshell in dry beans. Food Technol. 21:335.

Bourne, M.C. 1976. Texture of fruits and vegetables. In "Rheology and Texture in Food Quality," J.M. deMann, P.W. Voisey, V.F. Rasper, and D.W. Stanley (Ed.) p 275, AVI Publishing Co., Westport, CT.

Bray, H.G. and Thorpe, W.V. 1954. Analysis of phenolic compounds of interest in metabolism. In "Methods in Biochemical Analysis," D. Glich (Ed.).

Bressani, R. 1975. Legumes in human diets and how they might be improved. In "Nutritional Improvement of Food Legumes by Breeding," M. Milner (Ed.), John Wiley and Sons, New York, NY.

Bressani, R., Elias, L.G. and Braham, J.E. 1982. Reduction of digestibility of legume proteins by tannins. J. Plant Foods 4:43.

Brillouet, Jean-Marc and Mercier, C. 1981. Fractionation of wheat bran carbohydrates. J. Sci. Food Agric. 32:243.

Brillouet, Jean-Marc and Carre, B. 1983. Composition of cell walls from cotyledons of Pisum sativum, Viva faba and Glycine max. Phytochemistry 22:841.

Bukovac, M.J., Rasmussen, H.P., and Shull, V.E. 1981. The cuticle: Surface structure and function. Scanning Electron Microsc., III:213.

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

Cain, R.F. 1950. Relation of time and temperature of blanch to tenderness. The Canner 111:10.

Carpenter, K.J. 1981. The nutritional contribution of dry beans (*Phaseolus vulgaris*) in perspective. Food Technol. 35:77.

Cassab, G.I., Nieto-Sotelo, J., Cooper, J.B., Holst, G.J., and Varner, J.E. 1985. A developmentally regulated hydroxyproline-rich glycoprotein from the cell walls of soybean seed coats. Plant Physiol. 77:532.

Cerning-Beroard, J., Sapsonik, A. and Guilbot, A. 1975. Carbohydrate composition of horsebeans (*Vicia faba L.*) of different origins. Cereal Chem. 52:125.

Cerning-Beroard, J. and Filiatre, A. 1976. A comparison of the carbohydrate composition of legume seeds: horsebeans, peas, and lupines. Cereal Chem. 53:968.

Champ, M., Brillouet, Jean-Marc and Rouau, X. 1986. Nonstarchy polysaccharides of Phaseolus vulgaris, Lens esculenta, and Cicer arietinum seeds. J. Agric. Food Chem. 34:326.

Chang, K.C. and Satterlee, L.D. 1981. Isolation and characterization of the major protein from great northern beans (*Phaseolus vulgaris*). J. Food Sci. 46:1368.

Ching, T.M., and Schoolcraft, I. 1968. Physiological and chemical differences in aged seeds. Crop Sci. 8:407.

Coffey, D.G., Uebersax, M.A., Hosfield, G.L. and Brunner, J.R. 1985. Evaluation of the hemagglutinating activity of low-temperature cooked kidney beans. J. Food sci. 50:78.

Conklin, N.C. and Peacock, B. 1985. Dry bean markets. Bean Commission J. 9:8.

Crafts, A.F. 1944. Cellular changes in certain fruits and vegetables during blanching and dehydration. Food Res. 9:442.

Crean, D.E.C., and Haisman, D.R. 1963. The interaction between phytic acid and divalent cations during the cooking of dried peas. J. Sci. Food Agric. 14:824.

Dawson, E.H., Lamb, J.C., Toepfer, E.W. and Warren, H.W. 1952. Development of rapid methods of soaking and cooking dry beans. Technical Bulletin No. 1051, U.S. Dept. of Ag. Washington, D.C.

Deschamps, I. 1958. Peas and beans. In "Processed Plant Protein Foodstuffs," A.M. Altschul (Ed.), Academic Press, New York, NY.

Devries, J.A., Rombouts, F.M., Voragen, A.G.J., and Pilnik, W. 1983. Distribution of methoxyl groups in apple pectic substances. Carbohydr. Polym. 3:245.

Devries, J.A., Rombouts, F.M., Voragen, A.G.J., and Pilnik, W. 1984. Comparison of the structural features of apple and citrus pectic substances. Carbohydr. Polym. 4: 89.

Donovan, J.W. 1979. Phase transitions of the starch-water system. Biopolymers 18:263.

Dull, G.G. and Leeper, E.F. 1975. Ultrastructure of polysaccharides in relation to texture. In "Postharvest Biology and Handling of Fruits and Vegetables," N.F. Haard and D.K. Salunkhe (Ed.), p. 55-61, AVI Publishing Co., Westport, CT.

Ekpenyong, T.E. and Borchers, R.L. 1980. Effect of cooking on the chemical composition of winged beans (*Psophocarpus tetragonolobus*). J. Food Sci. 45:1559.

Elbert, E.M. 1961. Temperature effect on reconstitution of small white beans. Fifth Annual Dry bean Res. Conf., USDA.

Elias, L.G., de Fernandez, D.G., and Bressani, R. 1979. Possible effects of seed coat polyphenolics on the nutritional quality of bean protein. J. Food Sci. 44:524.

Eliasson, A.-C. and Bohlin, L. 1982. Rheological properties of concentrated wheat starch gels. Starch 34:267.

El-Refai, A.A., Gouda, M.S., and Ammar, K.A. 1987. Effect of processing and storage on protein and lipid composition of peas. Food Chem. 23:117.

Epstein, L., and Lamport, D.T.A. 1984. An intramolecular linkage involving isodityrosine in extensin. Phytochemistry 23:1241.

Evans, R.J. and Bandemer, S.L. 1967. Nutritive value of legume seed proteins. J. Agric. Food Chem. 15:439.

Fishman, M.L., Pfeffer, L., and Barford, R.A. 1984. Degree of polymerization of sodium polygalacturonate by membrane osmometry. J. Polym. Sci. 22: 899.

Fleming, S.E. 1981a. A study of relationships between flatus potential and carbohydrate distribution in legume seeds. J. Food Sci. 46:794.

Fleming, S.E. 1981b. Flatulence activity of the smooth-seeded field pea as indicated by hydrogen production in the rat. J. Food Sci. 47:12.

Fordham, J.R., Wells, C.E. and Chen, L.H. 1975. Sprouting of seeds and nutrient composition of seeds and sprouts. J. Food Sci. 40:552.

Fry, S.C. 1982. Phenolic components of the primary cell wall: feruloylated disaccharide of D-galactose and L-arabinose from spinach polysaccharide. Biochem. J. 203:493.

Fry, S.C. 1983. Feruloylated pectins from the primary cell wall: their structure and possible functions. Planta. 157:111.

Fry, S.C. 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. Ann. Rev. Plant Physiol. 37:165.

Garruti, R.D.S. and Bourne, M.C. 1985. Effect of storage conditions of dry bean seeds (*Phseolus vulgaris*) on texture profile parameters after cooking. J. Food Sci. 50:1067.

Geervani, P. and Theophilus, F. 1982. Influence of legumes starches on protein utilization and availability of lysine methionine to albino rats: A literature review. Nurt. Abstr. Rev. Ser. A. 52:1237.

Ghaderi, A., Hosfield, G.L., Adams, M.W. and Uebersax, M.A. 1984. Variability in culinary quality, component interrelationships, and breeding implications in navy and pinto beans. J. Amer. Soc. Hort. Sci. 109:85.

Gloyer, W.O. 1921. Sclerema and hard shell, two types of hardness of the bean. Assoc. Off. Seed Anal. No. Amer. Proc. 13:60.

Gomez-Brenes, R., Elias, L.G., Molina, M.R., de la Fuente,, G., and Bressani, R. 1975. Changes in the chemical and nutritive value of common beans and other legumes during house cooking. Arch. latinoam. Nutr. Proceedings of Annual Meeting, 1973:93-108.

Gomes, J.C., Koch, U. and Brunner, J.R. 1979. Isolation of a trypsin inhibitor from navy beans by affinity chromatography. Cereal Chem. 56:525.

Goodwin, T.W. and Mercer, E.I. 1983. The Plant Cell Wall. In "Introduction to Plant Biochemistry," 2nd edition, Pergamon Press, New York, NY.

Graessly, W.W. 1974. The entanglement concept in polymer rheology. Adv. Polymer Sci. 16:1.

Greenwood, M.L. 1935. Pinto beans: their preparation and palatability. N. Mesx. Agr. Expt. Sta. Bull. 231.

Greenwood, C.T. and Thompson, J. 1962. Studies on the biosyntheis of starch granules. II. The properties of the components of starches from smooth and wrinkled-seeded peas during growth. Biochem. J. 82:156.

Gupta, R.K. and Haslam, E. 1980. Vegetables tannins-structure and biosynthesis. In "Polyphenols in Cereals and Legumes," J.H. Hulse (Ed.) p. 15-24 IDRC-145e, Ottawa, Ontario.

Haard, N.F. 1985. Characteristics of edible plant tissues. In "Food Chemistry," 2nd ed., O.R. Fennema (Ed.), Marcel Dekker Inc., New York, NY.

Hahn, D.M., Jones, F.T., Akhavan, I. and Rockland, L.B. 1977. Light and scanning electron microscope studies on dry beans: Intracellular gelatinization of starch in cotyledons of large lima beans (*Phaseolus lunatus*). J. Food Sci. 42:1208.

Halbrook, W.U., and Kurtzman, R.H. 1975. Water uptake of bean and other starches at high temperatures and pressures. Cereal Chem. 52:156.

Hall, T.C., Sun, S.M., Ma, Y., McLeester, R.C., Pyne, J.W., Bliss, F.A., and Buchbinder, B.U. 1979. The major storage protein of French bean seeds: Characterization in vivo and translation in vitro. In "The Plant Seed: Development, Preservation, and Germination," I. Rubenstein, R.L. Phillips, C.E. Green, and B.G. Gengenbach (Ed.)

Hallam, N.D., Roberts, B.E., and Osborne, D.J. 1973. Embryogenesis and germination in rye (*Secale cereale L.*). Planta. 110:279.

Hammerschmidt, R. 1984. Rapid deposition of lignin in potato tubes tissue as a response to fungi non-pathogenic on potato. Physiol. Plant Pathol. 24:33.

Hammerschmidt, R., Lamport, D.T.A. and Muldoon, E.P. 1984. Cell wall hydroxyproline enhancement and lignin deposition as an early event in the resistance opf cucumber to Cladosporium cucumerinum. Physiol. Plant Pathol. 24:43.

Harborne, J.B. 1980. Plant phenolics. In "Secondary Plant Products," E.A. Bell and B.V. Charlwood (Ed.), Encyclopedia of Plant Physiology, Vol 8. Springer-Verlag, Berlin-Heidelberg, Germany.

Haslam, E. 1979. Vegetable tannins. Rec. Adv. Phytochem. 12:475.

Hickson, D.W., Dill, C.W., Morgan, R.G., Sweat, V.E., Suter, D.A. and Carpenter, Z.L. 1982. Rheological properties of two heat-induced protein gels. J. Food Sci. 47:783.

Hidalgo, R. 1988. The *Phaseolus* World Collection. In "Genetic Resources of Phaseolus Beans," M. Gepts (Ed.), Kluwer Academic Publishers, Dordrecht, Holland.

Hincks, M.J. and Stanley, D.W. 1986. Multiple mechanisms of bean hardening. J. Food Tech. 21:734.

Hoff, J.E. and Nelson, P.E. 1966. Methods for accelerating the processing of dry beans. Eighth Dry bean Research Conf. Bellair, MI. Aug. 11-13.

Hoff, J.E. and Nelson, P.E. 1967. Methods for accelerating the processing of dry beans. USARS 74-41:39.

Holberg, A.I. and Stanley, D.W. 1987. Hard-to-cook defect in black beans: Protein and starch considerations. J. Agric. Food Chem. 35:571.

Hoover, R. and Sosulski, F. 1985. Studies on the functional characteristics and digestibility of starchs from Phaseolus vulgaris biotypes. Starch 37:181.

Hosfield, G. and Uebersax, M.A. 1980. Variability in physio-chemical properties and nutritional components of tropical and domestic dry bean germplasm. J. Amer. Soc. Hort. Sci. 105:246.

Hsu, K.H. 1983. A diffusion model with a concentration dependent diffusion coefficient for describing water movement in legumes during soaking. J. Food Sci. 48:618.

Huang, H.M., Johanning, G.L., and O'Dell, B.L. 1986. Phenolic acid content of food plants and possible nutritional implications. J. Agric. Food Chem. 34:48.

Hulse, J.H. 1980. Polyphenols in cereals and legumes. p. 1-72. IDRC - 145e, Ottawa, Ontario.

Hymowitz, T., Collins, F.I., Panezner, J. and Walker, W. M. 1972. Relationship between oil, protein, and sugar in soybean seed. Agron. J. 64:613.

JAOAC 1985. Changes in Methods: Total Dietary Fiber in Foods, Enzymatic - Gravimetric method. J. Assoc. Off. Anal. Che. 68:399.

Jones, P.M.B. and Boulter, D. 1983a. The cause of reduced cooking rate in Phaseolus vulgaris following adverse storage conditions. J. Food Sci. 48:623.

Jones, P.M.B. and Boulter, D. 1983b. The analysis of development of hardbean during storage of black beans (*Phaseolus vulgaris L.*). Qual. Plant Foods Hum. Nutr. 33:77.

Jood, S., Mehta, U., and Singh, R. 1986. Effect of processing on available carbohydrates in legumes. J. Agric. Food Chem. 34:417.

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

Kay, D.E. 1979. Haricot bean. In "Food Legumes," D.E. Kay (Ed.), Tropical Products Institute, London, UK.

Koehler, H.H. and Burke, D.W. 1981. Nutrient composition, sensory characteristics, and texture measurements of seven cultivars of dry beans. J. Am. Soc. Hort. Sci. 106:313.

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

Kon, S., and Sanshuck, D.W. 1981. Phytate content and its effect on cooking quality of beans. J. Food Proc. Pres. 5:169.

Korban, S.S., Coyne, D.P. and Weihing, J.L. 1981. Rate of water uptake and sites of water entry in seeds of different cultivars of dry bean. Science 16:545.

Korytnyk, W. and Metzler, E.A. 1963. Composition of lipids of lima beans and certain other beans. J. Sci Food Agric. 14:841.

Koukol, J. and Conn, E.E. 1961. Purification and properties of the phyenylalanine deaminase of Hordeum vulgare. J. Biol. Chem. 236:2692.

Krygier, K., Sosulski, F., and Hogge, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. J. Agric. Food Chem. 30:330.

Kyle, J.H. and Randall, T.E. 1963. A new concept of the hard seed character in Phaseolus vulgaris L. and its use in breeding and inheritance studies. Amer. Soc. Hort. Sci. 83:461.

Lamport, D.T.A. 1967. Hydroxyproline-O-glycosidic linkage of the plant cell wall glycoprotein extensin. Nature 216:1322.

Lamport, D.T.A. 1969. The isolation and partial characterization of hydroxyproline-rich glycopeptides obtained by enzymic degradation of primary cell walls. Biochemistry 8:1155.

Lamport, D.T.A. and Miller, D.H. 1971. Hydroxyproline arabinosides in the plant kingdom. Plant Physiology 48:454.

Leach, H.W. McCowen, L.D. and Schoch, T.J. 1959. Structure of the starch granule. I. Swelling and solubility patterns of various starches. Cereal Chem. 36:534.

Liener, I.E. and Thompson, R.M. 1980. In vitro and in vivo studies on the digestibility of major storage protein of the navy bean (*Phaseolus vulgaris*). Qual. Plant. 30:13.

Loh, J., Breene, W.M. and Davis, E.A. 1982. Between species differences in fracturability loss: Microscopic and chemical comparison of potato and Chinese waterchestnut. J. Text. studies 13:325.

Lorenz, K.J. 1979. The starch of the faba bean (*Vicia faba L.*). Starch 31:181.

Lott, J.N. and Buttrose, M.S. 1978. Globoids in protein bodies of legume seed cotyledons. Aust. J. Plant Physiol. 5:89.

Ma, Y. and Bliss, F.A. 1978. Tannin content and inheritance in common bean. Crop Sci. 18:201.

Maddex, R.L. 1978. Handling and storage. In "Dry Bean Production Principles and Practices," Bull. E-1251, Agric. Expt. Sta., Michigan State University, East Lansing, MI.

Maga, J.A. 1973. A review of flavor investigations associated with the soy products raw soybeans, defatted flakes and flours, and isolates. J. Agric. Food Chem. 21:864.

Maga, J.A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1.

Mattson, S. 1946. The cookability of yellow peas. Acta Agric. Scand. 2:185.

Mazliak, P. 1983. Plant membrane lipids: Changes and alteration during aging and senescence. In "Postharvest Physiology and Crop Preservation," M. Lieberman (Ed.), p. 123. Plenum Press, New York, NY.

McEwen, T.J., Dronzek, B.L. and Bushuk, WS. 1974. A scanning electron microscope study of faba bean seed. Cereal Chem. 51:750.

Meiners, C.R., Derise, N.L., Lau, H.C., Ritchey, S.J., and Murphy, E.W. 1976a. Proximate composition and yield of raw and cooked mature dry legumes. J. Agric. Food Chem. 24:1122.

Mejia, E.G. 1980. Effect of various conditions of storage in general aspects of bean hardening. Final Rpt. UHU Newsletter 4(1):April.

Miles, M.J., Morris, V.J., Orford, P.D. and Ring, S.G. 1985. The roles of amylose and amylopectin in the gelation and retrogradation of starch. Carbohydr. Res. 135:271.

Monte, W.C. and Maga, J.A. 1980. Extraction and isolation of soluble and insoluble fiber fractions from the pinto bean (*Phaseolus vulgaris*). J. Agric. Food Chem. 28:1169.

Morrison W.R. and Laignelet, B. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. Cereal Sci. 1:9.

Morris, H.J. and Wood, E.R. 1956. Influence of moisture content on keeping quality of dry beans. Food Technol. 10:225.

Morris, H.J., Olson, R.L., and Bean, R.C. 1950. Processing quality of varieties and strains of dry beans. Food Technol. 4:247.

Morris, H.J. and Seifert, R.M. 1961. Constituents and treatments affecting cooking of dry beans. Proceedings of the 5th Dry Bean Research Conference. USDA Agr. Es. Service. p. 42.

Moscoso, W., Bourne, M.C., and Hood, L.F. 1984. Relationships between the hard-to-cook phenomenon in red kidney beans and water absorption: Puncture force, pectin, phytic acid, and minerals. J. Food Sci. 49:1577.

MSTAT. 1986. Version 4.0 Michigan State Univ., East Lansing, MI.

Muller, F.M. 1967. Cooking quality of pulses. J. Sci. Food Agric. 18:292.

Muneta, P.l. 1964. The cooking time of dry beans after extended storage. Food Technol. 18:130.

Murray, K.E., Shipton, J., Whitfield, F.B., and Last, J.H. 1976. The volatiles of off-flavored unblanched green peas (*Pisum sativum*). J. Sci. Food Agric. 27:1093.

Naivikul, O. and D'Appolonia, B.L. 1978. Comparison of legumes and wheat flour carbohydrates. I. Sugar analysis. Cereal Chem. 55:913.

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

Nene, S.P., Vakil, U.K. and Sreenivasan, A. 1975. Effect of gamma radiation on physico-chemical characteristics of red gram (*Cajanus cazan*) starch. J. Food Sci. 40:943.

Neukorn, H. and Markwalder, H-U. 1978. Oxidative gelation of wheat flour pentosans: A new way of cross-linking polymers. Cereal Foods World. 23:374.

Nordstrom, C.L. and Sistrunk, W.A. 1977. Effect of type of bean, soak time, canning media and storage time on quality attributes and nutritional value of canned dry beans. J. Food Sci. 42:795.

O'Dell, B.L., Burpo, C.E. and Savage, J.E. 1972. Evaluation of zinc availability in foodstuffs of plant and animal origin. J. Nutr. 102:653.

Ott, A.C. and Ball, C.D. 1943. Some components of the seedcoats of the common bean, *Phaseolus vulgaris*, and their relation to water retention. Arch. Biochem. 3:189.

Paredes-Lopez, O., Maza-Calvino, E.C., Gonzalez-Castaneda, J. and Montes-Rivera, R., Irapuato (Mexico), 1988. Physico-chemical and functional properties of common bean (*Phaseolus vulgaris*) starches. Starch 40:11.

Patel, K.M., Bedford, CL. and Youngs, C.W. 1980. Amino acid and mineral profile of air-classified navy bean flour fractions. Cereal Chem. 57:125.

Pearse, A.G. Everson, 1960 a. Cold Knife and Cold Microtome (cryostat) Methods. In "Histochemistry Theoretical and Applied". London J. & A. Churchill Ltd. (2nd edition), p. 22 - 23, 104 Gloucester Place, WI.

Pearse, A.G. Everson, 1960 b. Fixatives. In "Histochemistry Theoretical and Applied," London J. & A. Churchill Ltd. (2nd edition), p. 787 - 790, 104 Gloucester Place, WI.

Powell, D.A., Morris, E.R., Gidley, M.J., and Rees, D.A. 1982. Conformations and interactions of pectins. II. Influence of residue sequence on chain association in calcium pectate gels. J. Mol. Biol. 155:517.

Powrie, W.D., Adams, M.W. and Pflug, I.J. 1960. Chemical, anatomical and histochemical studies on the navy bean seed. Agron. J. 52:163.

Prattely, C.A. and Stanley, D.W. 1982. Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. J. Food Biochem. 6:243.

Pritchard, P.J., Dryburgh, E.A. and Wilson, B.J. 1973. Carbohydrates of spring and winter field beans (*Vicia faba L.*). J. Sci. Food Agric. 24:663.

Prosky, L., Nils-Georg, ASP., Furda, I., Devries, J.W., Schweizer, T.F. and Harland, B.F. 1984. Determination of total dietary fiber in foods, food products and total diets: Interlaboratory Study. J. Assoc. Off. Anal. Chem. 67:6.

Quast, D.C. and da Silva, S.D. 1977. Temperature dependence of the cooking rate of dry legumes. J. Food Sci. 42:370.

Raven, P.H., Evert, R.F. and Eichharn, S.E. 1986. The Plant Cell. In "Biology of Plants," 4th edition, Worth Publishers, Inc., New York, NY.

Reddy, N.R., Pierson, M.D., Sathe, S.K. and Salunkhe, D.K. 1984. Chemical, nutritional and physiological aspects of dry bean carbohydrates. A review. Food Chem. 13:25.

Reddy, N.R. and Salunkhe, D.K. 1980. Changes in oligosaccharides during germination and cooking of black gram and fermentation of black gram/rice blend. Cereal Chem. 57:356.

Rees, D.A., and Wight, A.W. 1971. Polysaccharide conformation. Part VII. Model building computations for alpha-1,4 galacturonan and the kinking function of L-rhamnose residues in pectic substances. J. Chem. Soc., Ser. B:1366.

Reeve, R.M. 1947. Relation of histological characteristics to texture in seed coats of peas. Food Res. 12:10.

Ring, S.G. 1985. Some studies on starch gelation. Starch 37:80.

Ring, S.J. and Selvendran, R.R. 1980. Isolation and analysis of cell wall material from beeswing wheat bran (*Triticum Aestivum*). Phytochemistry 19:1723.

Roberts, A.H. and Yudkin, J. 1960. Dietary phytate as a possible cause of magnesium deficiency. Nature 185:823.

Rockland, L.B. 1963. Chemical and physical changes associated with processing of large of large dry lima beans. Proceedings of the Sixth Annual Dry Bean Conference, Jan. 2-4, Los Angeles, CA, p. 9.

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

Rockland, L.B., Zaragosa, E.M. and Jracca-Tetteh, R. 1979. Quick-cooking of winged beans (*Psophocarpus tetragonolobus*). J. Food Sci. 44:1004.

Rolston, M.P. 1978. Water impermeable seed dormancy. Bot. Rev. 44:365.

Rozo, C. 1982. Effect of extended storage on the degree of thermal softening during cooking, cell wall components, and polyphenolic compounds of red kidney beans. Ph.D. Thesis, Cornell Univ., Ithaca, NY.

Sahasrabudhe, M.R., Quinn, J.R., Paton, D., Youngs, C.G. and Skura, B.J. 1981. Chemical composition of White bean (*Phaseolus vulgaris L.*) and functional characteristics of its air-classified protein and starch fractions. J. Food Sci. 46:1079.

Salimath, P.V. and Tharanathan, R.N. 1982. Carbohydrates of field bean (*Dolichos lablab*). Cereal Chem. 59:430.

Salunkhe, D.K., Jadhav, S.J., Kadam, S.S. and Chavan, J.K. 1982. Chemical, biochemical and biological significance of polyphenols in cereals and legumes. CRC Crit. Rev. Food Sci. Nutr. 17:277.

Salunkhe, D.K., Kadam, S.S., and Chavan, J.K. 1985. Chemical composition. Ch. 3. in "Postharvest Biotechnology of Food Legumes," p. 29. CRC Press, Inc., Boca Raton, FL.

Sarkanen, K.V., and Ludwig, C.H. 1971. Definition and nomenclature. In " Lignins," K.V. Sarkanen and C.H. Ludwig (Ed.), Wiley Interscience, New York, NY.

Sathe, S. K. and Krishnamurthy, K. 1953. Phytic acid and absorption of iron. Ind. J. Med. Res. 41:453.

Sathe, S.K., Iyer, V. and Salunhke, D.K. 1981. Functional properties of the great northern bean (*Phaseolus vulgaris*) proteins: Amino acid composition, in vitro digestibility, and application to cookies. J. Food Sci. 47:8.

Sathe, S.K. and Salunkhe, D.K. 1981. Isolation, partial characterization and modification of the great northern bean (*Phaseolus vulgaris L.*) starch. J. Food Sci. 46:952.

Sathe, S.K., Deshpande, S.S., and Salunkhe, D.K. 1984. Dry bean of Phaseolus. A review. Part 2. Chemical composition: Carbohydrates, fiber, minerals, vitamins, and lipids. Crit. Rev. Food Sci. Nutr. 21:41.

Saunders, R.M. and Betschart, A.A. 1980. The significance of protein as a component of dietary fiber. Am. J. Clin. Nutr. 33:960.

Schoch, T.J. and Maywald, E.C. 1968. Preparation and properties of various legume starches. Cereal Chem. 45:564.

Sefa-Dedeh, S. and Stanley, D.W. 1979. Microstructure of cowpea variety (*Adua ayera*). Cereal Chem. 56:367.

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

Sgarbieri, V.C. and Whitaker, J.R. 1982. Physical, chemical and nutritional properties of common bean (*Phaseolus*) proteins. Adv. Food Res. 28:93.

Sgarbieri, V.C. and Garruti, R.S. 1986. A review of some factors affecting the availability and the nutritional and technological quality of common dry beans, a dietary staple in Brazil. Can. Inst. Food Sci. Technol. J. 19:202.

Shewfelt, A.L., Paynter, V.A., and Jen, J.J. 1971. Textural and molecular characteristics of pectic constituents in ripening peaches. J. Food Sci. 36:573.

Silva, C.A.B., Bates, R.P., and Deng, J.C. 1981. Influence of soaking and cooking upon the softening and eating quality of black beans (*Phaseolus vulgaris*). J. Food Sci. 46:1716.

Silva, H.C. and Braga, G.L. 1982. Effect of soaking and cooking on the oligosaccharide content of dry beans (*Phaseolus vulgaris, L.*). J. Food Sci. 47:924.

Smith, A.K., and Nash, A.M. 1961. Water absorption of soybeans. J. Am. Oil Chem. Soc. 38:120.

Snyder, E.B. 1936. Some factors affecting the cooking quality of the pea and great northern types of dry beans. Nebraska Agric. Expt. Sta. Res. Bull. 85.

Sorochan, V.D., Dzizenko, A.K., Bodin, N.S., and Ovodov, Y.S. 1971. Light-scattering studies of pectic substances in aqueous solution. Carbohydr. Res. 20:243.

Sosulski, F.W. 1979. Organoleptic and nutritional effects of phenolic compounds on oil-seed protein products: A review. J. Am. Oil Chem. Soc. 56:711.

Srisuma, N., Ruengsakulrach, S., Uebersax, M.A. and Zabik, M.E. 1988. Characterization of navy, pinto and black bean starches. ASEAN Food Conference '88 Proceedings (in press).

Srisuma, N., Hammerschmidt, R., Uebersax, M.A., Ruengsakulrach, S., Bennik, M.R. and Hosfield, G.L. 1989. Storage induced changes of phenolic acids and the development of hard-to-cook in dry beans (*Phaseolus vulgaris*, var Seafarer). J. Food Sci. 54:311.

Stanley, D.W. and Aguilera, J.M. 1985. A review of textural defects in cooked reconstituted legumes: The influence of structure and composition. J. Food Biochem. 9:277.

StatView II. 1984. The Solution for Data Analysis and Presentation Graphics, Abacus Concepts Inc. Bonita Ave. Berkeley, CA 94704.

Steffe,.J.F., Castell-Perez, M.E., Rose, K.J., and Zabik, M.E. 1989. Rapid testing method for characterizing the rheological behavior of gelatinizing corn starch slurries. Cereal Chem. 66:65.

Stermer, B.A. and Hammerschmidt, R. 1987. Association of heat shock induced resistance to desease with increassed accumulation of insoluble extensin and ethylene synthesis. Physiological Molecular Plant Pathology 31:453.

Stevens, D.J. and Elton, G.A.H. 1971. Thermal properties of the starch/water system: Part I. Starch 23:8.

Sweat, V.E., Faubion, J.M., Palacios, L.G., Berry, G., Akingbala, L. and Rooney, W. 1984. Gelatinization energy and temperature of sorghum and corn starches. Transactions of the ASAE. 27:1961.

Takata, A., and Scheuer, P.J. 1976. Glyceryl esters of caffeic and p-coumaric from pineapple stems. Lloydia. 39:409.

Takayama, K.K., Muneta, P. and Wiese, A.C. 1965. Lipid composition of dry beans and its correlation with cooking time. J. Agr. Food Chem. 13:269.

Tobin, G. and Carpenter, K.J. 1978. The nutritional value of the dry bean (*Phaseolus vulgaris*). A literature review. Nutr. Abstr. and Rev. 48:920.

Tyler, R.T., Youngs, C.G. and Sosulski, F.W. 1981. Air-classification of legumes. I. Separation efficiency, yield and composition of the starch and protein fractions. Cereal Chem. 58:144.

Uebersax, M.A. 1972. Effects of storage and processing parameters on quality attributes of processed navy beans. M.S. Thesis. Michigan State University, East Lansing, MI.

Uebersax, M.A. and Ruengsakulrach, S. 1989. Structural and compositional changes during procesing of dry beans (*Phaseolus vulgaris*). In "Quality Factors of Fruits and Vegetables," ACS Symposium (in press).

Van Buren, J.P. 1979. The chemistry of texture in fruits and vegetables. J. Texture Studies 10:1.

Van Buren, J.P. 1980. Calcium binding to snap bean water insoluble solids calcium and sodium concentrates. J. Food Sci. 45:752.

Van Buren, J., Bourne, M., Downing, D., Queale, D., Chase, E., and Comstock, S. 1986. Processing factors influencing splitting and other quality characteristics of canned kidney beans. J. Food Sci. 51:1228.

Van Sumere, C.F., Cottenie, J., De Greef, J., and Kint, J. 1972. Biochemical studies in relation to the possible germination regulatory role of naturally occurring coumarin and phenolics. Recent Adv. Phytochem. 4:165.

Varriano-Marston, E., Zeleznak, K. and Nowotna, A. 1985. Structural characteristics of gelatinized starch. Starch 37:326.

Varriano-Marston, E. and DeOmana, E. 1979. Effects of sodium salt solutions on the chemical composition and morphology of black beans (*Phaseolus vulgaris*). J. Food Sci. 44:531.

Varriano-Marston, E. and Jackson, G.M. 1981. Hard-to-cook phenomenon in beans: Structural changes during storage and inhibition. J. Food Sci. 46:1379.

Villiers, T.A. 1972. Cytogical studies in seed dormancy. 2. Pathological aging changes during prolonged dormancy and recovery upon release. New Phytol. 71:145.

Vindiola, O.L., Seib, P.A., and Hoseney, R.C. 1986. Accelerated development of the hard-to-cook state in beans. Cereal Food Worlds. 31:538.

Vonsarnpiggon, S., Uebersax, M.A. and Hosfield, G.L. 1981. Effects of dry storage conditions on the quality of processed edible beans. Cereal Foods World 26:503.

Wang, C.R., Chang, K.C. and Grafton, K. 1988. Canning quality evaluation of pinto and navy beans. J. Food Sci. 53:772.

Watt. R.T. and Merrill, W. 1963. "USDA Handbook Number 8". Agric. Res. Service, Washington, D.C.

Wen, L.F., Chang, K.C., Brown, G. and Gallaher, D.D. 1988. Isolation and characterization of hemicellulose and cellulose from sugar beet pulp. J. Food Sci. 53:826.

Weston. W.J. and Morris, H.J. 1954. Hygroscopic equilibria of dry beans. Food Technol. 8:353.

Youssef, M.M., Bushuk, W., Murray, E.D., Zillman, R., and El-Tabey Shchata, A.M. 1982. Relationship between cookability and some chemical and physical properties of faba beans (*Vicia faba L.*). J. Food Sci. 47:1695.