CORRELATION OF PHYSIO-CHEMICAL CHARACTERISTICS IN THE SEED COAT AND CANNING QUALITY IN DIFFERENT DARK RED KIDNEY BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS

by

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ABSTRACT

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Correlation of Physiochmical Characteristics In The Seed Coat and Canning Quality In

(Title)

Different Dark Red Kidney Bean (*Phaseolus Vulgaris* L.) Cultivars

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The canned kidney bean (*Phaseolus vulgaris* L.) is one of the major consumption forms of this agricultural product. In the canning industry, seed coat splitting is considered one factor affecting the integrity of the appearance in the final product. Three different dark red kidney bean cultivars (85, 453 and Nickols) grown in the Wisconsin area were studied in this research. The physiochemical properties in this study included moisture content, the

seed coat to whole seed weight ratio, the weight per seed, ash, mineral (sodium, calcium, iron, potassium and magnesium) and total crude protein content in the seed coat. The correlations of these properties with the seed coat splits were investigated. In the canned product, highly significant differences ($P<0.01$) in percentage of split seed coat were found among the three cultivars studied. Canned cv. 85 had significantly fewer seed coat splits than the other two cultivars. Cv. 85 was significantly higher in moisture content, as well as ash, sodium, calcium and iron content in the seed coat. The seed coat of cv. 453 was significantly higher in magnesium, and total crude protein. Cv. Nickols was found to be significantly higher in the weight per bean and seed coat splits after the canning process. Significant negative correlations were found between the percentage of seed coat splits, sodium ($r = -0.89$, $p< 0.01$), calcium ($r = -0.74$, $P< 0.01$) and iron content ($r = -0.79$, $P< 0.05$) in the seed coat. A positive correlation was found between sodium content and calcium content ($r = 0.69$, $P< 0.05$) in the seed coat. Increased calcium content in the seed coat was accompanied with increased sodium content. A significant positive relationship ($r = 0.901$, $P< 0.01$) was found between seed weight and the percentage of seed coat splits. This research suggested that several physiochemical factors of the seed coat, including weight per seed, sodium, calcium, and iron content, might play important roles in the integrity of the seed coat during the thermal processing.

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CHAPTER I

INTRODUCTION

Statement of the Problem

Kidney bean, *Phaseolus vulgaris* L., is one of the common beans that evolved from an early ancestral *Phaseolus vulgaris* plant located in southwestern Mexico some 7,000 years ago. As a major world legume, kidney beans not only serve as a primary source of nutrients for many world populations, but also an important source of nutrients for many domesticated animals.

The canned kidney bean is one of the major consumption forms of this agricultural product. In the canning industry, seed coat splitting is considered one factor affecting the integrity of the appearance in the final product. This is often noticeable to consumers. While most of the current research focuses on the study of the processing damage on seed coat splitting, detection of splitting by water uptake kinetic, improvement in canned bean texture, optimizing canning conditions and genetic selection of strains with superior canning and growth quality, very few nutritional studies have been done to correlate with canning qualities among cultivars. More specifically, there is little research on analyzing canning quality with respect to the nutritional parameters presented in the seed coat from different cultivars.

Nutritional data show that the Legume family of plants (plants producing pods) is not only an important source of protein and carbohydrates, but also an important source

of several B-complex vitamins, minerals, and fiber. The various nutritional fractions are not evenly distributed through out the bean. Nutritional data, calculated as a percentage of the whole seed, indicates that most of the ash, protein and lipid are found in the cotyledon, while up to 80-93% of the crude fiber and 32-50% calcium are found in the seed coat (Deshpande & Damodaran, 1990). In this study, the correlations between physio-chemical properties and canning quality were examined. This data will provide insight on parameters that need to be considered when screening kidney beans in a breeding program, as well as predication of cultivars' performance in canning.

Objectives

This research was directed at studying several nutritional parameters in the seed coat of three different dark red kidney bean cultivars. These cultivars differ in disease resistance factors and canning qualities. This research also examined the nutritional differences in the seed coat and their correlation with the canning quality.

The objectives of this project include the following:

1) Investigate moisture content, ash content, crude protein content and mineral profile in the seed coat of different dark red kidney bean cultivars grown in the Wisconsin area.
2) Study the seed coat to whole seed weight ratio and the weight per seed.
3) Evaluate the relationship of the seed coat to whole seed weight ratio, seed weight and nutritional contents to canning quality.

4) Investigate the influences of the physiochemical properties in the seed coat to canning quality among different cultivars.

5) Provide information on factors related to canning qualities for cultivar screening.

Limitations

In this study, only three different cultivars were selected for the objective measurements. The correlation of physiochemical properties and canning quality were established based on statistical models. Further studies at the molecular level should be done to reveal the structural characteristic of the seed coat in response to different canning quality.

CHAPTER II

REVIEW OF LITERATURE

History and Application of Kidney Bean

History of kidney bean

Kidney bean, also known as *Phaseolus vulgaris* L., came under cultivation 7,000 years ago in southwestern Mexico. It is one of the common beans that have been developed into hundreds of varieties since then (Stone, S. & Stone, M., 1988). Kidney beans gained wide acceptance during the pre-Columbian period. Early chroniclers indicated that great importance was given to this species in the Aztec and Incan empires. It was even used to pay tributes (Debouck, 1994).

Application of kidney bean as food

Kidney beans were exported to Europe by Spanish explorers several thousand years later. They are often used in many standard recipes for the addition of eye appeal and a pleasant mouth feel, because of their color and texture (Stone, S. & Stone, M., 1988). The kidney beans provide a robust, full-bodied flavor and a rich, creamy texture (London S. & London M., 1992). Cooked kidney beans are widely used in Mexican dishes, bean salad, and baked beans (Stone, S. & Stone, M., 1988). Canned products include soups, chili, baked bean and others. They are enjoyed throughout the world due to their rich flavor and color.

Dry edible beans are a leading source of vegetable protein. They are inexpensive and also an excellent natural source of vitamins, minerals, soluble dietary fiber, and

phytochemicals. As a major world legume, kidney beans serve not only as a primary source of nutrients for many of the world populations, but also as an important source of nutrition for many domesticated animals (Young, Grummer & Hirschinger, 1977).

The importance of kidney bean in current market

Per capita dry bean use has been increasing in the U.S. in the past 20 years. Factors contributing to this continuous trend in the dry bean market include the increasing awareness and sophistication to the changes in the traditional American diet, the rising immigration of the Hispanic population and the widespread interests in ethnic foods featuring dry beans. In 1999, Chambers reported in U. S. Department of Agriculture [USDA]'s Agricultural Outlook, that about 61% of the total dark red kidney bean production was located in Minnesota and Wisconsin. The per capita consumption of kidney beans has increased by 59%, when the data of 1987-89 was compared to 1997-99 (Table 1). USDA's Economic Research Service in its 2001 Vegetable and Melons Outlook also reported a trend of increased consumption in Red Kidney Bean from 1978 to 2001 (Figure 1). According to the Continuing Survey of Food Intakes of Individuals, complied by USDA's Agricultural Research Service, about 4% of the population consumes kidney bean on any given day, which is among the highest of any dry bean consumption (Lucier, Lin, Allshouse & Kantor, 2000; Belshe, Boland, Daniel & O'Brien, 2001). Lucier et al. also have found that slightly more than 75% of all cooked beans are purchased in retail stores. Kidney beans are purchased and used mostly for at-home consumption. This data implies that canned and dry kidney beans are most likely to be selected by consumers.

Table 1

Dry edible beans consumed in U.S. in 1987-89 and 1997-99

	Pounds per capita		
Bean types	1987-1989	1997-1999	Percent change (%)
Kidney beans	0.37	0.59	59.0
Lima beans	0.23	0.22	4.3
Navy beans	1.20	1.26	5.0
Great northern	0.39	0.40	2.5

Source: Chambers, W. (1999, November). Role of traditional Ag markets: The dry edible bean industry. *Agriculture Outlook*. Retrieved December 21, 2001, from http://www.ers.usda.gov/publications/agoutlook/nov1999/ao266f.pdf

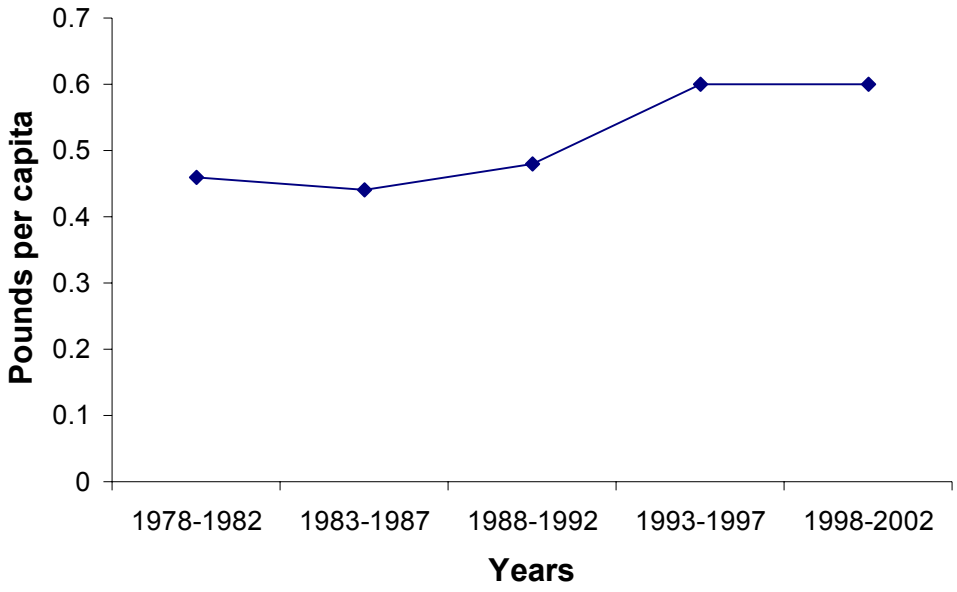

Figure 1. The average of pound per capita of US kidney bean consumption in every five years (1978 – 2002).

Source: Economic Research Service, USDA. (2001, December). Dry edible beans. *Vegetables and Melons Outlook*, VGS-288. Retrieved December 21, 2001, from http://www.ers.usda.gov/publications/vgs/tables/drvbn.pdf

Canning Technique for Kidney Bean Preservation

Canning as a preservation technique in food processing

Since the invention of canning by Nicholas Appert, a Frenchman who successfully developed this food preservation technique, for almost two centuries, canned foods have become widely accepted in daily lives. Canned foods provide a convenient food style free from food spoilage and natural deterioration with a long shelf life (Lopez, 1987). The thermal process greatly enhances the palatability of the edible dry beans, inactivates toxic factors, and increases the nutritional availability and digestibility of different nutrients (Uebersax, Ruengsakulrach & Occena, 1991). Current research also shows that thermal treatment can reduce heat-labile antinutritional factors and increase the digestibility of protein and amino acid in raw edible beans (*Phaseolus vulgaris* L.), therefore the nutritive value of beans is improved (Wu, et al., 1994). However, excessive cooking may introduce the reduced protein biological value and increased mineral loss (Uebersax, Ruengsakulrach & Occena, 1991).

The importance of canned bean product quality

Dry packaged kidney beans are available for consumers. Canned products from kidney bean, such as refried beans, soups and baked beans, are also common retail forms. Canners are often very particular about specific qualities of the beans. They look for beans with rapid expansion ability, higher drained weight, ease in cooking, and uniformity after the thermal process (Wassimi, Hosfield & Uebersax, 1990). The high standards requested by canners and customers force bean producers to actively participate

in breeding programs. From the stand point of breeders, they have to be concerned with not only the yields of the cultivars, disease resistance, weather tolerance ability and growth period, but also the culinary quality of the bean products as requested by the consumers (Wassimi, Hosfield & Uebersax, 1990).

Organoleptic properties within the final canned products are considered to be one of the major quality evaluation standards. However, not all the cultivars are blessed with equally acceptable quality. The problems affecting consumers are often related to the occurrence of bean discoloration, hardness of the beans and breakage of the seed coat after the canning process (Wassimi, Hosfield & Uebersax, 1990). When the seed coat splits, it affects more than just the appearance. It can also result in starchiness and excessive viscosity in the final product.

Standard canning procedure

A common process for canning kidney beans includes the following steps: cleaning, soaking, blanching, filling and sealing, cooking and cooling (Figure 2). Kidney beans with moisture ranging from 11 to 16% are selected as the optimal candidate for canning. In order to increase the permeability of the seed coat and reduce the possibility of incompletely hydrated beans, dry beans are stored under moderately high humidity conditions for several days prior to canning. Cleaning is done by passing the beans through a clipper cleaner or a destoner, where foreign objects and loose dirt are removed. The cleaned dried kidney beans are soaked in cold water for 10 to 12 hours at room temperature. By controlling the soaking period, the beans can reach uniform moisture

content. This can reduce the necessity to frequently adjust the blanching schedule and the ratio of beans to liquid in cans. Foreign particles are removed along with discolored and split beans after the soaking process (Lopez, 1987; Morrow, 1989).

The next phase of the process is hot water blanching. The kidney beans are blanched at 82° to 100° C for five to ten minutes. The soaking and blanching procedure provide beans with an optimal moisture range of 53-57%. The high temperature short time (HTST) blanching improves the firmness of the canned beans, which minimizes the undesirable clumping (Wang, Chang & Grafton, 1988), and also inactivates undesirable enzymatic reactions, removes foreign particles, improves the color and aids in filling the can (Nordstrom & Sistrunk, 1979; Drake & Kinman, 1984). Besides improving canning quality, HTST also reduces lab cost, improves efficiency of floor space usage, provides continuous flow and minimizes potential bacteriological problems (Uebersax, Ruengsakulrach & Occena, 1991). Reels and shakers are often used to remove splits or skin of the damaged beans during this operation. The blanched beans are inspected while running over an inspection belt, and hard, shriveled, broken, discolored beans are removed (Lopez, 1987; Morrow, 1989).

Brine, lightly sweetened sauce or heavy sauce, such as tomato sauce, are often used in the canning process. Beans are totally covered with the brine or sauce to prevent dark discoloration. The canning time varies, depending on the size of the cans and the operational temperature. A No.2 ½ can requires 45 minutes at 116°C. High temperature not only kills the microbes, but also completely tenderizes the beans. The canned beans

are immediately cooled by water bath to a temperature of 35° to 41°C. They are then ready to be labeled and shipped (Lopez, 1987; Morrow, 1989).

Beans are further hydrated during the thermal process. Equilibration with brine or sauce takes 2-4 weeks after canning. The final moisture content of the canned beans ranges from 65-70% (Uebersax, Ruengsakulrach & Occena, 1991).

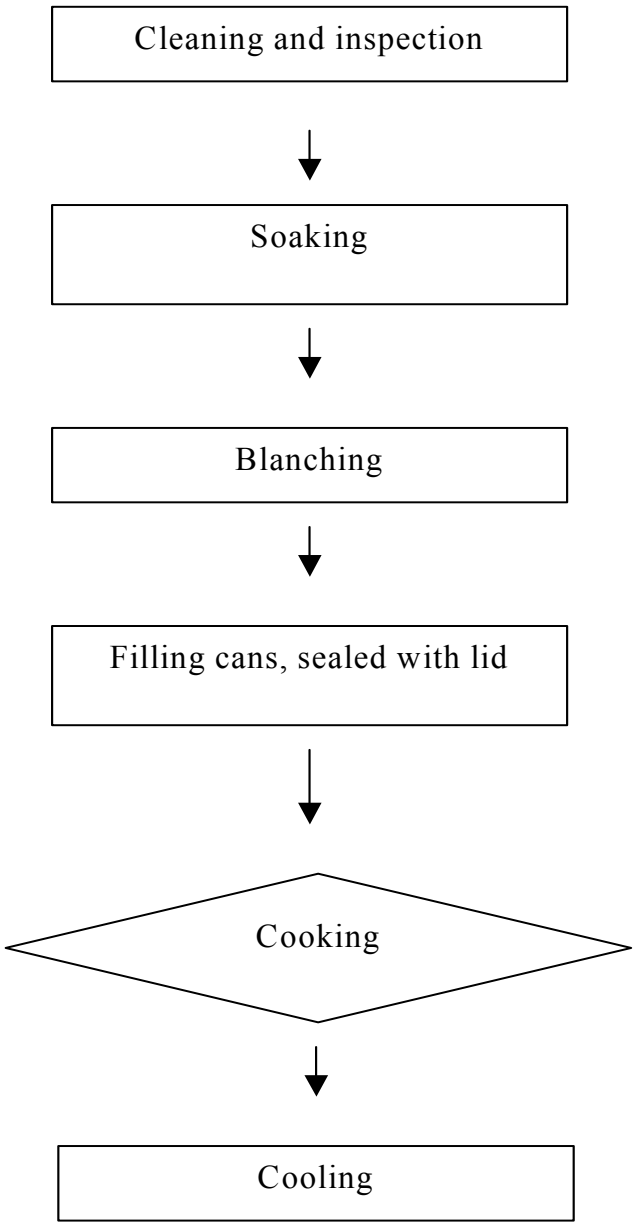

Figure 2. A general canning procedure for kidney beans.

Source: Lopez, A. (1987). Canning of dried pack products. *A complete course in canning and related processes: Vol.3, Processing procedures for canned food products* (Rev. 12th ed., pp. 301-305). Baltimore, MD: The Canning Trade, Inc.

Standards for Canned Beans Quality

Generally, the standard for kidney beans canning quality includes two categories. The first category involves USDA's specifications for dried bean grades. Beans are classified by moisture content, broken seeds, uniformity of size, color and status of foreign matter (Chambers, 1999; Belshe, Boland, Daniel & O'Brien, 2001). Grade A beans are usually preferred by canners. The second category involves post-canning quality (Chamber, 1999), in which several parameters are examined carefully. These parameters include the appearance of the canned beans, consistency of the canned products, and the flavor (USDA, 1976; Belshe, Boland, Daniel & O'Brien, 2001). Seed integrity after canning is one of the specifications used in evaluation. Grade A canned beans are required to be free from defects. These defects are referred to as vegetable material, loose skin, broken and mashed beans and blemished beans (USDA, 1976). According to USDA's consumer guidelines, a top-quality canned bean should have seed coats without separation or breakage from the cotyledon (U.S. Agricultural Marketing Service, 1994).

Two types of splits often occur in the canned kidney beans. The first type, known as the transverse crack, occurs in the region of the hilum and micropyle. The second type is longitudinal (Van Buren, et al., 1986). Seed coat splitting may have resulted from many different variables. Currently there is no standard pre-canning test available for canners. The canning industry and bean breeders widely apply a "Seed Coat Check" prior to canning. Seed coat check, or skin check, is a raw objective measurement of the small breaks in the seed coat after soaking. Kidney beans are soaked in warm water for a few

hours. The percentage of beans with broken seed coats is then determined. However, this test often does not predict post canning quality and the consensus of seed quality (Chambers, 1999). Research has shown that the damage exposed in the soak-tests does not correlate to the damage observed in the canned product (Heil, McCarthy & Özilgen, 1992b). Alternatively, an actual canning trial is often performed to identify the seed integrity after canning (Chambers, 1999).

The Influences of Seed Coat Splitting on Canning Quality

Consumers' responses towards to the appearance and texture of the processed beans are the primary considerations driving the standards used by the processors and bean breeders. The breeder, at the same time, is seeking traits that will improve the bean quality and result in the greater canning ease (Wassimi, Hosfield & Uebersax, 1990; Walters, Hosfield, Uebersax & Kelly, 1996). A cultivar may still be rejected by a processor and consumers if it has poor canning quality, even if it has superior agronomical ability.

Appearance

Consumers are very conscious of the bean color, appearance and wholesomeness. In the Lu & Chang's study (1996) for canned navy beans, a highly significant correlation was found between acceptance of the canned product and product appearance. The overall acceptance of the product decreased as the splits and the starchiness of the canned bean increased.

Starching & Texture

The water uptake rate is substantially higher in beans with damaged seed coats than in beans with undamaged seed coats. Exposing the cotyledon during thermal processing will finally cause excessive starch to dissolve in water (Heil, McCarthy & Özilgen, 1992a). Starch in the softened cotyledon can leach into brine through splits in seed coat (Lopez, 1987). This subsequently results in excessive viscosity, known as

starching. In the USDA standards for canned beans, matting is used to describe the result of starching. Matting is the excessive compaction of the beans within the can resulting in the inability of the beans to be removed from the cans without causing damage or excessive mushiness. Matting is not preferred for a Grade A canned kidney bean product (USDA, 1976). Decreasing starching and matting is fundamental in canning technology.

The direct exposure of cotyledon to cooking media also causes excessive softness in the canned beans. The texture of beans was also found to be significantly correlated with seed coat splits (Lange & Labuschagne, 2000).

Heat penetration

Heat penetration proceeds rapidly in the products with water, thin syrup or brine. During the canning process, the law of conduction and convection controls the penetration of heat. In conduction, heat is transferred between the adjacent molecules due to the vibration of the molecules. In convection, the movement of the molecules contributes to the heat transfer. Studies have shown that an increase in the starch concentration in the solution retards heat transfer and penetration. In a starch solution, the formation of a steady colloid system impedes the formation of convection currents, thus delaying heat penetration (Bigelow, 1961).

Current Studies On Seed Coat Splitting

Canning quality of dry edible beans, *Phaseolus vulgaris* L., has been found to be related to the factors, such as cultivars, maturity, environmental condition, storage condition, processing variables and handling procedure (Wang, Chang & Grafton, 1988; Occena, Cabello, Uebersax & Long, 1991; Balasubramanian, Slinkard, Tyler, & Vandenberg, 2000). Current research studies have focused on the improvement of canning quality in bean products through the identification of genetic traits by biochemical techniques. Today, the combined traditional selection strategies are coupled with marker-assisted selection (MAS) in the selection of cultivars with superior canning quality. In Walters' study, Randomly Amplified Polymorphic DNA was used to identify (RAPD) markers associated with canning quality traits and the degree of heritabilities of each trait (Walters, Hosfield, Uebersax & Kelly, 1996; Walters, Hosfield, Uebersax & Kelly, 1997). However, very few studies have focused on the influences from various chemical components in seed coat of the kidney bean and how they might relate to the bean canning quality among different cultivars. In those studies attempting to correlate links between chemical compounds and canning quality, only a single cultivar or different bean types in the *Phaseolus vulgaris* L family were often used.

In Lu, Chang, Grafton & Schwarz's study (1996) of navy bean canning quality, they suggested the possibility of a linkage between process methods, chemical composition and canning quality. Soluble pectin content, calcium content in the seed coat

and starch gelationization may play important roles in seed coat splitting (Lu, Chang, Grafton & Schwarz, 1996).

Research on edible dried bean canning quality suggested that the following factors directly contribute to the integrity of the seed coat during the processing:

Cultivars and environment

The types of cultivars and environmental factors have been found to be significantly related to variations in physiochemical attributes of dry beans. Lange & Labuschagne (2000) showed that the interaction of genotype and environment can significantly influence seed size, soaked bean mass, visual appearance, texture, protein, and seed coat splitting. Canning processors and breeders often experience some inconsistency in maintaining "good canning traits" which may be due to a change of cultivation location. Lange & Labuschagne's study suggested that a roadblock to successfully improve a genetic trait was the unpredictable environment. The consequence of growing a cultivar at a different site or at a different year under slightly changed environmental conditions can cause changes in the bean's physiological properties.

Storage time

Storage time was found to contribute to an increased percentage of splits in the canned bean products. Hydrolysis of polysaccharides may intensify after 6-month storage time. Therefore, the longer the beans are stored, the higher the percent of seed coat splits (Nordstrom & Sistrunk, 1979).

Mechanical injury on seed coat

Research has showed that mechanical damage of the seed coat often occurs during harvesting, transporting, storing and conditioning. The Red Kidney bean is most sensitive to seed coat damage during harvesting. Excessive abuse often results from the high speed of the combine cylinders. Moisture content of beans is another important factor that needs to be considered during harvesting. Beans with moisture content less than 18% are sensitive to mechanical damage during harvest. Bean seeds should be stored in aerated buildings with humidity less than 75 percent and temperatures ranging from 1.5 °C to 12°C. Handling beans at low temperature or low seed moisture must be avoided. Any of the above parameters when exceeded can result in mechanical damage to seed coat, such as cracking and splitting (Schwartz & Nuland, 1994). With adjustment in threshing conditions and gentler handling, mechanical damage of the seed coat can be minimized (Van Buren, et al., 1986).

The soaking process prior to canning can be used to discriminate the broken seed coat from mechanical injury. The rate of water uptake in the mechanically damaged seeds is greater than in the undamaged seeds, because of the direct contact of the cotyledons with water. This makes it possible to visualize the cracks on the fast swollen seed (Schwartz & Nuland, 1994).

Seed coat weight and seed density

The density of kidney bean and the seed coat weight are significantly correlated to the proportion of damaged beans in the cans after thermal processing (Heil, McCarthy &

Özilgen, 1992b). Density may be used to estimate the compactness and composition of the bean cells. In Heil's et al. (1992b) study, the percent of damaged kidney beans was found to increase with the increased density of the beans. Specific gravity (product density/water density at a certain temperature) was suggested as a method for measuring density (Heil, McCarthy & Özilgen, 1992b).

Seed coat weight was used to predict the ability of the seed coat to expand. The value was determined using the weight of seed coat per unit volume (g/mL). It has been shown that the proportion of damaged beans after the canning process increased dramatically, when the ratio of the seed coat weight to the volume of beans was lower than 10 g/mL. This may result from the inability of seed coat to further accommodate the fully expanded seed during thermal processing (Heil, McCarthy & Özilgen, 1992b).

Heil et al. also proposed that the content profile in the bean cells might also contribute to the maintenance of the seed coat during thermal processing, however, no further research has been done in this direction.

Processing temperature and time

The ultimate goal for soaking and blanching is to achieve a final moisture range of 53% to 57% in the beans. This moisture range is critical to achieve the desired fill ratio of the hydrated beans to brine volume. Insufficiently hydrated beans provide a high solid level per can, and consequently result in an increased firmness due to the tightly solidified pack, whereas, excessively soaked beans can't reach the desired solid level per

can. This results in an undesirable canned product (Uebersax, Reungsakulrach & Hosfield, 1989).

The soaking process prior to canning is necessary for reducing the length of thermal processing and minimizing toxic components found in dried beans (Ogwal & Davis, 1994). High soaking temperature was found to have a significant effect on reducing the percent of seed coat splitting. Kidney beans soaked at 71°C had 18% fewer seed coat defects than those soaked at 66°C, in spite of only a 5-degree difference in temperature (Van Buren, et al., 1986). However, prolonged soaking time produced an opposite effect. Kidney beans soaked beyond 6 hours at 15°C have a greater tendency to split during the canning process (Junek, Sistrunk & Neely, 1980).

Blanching methods have no significant affect on splits (Nordstrom & Sistrunk, 1979; Heil, McCarthy & Özilgen, 1992). However, overblanching may result in an increased number of seed coat splits. This results in excessive softening of the bean texture, and leaching of the starch into the brine. Ultimately, the canned products may obtain an undesirably excessive viscosity (Lopez, 1987).

Addition of Calcium Chloride in canning process

Calcium chloride is used in the canning industries to enhance the firmness of canned vegetables. In canned beans, the formation of metal-pectin complex may contribute to the toughening of seed coat and the turgidity of cell walls of the cotyledon tissue (Moscoso, Bourne & Hood, 1984; Uebersax & Ruengsakulrach, 1989;

Balasubramanian, Slinkard, Tyler, & Vandenberg, 2000; Lange & Labuschagne, 2000). Pectin substances cross-link with divalent cations, such as calcium, and form intercellular polyelectrolyte gels. The presence of calcium cross-links retards water intake, and increases the firmness due to the formation of calcium pectate (Uebersax, Reungsakulrach & Hosfield, 1989). This is a major factor contributing to the textural quality in food (Lange & Labuschagne, 2000). In Moscoso's et al. study (1984) for hard-to-cook red kidney beans, they proposed the possibility of an association between high calcium and magnesium values in the seed coat and high firmness in cooked beans. High mineral profiling also seemed to be related to the high value of total pectin substances and high water soluble pectin substances. However, potassium content was not shown to be correlated to bean firmness in this study (Moscoso, Bourne & Hood, 1984). In another study of canned kidney beans, the level of calcium in brine or soaking water was found to markedly affect the product quality. Increased quality was measured as increased firmness of the canned beans and a significantly decreased percentage of splits. The addition of calcium chloride at 66°C soaking water had the greatest effect on the reduction of splits. However, no significant difference in splitting was found in the samples with NaCl additions (Van Buren, et al., 1986).

Seed coat structure

The micropyle is a small visible pore in the seed coat; the hilum is a scar; and the raphe is the ridge on the seed (Raven, Evert & Curtis, 1982). The micropyle is one of the entrances for water penetration. In a microstructure study of Faba beans (*Vicia faba* L.), using Scanning Electron Microscope (SEM), researchers found that the palisade cells in

the seed coat of the better cookability cultivars were thinner and shorter (Youssef & Bushuk, 1984). The hard-to-cook beans also had smaller micropyle openings and thicker cell layers. These different aspects of microstructure suggested that the process of water uptake might be controlled by the structural differences in the seed coat (Youssef & Bushuk, 1984). A similar question can be derived from this study: If the water uptake can be influenced by the microstructure of the seed coat, will any structural variation of the seed coat also affect its integrity upon thermal processing? A further review on seed coat structure can be found in the following sections.

Pectin in cell wall structure

Recent studies reveal that a three-domain system exists in the cell wall structure. The first domain is the cellulose-hemicellulose structure with an embedded second domain of pectic substance. The third domain is composed of cross-linked proteins with covalent bounding. The integrity of cell wall and middle lamella attributes mostly to the texture of the processed vegetable and fruits. The presence of pectic compounds plays an important role in the strength and adhesiveness of cell walls. During the thermal process, both linear and branched pectic polymers are degraded. Thermal changes of hemicellulose and cellulose have only a minor impact on the structural texture (Stolle-Smits, Beekhuizen, Recourt, Voragen & Dijk, 1997).

The Principles of Water Uptake in Dry Beans

Water uptake ability plays an important role in the expansion of the seed coat. Water uptake during processing has been recognized as an important factor affecting the subsequent texture of the canned bean product (Stanley, Wu & Plhak, 1989). The water uptake of the undamaged beans is composed of two phases. In the initial phase, natural barriers, such as the seed coat and the hilum, control water uptake. Water enters the beans through three means: the micropyle, the hilum and the raphe (Ogwal & Davis, 1994). The seed coat is impermeable to water initially. As water enters at the hilum and micropyle region, the middle lamella, which bonds the seed coat and cotyledon, dissolves. The seed coat is then released from cotyledon and becomes permeable to water. Depending on the tightness of the cotyledon halves, water gradually reaches the middle lamella and seed coat opposite to the hilum region. Due to rapid hydration, wrinkles are often observable on the seed coat. When the cotyledon reaches full expansion, the seed coat stretches and becomes tautened. The second phase starts when the permeability of seed coat reaches saturation. Water uptake rate drops in this phase, as the water content in the beans approaching equilibrium (Heil, McCarthy & Özilgen, 1992a).

The rate of water uptake is proportional to the diffusivity of water, which is determined by a complex cluster of factors, such as microstructure, chemical composition, moisture, and the temperature of the seed. Both the seed coat microstructure and composition may affect the imbibition rate. The imbibition rate may also be related to seed coat thickness, numbers of seed coat pores, size of micropyle and hilum, and

cellular arrangement of the raphe. A darker-colored seed coat, which results from high phenolic content, has been reported to impede water permeability. Hydrophobic substances generated by the oxidative reaction of phenolic substance in seed coat are possibly responsible for the lowered water permeability (Del Valle & Stanley, 1992).

Physiochemical Properties of Kidney Beans

Edible dry beans of *Phaseolus vulgaris* L. are used as a major and inexpensive dietary protein source in many developing countries. Besides having high protein content, the *Phaseolus* family is also a very good source of vitamins, minerals (calcium, potassium, magnesium, copper, phosphor, and iron), complex carbohydrates and unsaturated fatty acids, which are beneficial to human health (Deshpande & Damodaran, 1990). Compared to ground beef on a 100 kcal basis, legumes contain 80% less total fat (Sathe, Deshpande & Salunkhe, 1984a). The consumption of legumes can significantly reduce excessive fat intake and avoid cholesterol intake. Both linoleic and linolenic acids were found as the predominant constituents of lipids in kidney bean. Unsaturated fatty acids in kidney bean consist of about 86% of the total lipid content (Sathe, Deshpande & Salunkhe, 1984b).

In general, legume seed weight fractions are: 8.5-10% seed coat, 85 – 90% cotyledon, and 1-3% embryo (Deshpande & Damodaran, 1990). The distribution of nutrients varies in different seed fractions as well as with different cultivars. Cotyledons have abundant proteins and lipids, and are high in ash content. Meanwhile 80-93% of the crude fiber and 32-50% calcium are often located in the seed coat (Deshpande & Damodaran, 1990). The chemical composition of kidney beans varies greatly among different genotypes. In order to improve the cultivar's physiological properties, breeders often genetically modify cultivated strains by cross-linking with wild species. This

further complicates the variation in physiochemical compounds among the commercial cultivars.

Structure and function of seed coats

The seed coat (testa) is more than a protection barrier against the intrusion of bacteria and fungi. It also transports minerals to the developing embryo, regulates nutrient flow, and controls dormancy (Yeung & Cavey, 1990; Kelly, Van Staden & Bell, 1992). The seed coat is composed of several layers of cells, which develop from the integuments of ovule. The epidermis is the outermost layer, also known as palisade cells, and is constructed of elongated cells. The hypodermis is the middle layer, containing hourglass cells. These cells are very small and inconspicuous. The third layer, called the mesophyll, contains large elongated parenchyma cells (Stanley, Wu & Plhak, 1989; Bhatty, 1995; Miller, Bowman, Gijzen & Miki, 1999). Bhatty (1995) reported that in the seed coat of lentils (*Lens culinaris*) with poor cooking quality, the epidermis was irregular and packed tightly, and the cell walls of the hypodermis were thicker. The seed coat consists of a complex mixture of chemicals, including polysaccharides, cellulose, hemicellulose, fats, waxes, protein and minerals (Noggle & Fritz, 1976).

Hydration of the bean is greatly influenced by physio-chemical properties in the cotyledon and the seed coat. The seed coat is a primary barrier for water imbibition by affecting the rate of water permeability. Studies have shown that cooking time could be reduced from 80 minutes to 30 minutes, when the seed coats were removed (Uebersax, Reungsakulrach & Hosfield, 1989). In a study on the cooking quality of lentils, the

removal of the seed coat increased the hydration coefficient and water absorption. Losing the natural barrier (seed coat), the solids lost from beans during soaking can increase significantly up to 100% (Bhatty, 1995).

During seed development, markable structural changes occur within the inner epidermis of the bean seed coat. Phenolic substances are responsible for the appearance of the electron-dense product in outer cell walls. Phenolic compounds are common components of cell walls. They strengthen the wall by cross-linking with other components. After the proembryo stage of development, no new cells are further generated. However, the inner surface of the seed coat is subjected to rapid stretching because of the rapid expansion of embryo. The phenolic compounds in the seed coat may contribute to its strength and prevent it from disrupting (Yeung & Cavey, 1990). In another study on the effects of the seed coat on cooked bean structure, researchers found that seed coat hardness was significantly related to water absorption (Stanley, Wu & Plhak, 1989). Seed coat hardening may be proceeded by a lignification mechanism, in which polymerization of phenolic substances occurs and hydrophobicity increases. Consequently, these contribute to the sensation of hardness of beans after cooking (Stanley, Wu & Plhak, 1989).

During cooking, the seed coat tends to separate at the mesophyll, and a gap between the seed coat layers can be observed by microscope. This is followed by hydration and swelling of complex carbohydrates (such as pectin, cellulose and hemicellulose) which lead to the softening of the seed coat (Stanley, Wu & Plhak, 1989).

Minerals

Similar to other legumes, kidney beans are also a very good source of minerals such as calcium, iron, copper, zinc, phosphor, potassium, and magnesium (Table 2). Potassium is a major mineral in kidney beans. It constitutes about 35-40% of the total mineral content of the kidney beans. Kidney beans are richer in calcium than most cereals, and their high iron content level makes them an excellent dietary source for iron. Their low sodium content is considered advantageous in maintaining a low sodium intake as recommended in Recommended Daily Allowance (RDA) diet (Sathe, Deshpande & Salunkhe, 1984b; Deshpande & Damodaran, 1990).

Table 2

Mineral content of different food legumes (mg/100g dry weight)

Type	Ca	Fe	Na	K	Mg	Cu
Kidney beans (*Phaseolus vulgaris* L.) [a]	143	8.2	24	1,406	140	0.96
Arachis hypogaea [b]	69	2.1	5	674	NA[c]	NA
Glycine max [b]	226	8.4	5	1,677	236	2.4
Phaseolus lunatus [b]	72	7.8	4	1,529	174	0.74
Phaseolus mungo [b]	154	9.1	40	NA	185	0.72

Note: The unit is expressed as mg per 100 g beans on dry weight basis

[c] Data is not available

Source: [a] USDA. (2001, July). USDA Nutrient database for standard reference: Release 14. Retrieved December 12, 2001, from http://www.nal.usda.gov/fnic/cgi-bin/list_nut.pl

[b] Deshpande, S. S., Damodaran, S. (1990). Food legumes: chemistry and technology. *Advances in cereal science and technology, 44* (10), 147-241.

According to the USDA's data, mineral content and ash content in kidney bean varies significantly between different cultivars (Table 3, Figure 3). This may be a reason for the inconsistency in mineral data from different research resources, as presented in Table 4. Some inconsistency may be due to different cultivars, climate, soil composition, and cultural practices. Very few reports have focused on the mineral profile in seed coat, most focused on the composition of the whole seed or the cotyledon.

Table 3

Mineral content in different kidney beans cultivars

	Ca	Cu	Fe	Mg	K	Na	Zn	P
California Red [a]	195	1.10	9.35	160	1,490	11	2.55	405
Royal Red [a]	131	1.11	8.70	138	1,346	13	2.66	406
Red Kloud [b]	131	NA[c]	5.16	128	1,038	NA	3.1	385

Note: The unit is expressed as mg per 100g beans on dry weight basis

[c] Data is not available

Source: [a] USDA. (2001, July). USDA Nutrient database for standard reference: Release 14. Retrieved December 12, 2001, from http://www.nal.usda.gov/fnic/cgi-bin/list_nut.pl

[b] Koehler, H. H., Chang, C., Scheier, G., Burke, D. W. (1987). Nutrient composition, protein quality, and sensory properties of thirty-six cultivars of dry beans (*Phaseolus vulgaris* L.). *Journal of Food Science, 52* (5), 1335-1340.

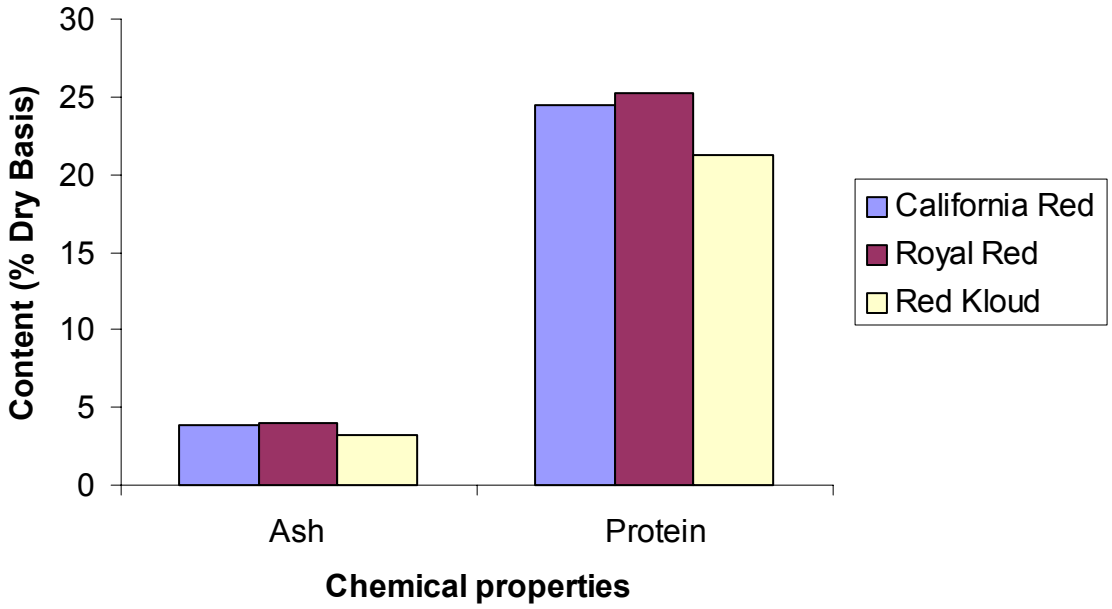

Figure 3. Comparison of ash and protein content (% Dry basis) in different kidney bean cultivars.

Note: [a] California Red and Royal Red

[b] Red Kloud

Source: [a] USDA. (2001, July). USDA Nutrient database for standard reference: Release 14. Retrieved December 12, 2001, from http://www.nal.usda.gov/fnic/cgi-bin/list_nut.pl

[b] Koehler, H. H., Chang, C., Scheier, G., Burke, D. W. (1987). Nutrient composition, protein quality, and sensory properties of thirty-six cultivars of dry beans (*Phaseolus vulgaris* L.). *Journal of Food Science, 52* (5), 1335-1340.

Table 4

Mineral content in different common dry beans (Phaseolus spp.) from various research

	Ca	Cu	Fe	Mg	K	Na	Zn	P
Great Northern bean	181	0.82	6.87	178	1,087	3.60	2.12	458
	190	0.85	3.83	220	1,630	7.40	2.50	480
Navy bean	136	0.80	5.34	163	821	1.66	2.17	453
	180	1.06	6.94	200	1,350	5.10	2.23	454
Red Kidney bean	90	0.77	6.90	180	1,650	6.10	3.70	500
	60	0.69	7.47	123	1,300	2.45	2.83	374
Pinto bean	111	0.67	7.04	171	1.156	2.51	2.21	510
	120	0.94	5.70	190	450	12.9	2.2	450

Note: The unit is expressed as mg per 100g beans on dry weight basis

Source: Sathe, S. K., Deshpande, S. S., & Salunkhe, D. K. (1984b). Dry beans of *Phaseolus*: A Review, Part 2: Chemical compositions: Carbohydrates, fiber, mineral, vitamins, and lipids. *CRC Critical Review in Food Science and Nutrition*, 21(1), 41-93.

In a study on canned navy bean, another strain of *Phaseolus vulgaris* L., a significant correlation ($r = 0.77$, $P<0.01$) between calcium content and ash content was also revealed. Even though the calcium content accounts for only 3% of the total ash content in raw navy beans, the data predicted that a possible ratio relationship between the two values might exist (Lu & Chang, 1996).

Protein

The protein content of kidney beans varies from 20-25% based on dry weight. Studies suggested that the protein content in the *Phaseolus vulgaris* group varied considerably among different cultivars (Figure 3). This data introduced the possibility of improving genetic germ plasm through the selection of cultivars with improved protein content.

Glycoprotein was found to be of great importance in growth control and disease resistance. Two types of glycoprotein have been isolated from kidney beans (Jaffe & Hannig, 1965). The differentiation of the two types glycoprotein is based on their distinct solubility in salt solution. Glycoprotein I is a strong-salt soluble protein, while glycoprotein II is dilute-salt soluble. Glycoprotein II is the major protein constituent in kidney beans. Very small quantities of methionine and cystine are presented in the glycoprotein II. Both proteins are resistant to enzymatic hydrolysis, even after heat denaturation. The stability may result from their complex tertiary and quaternary structure, which is constructed with cross-linked hydrogen bonds, hydrophobic interactions and electrostatic forces (Chang & Satterlee, 1982). The structural function of

glycoprotein in cell wall is still speculative. It is thought to form an intrinsically insoluble wall polymer, which constructs a complex network with pectin. This network may allow cellulose fiber to intercalate through and form a hardened cell wall (Cooper, Chen & Varner, 1984).

Principles of Kjeldahl Method

Crude protein content was determined using the Kjeldahl method in this study. Kjedahl is used for the determination of nitrogen content. In the presence of a catalyst, proteins in the food samples are digested with sulfuric acid, and organic nitrogen is converted to ammonium sulfate. When the digestant is neutralized and made basic with alkali, ammonia is formed and distilled into boric acid. The boric acid is then titrated with standardized hydrochloric acid, and the amount of borate anion, which is proportional to the amount of nitrogen, can be determined. During the reaction, a metallic catalyst, such as mercury, copper and selenium is added to sulfuric acid to complete the digestion (Chang, 1998).

The Kjeldahl method can be divided into three major steps: digestion, neutralization with distillation and titration.

Digestion

The sample is digested with acid in the presence of a catalyst. Digestion of all organic matter is complete when the digestant becomes clear. Nitrogen in protein is liberated, while carbon and hydrogen in the carbon chain are converted to carbon dioxide. The reaction between nitrogen in organic compound and sulfuric acid forms ammonium sulfate.

$$\text{Protein} \xrightarrow[\text{Heat \& catalyst}]{\text{Sulfuric acid}} (NH_4)_2SO_4$$

Neutralization

Alkali is added to neutralize the sulfuric acid. The excessive alkali reacts with ammonium sulfate and generates ammonia, which is distilled into boric acid. Methyl red is used as indicator.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$$

Titration

The amount of borate anion is determined by a standardized hydrochloride acid.

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3$$

Calculation

The amount of nitrogen is proportional to the amount of borate ion. Based on this relationship, nitrogen content is obtained using the following equation.

$$\%N = \frac{N \times (\text{Volume of HCl for sample} - \text{volume of HCl for blank}) \times 14}{\text{grams of sample}} \times 100\%$$

N is the normality of HCl in mole/1000 mL; volume is in mL; 14 is the atomic weight of nitrogen.

Most proteins contain 16% nitrogen. By applying this factor, the crude protein content can be calculated as:

$$\%N \times 6.25 = \% \text{ protein}$$

Due to the various amino acid profiles in different food sources, a conversion factor may be applied (Chang, 1998).

Along with nitrogen in proteins, the results of Kjeldahl determinations also include nitrogen from nonprotein compounds, such as free amino acid, amines, lipids, nucleic acids, and alkaloid. Studies have shown that nonprotein nitrogen accounted for about 8-15% of the total nitrogen in dried seed. This often leads to an overestimation of crude protein content (Deshpande & Damodaran, 1990).

Principles of Atomic Absorption

Atomic Absorption was used in this thesis project for mineral composition determination. Atomic Absorption Spectrophotometry is based upon the spectroscopic behavior of gaseous metal atoms. Bombarded by sufficient external energy, the vaporized metal atom can be promoted from a ground state to an excited state. This excitation is caused by the movement of electrons from a lower energy level to a higher one when the electrons absorb a photon of radiation (Pease, 1980). For a given energy and electronic transition, only radiation of a discrete wavelength can be absorbed. Each element has its specific set of electronic transitions, which allows a unique spectrum for atomic absorption. Atomization is the changing of atoms of an element into the atomic state, in which no other elements are combined. By exposing the analyte to a high temperature in a flame, ions from a food can be momentarily converted into atoms. The vaporized atoms can then be excited by radiation (Miller, 1998). The atoms absorb the energy from the emission radiation, which is generated by the source lamp. The radiation provides the identical energy and wavelength that is required to raise the atom to the excited state. After the energy is partially absorbed by the vaporized atom, the balance of the radiation is detected and measured (Pease, 1980).

Atomic absorption is used to quantify the absorption of electromagnetic radiation of the distinct atoms. It is perfectly suited for analytical measurements in food because the spectra consist of discrete lines and every element has its own spectral pattern. Atomic absorption has been particularly preferred for mineral composition studies in

foods since the sixties. It gives rapid, accurate and precise results in the identification of elements (Miller, 1998).

Flame atomization is a type of Atomic Absorption Spectroscopy. In this type, the sample solution is converted to vapor by a nebulizer-burner. After the sample is dispersed into tiny droplets, it is mixed with a fuel-oxidant mixture, and burned in a flame. Atoms and ions are produced as solvent evaporates and ionic compounds dissociate within the high temperature flame. Air-acetylene is the common fuel combination that results in efficient atomization. Once atomized in the flame, the absorbance at the analytical wavelength of the element being investigated is measured by determining attenuation of a beam of radiation passing through the flame. This radiation is produced by a source lamp, in which the element to be determined serves as the cathode. The radiation emitted from the lamp is identical to the emission spectrum of the element. The excited atoms absorb some of the radiation. The remaining radiation is separated by passing this beam through a monochromator, which causes only the radiation of a narrow bandwidth to reach the detector. The relationship between the measured absorbance and the concentration of the element being investigated is expected to obey the Beer's Law:

$$A = \log (I_0/I) = abc$$

A is absorbance; I_0 is the intensity of radiation incident on the flame; I is the intensity of radiation exiting the flame; a is molar absorptivity; b is path length through the flame; c is concentration of atoms in the flame (Miller, 1998).

The Flame Atomic Absorption System consists of the following units: radiation source, atomizer, monochromator, detector, and readout device. A hollow cathode lamp is often used as radiation source. The cathode is made of the metallic form of the element being measured. When voltage is applied, the lamp emits a spectrum of the metal. The monochromator is positioned in the optical path between the flame and the detector. It isolates the resonance line of interest from the rest of the radiation, so only the desired wavelength reaches the detector. The detector converts the radiant energy into an electrical signal that is processed to produce a digital readout. In a double beam detection system, the positioning of the constant rotating chopper is perpendicular to the light path between the flame and light source. This enables the radiation to be directed to the flame or around the flame at a constant frequency. A reference power and a sample power are alternatively detected by the detector. This constant comparison efficiently minimizes the variation in the intensity of the light source and fluctuation in the detector (Miller, 1998; Pease, 1980).

CHAPTER III

PROCEDURE

Objectives

The objectives of this project were to: 1) Investigate moisture content, ash content, crude protein content and mineral profile in the seed coat of different dark red kidney bean cultivars grown in the Wisconsin area. 2) Study the weight ratio of the seed coat to whole seed and the weight of per seed. 3) Evaluate the relationship of the seed coat to whole seed weight ratio, seed weight and nutritional contents to canning quality. 4) Investigate the influences of the physiochemical properties in the seed coat to canning quality among different cultivars. 5) Provide information on factors related to canning qualities for cultivar screening.

Methodology

In order to facilitate easy comprehension, the procedure was conveniently divided into five sections:

A. Sampling and storage of the cultivars

B. The evaluation of the canned kidney beans

C. The study of the ratio of seed coat to whole seed and seed weight

D. Other objective measurements

E. Statistical methods

All research was conducted in triplicate.

A. Sampling and Storage

Both of the dried and canned dark red kidney beans were provided by Chippewa Valley Bean Co., Menomonie, Wisconsin. Three different cultivars (85, 453 and Nickols) were selected for this study. All cultivars were planted in April 2001 in the Menomonie, Wisconsin area. In order to minimize mechanical injury (Heil, McCarthy & Özilgen, 1992b), samples were harvested manually in October 2001. During the research period, the dried beans were sealed within Ziploc® heavy-duty freezer bags, and stored in a low temperature incubator (Precision Scientific, Model Freds 815), with a temperature of 24°C and humidity of 12%. The dry bean samples were graded to size by using a standard bean sieve (No.12, Seeburo Equipment) prior to further experimentation. The fraction remaining in the sieve was selected for this study. The smallest, largest and broken seeds were further removed manually.

Canned beans of the three different cultivars were prepared by the Animal and Food Science Department, University of Wisconsin – River Falls in October 2001. The canned beans were stored at room temperature for 5 months.

B. The evaluation of the canned kidney beans

Three cans of each cultivar were randomly chosen for the evaluation set. The percent seed coat splitting was used to evaluate the seed coat integrity after the canning process. The percent splitting was defined as the number of beans with seed coat splitting per 100 beans. After the disclosure of the canned beans, the brine was removed and the samples were rinsed with distilled water to allow for better detection of the defects on the

seed coat. The percent of beans with seed coat splitting was determined using the following equation.

$$\text{Percent of seed coat splitting} = \frac{\text{Numbers of beans with splitting in one can}}{\text{Total numbers of beans in one can}} \times 100\%$$

C. The study of the seed coat to whole seed weight ratio and seed weight

The seed coat to whole seed weight ratio was defined as the ratio of the seed coat weight to the whole seed weight on a dry weight basis. Dark red kidney beans (50 g) were weighed on balance (Ohaus®, Galaxy™ 160 D) and then soaked with 150 mL Milli-Q water (Milli-Q® Water Purification System, Millipore Corporation) for 16 hours at 5°C. Water was drained off the seeds, and the seed coat and cotyledon were decorticated manually. Samples were placed into four evaporating bowls (one for seed coat, three for cotyledon) and dried in a vacuum oven (Fisher Scientific Isotemp® Vacuum oven, Model 282A) for 140 hours at 60°C, in order to remove the water in the samples. The weight of the dried seed coat and dried cotyledon were then determined. The calculation used was

$$\text{Ratio of seed coat to seed} = \frac{\text{Wt of dried seed coat}}{\text{Wt of dried cotyledon} + \text{Wt of dried seed coat}} \times 100\%$$

Seed weight was defined as the average weight per seed. Approximately 50 g of seeds was weighed using a balance (Ohaus®, Galaxy™ 160 D). The number of seeds was counted manually. The weight per bean was determined by dividing the weight of the seeds by the count of the seeds. The calculation used was:

$$\text{Weight per seed} = \frac{\text{Weight of beans}}{\text{Total count of the beans}}$$

D. Objectives Measurement

Preliminary trials were performed to optimize the procedures.

Moisture content

Moisture content measurement was modified according to the AOAC Official Method of Analysis 925.09 (1990). Approximately 50 g dark red kidney beans was weighed and evenly divided into two evaporating dishes. The dishes were then placed into a vacuum oven (Fisher Scientific Isotemp® Vacuum oven, Model 282A) for 72 hours at 60°C. Samples were transferred to a desiccator immediately after the drying process. Beans were cooled to room temperature, and the samples were weighed again. The differences between the weights of the samples prior to and after vacuum-drying were determined. Moisture content was obtained using the following equation:

$$\text{Moisture content} = \frac{\text{Wt of beans before drying} - \text{Wt of beans after drying}}{\text{Wt of beans before drying}} \times 100\%$$

The moisture content of each cultivar was expressed as the mean and standard deviation.

Ash content

Ash content measurement was modified and based on the AOAC Official Method of Analysis 923.03 and 942.05 (1990), and CEM Use Guidelines (CEM, 2001).

About 15 g of seed coats was ground using a commercial blender (Waring Blender 700, Model 31BL4) for 60 seconds. The powder was collected into an airtight sandwich bag (16.5×14.9 cm, Home Best^{TM}) and stored in a desiccator.

Prior to experimentation, CEM crucibles (CEM Quartz Fiber Ashing Crucibles 20ml) were heated for 10 minutes at an operating temperature of 800°C in a preheated furnace (Fisher Scientific Isotemp 650 Programmable Muffle Furnace). Loose fibers in the crucible were lightly brushed off, and the crucibles were stored in a desiccator. After being cooled to room temperature in a desiccator, each crucible weight was recorded. About 3 g of each ground sample was weighed in a crucible and placed in the preheated furnace. The samples were ignited in an 800°C furnace for 120 minutes to burn off the organic materials. The crucibles containing the samples were then placed in a desiccator, cooled to room temperature and weighed. The weight of ash was determined by subtracting the weight of samples prior to ignition from the weight after the ignition. The equation used in this calculation is shown as below:

$$\text{Ash content (\%)} = \frac{\text{Wt of ash}}{\text{Wt of sample before ignition}} \times 100\%$$

Mean and standard deviation were used to express ash content for each cultivar.

Protein content

The procedure used in determining the total protein content was adapted from the laboratory manual complied by Ondrus, M. G. (2001a) and the AOAC Official Method of Analysis. 2.055 to 2.059 (1980).

Chemicals used for the analysis included: 18 M sulfuric acid, potassium sulfate (analytic grade), 10 M sodium hydroxide, 50 g/L boric acid, methyl red – bromcresol green mixed indicator (American Public Health Association [APHA], American Water Works Association [AWWA] & Water Pollution Control Federation [WPCF], 1971), standardized (0.0995 N) hydrochloric acid, mossy zinc (analytical grade), and selenized hengar granules. Other materials consisted of polystyrene dishes, 250 mL erlenmeyer flasks, plastic bottles, pipets, filter paper, and distilled water. Equipment included a drying oven (Stabil-therm® Oven, Blue M electric company) maintained at 80°C, sets of the Kjedahl apparatus, electric burners (Corning hot plate stirrer, Model PC320), and desiccators. The procedures for preparing reagents are described in Appendix A.

The Kjeldahl method involves a two-step process: sample digestion and sample distillation. Ground seed coat samples prepared from the previous procedure were used. For sample digestion, sample powder (2 g) was weighed on a filter paper, and carefully added into a 500 mL Kjeldahl flask with filter paper. Concentrated (18 M) sulfuric acid (25 mL) was added to the flask, followed by 7 g potassium sulfate and 3-4 selenized hengar granules. Samples were held at a 30° angle from the vertical position and heated in the fume hood. Samples were then heated gently until frothing ceased. Further boiling

was continued until the solution became colorless. An additional 2-hour boiling period was extended to further digest all organic materials.

In sample distillation, the digestion solution was cooled slowly to room temperature. Cold distilled water (100 mL) was added to hasten cooling. A volume of 100 mL (10 M) sodium hydroxide was poured down the side of the digestion flask to form two liquid layers. Three pieces of zinc metal were dropped in the digestion flask to prevent vigorous bumping. After the flask was connected to the trap and condenser, the two layers of liquid in the digestion flask were mixed with gentle swirling. The solution was brought to a boil and distilled to half the previous volume. The ammonium nitrogen produced in the digestion flask was distilled into an erlenmeyer flask containing 50.0 mL 50 g/L boric acid. The collected solution was then titrated with standardized 0.0995 N hydrochloric acid. Digestion was complete when the indicator in the boric acid solution changed from blue to red.

A blank sample with the same-size filter paper only was run to eliminate system errors. A National Bureau of Standard (NBS) Orchard Leaves sample (Standard Reference Material 1571, Office of Standard Reference Material) with known nitrogen content (2.76 ± 0.05 %) was also run with the samples for accuracy comparison. Three replicates were analyzed using the same procedures used in this work. The difference between the official value and the mean value (2.79 ± 0.01 %) obtained in this work was less than 2%.

Mineral profiling with Atomic Absorption

All the procedures used for the mineral profile were adapted from the laboratory manual complied by Ondrus, M. G. (2001b), Klahr, K. A. (1987) and the AOAC Official Method of Analysis 2.055 to 2.059 (1980).

Chemicals used for the analysis included: calcium carbonate ($CaCO_3$), potassium chloride (KCl), sodium chloride (NaCl), Lantham oxide (La_2O_3), magnesium metal, metallic iron, and concentrated hydrochloric acid (6 M HCl). Other materials used consisted of polystyrene dishes, 250 mL beakers, 100 mL, 250 mL and 1,000 mL volumetric flasks, pipetters, funnels, pipettes, and graduate cylinders. Equipment used included a drying oven (Stabil-therm® Oven, Blue M electric company) maintained at 80°C, a furnace (Fisher Scientific Isotemp 650 Programmable Muffle Furnace), Atomic Absorption/Atomic Emission (AA/AE) Spectrophotometer (Instrumentation Laboratory, Model S12), hollow cathode lamps (Table 6), electric burners (Corning hot plate stirrer, Model PC320), and desiccators.

Table 5

Different hollow cathode lamps applied in the research

	Company
Potassium Hollow Cathode Lamp	Fisher Scientific
Sodium & Potassium Hollow Cathode Lamp Visimax ® I	Thermo Jarell Ash Corp.
Calcium Hollow Cathode Lamp	VWR Specific
Magnesium Hollow Cathode Lamp	VWR Specific
Iron Hollow Cathode Lamp	VWR Specific

The atomic absorption method procedure used in this research involved four phases: stock solution preparation, standard solution preparation, sample preparation and mineral content determination.

The preparation of the stock solution is described in Appendix B. Five different standard solutions were established, including 1.0 mg/L, 2.5 mg/L, 5.0 mg/L, 7.5 mg/L, and 10 mg/L. At each concentration, the standard matrix contained the same concentration of each of the minerals: calcium, magnesium, potassium, sodium, and iron. As shown in Table 6, the volumes of the stock solutions used were transferred to 100 mL volumetric flasks. In each flask 10.0 mL of lanthanum stock solution was also added. Milli-Q water was added to bring the volume to 90 mL. While swirling, each solution was further diluted by adding Milli-Q water to reach a final volume of 100 mL. The presence of phosphate can interfere with calcium and magnesium detection; therefore lanthanum stock solution was added to the standards and samples to eliminate this interference (AOAC, 1980). A blank was prepared by diluting 10.0 mL lanthanum stock solution in a 100 mL volumetric flask.

Table 6

Volumes of different stock solutions used to prepare a standard solution series

Concentration of the standard (mg/L)	1.0	2.5	5.0	7.5	10
Volume used from 1000.0 mg/L solutions (mL)	0.10	0.25	0.50	0.75	1.00
Volume used from 500.0 mg/L solutions (mL)	0.20	0.50	1.00	1.50	2.00

Approximately 3 g of vacuum-dried seed coat was weighed, and placed into CEM crucibles. The samples were placed into an 800°C furnace for 2 hours to ignite and burn off all organic matters. Upon moving the crucible from the furnace, the ash was placed into a desiccator and cooled to room temperature. The ash samples were then placed into a 250 mL beaker. Fifteen mL 6 M HCl was added and the solution was boiled for 5 minutes to digest the ash samples. Following the initial acid digestion, further digestion was accomplished by adding an additional 10 mL of 6 M HCl and boiling for another 5 minutes (CEM, 2000). The digested solution and crucible were then transferred to a 100 mL volumetric flask using a funnel. In each flask, 10.0 mL of lanthanum stock solution was added. Milli-Q water was used to rinse the crucible and bring the volume up to 90 mL. The samples were swirled, well mixed, and additional Milli-Q water added to bring the volume to 100 mL. As indicated by preliminary tests, solutions were further diluted 10-fold to 100-fold for more efficient reading of absorbance.

Samples were analyzed for their iron, sodium, calcium, magnesium, and potassium content by using AA/AE Spectrophotometer with an air/acetylene flame. A sodium/potassium hollow cathode lamp was used for sodium determination, and a single element hollow cathode lamp was also used for the other mineral detections. The parameters used in instrument settings were in accordance with the manufacturer's specification, as shown in Table 7. A larger slit width than the recommended value was used for potassium, due to the lack of a "red-sensitive" photomuliplier tube. The instrument was zeroed using a blank solution. The linearity of each mineral standard solution was determined by measuring the absorbance of standard solution series. The concentrations and respective standard curves for each are shown in Table 8 and Figures 4-9. Mineral content for each sample was measured and the mineral concentration was determined by plotting the absorbance within the standard curves.

Table 7

The parameters used for mineral analysis with atomic absorption

	Wavelength (nm)	Bandpass	Lamp current (mA)
Magnesium	285.2	1.0	3.0
Sodium	589.0	0.5	8.0
Calcium	422.7	1.0	7.0
Potassium	766.5	2.0	7.0
Iron	248.3	0.3	8.0

Table 8

The absorbance of the standard solution series

Standard Concentration (mg/L)	Absorbance				
	Na	Ca	Fe	K	Mg
1.0	0.032	0.013	0.008	0.012	0.099
2.5	0.089	0.028	0.016	0.030	0.242
5.0	0.182	0.057	0.030	0.058	0.469
7.5	0.276	0.086	0.039	0.086	0.667
10.0	0.363	0.120	0.049	0.119	0.883

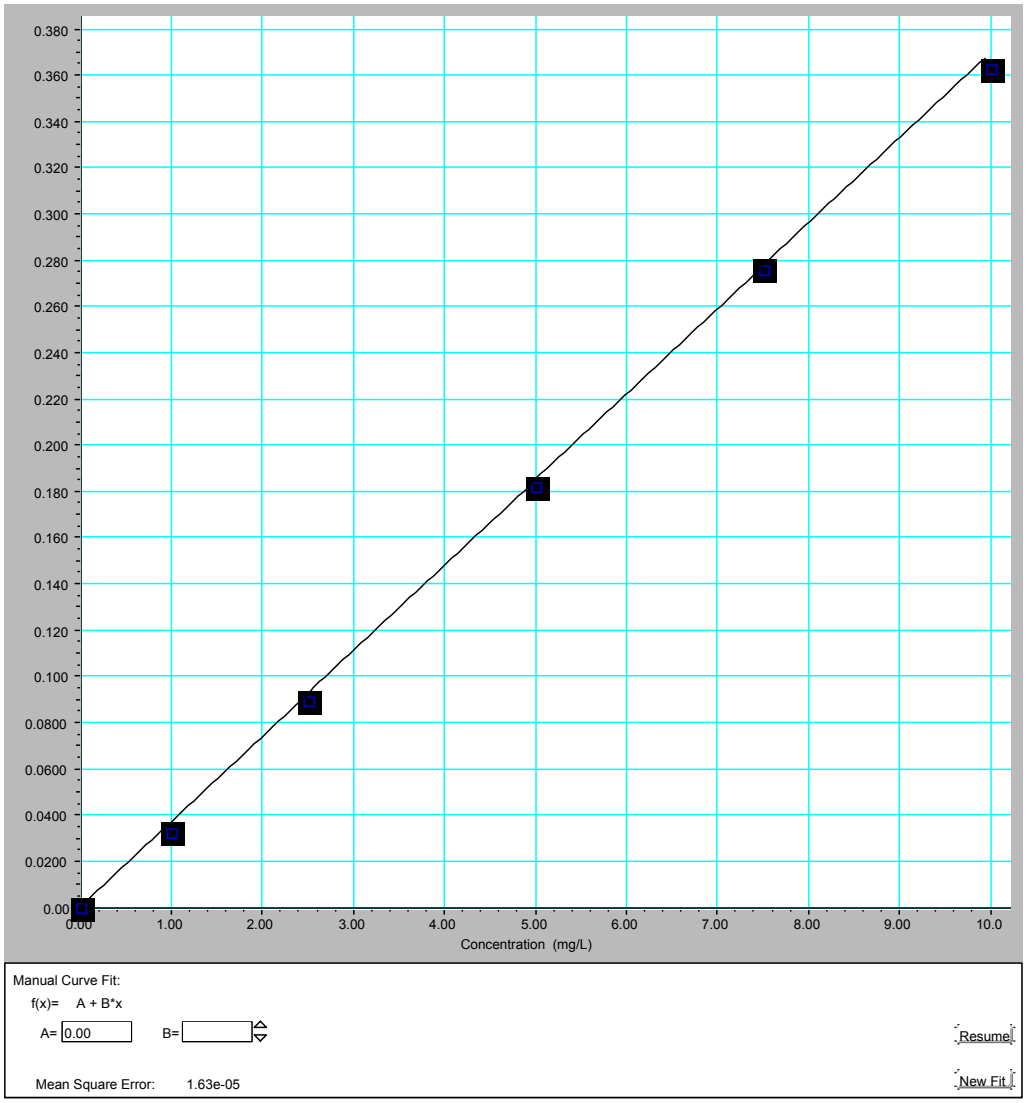

Figure 4 Standard curve for sodium content determination (Y= 0.037X)

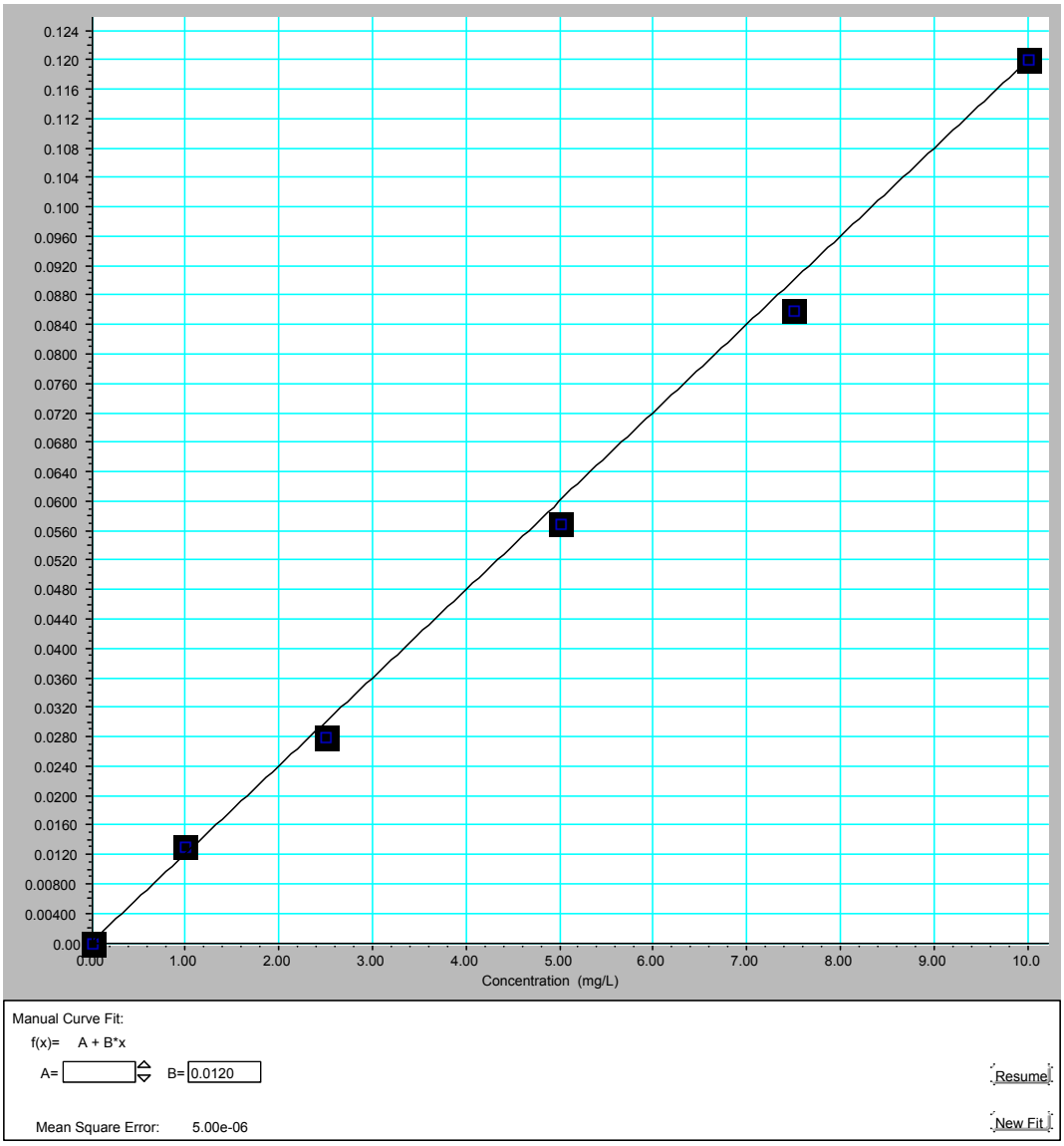

Figure 5 Standard curve for calcium content determination (Y= 0.012X)

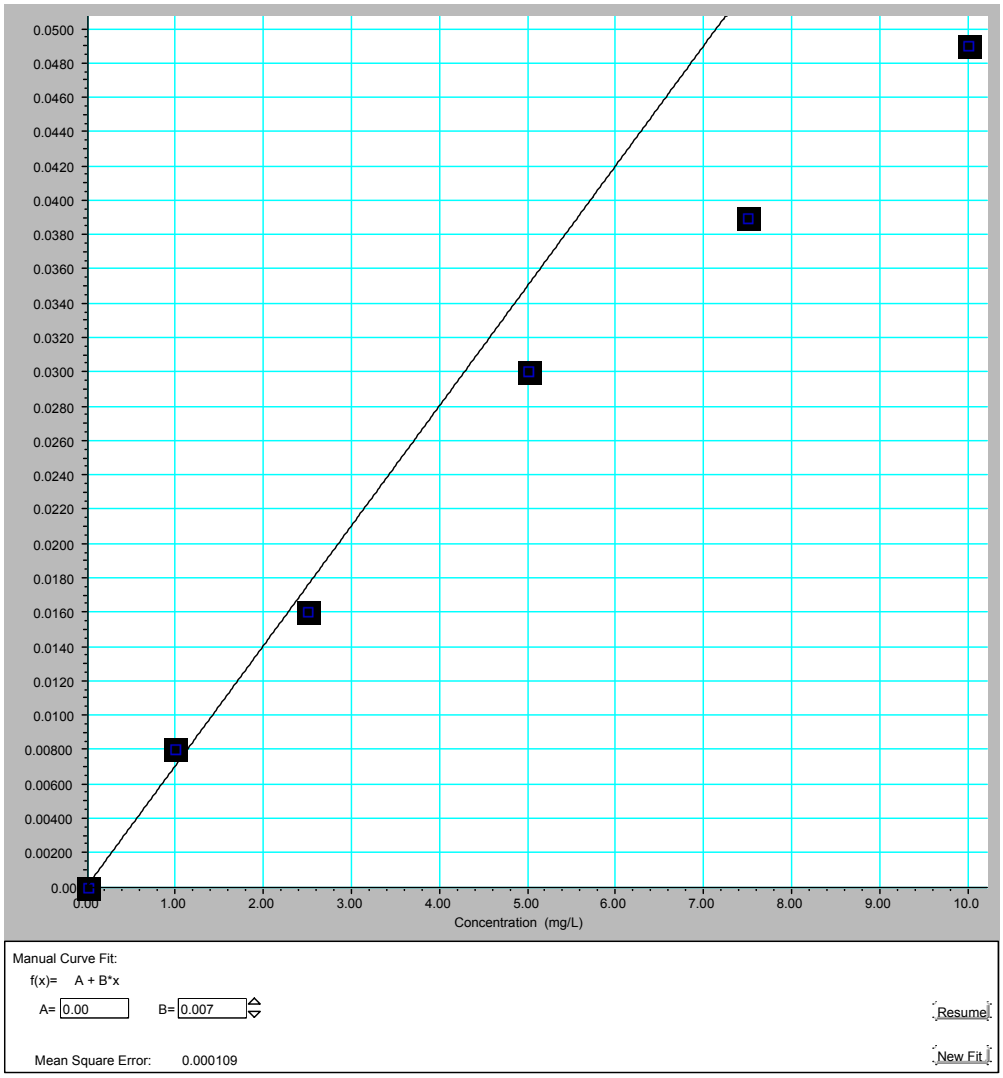

Figure 6 Standard curve for iron content determination (Y= 0.007X)

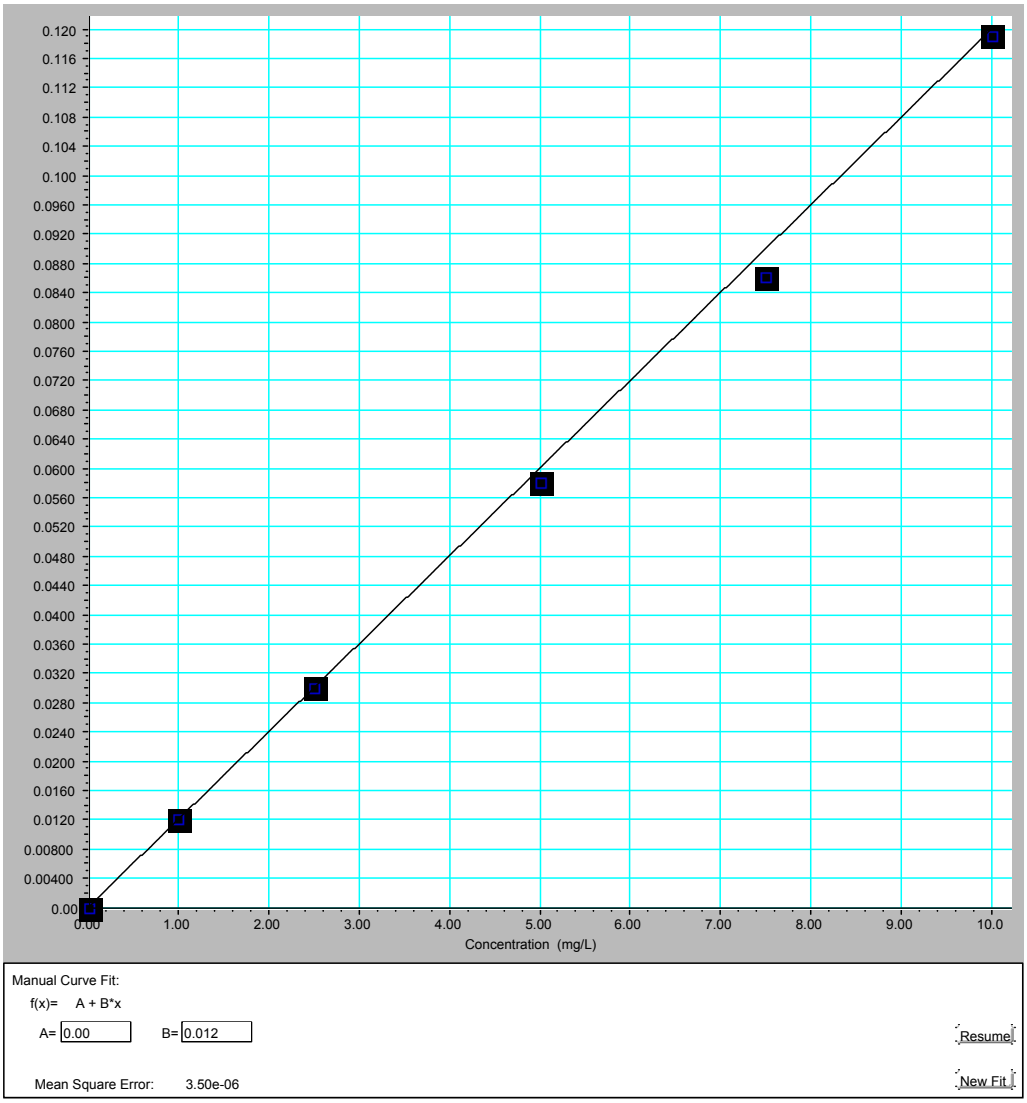

Figure 7 Standard curve for potassium content determination (Y= 0.012X)

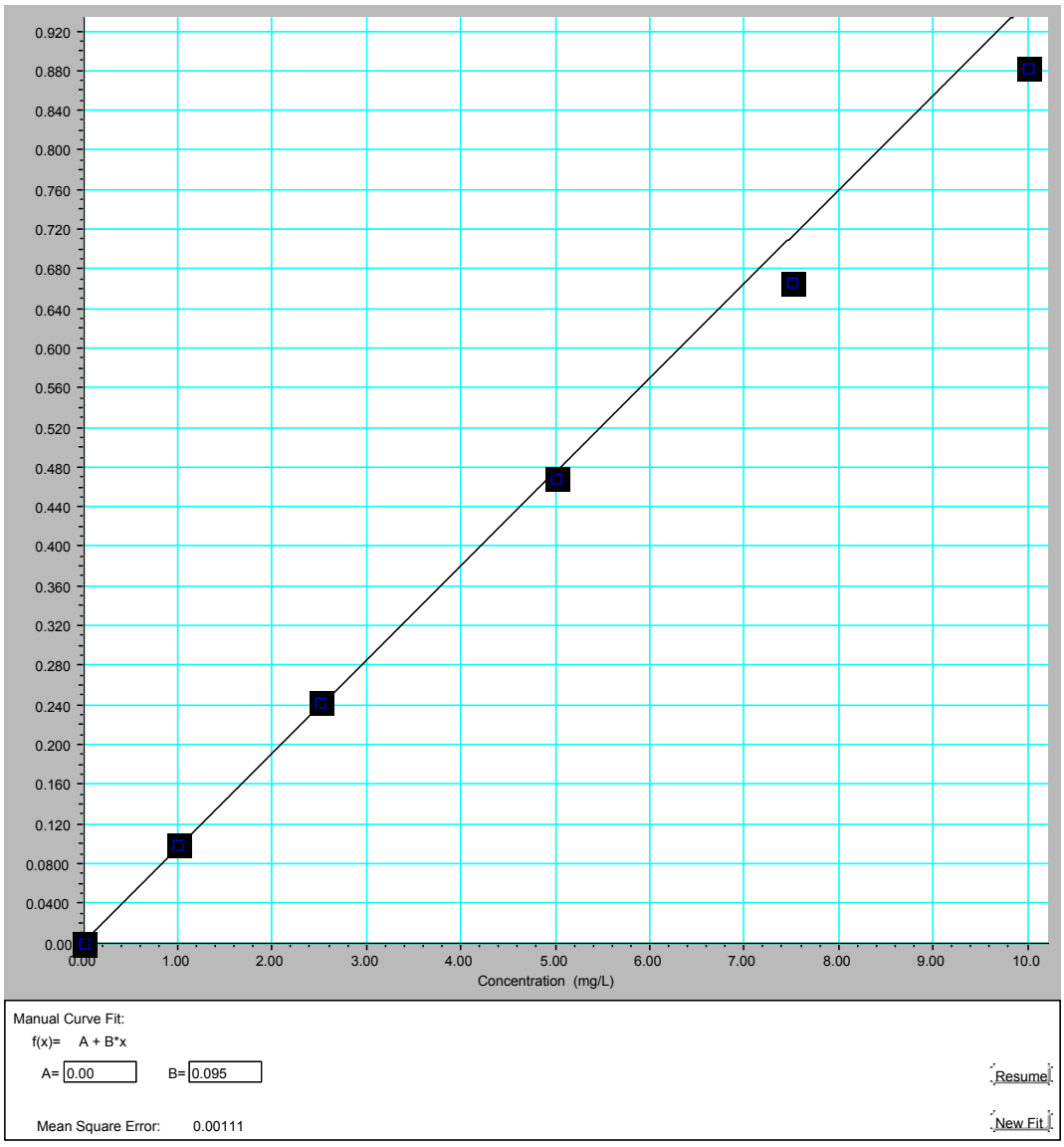

Figure 8 Standard curve for magnesium content determination (Y= 0.095X)

E. Statistical analysis

Results were expressed as mean ± the standard deviation of the triplicate experiment results. Data sets were evaluated by using the one-way analysis of variance (ANOVA) or Error Bar graphic analysis. Mean separation was done by using the Fisher Least Significance Difference (LSD). Pearson correlation was used to evaluation the relationship of canning quality and physiochemical properties.

CHAPTER IV

RESULT AND DISCUSSION

The raw data are presented in appendix C for easy comprehension.

Percentage of splits

Percentage of splits in canned kidney beans is defined as the number of beans with split seed coat per 100 canned beans. The ANOVA data indicated that highly significant statistical differences ($P \leq 0.001$) in percent splits existed among the cultivars (Table 9, 10). The mean value of split in percentage from different canned kidney bean cultivars is shown in Table 11.Canned products of cultivar (cv.) 85 had significantly fewer splits than the other two cultivars, while canned cv. Nickols had the highest number of seed coat splits per can. This indicated that the seed coat of cv. 85 had a higher ability to maintain its integrity during the canning process. Compared to the other two cultivars, the seed coat of cv. Nickols was most susceptible to thermal process as indicated by the number of seed coat splits.

Table 9

Analysis of variance for the mean of the percentage of splitting among the different cultivars

	Sum of Squares	df	Mean Square	F	Significance (P value)
Between groups	9.87	2	4.93	898.66	0.000*
Within groups	1.29	6	0.214		

Note: * indicates significance at 0.001 level.

Table 10

Analysis of the mean difference of the percentage of splitting among the different cultivars

					95% Confidence Interval	
Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	Lower Bound	Upper Bound
453	85	1.88*		0.002	0.96	2.81
Nickols	85	8.43*	0.378	0.000	7.51	9.36
Nickols	453	6.55*		0.000	5.62	7.47

Note: * indicates significance at 0.01 level.

Table 11

The percentage of seed coat splits in the different canned cultivars

Cultivar	85	453	Nickols
Percent splits (splits/per 100 seeds)	2.79 ± 0.22^{c}	4.67 ± 0.38^{b}	11.22 ± 0.67^{a}

Note: Samples were expressed in mean ± standard deviation.

Triplicates were conducted for each cultivar.

Means followed by different superscripts indicate significant differences ($P \leq 0.01$).

A study on the correlation between physiochemical properties of kidney bean seed coat and splits could provide important information on the influence of compositional or structural differences on maintaining seed coat integrity during the thermal processing. It may also suggest possible parameters associated with the seed coat integrity during canning and rapid identification methods for those parameters.

Ratio of seed coat to whole seed

Research has revealed a possible correlation between seed coat weight and canning quality (Heil, McCarthy & Özilgen, 1992b). The seed coat to seed weight ratio can be used to predict the thickness and heaviness of the seed coat. A highly significant difference ($P \leq 0.001$) was observed between the three different cultivars, when the data was compared by one-way ANOVA (Table 12). Cv. 453 had a significantly higher seed coat ratio than the other cultivars, while cv. 85 had a significantly lower number than the

others (Table 13). Based on dried weight, the seed coat to whole bean weight ratio was shown in Table 14.

Table 12

Analysis of variance for the mean of the ratio of seed coat to whole seed among the different cultivars

	Sum of Squares	df	Mean Square	F	Significance (P value)
Between groups	8.94×10^{-5}	2	4.47×10^{-5}	297.87	0.000*
Within groups	9.00×10^{-7}	6	1.50×10^{-7}		

Note: * indicates significance at 0.001 level.

Table 13

Analysis of the mean difference of the ratio of seed coat to whole seed among the different cultivars

Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
453	85	$7.40\times10^{-3*}$		0.000	6.63 $\times10^{-3}$	8.17 $\times10^{-3}$
453	Nickols	$5.60\times10^{-3*}$	3.16×10^{-4}	0.000	4.83 $\times10^{-3}$	6.37 $\times10^{-3}$
Nickols	85	$1.80\times10^{-3*}$		0.001	1.03 $\times10^{-3}$	2.57 $\times10^{-3}$

Note: * indicates significance at 0.001 level.

Table 14

The ratio of seed coat to whole seed from the different dried cultivars

Cultivar	85	453	Nickols
Ratio	0.0912 ± 0.0004^{c}	0.0987 ± 0.0004^{a}	0.0931 ± 0.0004^{b}

Note: Samples were expressed in mean ± standard deviation. The values were based on dried weight.

Triplicates were conducted for each cultivar.

Means followed by different superscripts indicate significantly differences ($P \leq 0.001$).

No significant correlation between bean splits and the seed coat to whole seed weight ratio was found in this study (Table 15). However, when measuring the ratio of seed coat dry weight to bean volume, Heil's et al. (1992b) discovered that the proportion of broken kidney beans increased dramatically when the weight of seed coat per unit volume was less than 10 g/mL. When this ratio is above 10 g/mL, the number of beans with damaged seed coat remained relatively consistent. Heil's finding suggested that the relationship of the seed coat weight to the bean size might play an important role predicting breakage of the seed coat during canning processing.

Table 15

Pearson's correlation between bean properties and seed coat ratio

	r value	Significance of r
Bean splits vs. seed coat ratio	- 0.073	0.851
Magnesium content vs. seed coat ratio	0.889*	0.001
Total protein content vs. seed coat ratio	0.938*	0.000

Note: * indicates significance at 0.001 level.

In this study, highly significant correlations were found between the seed coat to whole seed weight ratio and magnesium content, as well as total protein content (Table 15). The correlation coefficients (r) of ratio versus magnesium and total protein content were 0.89 ($P \leq 0.001$) and 0.94 ($P \leq 0.001$) respectively, indicating a very good agreement. This result strongly suggested that the higher the magnesium and total protein content, the higher the seed coat to whole seed weight ratio became. This outcome has suggested the possibility of using the seed coat to whole seed weight ratio as a predictor of magnesium and total protein content in the seed coat of raw beans.

Weight per bean

Kidney bean breeders and canners often indicate that the seed coats of larger seeds tend to split more often than the seed coat of smaller seeds during the canning process. In this study, the weight per bean of the three different cultivars was: 0.423 ±

0.007 (cv. 85), 0.417 ± 0.003 (cv. 453), 0.454 ± 0.005 (cv. Nickols) respectively (Figure 9). A highly significant difference ($P \leq 0.001$) was observed among the three cultivars (Table 16). Error Bar graphic analysis showed that cv. Nicokls was significantly heavier and larger than the other two cultivars. Statistically cv. 85 and cv. 453 did not differ in their seed weight.

Table 16

Analysis of the difference in the weight per bean among the different cultivars

	Sum of Squares	df	Mean Square	F	Significance (P value)
Between groups	2.41×10^{-3}	2	1.21×10^{-3}	51.43	0.000^{*}
Within groups	1.41×10^{-4}	6	2.34×10^{-5}		

Note: * indicates significance at 0.001 level.

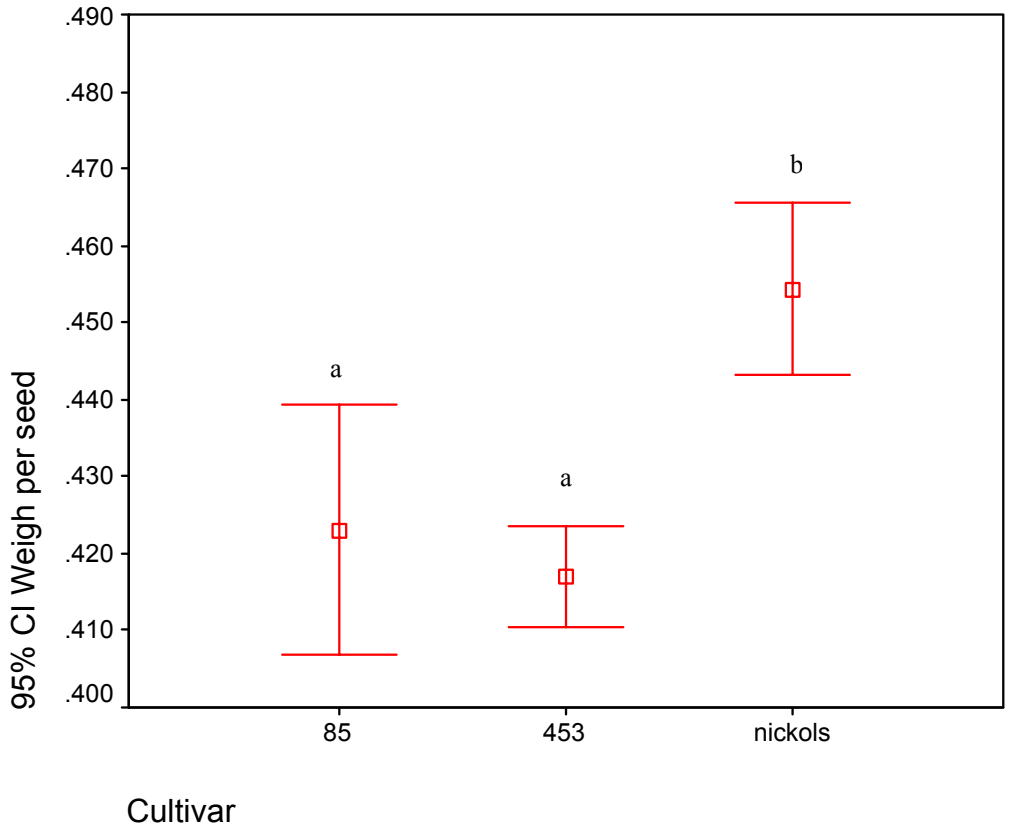

Figure 9 The weight per bean of the three different cultivars

Note: Means followed by different superscripts indicate significantly differences (P≤0.001).

This study found a strong correlation between weight per seed and the number of bean splits after canning (r = 0.901, P≤ 0.001). This inferred that the size of the seed affected the expansion of cotyledon during seed hydration. The seed coat is a natural barrier to this seed expansion. The inability of the seed coat to accommodate the fully expanded cotyledon, especially in the case of larger seeds, is somehow associated with the number of seed coat splits. This finding was in agreement with Heil's et al. (1992b) study on the parameters for predicting canned kidney beans quality. Seed coat rigidity

and how it functions as a barrier during seed germination have been widely studied (Yeung & Cavey, 1990; Kelly, Van Staden &Bell, 1992). The expansion of cotyledon seemed to be controlled by the seed coat. During seed hydration, rapid expansion of the bigger cotyledon results in a rapid stretching of the seed coat, which is caused by a higher pressure against the inner wall of the seed coat.

Moisture content

The moisture content of the dried beans varied among the three different cultivars. Cv. 85 contained 11.92 ± 0.05 % moisture; cv 453 consisted of 9.51 ± 0.01% moisture. The moisture content in cv. Nickols was 9.94 ± 0.12%. At the 95% confidence level, a significant difference ($P \leq 0.001$) could be observed in the moisture content among the three cultivars (Figure 10, Table 17). However, no statistically significant correlation was found between the moisture content and percent of bean splits after thermal processing.

Table 17

Analysis of the difference of the moisture content among the different cultivars

	Sum of Squares	Df	Mean Square	F	Significance (P value)
Between groups	9.86	2	4.95	836.94	0.000*
Within groups	3.55×10^{-2}	6	5.91×10^{-3}		

Note: * indicates significance at 0.001 level.

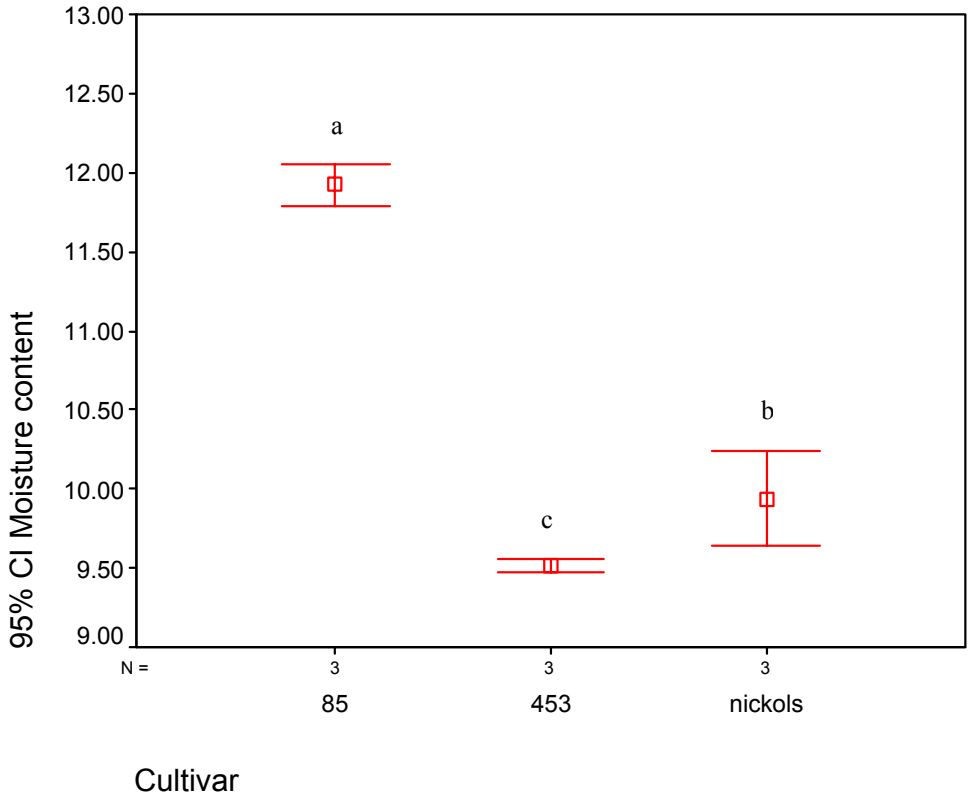

Figure 10 Moisture content of different cultivars at 95% confidence interval.

Note: Means followed by different superscripts indicate significantly differences (P≤0.001).

Ash content and mineral profile in the seed coat

This study showed that both calcium and potassium were the major minerals present in the seed coat of kidney beans. This was followed by magnesium, sodium and iron in a descending sequence (Table 18 & 19). Compared to the other studied minerals, calcium was found to comprise the highest content in seed coat.

Table 18

The ash and mineral content of seed coat in different cultivars based on dried weight (per 100 g seed coat)

Cultivar	Ash (g)	Calcium (mg)	Potassium (mg)	Magnesium (mg)	Sodium (mg)	Iron (mg)
85	2.71 ± 0.08^{a}	793 ± 34^{a}	590 ± 18	274 ± 11^{b}	13.87 ± 1.16^{a}	7.43 ± 0.74^{b}
453	2.47 ± 0.09^{b}	626 ± 57^{b}	526 ± 29	359 ± 32^{a}	11.90 ± 0.66^{b}	7.43 ± 0.99^{b}
Nickols	2.46 ± 0.02^{b}	618 ± 41^{b}	535 ± 32	269 ± 15^{b}	9.36 ± 0.81^{c}	5.54 ± 0.55^{a}

Note: Samples were expressed in mean ± standard deviation.

Triplicates were conducted for each cultivar.

For one set of mineral, means followed by different superscripts were significantly different ($P \leq 0.05$).

Table 19

The percent of minerals in the ash among the different cultivars (per 100 g ash)

Cultivar	Calcium (g)	Potassium (g)	Magnesium (g)	Sodium (g)	Iron (g)
85	29.25 ± 1.10	21.77 ± 0.64	10.14 ± 0.46[b]	0.513 ± 0.059[a]	0.274 ± 0.029
453	25.51 ± 3.29	21.32 ± 0.62	14.64 ± 1.91[a]	0.483 ± 0.029[a]	0.301 ± 0.038[a]
Nickols	25.11 ± 1.81	21.73 ± 1.24	10.94 ± 0.66[b]	0.380 ± 0.035[b]	0.225 ± 0.023[b]

Note: Means followed by different superscripts indicate significantly differences (P≤0.05).

Significant differences were found in the seed coat ash and some mineral contents among various cultivars; an exception was noted in the potassium content (Table 20). Mean separation (Table 21-23) showed that the seed coat of cv. 85 contained significantly higher ash content, calcium content and sodium content than the other two cultivars. Cv. 453 and cv. Nickols did not differ significantly in ash and calcium content. However, cv. 85 and cv. 453 were also significantly higher in sodium content than cv. Nickols.

The seed coat of cv.453 was significantly higher in magnesium content than either cv. 85 or cv. Nickols. Mean difference (Table 24) in magnesium content was not significant between cv.85 and cv. Nickols. The seed coat of cv. Nickols was found to have a significantly lower level of iron than the other two cultivars (Table 25). Cv. 85 and cv. 453 showed no significant difference in seed coat iron content. No significant correlation was found between bean splits and ash content, but a strong positive correlation was found between ash, potassium and calcium content (Table 26).

Table 20

Analysis of the ash and mineral content among the different cultivars

	Sum of Squares	df	Mean Square	F	Significance (P value)
Ash content					
Between groups	0.123	2	6.15×10^{-2}	12.65	0.007^{**}
Within groups	2.91×10^{-2}	6	4.86×10^{-3}		
Calcium content					
Between groups	5.84×10^{4}	2	2.92×10^{4}	14.36	0.005^{**}
Within groups	1.22×10^{4}	6	2.03×10^{3}		
Potassium content					
Between groups	7.30×10^{3}	2	3.65×10^{3}	5.03	0.052
Within groups	4.36×10^{3}	6	7.26×10^{2}		
Magnesium content					
Between groups	1.54×10^{4}	2	7.71×10^{3}	16.46	0.004^{**}
Within groups	2.81×10^{3}	6	4.68×10^{2}		
Sodium content					
Between groups	30.58	2	15.29	18.82	0.003^{**}
Within groups	4.87	6	0.812		
Iron content					
Between groups	7.16	2	3.58	5.89	0.038^{*}
Within groups	3.64	6	0.607		

Note: * indicates significance at 0.05 level; ** indicates significance at 0.01 level.

Table 21

Analysis of the mean difference of ash content in the seed coat among the different cultivars

Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
85	453	0.24*	5.69×10^{-2}	0.005	0.11	0.39
85	Nickols	0.25*		0.005	0.11	0.39
453	Nickols	0.01*		0.973	-0.14	0.14

Note: * indicates significance at 0.01 level.

Table 22

Analysis of the mean difference of calcium content in the seed coat among the different cultivars

Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
85	453	167*		0.004	77	257
85	Nickols	175*	36.8	0.003	85	265
453	Nickols	8		0.835	-82	98

Note: * indicates significance at 0.01 level.

Table 23

Analysis of the mean difference of sodium content in the seed coat among the different cultivars

Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
85	453	1.97*		0.037	0.17	3.77
85	Nickols	4.51**	0.736	0.001	2.70	6.30
453	Nickols	2.54*		0.014	0.74	4.34

Note: * indicates significance at 0.05 level; ** indicates significance at 0.001 level.

Table 24

Analysis of the mean difference of iron content in the seed coat among the different cultivars

Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
85	453	0.00		0.996	-1.55	1.56
85	Nickols	1.89*	0.636	0.025	0.33	3.45
453	Nickols	1.89*		0.025	0.33	3.45

Note: * indicates significance at 0.05 level.

Table 25

Analysis of the mean difference of magnesium content in the seed coat among the different cultivars

Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
453	85	85*		0.003	42	128
453	Nickols	90*	17.7	0.002	47	133
85	Nickols	5		0.773	-49	38

Note: * indicates significance at 0.01 level.

Table 26

Pearson's correlation between bean splits and mineral content studied

	r value	Significance of r
Bean splits vs. ash content	- 0.584	0.099
Bean splits vs. calcium content	- 0.668	0.049*
Bean splits vs. sodium content	- 0.891	0.001**
Bean splits vs. iron content	- 0.794	0.011*
Bean splits vs. magnesium content	- 0.347	0.361
Bean splits vs. potassium content	- 0.400	0.287

Note: * indicates significance at 0.05 level; ** indicates significance at 0.01 level.

Calcium

Calcium accounts for 20-30% of the total ash content in the seed coat. In this study, calcium was found to vary significantly among different cultivars (Table 19). Calcium content in the seed coat of cv. 85 was the highest (793 ± 34 mg/100g seed coat) among the three different cultivars. The smallest number of seed coat splits was found in canned kidney beans from cv. 85 (2.79 ± 0.22 splits/100 seeds), while canned cv. Nickols contained the highest number of splits and lowest amount of calcium in seed coat. Pearson's correlation showed that the calcium content was significantly correlated with beans splits ($r = -0.74$, $P \leq 0.05$), (Table 20). This indicated that higher calcium content in seed coat might result in fewer seed coat splits during thermal processing.

In the legume family, 30-50% of the total calcium is contributed to the seed coat (Deshpande & Damodaran, 1990). Calcium is particularly abundant in the plant cell wall. A study on horse beans (*Vicia faba minor* L.) showed that 60% of the total calcium was associated with the cell wall. The presence of calcium ions was believed to greatly increase the stability and cohesion of cell walls due to its formation of insoluble complexes with pectin (Demarty, Morvan & Thellier, 1984). The cell wall is composed of proximately 60% water and 40% polymers. Pectins make up 20-35% of the total polymers and are present in a high concentration at 8-14 % (w/w) in cell wall (Van Buren, 1991). In the cell wall, the cellulose microfibrils are imbedded in a gel-like amorphous structure consisting of a network of pectin and hemicellulose along with proteins (Bidwell, 1974). The outer part of the cell wall is connected by the middle lamella, which has a gel-like structure serves as intercellular bridges between cells. Pectin

predominates in the middle lamella with a concentration varying between 10-30%. The chemical cross-linking between calcium ions and pectin creates a network structure and additional constraints, which results in strong cohesiveness in the structure (Van Buren, 1991).

Pectins are polymers of polygalacturonic acids (Kertesz, 1951), which are composed mainly of 1,4 linked α-D- galacturonopyranosyl units. Pectin can be categorized into three classes: Homogalacturonan (HGA), Rhamnogalaturonan I (RGI), and Rhamnogalaturonan II (RGII). The HGA is comprised of a 1,4-linked α-D-galacturonic acid backbone, with few side chains. RGI has a highly branched backbone of alternating rhamnose and galacturonic acid residues. RGII consists of a 1,4-linked α-D- galacturonic acid backbone, with complex sugar side chains. RGII concentration is low in cell walls (Steele, McCann & Roberts, 1997; McCann, et al., 2001). In a study of genotype and environmental variation in soybean cell wall polysaccharides (Stombaugh, Jung, Orf & Somers, 2000), the author found that variation in cell wall pectin constituents was more prevalent in the cotyledon than in the seed coat. Pectin content and total cell wall polysaccharides content in seed coat was not significantly different among five studied cultivars of soybeans (*Glycine max* L.), (Stombaugh, Jung, Orf & Somers, 2000). This suggested the importance of other components in the structural maintenance of the cell wall.

Calcium can bind with unesterified pectins by forming a cross bridge between the negatively charged carboxyl groups of the galacturonic acid (Steele, McCann & Roberts,

1997), (Figure 11). The dimerization of homopolymeric chain sequences is the first event in the induction of interchain association by calcium. In this mechanism, calcium ions are sandwiched between pectin monomers at specific sites by electrostatic interactions with the carboxyl groups of the pectin (Demarty, Morvan & Thellier, 1984).

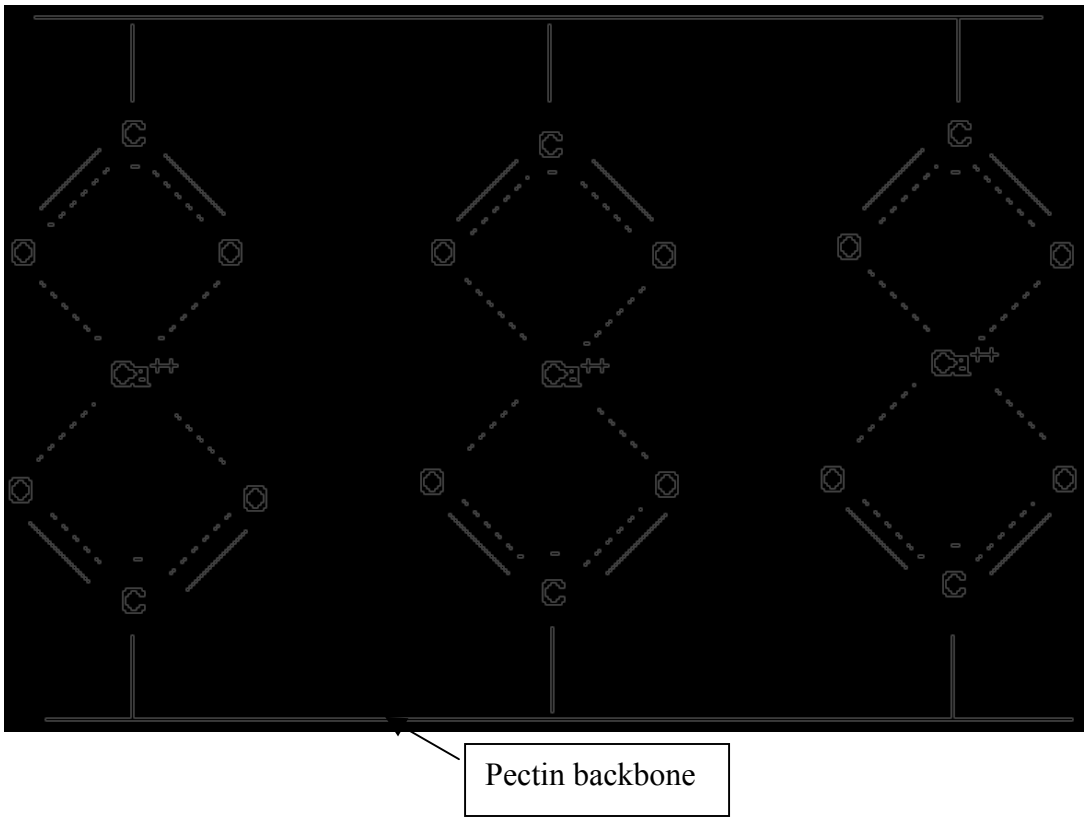

Figure 11 The network structure of calcium and pectin.

Source: Whistler, R.L. & Daniel, J.R. (1985). Carbonhydrates. In O. R. Fennema (Eds.), Food chemistry (pp. 125). New York, New York: Marcel Dekker, Inc.

Calcium linkages with pectin involve many other functional groups besides the carboxyl groups. Strong interaction between calcium and other oxygen atoms on pectin has been reported. The effectiveness of the complexes between calcium and carbohydrate is due to the atomic properties of the calcium ion. The radius of the calcium ion is 0.1nm,

which makes it large enough to coordinate with oxygen atoms in carbohydrate. The coordination bonds in the complex involve sharing the unfilled orbital of the calcium ion (Van Buren, 1991). Calcium is also linked with diverse polysaccharides at hydroxylic groups. These cross bridges can connect the pectin components to one another or to other polysaccharides. Calcium is well fixed into the cell walls. In the studies of plant cell growth, calcium is identified as a major factor in preventing cell wall weakening and subsequent cell extension, due to its large affinity for uronic groups on the pectin molecules. This is considered to result in the displacement of cell wall protons, and hence modifying the local pH, which may interfere the enzymatic activity on cell enlargement (Demarty, Morvan & Thellier, 1984).

Pectin may be initially produced in a highly esterified form in the cell. Deesterification of pectin often occurs when it is inserted into the cell walls or middle lamella. The degree of esterification (DE) of the uronide carboxyl groups with methyl alcohol is an important indicator in the degree of cell cohesiveness and tissue firmness of the beans. The reduction of DE accompanies the improvement in cohesion. Demethylation results in an increased number of free carboxyl groups in pectin, which offers greater opportunities for calcium to bind with pectin polymers. Pectin methylesterase (PME) carries out the demethylation in the tissues. The optimal working temperature for this enzyme is in the range of 50 to 80°C. The temperature for blanching often falls into or overlaps with the optimal working temperatures. As the tissue is heated, the susceptibility of pectin depolymerization decreases. Studies have shown that the dry weight of calcium in cell wall increased 100% when potato tissues were preheated at

70°C rather than at 27°C. The DE value also dropped about 100% as the firmness of the tissue increased (Van Buren, 1991).

Another form of calcium deposition in the seed coat is the presence of calcium oxalate crystals. Calcium oxalate crystals in plants are often euhedral, which means that the crystals have well-formed faces. A sheathing material surrounding the crystals has been identified as cellulose, cellulose-lignin or cellulose-pectin. In the bean seed coat, calcium oxalate monohydrate has been found present in abundance. This monohydrate is stable in water. Total erosion of crystals when exposed to water may take several days, while the sheath may remain even after the entire dissolution of the crystal. The growth of crystals is generally considered to be controlled genetically. The production of specific crystals and their location can vary considerably among cultivars (Grimson, Arnott & Webb, 1982). The crystal growth in the seed coat starts in the parenchyma cells near the hilum, and finished in hypodermal cells. The growth is accompanied by thickening of the cell wall. The exact biological role of calcium oxalate crystals in plants is still unclear, although the possible functions include regulating intracellular pH balance, and protecting the embryo from microbial invasion. In addition, crystal growth serves as building bricks, which parallels with cell wall thickening. This enhances the protective role of the seed coat (Barnabas & Arnott, 1990).

Commercially $CaCl_2$ is often used to reduce seed coat splitting and the consequent matting in canned beans. Studies have shown that the addition of $CaCl_2$ during the canning process improves seed coat integrity and reduces problems in appearance. Lopez

and Williams (1988) found that by adding calcium chloride in the cooking media, the calcium of canned beans increased. The addition of calcium might play an important role on the network formation with pectin during the process.

Sodium

The seed coat contains a very small amount of sodium, as shown in Table 18 & 19. Sodium content in the seed coat accounts for only 0.30 – 0.50% of the total ash content. Statistically, however, highly significant differences ($P \leq 0.01$) were found in the sodium content of the seed coats among the three cultivars (Table 20). Cv. Nickols had significantly lower sodium than the other two cultivars. Cv. 85 had significantly higher sodium content than cv. 453. A strong negative correlation was found between sodium content and seed coat splits ($r = -0.89$, $P \leq 0.01$), (Table 26). This indicated that an increased sodium content presented in seed coat might reduce the possibility of seed coat splitting during the canning process. Interestingly, a positive correlation was found between sodium content and calcium content ($r = 0.69$, $P \leq 0.05$). Increased calcium content was accompanied with increased sodium content in the seed coat.

The function of sodium interaction with divalent ions on the stability of pectin polymerization is still unclear. Some studies have found that the presence of monovalent salt (such as NaCl) at high concentration, can remove calcium from the pectin-calcium network (Van Buren, 1986; Coimbra, Waldron, Delgadillo & Selvendran, 1996). However, other studies have found that the presence of 0.1 M NaCl reduces the critical concentration of calcium required for the occurrence of precipitation in the stable pectin solution (Axelos, Garnier, Renard & Thibault, 1996).

Iron

A significant difference ($P \leq 0.05$) in iron content of the seed coat was found among the three different cultivars (Table 20). Cv. Nickols had a significantly lower amount of iron in the seed coat than the other two cultivars. No significant difference was found in the mean difference of the seed coat iron contents of cv.85 and cv. 453 (Table 24). Iron content in the seed coats was found to be strongly correlated with the bean splits ($r = -0.79$, $P \leq 0.05$), (Table 26). This suggested that higher iron content might result in fewer seed coat splits during the canning process. A positive correlation between iron and sodium was also observed in the seed coat of the three cultivars ($r = 0.67$, $P \leq 0.05$). Increased iron content in the seed coat could be accompanied by an increase in sodium.

The mechanism of iron transportation in the bean seed is not fully understood. For peas (*Pisum sativum* L.), iron is transported to the seed coat where it is released and then absorbed by the embryo during the seed development. The form of the iron when released in seed coat has not yet been identified. When absorbed by the embryo, iron complexes with a high molecular weight protein, known as ferritin. As many as 2000 iron atoms can be stored in each ferritin molecule. However, ferritin has not been detected in the seed coats of the developing pea seeds, which suggested that iron might be adequately chelated in order to prevent oxidative stress in the seed coat (Marentes & Grusak, 1998).

Magnesium

Highly significant differences ($P \leq 0.01$) were found in the magnesium content of seed coat among the three different cultivars (Table 20). The seed coat of cv.453 had the

highest seed coat content. Magnesium content in the seed coat of cv.453 was significantly higher than the other two cultivars. Cv. 85 and cv. Nickols had no statistical difference in their means of magnesium content (Table 25). As a divalent ion, magnesium functions similar to calcium in pectin polymerization. In a study on the magnesium fertilization on potato firmness (Klein, Chandra & Mondy, 1982), the authors found that increased magnesium fertilization resulted in increased firmness of the potatoes. They suggested that the increased firmness was associated with the formation of metal bridges between pectins (Klein, Chandra & Mondy, 1982). However, no significant correlation was found between present seed coat splits and magnesium content in this research (Table 26). A study on soybeans found that magnesium could only weakly bound by the seed coat cell walls. Compared to calcium, the high mobility of magnesium in the seed coat during seed maturation leads to less accumulation of this mineral in cell walls (Laszlo, 1990). In another study, which examined the mineral loss in kidney bean during the canning process, the author found that the canned kidney beans were significantly lower in magnesium than the dry kidney beans. The reduction of magnesium content on a dry weight basis was about 37.7% (Lopez & Williams, 1988).

During the canning process, the softening of seed coat was found to be slower than the softening of cotyledon (Stanley, Wu & Plhak, 1989). The presence of certain more highly concentrated minerals (calcium, sodium, iron) in seed coat may well control the process of softening and maintain the integrity of the seed coat during the processing. Studies have revealed that the sensitivity of precipitation of pectin varied according to the divalent irons present. Decreased order sensitivity was found to be: Pb ≈ Cu >> Zn > Cd ≈ Ni > Ca (Axelos, Garnier, Renard & Thibault, 1996). In this study, only selected

mineral contents in the seed coat were investigated. It could be expected that interactions of other minerals with pectin might also influence the structural integrity during the thermal processing.

Crude protein content in the seed coat

The crude protein content in the seed coat of the three kidney bean cultivars varied. A significant difference in the crude protein content of the seed coats was found between the different cultivar groups (Table 27). The crude protein content in the seed coat of cv. 453 was significantly higher than cv. 85 and cv. Nickols (Table 28). And the protein content in the seed coat of cv.85 was significantly lower than cv. Nickols (Table 28). The means of the crude protein contents in the seed coat are shown in Table 29.

Table 27

Difference in the protein content in seed coat among the three different cultivars

	Sum of Squares	df	Mean Square	F	Significance (P value)
Between groups	0.658	2	0.329	25.66	0.001*
Within groups	7.69×10^{-2}	6	1.28×10^{-2}		

Note: * indicates significance at 0.01 level.

Table 28

Analysis of the mean difference of crude protein content in the seed coat among the different cultivars

Type of the cultivar (I)	Type of the cultivar (J)	Mean Difference (I-J)	Std. Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
453	85	0.66^{***}		0.000	0.43	0.88
85	Nickols	0.40^{**}	9.25×10^{-2}	0.005	0.17	0.63
453	Nickols	0.26^{*}		0.034	0.03	0.48

Note: * indicates significance at 0.05 level;

** indicates significance at 0.01 level;

** * indicates significance at 0.001 level.

Table 28

The crude protein content in the seed coat of the three different kidney bean cultivars

Cultivar	85	453	Nickols
Crude protein content (g/100g seed coat)	4.64 ± 0.14^{c}	5.30 ± 0.11^{a}	4.90 ± 0.08^{b}

Note: Samples were expressed in mean ± standard deviation. The values were based on dried weight.

Triplicates were conducted for each cultivar.

Means followed by different superscripts indicate significantly differences ($P<0.05$).

Some studies showed that the crude protein content might be related to the cookability of some edible dry beans (Youssef & Bushuk, 1984); in this research the crude protein content of the seed coat of different kidney bean cultivars was not found to be significantly correlated to the seed coat split during the canning process.

In this research, glycoprotein was not examined. It appears that glycoproteins are highly involved in the physical characteristics of the cell wall. Glycoprotein comprises about 10% of the dry weight of the primary cell wall. Extensins formed in the cell walls are a family of hydroxyproline-rich glycoprotein, which are involved in the extensibility of the cell walls; however, the deposition of extensins may strengthen the cell wall but limit extensibility (Raven, Evert & Eichhorn, 1999). Cooper, Chen & Varner (1984) found glycoprotein to be involved with the formation of a network with pectin in the cell wall. Biggs & Fry's study (1987) also found that the cross-linking between the cell wall glycoprotein extensin and isodityrosine could further enhance the rigidity of the cell wall.

Isodityrosine is a phenolic dimer of tyrosine, which links through a diphenyl ether bridge. This bridge acts as an inter-polypepetide cross-link between paired glycoprotein molecules. The tightening of the cell wall consequently provides less scope for wall expansion.

In further studies, more specific research needs to examine the glycoprotein and phenolic compounds in the seed coat. This data may reveal possible relationships between seed coat splitting and seed coat compositional properties.

CHAPTER V

SUMMARY AND CONCLUSIONS

Restating the objectives, this study was carried out to:

1) Investigate moisture content, ash content, crude protein content and mineral profile in the seed coat of different dark red kidney bean cultivars grown in the Wisconsin area.
2) Study the weight ratio of the seed coat to whole seed and the weight of per seed.
3) Evaluate the relationship of the seed coat to whole seed weight ratio, seed weight and nutritional contents to canning quality.
4) Investigate the influences of the physiochemical properties in the seed coat to canning quality among different cultivars.
5) Provide information on factors related to canning qualities for cultivar screening.

Three different cultivars (85, 453 and Nickols) grown in the Wisconsin area were selected for comparison of their physiochemical properties and the degree of seed coat split during the canning process. The physiochemical properties in this study included moisture content, the weight ratio of seed coat to whole seed, the weight per seed, ash, mineral (sodium, calcium, iron, potassium and magnesium) and total crude protein content in the seed coat. Atomic Absorption Spectrophotometry was used for

determination of mineral profiling. Kjeldahl method was used to assess the crude protein content.

The study showed significant differences in the ratio of seed coat to whole seed, the weight per seed, ash content, moisture content, sodium, calcium, iron and magnesium content, and total protein content between the three cultivars. Highly significant differences ($P<0.01$) were found in percentage of split seed coat among the three cultivars in the canned product. Canned cv. 85 had significantly fewer seed coat splits than the other two cultivars, while canned cv. Nickols contained the highest number of splits. Cv. 85 was significantly higher in moisture content, as well as ash, sodium, calcium and iron content in the seed coat. Cv. 453 and cv.85 had similar amounts of iron in their seed coats. The seed coat of cv. 453 was significantly higher in magnesium, and total crude protein. The seed coat to whole seed weight ratio for cv. 453 was also significantly higher than the other two cultivars. Cv. Nickols was found to be significantly higher in the weight per bean and seed coat splits following the canning process. However, the seed coat of this cultivar had significantly lower sodium, and iron content than the other two cultivars.

Some strong correlations between physiochemical properties and canning quality were observed. Significant negative correlations were found between the percentage of seed coat splits, sodium ($r = -0.89$, $p< 0.01$), calcium ($r = -0.74$, $P< 0.01$) and iron content ($r = -0.79$, $P< 0.05$). Calcium accounts for 20-30% of the total ash content in seed coat. The formation of a strong network between calcium and pectin may contribute to a rigorous seed coat structure, which consequently results in fewer seed coat splits during

thermal processing. The presence of sodium may influence the ratio of monvalent to divalent metal ions, which may influence the textural characteristics of the canned product. Interestingly, a positive correlation was found between sodium content and calcium content ($r = 0.69$, $P < 0.05$). Increased calcium content in the seed coat was accompanied with increased sodium content.

The storage form of iron in the seed coat is still unknown. Unlike what has been found in cotyledons, studies have shown no ferritin, a protein storing iron, present in the seed coat (Marentes & Grusak, 1998). A positive correlation between iron and sodium content was also shown in the seed coat of kidney beans ($r = 0.67$, $P<0.05$). Increased iron content in seed coat was accompanied by increased sodium content.

A significant positive relationship ($r = 0.901$, $P < 0.01$) was found between seed weight and the percentage of seed coat splits. The excessive pressure introduced to the seed coat due to the expansion of cotyledon during hydration may enhance the breakage of the seed coat.

No significant correlation was found between the percentage of seed coat splits, moisture, potassium, magnesium and total crude protein content.

In conclusion, this thesis project showed that some physiochemical factors within the seed coat might play an important role in the integrity of the seed coat during the thermal processing. These factors include weight per seed, sodium, calcium, and iron

content in the seed coat. However, other physiochemical characters of the seed coat, as yet not examined, may also play important roles in seed coat integrity.

Recommendations for future study

The ability of seed coat to expand and maintain its integrity spontaneously during thermal processing is a complex mechanism involving many different physical and chemical prospects. More information is needed to determine the fundamental causes of seed coat splitting during canning. Recommendations for future stduy might include the following:

1. Determination of mineral content in the seed coat in relationship to polyssacharide, phytic acid and phenolic acid, since the polymerization of the above molecules often has mineral involvement.

2. Examination of the microstructures of the seed coats, and their influences on the seed coat integrity during the canning process.

3. Examination of factors involved with the expansion ability of the seed coat during thermal process.

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APPENDIX A

Solutions for the determination of crude protein content

1. Preparation of 10 M sodium hydroxide solutions

Exactly 200.00 g of sodium hydroxide pellets were weighed and dissolved with 1.0 L distilled water in an ice water bath using constant agitation. The solution was then cooled to room temperature.

2. Preparation of 50 g/L boric acid solutions

Exactly 50.00 g of boric acid were weighed and dissolved with 1.0 L hot distilled water. The solution was then cooled to room temperature.

3. Preparation of methyl red – bromcresol green mixed indicator

Exactly 100 mg bromcresol green and 20 mg methyl red were and dissolved in 100 mL 95% ethylalcohol.

APPENDIX B

Stock solutions for the determination of mineral content

1. 500 mg/L Calcium stock solution

Exactly 1.249 g of oven-dried primary standard calcium bicarbonate was weighed and a 1.0 L volumetirc falsk with approximately 50 mL Milli-Q water was used to dissolve the chemical. Dropwise a minimum volume of 6 M hydrochloric acid (about 10 mL) was added to completely dissolve the chemical. The solution was further diluted to 1.0 L with Milli-Q water.

2. 500 mg/L Magnesium stock solution

Exactly 0.500 g of magnesium metal was weighed and placed into 50 mL Milli-Q water in a 1.0 L volumetric flask. Dropwise a minimum volume (about 10 mL) of 6 M hydrochloric acid was added to completely dissolve the metal. The solution was further diluted to 1.0 L with Milli-Q water.

3. 500mg/L Iron stock solution

Exactly 0.500 g of iron metal wire was weighed and placed into 150 mL 6 M hydrochloric acid. The metal was completely dissolved when left overnight. The solution was further diluted with Milli-Q water in a 1.0 L volumetric flask.

4. 1,000 mg/L potassium stock solution

Exactly 1.907 g of oven-dried primary standard potassium chloride was weighed and placed into a 1.0 L volumetric flask. The solution was further diluted to 1.0 L with Milli-Q water.

5. 1,000 mg/L sodium stock solution

Exactly 2.542 g of oven-dried primary standard sodium chloride was weighed and placed into a 1.0 L volumetric flask. The solution was further diluted to 1.0 L with Milli-Q water.

6. 10,000 mg/L lanthanum stock solution

Exactly 2.933 g of oven-dried primary standard lantham oxide was weighed and placed into a 250 mL volumetric flask. Approximately 15 mL Milli-Q water was added, and dropwise a minimum volume (about 10 mL) of 6 M hydrochloric acid was added to completely dissolve the solid. The solution was further diluted to 250 mL with Milli-Q water.

APPENDIX C

Raw Data

Table C1

Raw data for the percentage of seed coat splits in canned different cultivars

	85			453			Nickols		
Cultivar samples	1	2	3	1	2	3	1	2	3
Beans without splits (number/ can)	256	255	255	243	246	245	199	197	197
Beans with splits (number/ can)	7	8	7	12	11	13	26	23	26
Total beans (number/ can)	263	263	262	255	257	258	225	220	223
Percent splits (splits/per 100 seeds)	2.66	3.04	2.67	4.71	4.28	5.04	11.56	10.45	11.66

Note: Triplicates were conducted for each cultivar.

Table C2

Raw data for the ratio of seed coat to the whole seed for different cultivars (on dry weight basis)

	85			453			Nickols		
Cultivar samples	1	2	3	1	2	3	1	2	3
Wt of dried seed coat (g)	4.06	4.01	4.03	4.46	4.47	4.51	4.24	4.20	4.20
Wt of dried cotyledon (g)	40.32	40.17	40.05	40.92	40.88	41.03	41.10	40.88	41.09
Wt of dried whole seed (g)	44.38	44.18	44.08	45.38	45.35	45.54	45.34	45.08	45.29
Ratio	0.0915	0.0908	0.0915	0.0983	0.0986	0.0991	0.0934	0.0931	0.0927

Note: Triplicates were conducted for each cultivar.

Table C3

Raw data for the weight of per bean for different cultivars

	85			453			Nickols		
Cultivar samples	1	2	3	1	2	3	1	2	3
Wt of seeds (g)	50.35	50.17	50.07	50.16	50.13	50.32	50.35	49.99	50.35
Numbers of seeds (g)	121	117	118	120	121	120	112	109	111
Wt of per bean (g)	0.416	0.429	0.424	0.418	0.414	0.419	0.450	0.459	0.454

Note: Triplicates were conducted for each cultivar.

Table C4

Raw data for the moisture content for different cultivars

	85			453			Nickols		
Cultivar samples	1	2	3	1	2	3	1	2	3
Wt of harvested beans (g)	50.12	50.18	50.00	50.16	50.04	50.12	50.28	50.03	50.26
Wt of dried beans (g)	44.18	44.19	44.02	45.39	45.27	45.36	45.27	45.12	45.21
Moisture content (%)	11.86	11.94	11.96	9.51	9.53	9.50	9.96	9.81	10.05

Note: Triplicates were conducted for each cultivar.

Table C5

Raw data for the ash content for different cultivars

	85			453			Nickols		
	1	2	3	1	2	3	1	2	3
Wt of crucible (g)	0.9635	0.8316	0.8462	0.9857	0.941	0.8111	0.9807	0.9175	0.9794
Wt of dried seed coat (g)	3.0147	3.0188	3.0029	3.0105	3.0185	3.0093	3.0117	3.0134	3.0029
Wt of ash and crucible (g)	1.0426	0.9148	0.929	1.0614	1.0122	0.887	1.0547	0.9914	1.0539
Wt of ash (mg)	79.100	83.200	82.800	75.700	71.200	75.900	74.000	73.900	74.500
Ash content (%)	2.624	2.756	2.757	2.515	2.359	2.522	2.457	2.452	2.481

Note: Triplicates were conducted for each cultivar.

Table C6

Raw data for the absorbance of the sample solutions (100 mL) by different cathode lamps

	Dilution of	85			453			Nickols		
	sample	1	2	3	1	2	3	1	2	3
Sodium	1:1	0.170	0.149	0.145	0.126	0.132	0.140	0.115	0.100	0.098
Calcium	1:100	0.028	0.028	0.030	0.021	0.025	0.022	0.022	0.024	0.021
Iron	1:1	0.016	0.014	0.017	0.018	0.015	0.014	0.013	0.011	0.011
Potassium	1:100	0.021	0.021	0.022	0.020	0.018	0.019	0.020	0.018	0.020
Magnesium	1:100	0.078	0.076	0.082	0.097	0.114	0.098	0.080	0.079	0.072

Note: Triplicates were conducted for each cultivar.

Table C7

Raw data for the mineral content in the samples for different cultivars (mg)

	85			453			Nickols		
	1	2	3	1	2	3	1	2	3
Sodium	0.459	0.403	0.392	0.341	0.357	0.378	0.311	0.270	0.265
Calcium	23.333	23.333	25.000	17.500	20.833	18.333	18.333	20.000	17.500
Iron	0.229	0.200	0.243	0.257	0.214	0.200	0.186	0.157	0.157
Potassium	17.500	17.500	18.333	16.667	15.000	15.833	16.667	15.000	16.667
Magnesium	8.211	8.000	8.632	10.211	12.000	10.316	8.421	8.316	7.579

Note: Triplicates were conducted for each cultivar.

The mineral content was determined by the following equation:

Wt of mineral in the sample (mg) = Absorbance ÷ K × Dilution factor × 0.1

K is the slope of the standard curve. 0.1is the volume (L) in which the ash were diluted.

Table C8

Raw data for the mineral content per 100 g seed coat samples for different cultivars (mg/100g seed coat)

	85			453			Nickols		
	1	2	3	1	2	3	1	2	3
Sodium	15.241	13.340	13.050	11.312	11.819	12.574	10.320	8.969	8.820
Calcium	773.985	772.934	832.529	581.299	690.188	609.223	608.737	663.702	582.770
Iron	7.582	6.625	8.087	8.542	7.099	6.646	6.166	5.215	5.233
Potassium	580.489	579.701	610.521	553.618	496.936	526.147	553.397	497.777	555.019
Magnesium	272.350	265.006	287.441	339.164	397.548	342.797	279.611	275.960	252.388

Note: Triplicates were conducted for each cultivar.

The mineral content was determined by the following equation:

Wt (mg) of mineral per 100g seed coat = Wt of mineral in the samples (mg) ÷ Wt of seed coat (g) × 100

Table C9

Raw data for the percentage of mineral in ash for different cultivars (%)

	85			453			Nickols		
	1	2	3	1	2	3	1	2	3
Sodium	0.581	0.484	0.473	0.450	0.501	0.499	0.420	0.366	0.356
Calcium	29.499	28.045	30.193	23.118	29.260	24.155	24.775	27.064	23.490
Iron	0.289	0.240	0.293	0.340	0.301	0.264	0.251	0.213	0.211
Potassium	22.124	21.034	22.142	22.017	21.067	20.861	22.523	20.298	22.371
Magnesium	10.380	9.615	10.425	13.488	16.854	13.591	11.380	11.253	10.173

Note: Triplicates were conducted for each cultivar.

The percentage was determined by the following equation:

Percentage of mineral in ash (%) = Wt of mineral in the samples (mg) ÷ Wt of ash (mg) × 100%

Table C10

Raw data for the crude total protein content for different cultivars

	85			453			Nickols		
	1	2	3	1	2	3	1	2	3
Weight of dry seed coat (g)	1.0008	1.0006	1.0005	1.0005	1.0013	1.0010	1.0018	1.0009	1.0033
Begin Volume of HCl (mL)	0.00	5.70	5.70	6.25	12.25	11.00	1.76	17.32	0.01
End Volume of HCl (mL)	5.52	11.00	10.90	12.23	18.32	17.23	7.30	23.00	5.70
Total Volume of HCl (mL)	5.52	5.30	5.20	5.98	6.07	6.23	5.54	5.68	5.69
% Nitogen	0.77	0.74	0.72	0.83	0.84	0.87	0.77	0.79	0.79
% Protein	4.80	4.61	4.52	5.20	5.28	5.42	4.81	4.94	4.94

Note: Triplicates were conducted for each cultivar.