

EFFECTS OF PRETREATMENTS ON SEPARATING THE SEED COAT FROM THE
COTYLEDON OF BLACK BEAN

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Effects of pretreatments on separating the seed coat from the cotyledon of
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ABSTRACT

Separation of seed coat from the cotyledon could result in an additional black bean food ingredient. The objective of this study was to develop a standard milling procedure that can achieve optimum seed coat removal from black bean seed. Black beans were cooked in boiling water for 0, 5, 10, and 20 min or were tempered to 10, 20, 30, 40 and 50% moisture. Then all samples were dried to the original moisture content in ambient air or at 90°C. Pretreated black beans were milled using a burr mill and a roller mill. Seed coat was removed by aspiration. Seed coat yield was greater with tempered than with boiled seed dried at 90°C. The chemical and physical changes in the bean flours were less in tempered-dried pretreatment than with cooked-dried pretreatments. Higher seed coat separation with less changes in flour is important in food applications with health benefits.

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To Almighty God, thank you for blessing me with such a wonderful life.

DEDICATION

I would like to dedicate this thesis to my loving parents and wife, who are my strength and inspiration.

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INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are divided into the following eleven market classes that are grown in the United States (US): black beans, kidney beans (light red and dark red), navy beans, pinto beans, great northern beans, small red beans, pink beans, small white beans, cranberry beans, and yellow-eye beans. Pinto and navy beans are the two leading classes of dry beans produced in the US (USDA-ERS 2016a). Although dry beans of different market classes possess a similar seed structure, they vary widely among classes for color, size and shape (Siddiq and Uebersax 2013).

Globally, dry beans are important staple food in human nutrition, especially among the low-income groups of people in developing countries where protein energy malnutrition is often prevalent. Dry beans are a low cost source of protein and important source of carbohydrates, dietary fiber, certain minerals and vitamins in the human diet (Sathe 2002; Van Heerden and Schonfeldt 2004; Iqbal et al. 2006). In addition, secondary metabolites, such as phenolic compounds that possess antioxidant properties, are known to contribute to the health benefits of dry beans (Madhujith and Shahidi 2005).

Dry bean utilization by the food industry can be increased by developing value-added processing applications. The new trend of incorporating non-wheat flours into food products is driven by the increased demand for nutrient dense foods. Several studies have explored the utilization of dry beans in traditional products, such as bread, spaghetti, and snacks (Aguilera et al. 1982; Chillo et al. 2008; Han et al. 2010). Hence, the inclusion of bean flour in these products will improve their nutritional value.

Use of dry beans in processing and implementing research findings in commercial operations have been hindered by the presence of seed coat, anti-nutrients and beany flavor

(Siddiq and Uebersax 2013). Removal of these factors can be challenging and requires treatment methods such as cooking, soaking, tempering, fermentation and germination (Abd El-Hady and Habiba 2003; Martin-Cabrejas et al. 2004). Interestingly, seed coat removal has been reported to reduce cooking time and increase in *in vitro* protein digestibility (Kon et al. 1973; Deshpande et al. 1982).

In general, dehulling or the seed coat removal is a very difficult process due to strong attachment of seed coat to the cotyledons. Dehulling produces refined cotyledons with good appearance, texture, and cooking qualities (UDPLC 2010). Separation of seed coat from the cotyledon can expand the use of bean flour in food product applications by creating different food ingredients such as cotyledon flour and seed coat flour. Other than the economic advantages, seed coat removal will result in improving the cotyledon flour digestibility and palatability as the content of some of the antinutrients, such as tannins, would be reduced by removing the seed coat (Towo et al. 2003).

Cooking and tempering as pretreatments to aid in removal of the seed coat was the focus of the reported research. Dry bean seeds cooked in boiling water increased the seed plasticity and water absorption (Abu-Ghannam and McKenna 1997). Cooking dry beans improves the texture and inactivates undesirable enzymes such as protease inhibitors (Siddiq and Uebersax 2013). Starch gelatinization, swelling, leaching of amylose and loss of crystalline structure occurs during cooking (Ovando-Martinez et al. 2011). Even though some antinutrients are somewhat heat resistant (i.e. phytate), cooking reduces the majority of antinutrients to acceptable levels and can improve organoleptic quality of dry beans (Rehman et al. 2004).

Tempering brings the seed to a desired moisture content before milling. Relatively few studies have been published concerning tempering of dry beans. Tempering dry beans can result in smaller nutritional losses when compared to cooking.

Carter (2014) used a burr mill in the pre-break step in milling black beans. A burr mill is a device that consists of a set of burrs or raised edges that use cutting, shearing, and crushing action for particle size reduction (Haque 1991). A burr mill has two roughened chilled cast iron plates that rub together, one plate is stationary and the other one rotates on a shaft with operation speed usually less than 1,200 rpm. Grain fed between the plates is crushed and sheared. The fineness of ground product is controlled by the size and quantity of burrs on the plate and the clearance between the two plates. The efficiency of a burr mill depends on the moisture content of the food-materials as well as the mechanical strength of its constituent composition (Shakiru and Babasola 2014).

After the pre-break step with the burr mill, the bean pieces can be further milled on a roller mill. The roller mill has the potential of further mechanically removing any remaining seed coat from cotyledon. Each roll in a pair can rotate at different speed (Posner and Hibbs 2005). Roll speed differential results in a shearing action that aids in removing the seed coat from the cotyledon. A roller mill uses multiple stage approach to achieve desired size reduction.

Dry bean milling has attracted interest due to the increasing need of non-wheat food ingredients available for food applications (Siddiq and Uebersax 2013). Black beans were selected for this research since the dark seed coat offered a stark contrast against the cream color of the cotyledon, which aided in the assessment of efficiency of removing the seed coat. Any articles were not found in the literature search that reported on physical separation of seed coat from cotyledon during milling of black bean seeds nor on the effect of pretreatments on the

physical quality, chemical composition, and pasting properties of black bean flour. The effect of cooking and tempering pretreatments in combination with drying on burr mill/roller mill system and on seed and flour physical and chemical composition was the focus of this research.

LITERATURE REVIEW

Background

After cereals, the *Fabaceae* family (which includes legumes) is the second largest source of human food and feed for livestock (Graham and Vance 2003; Berrios 2006). *Fabaceae* represents one of the most diverse families of plants that are grown in different parts of the world (Deshpande 1992). More than 7,000 years ago, dry beans were domesticated in tropical and subtropical areas of Central and South America (Kaplan 1965). Today, dry beans (*Phaseolus vulgaris* L.) are the world's second most important legume class, after soybeans, and are one of the basic foods in Africa, India and Latin America (Xu and Chang 2008). The regions of highest dry bean consumption include all of Latin America, where legume consumption ranges from 1-25 kg per capita per year, and where dry beans dominate and account for 87% of the total legume products consumed (Leterme and Munoz 2002). Dry beans are not a staple in the US, and per capita consumption had been declining since the mid-1960s with a recent figure of 2.6 kg in 2014 (USDA-ERS 2016b).

The world production of dry beans was 26.5 million metric tons and Myanmar, India, Brazil, US and United Republic of Tanzania were the top five dry beans producing countries in 2014 (FAOSTAT 2016). North Dakota and Michigan states were the two leading dry bean producing states in 2014; together they represent about 45% of total US production with a 30.4% and 15.1% share, respectively (USDA-NASS 2016). Pinto bean (43%), navy bean (18%), black bean (17%), and great northern (11%) are the leading dry bean market classes in US (USDA-ERS 2016b). In US, total metric tons of black bean production were about 2.6 times more in 2014 compared to 1980 and it was the only bean class that has seen such a tremendous growth (USDA-ERS 2016a).

Dry beans are a good source of protein, dietary fiber, starch (Osorio Diaz et al. 2003), and minerals and vitamins (Kutos et al. 2002). From a nutritional perspective, dry beans are considered to be important in countries where protein energy malnutrition is prevalent (Van Heerden and Schonfeldt 2004). Dry beans contain 20-30% protein on dry weight basis, which is twice as much as found in cereal grains (7-14%) (Tosh and Yada 2010). Presence of fiber, bioactive proteins and secondary metabolites such as phenolic compounds that possess antioxidant properties, contribute to the health benefits associated with dry beans (Madhujith and Shahidi 2005). Some of these antioxidants, such as flavonoids, are heat resistant and could survive extreme processing conditions (Kon 1979). Several researchers have reported on potential health benefits of dry bean consumption including reduced risk of hyperglycemia (Anderson et al. 2009), diabetes (Sievenpiper et al. 2009), cardiovascular disease (Bazzano et al. 2001), obesity (Anderson et al. 2009) and cancer (McCann et al. 2010).

Morphology and Anatomy of Dry Bean Seed

All dry beans possess a similar seed structure, which includes a seed coat, cotyledon and embryonic axis. The structural features of seed tissues and the cellular and sub-cellular components greatly influence hydration, cooking and processing performance of the dry bean (Siddiq and Uebersax 2013). The outermost layer of the seed is the seed coat, which protects the embryonic structure. Phytic acid, tannins, and phenolic compounds are concentrated in the dry bean seed coat. These compounds exhibit antioxidant activities and protect the seed from oxidative damage (Adebooye and Singh 2007). The cotyledon serves as energy storage and it comprises the largest mass of the seed. The embryonic structure is relatively small, high in lipid and can have a dramatic influence on seed quality as optimum conditions can activate the

enzymes and lead to seed germination. Hilum and micropyle are two external anatomical features, which help in water absorption and seed gas exchange.

Seed Coat

The seed coat consists of approximately 8% of the total dry weight of the dry bean (Rahman 2007). The seed coat is mainly composed of cellulose and hemicellulose, which contribute to the high level of total dietary fiber found in the seed coat (Aguilera et al. 1982). Main function of the seed coat is to protect the cotyledon and embryo from microbial contamination and diseases especially during harvest and storage. The major components of the seed coat microstructure are; the waxy cuticle layer, palisade cell layer, the hour-glass cells, and the parenchyma layer (Ruengsakulrach 1990). The waxy cuticle layer is the outer most layer of the seed coat. Bukovac et al. (1981) reported that its primary function was to prevent water penetration through its hydrophobic layers, though the cuticle layer does allow some polar and non-polar molecules to permeate the seed coat. The palisade layer has been reported to have two layers of cells (Sefa-Dedeh and Stanley 1979a). Hourglass cell layer is immediately beneath the palisade layer, which Sefa-Dedeh and Stanley (1979a) reported it to be the second layer in palisade layer. The parenchyma cells have thick cell walls, which can be easily distinguish after hydration as the water imbibition rate increases and cells become spongy (Siddiq and Uebersax 2013).

Cotyledon

Cotyledon represents the largest portion of the black bean seed, accounting for 90% of the seed weight (Rahman 2007) and contributes to the texture and nutritive value of the bean. Dry bean cotyledons had about 39% starch, 28% protein, 2% lipids, 4% ash and 20-30% non-

starch polysaccharides (Powrie et al. 1960; Harvard 2015). Cotyledons of dry beans are botanically classified as a segment of the embryo (Siddiq and Uebersax 2013). During the seed maturation period, cotyledon works as a storage unit and upon germination, the seedling uses it as an energy source. Cotyledon is also a photosynthetic structure that is responsible for the embryonic leaf tissue. Zimmermann et al. (1967) demonstrated the partitioning of nutrients within the cotyledon, with higher levels of protein and trypsin inhibitors in the outer layers compared to inner layers. The epidermal layer is the outer most layer, which consists of outer cells that appear to be cubical and inner cells that are elongated. Siddiq and Uebersax (2013) surmised that the epidermal layer did not contain starch, as all cells appeared to contain granular like protein. The hypodermis, which consists of large elliptical cells, is the next apparent layer. The remaining parenchyma cells are bound by distinct cell walls and middle lamellae. They have thick walls that give rigidity to the cotyledon. The parenchyma cells in the cotyledon are packed with starch granules that are embedded in a matrix of storage proteins (Tiwari and Singh 2012). Mature parenchyma cells are very thick with the secondary wall and pits in the wall facilitate the water diffusion during soaking. The middle lamella is a pectin layer, which binds cells together (Siddiq and Uebersax 2013).

Embryo

Embryonic axis serves as a nutrient-absorbing organ for the embryo during germination. Raphe, micropyle and hilum work as entry points for water diffusion into the seeds during water imbibition by the seed coat (Tiwari and Singh 2012). Water permeability is greatest in the hilum or micropyle areas. The hilum is the scar left when the ovule separated from the funiculus (stalk) which had supported and attached the seed to the pod during development. The micropyle is the site of pollen tube entry during fertilization. Raphe is the ridge on seed coat formed by the

attachment of the funiculus to the seed coat (Helm et al. 1990). The embryo is living tissue, relatively small and represents only 2% or less of the seed weight (Rahman 2007). The embryo is rich in lipids, vitamins and enzymes required in growth and development (Siddiq and Uebersax 2013).

Optimum moisture and temperature conditions can activate the embryonic enzymes. The optimum temperature for most seeds is between 15 and 30° C and optimum moisture level is greater than 18% (Siddiq and Uebersax 2013). During storage, high temperature can damage the embryo with irreversible loss of seed vitality. Ambient water soaking conditions have been commonly recognized to initiate the precursors of the germination process. The water hydrating and water holding capacity of the dry bean are enhanced with traditional overnight soaking as it activates many embryonic enzymes that are utilized in seed sprouting.

Macro Nutrients

Carbohydrate

Carbohydrates are an important chemical component that accounts for 55-65% of dry bean weight (Siddiq and Uebersax 2013). The carbohydrate portion consists of starch and nonstarch polysaccharides with small amounts of oligosaccharides (Bravo et al. 1998).

Starch

Starch is the main nutrient in dry bean and accounts for approximately 60% of total carbohydrates present in the seed (Reddy et al. 1984). Raw dry bean starches appear to be smooth with an oval or elliptical shape (Gujska et al. 1994; Hoover and Ratnayake 2002). Starch is mainly composed of amylose and amylopectin. The total amylose in black beans can range from 27-39% (Hoover et al. 2010) and is higher than the amylose content \approx 20% in cereal grains

(Hu et al. 2010). Dry bean starches are known to develop greater viscosity than cereal starches (Lineback and Ke 1975).

The shape, size, and other morphological characteristics of starch granules vary depending primarily on factors such as the botanical source and environmental conditions under which the crop was grown (Wang 2013). Smaller granules gelatinized at higher temperature with lower gelatinization enthalpy than larger granules (Chiotelli and Le Meste 2002). Large granules are more likely to be damaged during milling than are small ones and the degree of starch damage will influence flour functionalities, such as water absorption and flour dough properties because damaged starch absorbs considerably more water than undamaged starch (Oh et al. 1985; Dexter et al. 1994).

Based on both *in vitro* and *in vivo* studies, the digestion rate of dry bean starches is slow and relatively incomplete compared to common cereal starches, but more digestible than potato or high amylose maize starch (Tovar et al. 1992; Madhusudhan and Tharanathan 1995; Hoover and Zhou 2003; Liu et al. 2006; Sandhu and Lim 2008). The slow digestion rate, in turn, makes beans a low glycemic food, compared to cereals (Jenkins et al. 1983). Starch structure, amylose and amylopectin ratio, presence of antinutrients that effect enzyme activity, and cell structure also can influence the low glycemic response of dry beans (Bjorck et al. 1994).

Non-Starch Polysaccharides

Dry beans contain complex polysaccharides such as celluloses, hemicelluloses, pectins and gums (Siddiq and Uebersax 2013). Less hemicellulose and more pectin are present in dicots than monocots (Caffall and Mohnen 2009). Dietary fiber (DF) represents non-starch polysaccharides. Significant amounts of dietary fiber present in dry beans have attracted food processors to incorporate dry bean into novel foods (Bressani 1993). In dry beans, dietary fiber

can range from 5-20% with a high portion being insoluble (Tiwari et al. 2011). The seed coat is comprised of cellulose, which was found to be more than 60%, hemicellulose (20%), and small amount of lignin (2%) (Srisuma et al. 1991). The pectin layer can be found in the plant primary cell wall and the middle lamellae. Non-starch polysaccharides in foods are known for their swelling capacity, water-holding capacity, oil-binding capacity, and cation exchange capacity.

Oligosaccharides

Dry beans contain various oligosaccharides, mainly raffinose and stachyose, which cannot be hydrolyzed within human digestive system (Shimelis and Rakshit 2007). Microflora in the colon, metabolize the oligosaccharides and result in intestinal discomfort and flatulence. Different processing methods can reduce the oligosaccharides in significant amounts (Barampama and Simard 1994; Matella et al. 2005; Kelkar et al. 2012). The loss of these oligosaccharides (like raffinose and stachyose) from the beans may increase the digestibility of the beans which leads to less gas formation since bacteria in human large intestine can break these oligosaccharides down into carbon dioxide and water (Roberfroid and slavin 2000). However, this loss of oligosaccharides from the beans will mean fewer oligosaccharides reaching the large intestine and serving as an energy-source for bacteria like Bifidobacteria or Lactobacilli that live there (Bornet and Brouns 2002). Since robust concentrations of these bacteria are usually helpful to human health, this loss of oligosaccharides may not be desirable from an intestinal health standpoint.

Protein

In dry beans, most of the protein is found in protein bodies located in cells of the cotyledon and embryonic axis (Van Der Poel 1990). The protein bodies of dry beans are in a cell matrix between the starch granules and individual protein bodies are small (1-10 μm) and

spherical to oval (Berrios and Swanson et al. 1998; Wood et al. 1998). Phaseolin and phytohemagglutinin are the main seed storage proteins of dry beans (Siddiq and Uebersax 2013). Phaseolin is a globulin and highly soluble in all pH values in salt solutions. Phytohemagglutinin has carbohydrate-binding specificity for a complex oligosaccharide containing galactose, N-acetylglucosamine, and mannose (Sharon and Lis 1989).

Even though dry beans contain high levels of protein, the protein contains low amounts of some essential amino acids and low true digestibility of protein due to antinutrients; thus, dry bean protein quality is considered low, compared to animal protein (Belitz et al. 2009). Dry beans are rich in amino acid lysine, but some amino acids like methionine, cysteine and tryptophan are present in low frequency (Deshpande and Nielsen 1987).

Dry bean proteins have gained increased importance in food systems because they are used to provide functional properties including gelling, emulsifying properties, fat binding and water holding (Tiwari et al. 2011). Proteins in bean flour restrict starch granule swelling and reduce the amylogram viscosity.

Lipids

Dry beans have low lipid content, typically ranging from 1.8-2.6% and includes triacylglycerides, free fatty acids, sterols, glycolipids and phospholipids (Drumm et al. 1990). Lipid content may vary depending upon origin, location, climate and other growing conditions (Worthington et al. 1972). The most important polyunsaturated fatty acids in black beans are linolenic (C_{18:3}) and linoleic (C_{18:2}) acids. Palmitic (C_{16:0}) and oleic (C_{18:1}) acids were detected in significant quantities in black beans (Sutivisedsak et al. 2010). Lipids in black beans are not a function of storage as in soybeans, which contain high fat content of ≈20% (Coelho and Benedito 2008). The lipid fraction contains the essential vitamins, E and K (Campos-Vega et al. 2010).

Micro Nutrients

Vitamins

Black beans have water soluble vitamins especially thiamine, niacin, riboflavin and folate that can mostly be found in the cotyledons (Siddiq and Uebersax 2013). Gregory and Kirk (1981) reported the presence of nondigestible polysaccharides and lignin may reduce the availability of Vitamin B for absorption.

Minerals

Minerals can be classified as macro-mineral nutrients and micro-mineral nutrients. Macro-mineral nutrients include: calcium, magnesium, phosphorus, potassium, and sodium. Micro-mineral nutrients include: copper, iron, manganese, selenium and zinc. Black beans are rich in minerals such as calcium, iron, copper, zinc, potassium, phosphorus and magnesium (Siddiq and Uebersax 2013). Dry beans contain low sodium levels and contain high amounts of iron, calcium, and zinc. Most of the phosphorus and iron are found in the cotyledons. Phosphorus in beans is present in the form of phytic acid. Storage iron in legumes is sequestered in ferritin, which is the major iron storage protein. However, 70–85% of the iron present in beans is in the form of non-ferritin-bound iron possibly bound to phytic acid (Petry et al. 2015). Calcium, copper, zinc, potassium, phosphorus and magnesium are found mainly in the seed coat (Deshpande and Damodaran 1990). The difference in mineral content is due to different soil types and fertilizers used to grow dry beans (Tiwari and Singh 2012).

Phytochemicals

Dry beans contain various bioactive phytochemical compounds that are referred to as “antinutritional factors” due to their adverse impact on nutrient bioavailability (Siddiq and Uebersax 2013). Black bean has phytochemicals such as enzyme inhibitors, lectins, phytates, and phenolic compounds. In the dry bean seed, enzyme inhibitors such as trypsin and chymotrypsin could affect protein digestibility. Proteins with high digestibility are desired owing to their positive contribution to nutritional value (Boye et al. 2010).

Phytic acid is a major storage form for phosphorus and inositol in dry bean seeds. Phenolic compounds such as phenolic acids and polyphenols such as tannins, flavonoids and anthocyanins are responsible for the seed coat pigmentation. Diaz et al. (2010) reported the relationship between tannins and seed coat color, where black beans with a dark purple color have more tannins than seed coats of white or light colored beans. Flavonoids are present in the seed coat, whereas non-flavonoid compounds are present in the cotyledons.

These antinutritional factors collectively contribute to several health benefits on hyperglycemia, diabetes, cancer and cardiovascular disease, as well as some adverse effects on nutrition (Siddiq and Uebersax 2013). The inactivation of antinutritional factors is important to ensure nutrient absorption and proper contribution of health benefits by consuming beans (Uebersax 2006).

Black Bean Processing

Cooking

Dry bean seeds can be cooked in boiling water, which increases seed plasticity and water absorption (Abu-Ghannam and McKenna 1997). Cooking softens plant tissues, improves the texture and palatability of plant-based foods and helps to increase the access of digestive

enzymes to the starch and protein present inside the cell (Siddiq and Uebersax 2013). Aguilera and Stanley (1985) described cooking as a two-phase process. During the first phase, the middle lamella breaks down and the cells separate (Rockland and Jones, 1974). During the second phase, the predominant phenomenon is starch gelatinization. Starch gelatinization, swelling, leaching of amylose and the loss of the crystalline structure occur during cooking (Ovando-Martinez et al. 2011). Starch susceptibility to enzyme digestion can increase due to loss of the granular structure during phase transition by starch during processing operations and cooking. During thermal treatments, middle lamellae is degraded and pectin is broken down (Aguilera et al. 2009). The polysaccharide structure can be affected by the high temperature by breaking linkages and promoting de-polymerization (Ilker and Szczesniak 1990; Del Valle and Stanley 1995). Thermal treatments increase the water solubility by de-polymerization of polysaccharides and changes the nutritional properties of the dietary fiber (Brett and Waldron 1996).

Dry bean processing methods such as soaking and thermal treatments can reduce the protein content while increasing the digestibility (Rehman and Shah 2005). For proteins, the exact mechanism underlying the influence of heating on legume protein digestibility is still unclear and conflicting (Siddiq and Uebersax 2013). Starch gelatinization, protein denaturation, and swelling may further facilitate cell separation and the development of the uniform, smooth texture in fully cooked beans (Rockland and Jones, 1974).

Wang (2010) reported that cooking dry beans in boiling water increased the amount of available Mn and P (on a dry weight basis), but decreased K and Mg. In general, the ash content of dry beans was found to decrease after cooking due to diffusion of certain minerals into the cooking water (Wang et al. 2010). Vitamins of cooked dry beans' bioavailability and interaction with other food components are not well known.

Cooking inactivates Antinutritional Factors (ANF) such as protease inhibitors and lectins. Cooked beans with acceptable firmness are not necessarily free of ANFs (Wang et al. 1988). Even though some ANFs are somewhat heat resistant (i.e. phytic acids), cooking reduces the majority of ANFs to acceptable levels, and improves organoleptic quality of dry beans (Rahman et al. 2004).

Tempering

Tempering is used to bring the seed to desired moisture content before milling by adding water depending on the initial seed moisture level. Tempering beans can result in smaller nutritional losses when compared to cooking; however, relatively few studies have been published concerning tempering of dry beans.

Drying

Drying step is essential due to the high moisture content in the seed at harvest (18-25%) as well as after some pre-treatments. Drying reduces the moisture content of the dry bean seed to 9 -12%, which is considered an optimum range for safe storage (Tiwari et al. 2011). Different traditional and modern techniques can be used to dry the beans. In developing countries, sun drying is the most common drying method. Other artificial and commercial methods can be utilized, such as thin-layer drying and fluidized-bed drying. Temperature and moisture content of the seed will affect the drying rate and the milling properties (Kundu et al. 2005).

Dehulling

Removal of the seed coat before processing and consumption is referred to as dehulling or decortication. The dehulling of legumes generally consists of two steps: 1) loosening the seed coat (often by a dry or wet treatment) and 2) removing the seed coat and cleaning. Dehulling

produces refined cotyledons with good appearance, texture, and cooking qualities. Legumes that have gone through this process are more easily digested and are efficiently utilized by the body (Deshpande et al. 1982). Dehulling can be a time-consuming procedure depending on how tightly the legume seed coat adheres to the endosperm or cotyledon. For legumes in general, large grain legumes are easier to dehull and give a higher yield, making them the preferred legume among millers (UDPLC 2010). Small seeded legumes, meanwhile, require repeated pre-dehulling treatments and other complex procedures. Due to the higher moisture content, freshly harvested legumes are more difficult to process. Legumes of this kind are either stored for some time to reduce moisture, or are treated with lime-water or a solution of sodium carbonate to loosen the hull (UDPLC 2010).

Dry beans can be dehulled by several methods. Drying seeds in the sun or mixing seeds with water and then pounding them in a mortar with a pestle are the oldest and most common techniques to remove seed coat (UDPLC 2010). The seed coat is winnowed-off to get the clean cotyledons. Similar methods are used in commercial mills, though on a larger scale. Dry bean dehulling on a commercial scale generally is based on dry-processing techniques. Smaller processors can expect less removal with the first effort and the process is then repeated several times until almost all the grain is converted into dehulled, split cotyledons. It can be difficult with this approach to achieve complete removal of the seed coat from the grain (UDPLC 2010). Another method for dehulling is based on adjusting the moisture of the grain to loosen the seed coat. First, the grain is exposed to heated air in a tempering bin for a pre-determined time based on the market class. Through gradual aeration, it reaches a critical moisture level. The seed coat is then removed in an abrasion type-hulling machine (UDPLC 2010).

Kon et al. (1973) reported a reduction of 42, 53 and 70% in cooking times for dehulled, unsoaked California small white, navy, and pinto beans, respectively, compared to the controls. According to Deshpande et al. (1982), dehulling significantly increased the amount of phytic acid, trypsin, chymotrypsin and α -amylase inhibitory activities in several dry beans while it decreased the tannin content of colored beans by 68-95% and improved the *in vitro* protein digestibility (Deshpande et al. 1982). Since phytates are mainly located in cotyledon and tannins in the seed coat, the physical removal of seed coat increases the phytic acid while decreasing tannin and polyphenol levels (Alonso et al. 2000). Similarly, dehulling increases the measured protein content, as the seed coat of dry beans contain less protein than do cotyledons (Alonso et al. 2000). Dehulling not only improves the cooking utility and reduces some of the antinutritional factors, but also improves protein quality, palatability, and digestibility of pulses (Salunkhe et al. 1986).

Milling

Milling is a process where grains are reduced to meal or flour by processes involving grinding, sieving, and purifying (Limsangouan and Isobe 2009). Milling process can be of two kinds, (1) wherein the whole grain is converted into flour without abstracting any parts or, (2) it could undergo differential milling to separate the grain into different parts (Oghbaei and Prakash 2016). During milling, the seed absorbs force as strain energy. When the local strain energy in the seed exceeds a critical level, fractures occur along lines of weakness and the stored energy is released as heat (Earle and Earle 2004; Schorno 2006). The efficiency of milling is related to uniformity of the particle size distribution and the differences in particle sizes, which can impact the bioavailability of macronutrients and their digestion (Wondra et al. 1995). Starch granules are damaged by the disruption of the granular structure of starch by the effect of particle size

reduction (Tran et al. 2011). Heat generated during milling, can change flour characteristics, such as increase starch damage, protein denaturation and lipid oxidation.

Seed parameters like size, shape, weight, volume, porosity, density, and coefficient of friction are relevant to milling yields of beans and these factors influence the performance and quality of dry bean milling (Altuntas and Demirtola 2007). Assuming other seed attributes are the same, when the seed coat fraction is low, the milling yield will increase. Seed size should be uniform to obtain higher milling yields. Seed shape should be uniform to reduce broken seeds (Wang 2005; Wood et al. 2008; Goyal et al. 2009; Wood 2010).

Seed hardness is important in milling, because it affects the milling time and energy expenditure as well as the final ground product properties and appearance (De Francisco et al. 1982). An incremental increase in seed moisture level reduces the seed hardness and decrease the impact milling efficiency (Tyler and Panchuk 1982). Most researchers have concluded that moisture of the seed is one of the most important factor affects hardness (Morris 2002; Tranquilli et al. 2002), which will finally affect the milling ability.

Burr Mill

A burr mill (Figure 1) consists of a set of burrs or raised edges that use a cutting, shearing, and crushing action for particle size reduction (Haque 1991). Burr mills, a type of plate mill, have two circular plates where material is fed between them. One of the circular plates is fixed and the other rotates. The material comes in contact with the two plates where it is sheared and crushed and exits through the edge of the plates. The plates of the burr mill are vertically mounted. Particle size principle of the burr mill is mainly due to cutting and shearing forces. Burr mill is suitable for dry grains and beans, but does not grind oily, wet or fibrous materials very well.

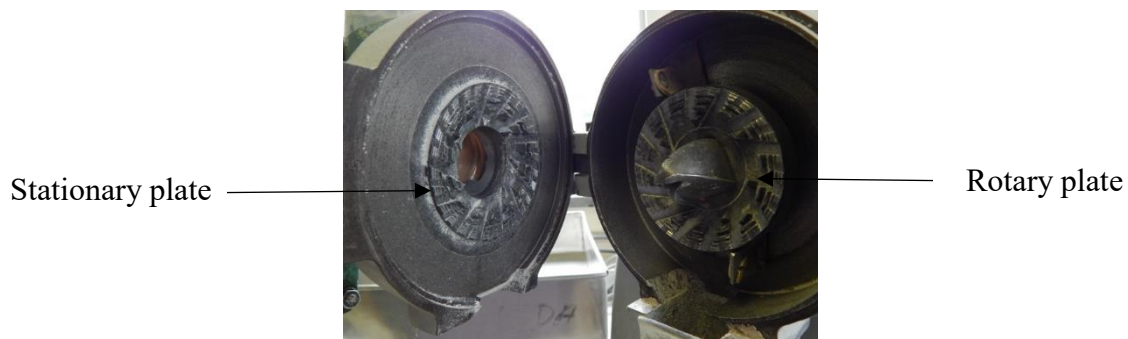


Figure 1. Burr mill

Roller mill

Roller mill is a common mill used to mill wheat into bran and flour. Roller mills use cylindrical rolls, either in opposing pairs or against flat plates, to crush or grind various materials. The roller mill has the potential of mechanically removing the seed coat from cotyledon. Size reduction in roller mill is done by multiple stage approach. Roller mill has a set of paired rolls that can be corrugated or smooth. The corrugated rolls are cut with a slight spiral and are not parallel to the roll axis. Increasing the spiral corrugation also increases the slicing action (Creason 1975). The corrugations have sharp or dull angles that can have different orientation configurations such as: sharp: sharp, sharp: dull, dull: sharp, or dull: dull. The orientation of roll configurations impact shear and compression forces. For example, dull: dull to sharp: sharp, shear force increases and compression forces decreases (Schorno 2006). Each roll in a pair can rotate at the same speed or can rotate at different speeds (Posner and Hibbs 2005). Differences in roller speed results in a shearing action that can aid in removing seed coat from the cotyledon. When feed material is drawn between the rolls having similar rotational speed, compression force is applied whereas, shearing forces result when roll-speed differential and roll corrugation are used (Schorno 2006).

The functional and physiochemical properties of the milled pulse flour are influenced by the particle size, which is an important variable of flour quality (Kerr et al. 2000). According to Kerr et al. (2000; 2001) and Singh et al. (2015), the particle size affects end product attributes. According to Singh et al. (2015), Navy bean flour particle size affected the cake baking and batter quality. Fine bean flour fractions were less sticky, and had storage modulus similar to wheat flour batters. Therefore, it is important to select a suitable mill, which achieves the desired particle size reduction for a specific food product and at a minimum cost.

OBJECTIVES AND NEEDS STATEMENT

Needs Statement

Dry bean milling, has not been studied extensively. However, dry bean milling has attracted interest due to the increasing need of non-wheat food ingredients available for food applications. Separation of seed coat from the cotyledon can expand the use of dry bean flour in food product applications by creating different food ingredients such as cotyledon flour and seed coat flour. This study developed a standard milling procedure that can achieve optimum yields of different flours (cotyledon and seed coat) for black bean by determining the effect of cooking and tempering pretreatments in combination with drying on seed and flour physical and chemical composition using a burr mill/roller mill system.

Research Objectives

The present study was conducted to determine the effect of cooking and tempering pretreatments in combination with drying on:

1. Achieving optimum yields of black bean seed coat and cotyledon using a burr mill/roller mill system.
2. Black bean seed and flour physical, chemical composition and functional properties.

Hypothesis

Cooking and tempering pretreatments in combination with drying will affect the seed coat yield, flour chemical composition, physical and functional properties after milling.

MATERIALS AND METHODS

Black beans were obtained from three dry bean companies. Seeds from each company were kept separate and treated as replications in the ensuing experiments. Cooked-dried and tempered-dried pretreatments were evaluated. For the cooked-dried pretreatments, 400 g of clean black beans were cooked in 2 L boiling distilled water for 0, 5, 10 and 20 min. Each cooked sample was divided into two sets. One set was air-dried (ambient conditions, 22°C, 30% rh) and one set was dried at 90°C in a forced-air oven. Seeds were dried to the original pretreated moisture content (6.5%). For the tempered-dried pretreatments, 400 g of clean dry beans were tempered with distilled water to 'as is' and to 10, 20, 30, 40 and 50% moisture. Samples were allowed to equilibrate in a closed plastic container for 1 day. Each tempered sample was divided into two sets. One set was air-dried and one set was dried at 90°C in a forced-air oven. Seeds were dried to the original pretreated moisture content. Cooked-dried and tempered-dried pretreatments were separate experiments. A sub set of each pretreated bean was cooked to determine the cooking loss by AACC International method 66-50.01 for pasta.

Physical Seed Properties

Seed Weight and Size

Seed weight before and after cooking or tempering was recorded. Wet seed appearance for both cooked and tempered seeds were visually determined. Also, seed dimension was measured for seeds dried after cooking or tempering. Seed dimension was calculated using a caliper by measuring the seed length, width, and thickness. Estimated seed volume was calculated by determining the product of length x width x thickness. Mass of 100 dry black bean seeds was determined by taking 100 seeds randomly and measuring the mass of seeds for all the different treatments.

Seed Hardness

Seed hardness was determined before and after all seed treatments. A compression test was conducted using a Texture Analyzer (TA-XT2 texture analyzer, Texture Technologies Corp., Scarsdale, NY) to measure black bean seed hardness. The fracture force of the seed in terms of force (N) required to break the seed along its principal axis was measured. The parameters calculated from the force-distance curve were fracture force and maximum compression force. The Texture Analyzer had a TPA-probe fitted onto a 50 kg load cell and it moved downwards with a speed of 1 mm/sec. The seed sample was placed on the fixed base. The seed samples were compressed with a 40% strain and a trigger force of 4 g. The fracture force was associated with a non-linear deformation zone as the first crack of the seed, and the maximum compression force was associated with the final force. These determinations were done on 10 seeds per dry bean pretreatment.

Milling Procedure

A laboratory-type burr mill (Model 289, Laboratory Constructions Co., KS) was used as a prebreak system before milling with a roller mill (Model MLU202, Buhler, Minneapolis, MN, USA) configured for milling durum wheat into semolina and bran/germ. The burr mill settings, such as gap and feed rate, were fixed. A commercial aspirator (Model 63-115-60-VS, Grain Machinery Mfg. Corp., FL) was used to remove the seed coat from the pre-break seed fraction. The air flow use was set on setting 3 of the aspirator. Samples without seed coat were sieved through 6.73, 2.83 and 2.38 mm sieves and the particles retained on sieves with screen mesh of 6.73 and 2.83 mm were combined and milled together on the roller mill using only the 1st break. Seed coat rich percent was the portion recovered by the aspirator.

The cotyledon fraction was milled using the first break rolls. The milled product was sieved through the 1.19 mm mesh sieve and the commercial aspirator was used to remove the seed coat from the first break seed fraction that remained on the 1.19 mm mesh sieve. Whole bean, cotyledon and seed coat fractions were further milled into flour using a UDY mill (3010-030, UDY Corp., Colorado) configured with a 1 mm mesh screen. These flours were assayed for chemical, physical and functional properties.

Physical Quality of Flour

Flour fractions were evaluated for color (CIE L, a, b values) using Minolta 410 colorimeter. Flour was placed in a round black measurement cell (6 cm diameter x 2 cm depth) with a quartz glass window. Color difference was determined, which is defined as “the magnitude and character of the difference between two colors under specified conditions”.

Color difference (ΔE^*ab) was calculated using the equation:

$$\Delta E^*ab = \sqrt{(L2 - L1)^2 + (a2 - a1)^2 + (b2 - b1)^2}$$

Where L1, a1, and b1 were CIE L, a, b values for control sample, while L2, a2, and b2 were CIE L, a, b values of a pretreated sample.

Chemical Composition of Flour

Ash content, moisture content and protein content were determined according to AACC International Approved Methods 08-01.01, 44-15.02, and 46-30.01, respectively. Nitrogen content of samples was determined using Leco FP 528 combustion nitrogen analyzer (LECO Corp, St. Joseph, MI). Protein content was calculated as % N \times 6.25. Total lipid content was determined using 16 h Soxhlet extraction with hexane according to Method Ba 3-38 (AOCS 1998). Total starch content was determined using an enzymatic total starch assay kit (Megazyme

International, Co. Wicklow, Ireland) according to AACC International Approved Method 76.13.01 and the amount of starch damage was determined using an enzymatic starch damage assay kit according to AACC International Approved Method 76-31.01.

Pasting Properties

Pasting properties were determined using a Rapid Visco-Analyzer (Perten Instruments, Springfield, IL, USA). Black bean flour (3.5 g, 14% moisture basis) was added to 25 ml deionized water in a RVA canister. The flour slurry was held at 50 °C for 1 min before heating to 95 °C at a rate of 12 °C/min and held at 95 °C for 2 min. The slurry was cooled at a rate of 12 °C/min to 50 °C and held for 2 min.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was conducted by the Electron Microscopy Center located at North Dakota State University, Fargo. Samples were mounted on aluminum mounts with silver paint (SPI Supplies, Structure Probe Inc., West Chester, PA, USA). They were then sputter coated (Model SCD 030, Balzers, Liechtenstein) with gold-palladium to make them electrically conductive. The samples were viewed and images obtained with a JEOL JSM-6490LV scanning electron microscope (JEOL USA, Peabody, Massachusetts USA) at an accelerating voltage of 15 kV.

Experimental Design and Data Analysis

The experimental design was a randomized complete block with a factorial arrangement of cooking time (4) and drying method (2) or of tempering level (6) and drying method (2). Each treatment was replicated three times. Data were analyzed using SAS 9.3 package. The data were

subjected to analysis of variance. F-Test was significant at $P < 0.05$. Treatment means were separated by Fisher's protected Least Significant Difference test calculated at $P=0.05$.

RESULTS AND DISCUSSION

Black Bean Seed Structure

Black beans are small to medium, oval shaped beans with a shiny black seed coat, a small white spot (hilum), and a creamy white interior (cotyledons). Black beans possess a seed structure, which contains a seed coat, two cotyledons and embryonic axis (Figure 2). Raphe, micropyle and hilum in seed, work as entry points for water diffusion into the seeds during water imbibition by the seed coat (Tiwari and Singh 2012). The hilum is the scar left when the ovule separates from the funiculus (stalk), which had supported and attached the ovule to the pod during development. The micropyle is the site of pollen tube entry during fertilization.

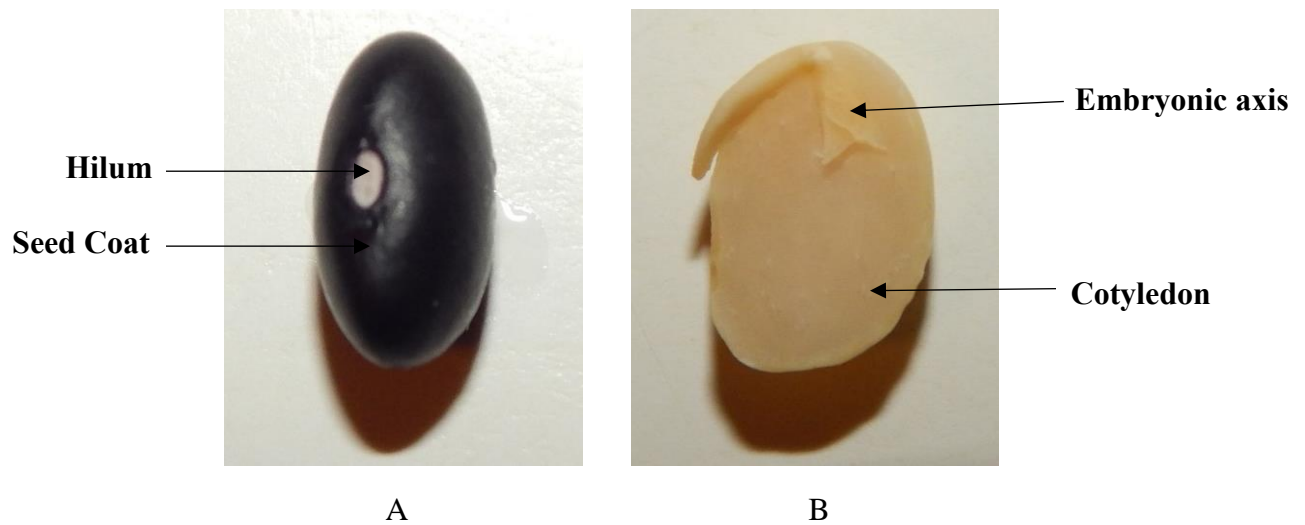


Figure 2. Black bean seed macrostructure (A) Intact seed and (B) Split seed

The black appearance of black bean is due to high concentration of anthocyanins in the seed coat (Siddiq and Uebersax 2013). The seed coat accounts for about 8% of the seed weight (Carter 2014) and it protects the cotyledon and embryo from physical damage, microbial contamination and diseases especially during harvest and storage. Also during the seed development stage, the seed coat supplies nutrients that are imported through the phloem, using

its vascular network (Boesewinkel and Bouman 1995; Ammerlaan et al. 2001; Van Dongen et al. 2003). High level of dietary fiber can be found in the seed coat as it mainly is composed of cellulose and hemicellulose (Aguilera et al. 1982).

The major components of the seed coat microstructure are; the waxy cuticle layer, palisade cell layer (epidermal layer), the hour-glass cells (hypodermis), and the parenchyma layer (Figure 3) (Ruengsakulrach 1990). The waxy cuticle layer is the outer most layer (Figure 4A) of the seed coat and its primary function is to prevent water penetration through its hydrophobic layers (Bukovac et al. 1981). The palisade layer has been reported to have two layers of cells (Sefa-Dedeh and Stanley 1979b) that are perpendicularly oriented to the surface. Hour-glass cell layer (Figure 4B) is immediately beneath the palisade layer and cells are oriented parallel to the surface. The parenchyma cells are elongated cells that are parallel to the surface of cotyledon and have thick cell walls (Tiwari and Singh 2012). These cells can be easily distinguished after hydration as the cells swell and become spongy (Siddiq and Uebersax 2013).

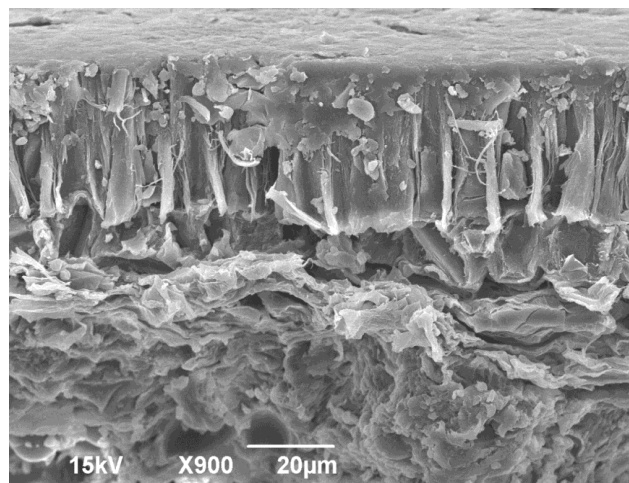


Figure 3. Scanning Electron Micrograph of raw black bean seed coat cross section
Source-Carter 2014

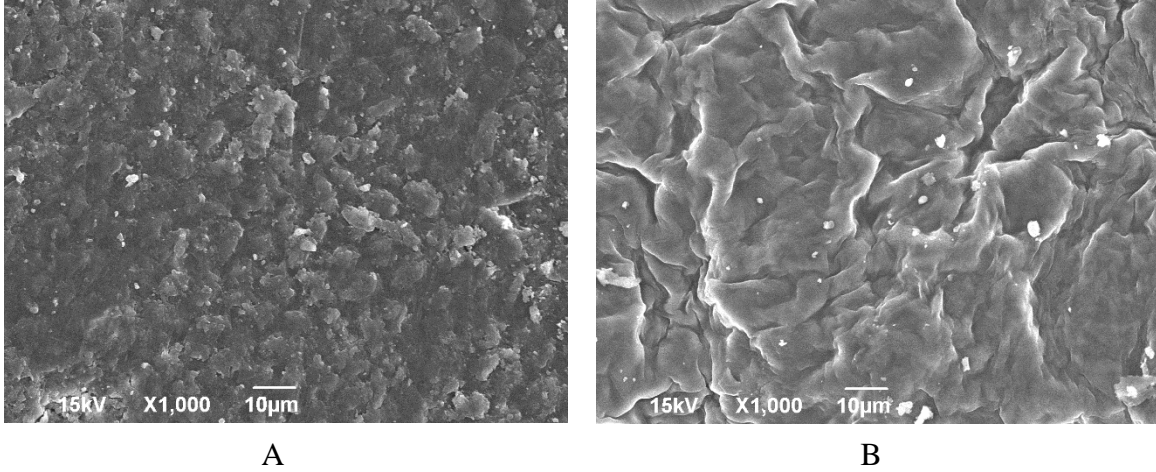
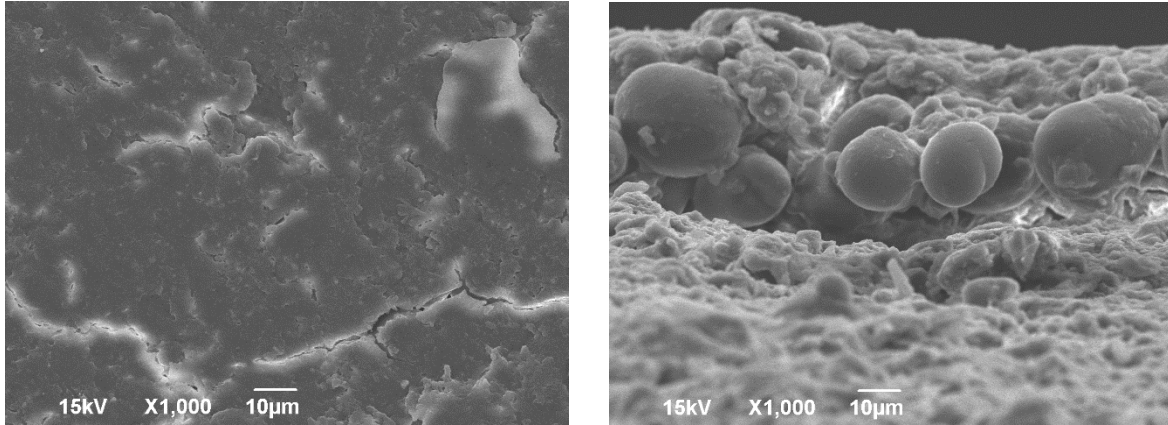


Figure 4. Scanning Electron Micrograph of untreated black bean seed coat (A) Outer surface and (B) Inner surface

The cotyledon serves as energy storage and it comprises the largest mass of the seed accounting for 90% of the seed weight (Rahman 2007). The outer most layer is a tightly packed epidermal layer (Figure 5A), which consists of outer cells that appear to be cubical and inner cells that are elongated. Epidermal layer does not contain starch, as all cells appeared to be granular like protein (Siddiq and Uebersax 2013). The next apparent layer is the hypodermis, which consists of large elliptical cells. The remaining parenchyma cells have thick walls that give rigidity to the cotyledon and bound by distinct cell walls and a pectin rich middle lamellae layer. Starch granules in the parenchyma cells are embedded in a matrix of storage proteins (Figure 5B) (Tiwari and Singh 2012).



A

B

Figure 5. Scanning Electron Micrograph of untreated black bean (A) cotyledon outer surface which contact with seed coat and (B) starch granules embedded in a protein matrix

The embryo is relatively small and represents only 2% or less of the seed weight (Rahman 2007). Embryonic axis (Figure 6) serves as a nutrient- absorbing organ for the embryo during germination. The embryonic axis has the radicle, hypocotyl and epicotyl. Raphe, micropyle and hilum in seed, work as entry points for water diffusion into the seeds during water imbibition by the seed coat (Tiwari and Singh 2012). The hilum is the scar left when the ovule separates from the funiculus (stalk), which had supported and attached the ovule to the pod during development. The micropyle is the site of pollen tube entry during fertilization.

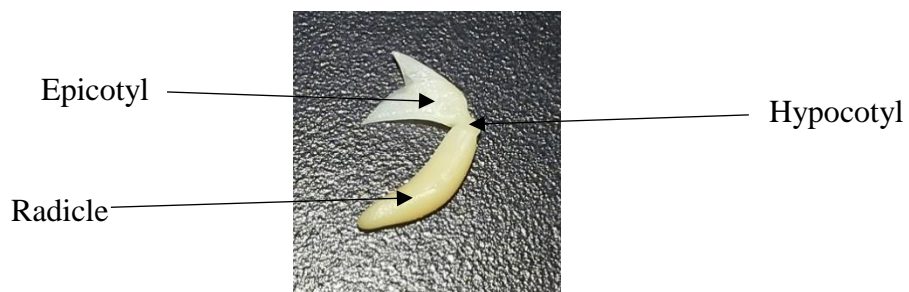


Figure 6. Dissected embryo of black bean seed

Proximate Chemical Composition of Untreated Black Beans

The proximate chemical composition of the untreated black bean flour fractions is shown in Table 1. Manual dehulling of the untreated beans enabled the separation of 8% of seed coat from the cotyledon fraction (90%) for the proximate tests. Embryo came off with the cotyledon, where further dissecting allowed separating 2% of embryo. In general, protein, ash, lipid and starch content values were greater for cotyledon flour than for whole bean flour (Table 1). An increase from 22.9 to 24.5%, 1.9 to 2.7%, and 4.5 to 4.8% in protein, fat, and ash contents, respectively, in cotyledon flour compared to whole flour was reported by Deshpande et al. (1982). Other researchers have reported similar results (Dzudie and Hardy 1996; Eknayake et al. 1999; Wang et al. 2009; Mugendi et al. 2010; Siddiq et al. 2010; Akinjayeju and Ajayi 2011). These results reflect the composition of the seed coat, which is composed mainly of fiber, while the cotyledon is the storage organ, which contains protein, starch, lipid and ash.

Table 1. Proximate composition^a of untreated black bean fractions.

| Black bean fraction | Moisture content | Ash | Protein (%) | Total lipids | Total starch |
|---------------------|------------------|------|-------------|--------------|--------------|
| Whole seed | 6.4 | 4.01 | 21.8 | 1.9 | 38.0 |
| Cotyledon | 6.7 | 4.12 | 23.5 | 3.1 | 38.4 |

^aWet weight basis.

Black beans contain 70% of carbohydrates (Belitz et al. 2009). The major carbohydrate component of the cotyledon fraction is starch (Bravo et al. 1998). Whole black bean flour and cotyledon fraction total starch was 38.0% and 38.4%, respectively (Table 1). The difference between the carbohydrate level and the total starch values reported here is due to the non-starch polysaccharides that was not determined. Total starch of whole black bean flour is in agreement with values found by Carmona-Garcia et al. (2007). Since seed coats reportedly contain little protein and starch, it is suggested that dehulled seeds would proportionately contain more protein

and starch (Wang et al. 2009). The seed coat is composed mostly of fiber and waxes. Starch in seed coat has not been reported in published literature.

Effect of Cooking and Tempering Pretreatments on Physical Seed Properties

Cooked-Dried Pretreatment

Cooked Seed Appearance

After cooking, seeds were bigger in size compared to untreated seeds but did not swell equally probably due to differences in individual water absorption rates. The seed coat of cooked beans was lighter in color, wrinkled and most remained intact with the cotyledon. Some seed had ruptured seed coat and split cotyledon (Figure 7). Cooked beans felt rubbery, soft and seed coat came off easily after cooling.

According to Abu-Ghannam and McKenna (1997), cooking increases seed plasticity and water absorption. They reported that the cooking water (≥ 40 °C) had a plasticizing effect on the seed coat, which resulted in the seed coats developing a rubbery texture and that there was a positive relationship between the plasticity of the seed coat and the water absorption rate.

Rehman et al. (2004) reported that cooking improved the protein and starch digestibility. The cell wall of dry beans has a pectin-rich structure and is rich in arabinan with high hydroxide concentrations indicating a strong association (Gooneratne et al. 1994).



Figure 7. Appearance of cooked black beans after 20 min

Cooking generally rendered black beans pale and opaque with a gray or brown appearance. The leaching of pigment (anthocyanins) during cooking is a major quality problem associated with all colored beans, particularly black beans. The cooking water left in the beaker was very dark due to high amounts of anthocyanins (Bushey and Hosfield 2007). Pigments found in the seed coat are typically phenolic compounds, particularly anthocyanins that impart a distinctive color (Siddiq and Uebersax 2013).

The cooking quality of dry beans is dependent on the thermal degradation and solubilization of cell wall polymers and consequently on the structure and chemical composition of their pectic polysaccharides (Gooneratne et al. 1994; Talbott and Ray 1992). The cooking process involves gelatinization of starch granules contained within integral cell units and concurrent dispersion of intercellular components of the middle lamella, which facilitates separation of intact cells without rupture of cell walls. Mechanical stresses due to starch gelatinization, protein denaturation, swelling and heat convection can promote cell separation.

The cotyledon splitting is related to the high hydration capacity and swelling capacity of the seeds and results in more exudation of starch into the cooking water (Tiwari and Singh

2012). The pectin content and calcium content in the seed coat and starch gelatinization behavior determine the tendency of seed coat to split during cooking (Lu and Chang 1996). Hard-to-cook phenomenon is the occurrence of seeds that do not hydrate completely and remain hard even after extensive cooking (Siddiq and Uebersax 2013). The formation of insoluble pectate, polymerization and cross-linking of phenolic compounds, lignification of middle lamellae, and protein-starch interactions may contribute to the hard-to-cook phenomena and these factors make adhesion of the seed coat to the cotyledon surface strong (Sefa-Dedeh et al. 1978).

Cooking Loss and Cooked Weight Gain

Cooking impacted cooking loss and cooked weight gain of black beans (Table 2). Cooking loss increased with increased cooking time up to 10 min. No additional cooking loss was detected between 10 and 20 min of cooking because near maximum level of materials has leached out after 10 min cooking. Cooking loss probably reflects the loss of solids from disrupted starch granules during gelatinization and from soluble oligosaccharides such as raffinose (Siddiq and Uebersax 2013). These oligosaccharides can be identify by the mass spectroscopy and Nuclear Magnetic Resonance methods.

Table 2. Mean cooking loss and cooked weight gain values^a of cooked seeds.

| Cooking time (min) | Cooking loss (%) | Cooked weight gain (%) |
|--------------------|------------------|------------------------|
| 5 | 0.7b | 145c |
| 10 | 1.8a | 158b |
| 20 | 1.8a | 185a |

^aValues followed by same letter are not significantly different at $P=0.05$.

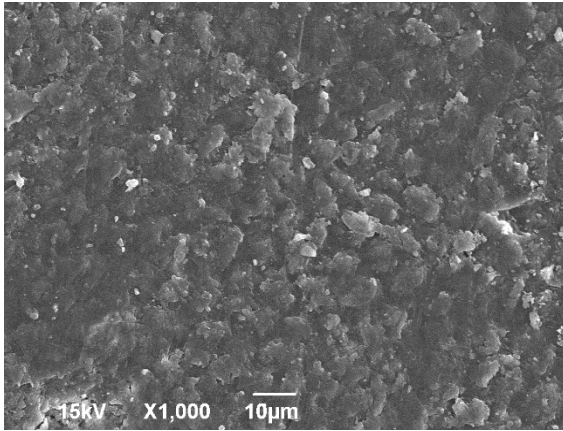
Cooked weight gain was due to the water absorbed by the seeds during cooking. Most of the weight gain (45 percentage units) occurred by 5 min of cooking. The cooked weight gain increased by an additional 40 percentage units as cooking time increased from 5 to 20 min (Table 2).

Cooked-Dried Seed Appearance

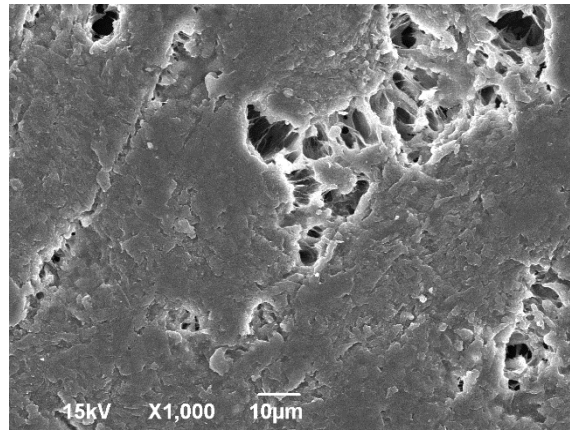
The cooked-dried whole beans were less dark in color while the cotyledons looked mostly dark due to pigments leached from the seed coat into the cooking water and onto the cotyledons (Figure 8). After drying, the cooked seeds looked similar to their original non-treated dry seeds. Seed coat had ruptured and cotyledons were split in some seeds, as the forces associated with drying deformed the shape of the seeds. After the cooked dried pretreatment, holes and cracks, were visible in outer and inner surfaces of the seed coat micro structure (Figure 9 & 10). Cooked dried cotyledon outer surface was also different to the untreated cotyledon (Figure 11).



Figure 8. Appearance of cooked-oven dried black beans

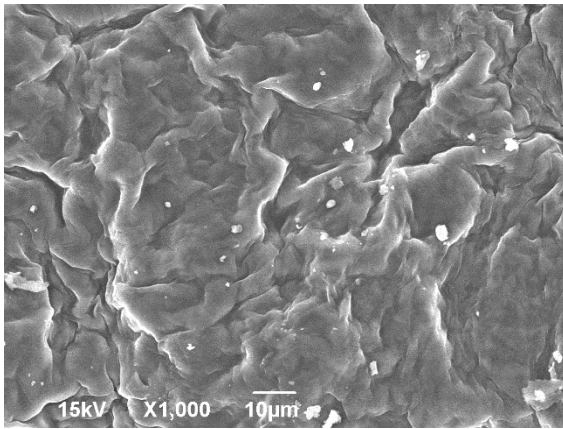


A

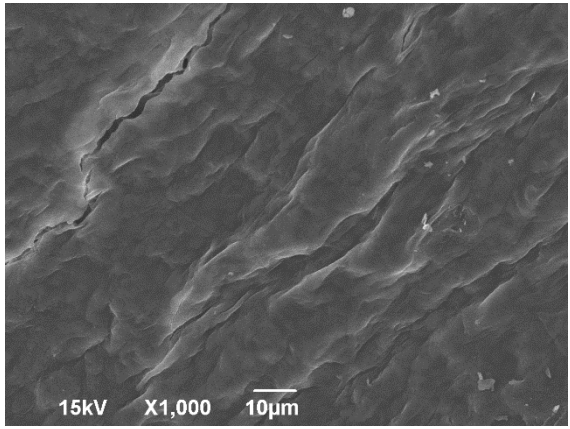


B

Figure 9. Scanning Electron Micrograph of black bean seed coat outer surface (A) untreated and (B) 20 min cooked and oven dried



A



B

Figure 10. Scanning Electron Micrograph of black bean seed coat inner surface (A) untreated and (B) 20 min cooked and oven dried

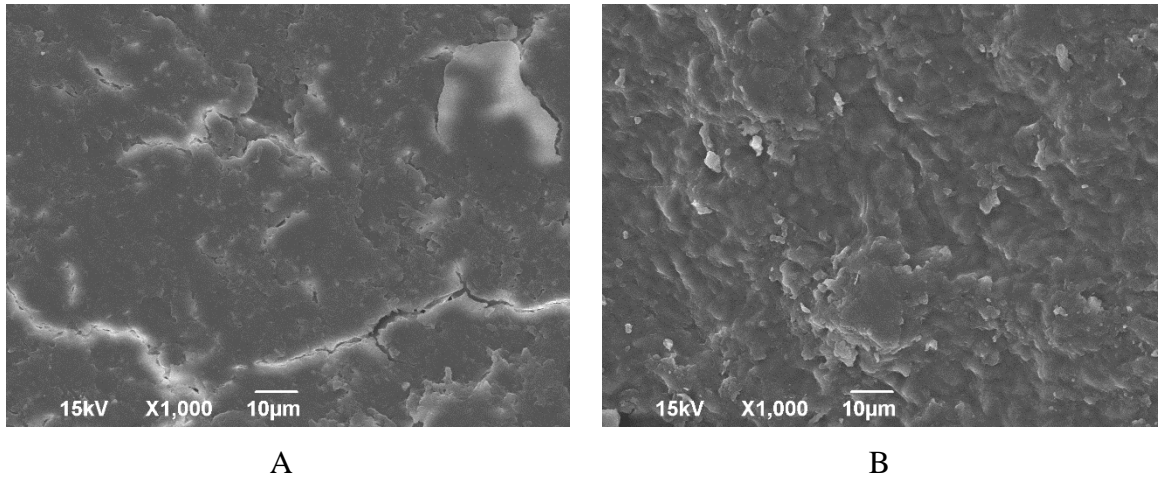


Figure 11. Scanning Electron Micrograph of black bean (A) untreated and (B) 20 min cooked and oven dried cotyledon outer surface

Cooked-Dried Seed Physical Tests

Cooked-dried pretreatment significantly affected the seed length, width thickness, and volume (Table 3). As cooking time increased, seed dimension values (length, width, thickness) increased. Although not statistically different, the 100-seed weight for black bean seeds tended to decrease. The small decline in seed weight reflects the relatively low level of cooking loss detected in the cooking water after cooking 20 min (Table 2). Drying temperature had no significant effect on the 100-seed weight, but seed length and thickness were greater with seed dried at 90°C compared to seed that was air dried. The increase in seed length and thickness resulted in an increase in seed volume. The effect of drying on seed size is attributed to the possibility that slow drying allowed for contraction of the seed, which did not occur when the seed was dried rapidly.

Table 3. Mean physical properties values^a of cooked-dried seeds.

| | Length | Width | Thickness | Estimated volume (mm ³) | 100-seed weight (g) |
|---|--------|--------|-----------|--|---------------------------|
| | (mm) | | | | |
| Cooking time (min) ^b | | | | | |
| 0 | 8.8c | 6.0b | 4.7b | 245c | 19.7 ns |
| 5 | 9.1c | 6.1b | 4.9a | 271b | 19.5 ns |
| 10 | 9.6b | 6.2ab | 4.9a | 292b | 19.5 ns |
| 20 | 10.1a | 6.4a | 4.9a | 319a | 19.0 ns |
| Drying temperature (°C) ^c | | | | | |
| 20 | 9.1b | 6.1 ns | 4.7b | 263b | 19.3 ns |
| 90 | 9.7a | 6.2 ns | 5.0a | 300a | 19.5 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^b Values averaged over cooking time

^c Values averaged over drying temperature

ns – Not Significant

Cooked-Dried Seed Hardness

Cooked-dried pretreatment significantly affected bean fracture force (Table 4). For cooked samples, fracture force decreased as cooking time increased. The fracture force decreased $\approx 63\%$ for seeds cooked for 20 min compared to the non-treated seeds. Drying temperature had no significant effect on the fracture force.

Table 4. Mean seed fracture force^a of cooked-dried seeds.

| | Fracture point (N) |
|--------------------------------------|--------------------|
| Cooking time (min) ^b | |
| 0 | 162a |
| 5 | 128b |
| 10 | 86c |
| 20 | 60d |
| Drying temperature (°C) ^c | |
| 20 | 113 ns |
| 90 | 106 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^b Values averaged over cooking time

^c Values averaged over drying temperature

ns – Not Significant

Peak force had an interaction effect of cooking time and drying temperature on cooked-dried seeds (Table 5). Peak force for black beans dried at 90°C decreased nearly 50% as cooking time increased from 0 to 20 min. Conversely, peak force for black beans cooked up to 20 min and then air-dried did not vary greatly. The effect of cooking time on peak force was more pronounced when dried at 90°C than when air-dried. This could reflect the contraction of seed with air drying making the seed denser or conversely the lack of contraction with high temperature drying resulted in less dense seed and easier to crack seed.

Table 5. Mean seed peak force values^a for cooked-dried seeds.

| Cooking time | Peak Force (N) | |
|--------------|----------------|--------|
| | 20 °C | 90 °C |
| 0 | 178a,A | 183a,A |
| 5 | 158ab,A | 150b,A |
| 10 | 137b,A | 123c,A |
| 20 | 114c,A | 94d,B |

^aDifferent uppercase letters across rows indicates significant differences between drying temperature ($P=0.05$). Different lowercase letters across column indicates significant differences between cooking time ($P=0.05$)

Tempered-Dried Pretreatment

Tempered Seed Appearance

Physical appearance of black bean seed was not affected by tempering and remained similar to non-treated seeds. The cotyledon retained its color and did not split (Figure 12). The seed coat and cotyledon remained intact without loosening or seed coat being ruptured. The seed coat inner surface appeared brownish in color and the cotyledon remained white in color. No major changes occurred to tempered seeds on the surface and interior of the cotyledon.



Figure 12. Appearance of tempered black beans

Tempered Weight Gain

Tempered weight gain was due to the water absorbed by the seeds during moisture conditioning. The tempered weight gain was increased by 79% as tempering moisture levels increased from 10% to 50 % and the increment was significantly different between every moisture level (Table 6). It is interesting to note that weight gained with 40 and 50% temper was similar to cooking for 10 and 20 min, respectively (Tables 2 and 6).

Table 6. Mean weight gain^a of tempered seeds.

| Tempered Seeds (%) | Tempered Weight Gain (%) |
|--------------------|--------------------------|
| 10 | 102e |
| 20 | 115d |
| 30 | 131c |
| 40 | 154b |
| 50 | 181a |

^aValues followed by same letter are not significantly different at $P=0.05$.

Tempered-Dried Seed Appearance

The tempered-dried whole beans retained its color (Figure 13). No major changes occurred to tempered seeds on the surface (Figure 14 & 15) and interior of the cotyledon (Figure 16) and looked similar to their original non-treated dry seeds. Seed coat was ruptured and cotyledon was split in some seeds, as the forces associated with drying deformed the shape of the seeds.

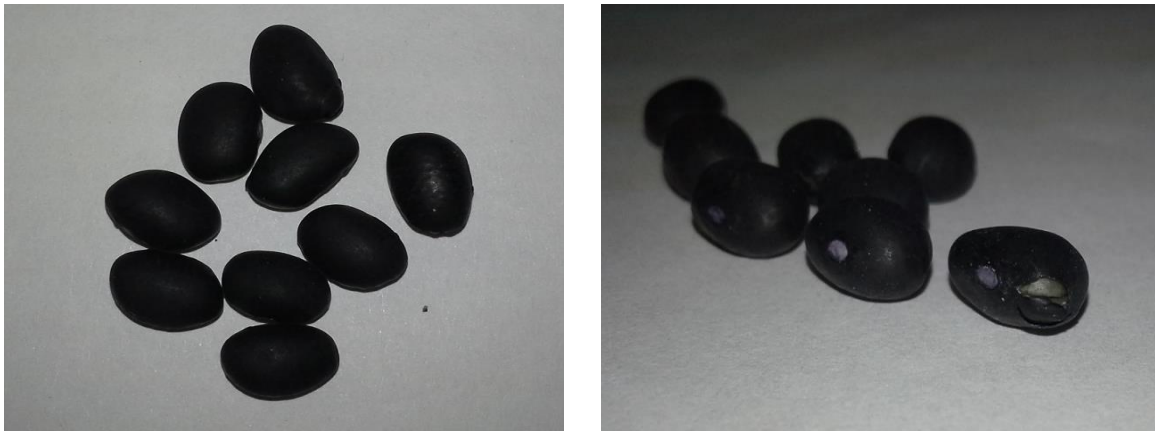
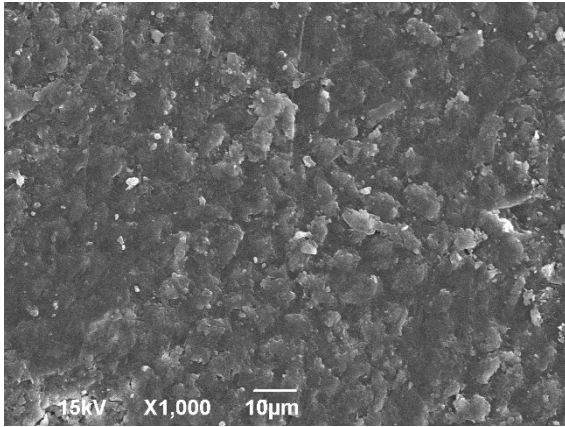
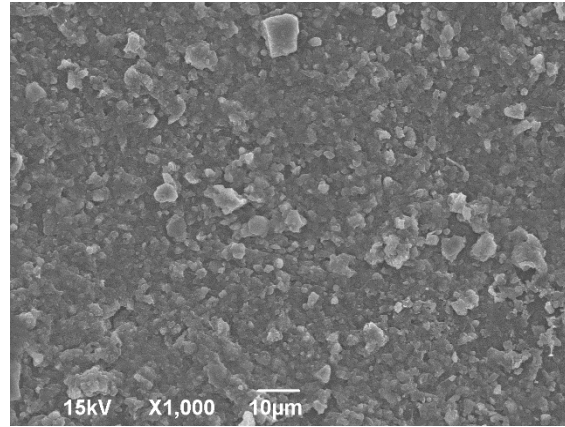


Figure 13. Appearance of tempered-dried black beans

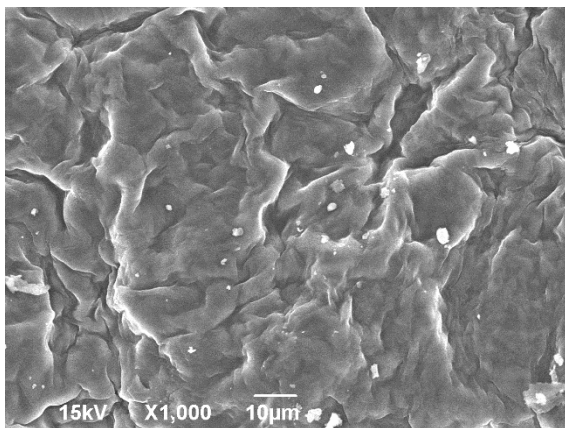


A

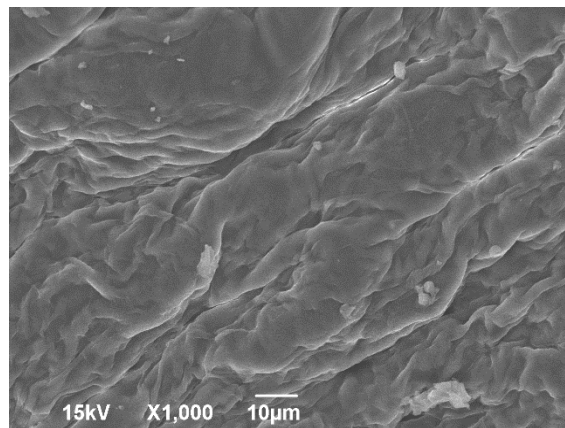


B

Figure 14. Scanning Electron Micrograph of black bean seed coat outer surface (A) untreated and (B) 30% tempered black bean oven dried



A



B

Figure 15. Scanning Electron Micrograph of black bean seed coat inner surface (A) untreated and (B) 30% tempered black bean oven dried

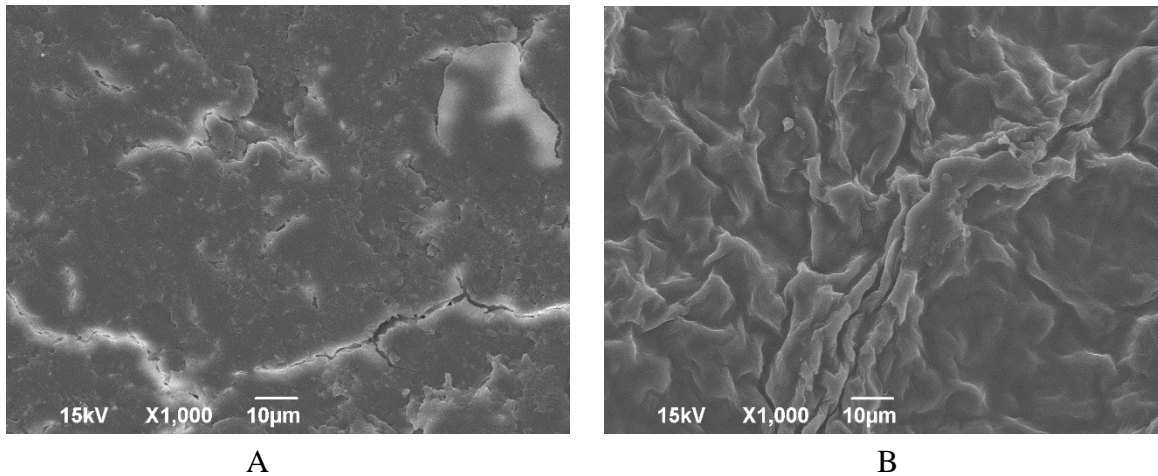


Figure 16. Scanning Electron Micrograph of black bean cotyledon outer surface (A) untreated and (B) 30% tempered black bean oven dried

Tempered-Dried Seed Physical Tests

Tempered-dried pretreatment significantly affected seed dimension (i.e., length, width, and thickness) (Table 7). Drying temperature had no significant effect on the seed dimension and 100-seed weight. As tempering level increased, length and width values continuously increased, while thickness was lower in the beans tempered to the 50% moisture level. Altuntas and Demirtola (2007) also reported that as moisture content increased in kidney bean seeds, length, width, and thickness slightly increased. The effect of tempering on 100-seed weight was variable and was not significant. Altuntas and Demirtola (2007) reported as moisture content increased for pea, kidney beans, and black-eyed, 1000-seed weight was increased and showed a linear relationship. This positive linear relationship of 1000-seed weight and moisture content of the seed were also reported by Aviara et al. (1999) in guna seeds and Vilche et al. (2003) in quinoa seeds. In this study, the tempered seeds were dried to the initial moisture level as to facilitate the final milling and drying may be the reason for not having such a significant increment and linear relationship in black beans compared to previous reports.

Table 7. Mean physical properties values^a of tempered-dried seeds.

| | Length | Width | Thickness | Estimated Volume | 100-seed weight |
|--------------------------------------|--------|--------|-----------|--------------------|-----------------|
| | (mm) | | | (mm ³) | (g) |
| Temper moisture (%) ^b | | | | | |
| 7.7 | 8.7d | 6.0b | 4.7e | 244c | 19.7 ns |
| 10 | 8.9dc | 6.0b | 4.7de | 251c | 19.2 ns |
| 20 | 8.9dc | 6.1b | 4.9bc | 260bc | 19.9 ns |
| 30 | 9.0c | 6.1b | 5.0ab | 277bc | 19.6 ns |
| 40 | 9.5b | 6.4a | 5.1a | 308a | 19.2 ns |
| 50 | 10.2a | 6.6a | 4.8cd | 321a | 19.7 ns |
| Drying temperature (°C) ^c | | | | | |
| 20 | 9.1 ns | 6.2 ns | 4.8 ns | 272 ns | 19.8 ns |
| 90 | 9.3 ns | 6.3 ns | 4.9 ns | 283 ns | 19.3 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over temper moisture

^cValues averaged over drying temperature

ns – Not Significant

Tempered-Dried Seed Hardness

Tempered-dried seed pretreatment significantly affected bean fracture force (Table 8). The fracture force was the first major rupture point where the seed first cracks. As moisture content increased, the fracture force decreased, particularly between 20 and 30% temper moisture. Peak force had an interaction effect of tempering moisture and drying temperature on tempered-dried seeds (Table 9).

Table 8. Mean seed fracture force^a of tempered-dried seeds.

| | Fracture point (N) |
|--------------------|-----------------------|
| Tempered moisture | |
| (%) ^b | |
| 7.7 | 161a |
| 10 | 162a |
| 20 | 129b |
| 30 | 60c |
| 40 | 40c |
| 50 | 40c |
| Drying temperature | |
| (°C) ^c | |
| 20 | 105 ns |
| 90 | 92 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over temper moisture

^cValues averaged over drying temperature

ns – Not Significant

Table 9. Mean seed peak values^a for tempered seeds.

| Tempered moisture level | Peak Force (N) | |
|-------------------------|----------------|---------|
| | 20 °C | 90 °C |
| 7.7 | 190ab,A | 187a,A |
| 10 | 186bc,A | 187a,A |
| 20 | 165bcd,A | 125bc,A |
| 30 | 118d,A | 130b,A |
| 40 | 153cd,A | 94cd,B |
| 50 | 212a,A | 75d,B |

^aDifferent uppercase letters across rows indicates significant differences between drying temperature ($P=0.05$). Different lowercase letters across column indicates significant differences between temper moisture ($P=0.05$)

Frączek et al. (2005) stated that at low moisture content, the seed coat is “relatively hard and brittle, whereas at greater moisture contents it acts like an elastic membrane”. Based on morphological differences, bean seed coat, which possesses several layers of cells, absorbs water slower than the cotyledon or embryo (Frączek et al. 2005).

Effect of Cooking and Tempering Pretreatments on Milling Yield and Physical Flour Quality

Cooked-dried Pretreatment

Cooked-dried Burr Mill - Milling Yield

Cooked-dried pretreatment affected the separation of cotyledon and seed coat during burr milling (Table 10). The yield of these fractions was inversely proportional since as cotyledon rich fraction decreased, the seed coat rich fraction increased.

The cooking time by drying temperature interaction was significant for each milling fraction. Cooking for 20 min and drying at 90 °C treatment, resulted in the highest seed coat rich fraction among all the treatments (Figure 17). It had a 4.7% increment in seed coat rich fraction yield, compared to the control, which was non-treated. Dry bean seed consists of about 8% seed coat on a dry basis (Rahman 2007). The seed coat fraction was higher when dried at 90 °C than at ambient (22 °C) conditions. The separation of the seed coat from the cotyledon was not very efficient step. During aspiration, embryo and light cotyledon particles were moved into the seed coat fraction.

Cooking time by drying temperature was significant for seed coat fraction and particle size distribution values. Increasing the cooking time and drying at 90 °C increased the amount of particle size < 2.38 mm fraction. Cooking and drying at 20°C treatments did not have a clear relationship. By visual evaluation and comparing seed coat yields, it was apparent that, whatever the drying method, cooking improved seed coat removal.



A



B

Figure 17. (A) Seed coat and (B) Cotyledon from cooked-dried pretreatment after Burr mill

Table 10. Mean fractions (%) and cotyledon fraction particle size distribution values^a from cooked-dried seeds following burr milling.

| Cooking time | Seed Coat Fraction | | 2.83-6.73 mm | | < 2.38 mm | |
|--------------|--------------------|---------|--------------|---------|-----------|---------|
| | 20 °C | 90 °C | 20 °C | 90 °C | 20 °C | 90 °C |
| 0 | 0.9c,A | 0.9d,A | 99.2ab,A | 99.1a,A | 0.5ab,B | 0.6c,AB |
| 5 | 3.2a,B | 4.2c,AB | 98.8c,A | 98.8b,A | 0.6a,B | 0.9b,A |
| 10 | 2.9ab,B | 4.8b,A | 99.0bc,A | 98.6b,A | 0.6ab,B | 0.9b,A |
| 20 | 2.6b,B | 5.6a,A | 99.4a,A | 98.5b,A | 0.4b,B | 1.1a,A |

^aDifferent uppercase letters across rows indicates significant differences between drying temperature ($P=0.05$). Different lowercase letters across column indicates significant differences between cooking time ($P=0.05$)

Cooked-dried Roller Mill - Milling Yield

Cooked-dried pretreatment was not significant for further seed coat removal in the roller mill (Table 11). Also the seed coat fraction was highly contaminated with light cotyledon particles.

Table 11. Mean fractions (%) and cotyledon fraction particle size distribution values^a from cooked-dried seeds following roller milling.

| | > 1.19 mm | Light fraction | Heavy fraction |
|--------------------------------------|-----------|----------------|----------------|
| <hr/> | | | |
| Cooking time (min) ^b | | | |
| 0 | 57.0c | 8.7 ns | 91.3 ns |
| 5 | 56.9c | 9.7 ns | 90.3 ns |
| 10 | 59.2b | 9.6 ns | 90.4 ns |
| 20 | 61.6a | 9.7 ns | 90.3 ns |
| Drying temperature (°C) ^c | | | |
| 20 | 60.9a | 12.6a | 87.4b |
| 90 | 56.4b | 6.2b | 93.8a |

^aValues followed by same letter are not significantly different at $P=0.05$.

^b Values averaged over cooking time

^c Values averaged over drying temperature

ns – Not Significant

Cooked-Dried Color

Cooked-dried seed pretreatments significantly affected L-value and color difference (Table 12). Cooked-dried pretreatments decreased L-value for the intact seed and whole bean flour. The L-value of cotyledon flour was not affected by the cooked-dried pretreatments. The most significant color difference (ΔE^*ab) of intact seed, whole bean flour and cotyledon flour was between the flours of the 5 min cooking and control. Greatest reduction in lightness, and highest color difference was observed in the 5 min cooked seeds and respective flours. Drying temperatures did not affect the L-value or color differences of whole bean flour and cotyledon flour. However, L-value was greater for intact beans dried at 90°C than at 20°C.

Table 12. Mean L values^a of intact seed, whole bean flour and cotyledon flour from cooked-dried seeds.

| | Intact Seed | | Whole bean flour | | Cotyledon flour | |
|--------------------------------------|-------------|----------------|------------------|----------------|-----------------|----------------|
| | L | ΔE^*ab | L | ΔE^*ab | L | ΔE^*ab |
| Cooking time (min) ^b | | | | | | |
| 0 | 26.21a | 0.00b | 80.05a | 0.00b | 82.68 ns | 0.00b |
| 5 | 22.71b | 3.86a | 77.00c | 3.40a | 82.80 ns | 2.33a |
| 10 | 23.42b | 3.44a | 79.12ab | 1.50ab | 83.74 ns | 1.85a |
| 20 | 24.08b | 3.54a | 77.4bc | 2.68a | 82.88 ns | 1.35a |
| Drying temperature (°C) ^c | | | | | | |
| 20 | 22.99b | 3.15 ns | 78.38 ns | 1.72 ns | 82.83 ns | 1.30 ns |
| 90 | 25.21a | 2.27 ns | 78.39 ns | 2.07 ns | 83.22 ns | 1.47 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over cooking time

^cValues averaged over drying temperature

ns – Not Significant

The L-value for flour color is commonly used to measure the degree of lightness (Siddiq et al., 2013). It was expected that lightness of intact seed and whole seed flour fractions would decrease, as cooking time increased. But in this study, 5 min cooking time had the lowest lightness. It was observed that during cooking of black beans, soluble color compounds, anthocyanins, leached into the cooking water medium and stained the seeds. Flour color is an important quality factor because it transfers to the final product and defines its acceptability, marketability and freshness. Cooking treatment greatly affected the color difference (ΔE^*ab) due to leaching of the seed coat color.

Tempered-Dried Pretreatment

Tempered-dried Burr Mill - Milling Yield

Tempering moisture level by drying temperature interaction was significant for seed coat yield (Table 13). Tempering to 40% moisture content and drying at 90 °C treatment, resulted in the highest seed coat rich fraction (7.4%) among all the treatments (Figure 18). It had a 6.3% increment in seed coat rich fraction yield, compared to the non-treated control. At each tempering moisture, the seed coat fraction was higher when dried at 90 than at 20 °C. The separation of the seed coat from the cotyledon was not very efficient step, where an aspirator was used. In that separation process, air blowing could have separated the embryo parts and light cotyledon particles into the seed coat fraction.

Temper moisture level by drying temperature interaction was significant for particle size distribution of cotyledon fraction (Table 13). At the 30% temper moisture level, particle size < 2.38 mm fraction resulted in the highest yield at both the drying temperatures. Other cotyledon fraction particle size distribution values did not have a clear relationship with temper moisture level and drying temperature. In both drying temperatures, tempering made the seed coat removal easier compared to the control. Also, visual evaluation and seed coat yield comparison showed that tempered-dried pretreatment was more effective than the cooked-dried pretreatment.



A

B

Figure 18. (A) Seed coat and (B) Cotyledon from tempered-dried pretreatment after burr mill

Table 13. Mean seed coat fraction (%) and cotyledon fraction particle size distribution values^a from tempered-dried seeds following burr milling.

| Temper moisture level | Seed Coat Fraction | | 2.83- 6.73 mm | | < 2.38 mm | |
|--------------------------|--------------------|--------|---------------|----------|-----------|--------|
| | 20 °C | 90 °C | 20 °C | 90 °C | 20 °C | 90 °C |
| 7.7 | 1.0e,A | 1.1c,A | 99.2a,A | 99.2a,A | 0.5b,A | 0.5c,A |
| 10 | 1.3de,A | 1.6c,A | 98.8ab,A | 98.9ab,A | 0.7ab,A | 0.7c,A |
| 20 | 2.4bc,AB | 3.6b,A | 98.6bc,A | 96.0d,AB | 0.8a,AB | 1.4b,A |
| 30 | 3.2ab,B | 6.8a,A | 98.3c,A | 96.6c,AB | 0.9a,B | 1.8a,A |
| 40 | 3.3a,B | 7.4a,A | 98.4bc,A | 98.5b,A | 0.9a,AB | 1.2b,A |
| 50 | 2.1cd,B | 7.4a,A | 98.6bc,A | 98.5b,A | 0.7a,AB | 1.1b,A |

^aDifferent uppercase letters across rows indicates significant differences between drying temperature ($P=0.05$). Different lowercase letters across column indicates significant differences between temper moisture ($P=0.05$)

Tempered-dried Roller Mill - Milling Yield

Seed coat extraction was greatest and cleanest with beans tempered to 40% moisture and then dried at 90 °C, in the burr mill. Roller mill tended to extract more contaminated seed coat with cotyledon (Table 14).

Seed coat removal efficiency is important to ensure the economic potential of intact seed coat and cotyledon fractions. Seed coat fraction represents an opportunity for an intact and clean source of dietary fiber, which has been shown to provide physiological benefits such as the

reduction of glycemic index (Hangen and Bennink 2002, Han et al. 2004). The seed coat removal from cotyledons contribute to reduced tannin content, better appearance, texture, palatability and digestibility for the cotyledon flour (Deshpande et al. 1982; Ehiwe and Reichert 1987, Towo et al. 2003).

Table 14. Mean cotyledon fraction particle size distribution values^a (%) from tempered-dried seeds following roller milling.

| | > 1.19 mm | Light fraction | Heavy fraction |
|--------------------------------------|-----------|----------------|----------------|
| | (%) | | |
| Cooking time (min) ^b | | | |
| 7.7 | 58.6c | 11.7 ns | 88.3 ns |
| 10 | 58.5c | 11.0 ns | 89.0 ns |
| 20 | 59.3bc | 11.7 ns | 88.3 ns |
| 30 | 60.2abc | 10.9 ns | 89.1 ns |
| 40 | 60.3ab | 10.8 ns | 89.2 ns |
| 50 | 61.2a | 11.7 ns | 88.3 ns |
| Drying temperature (°C) ^c | | | |
| 20 | 59.2 ns | 11.7 ns | 88.3 ns |
| 90 | 60.2 ns | 11.0ns | 89.1 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^b Values averaged over cooking time

^c Values averaged over drying temperature

ns – Not Significant

Tempered-Dried Color

Tempered pretreatment significantly affected L-value and color difference (Table 15). In general, L values decreased compared to control, for whole seeds and whole bean flour, when moisture content increased from 7.7 to 50%. Highest L value for cotyledon flour was obtained in the 30% moisture pretreatment. Color of flour compared to whole seeds, had the highest L-value or lightness due to finer particle size. It was expected that lightness of the cotyledon flour fractions are higher in temper moisture levels where seed coat removal was easy. Drying

temperatures did not affect the L-value and color differences of whole seed, whole bean flour and cotyledon flour.

Table 15. Mean L values^a of whole seed, whole bean flour and cotyledon flour from tempered-dried seeds.

| | Whole Seed | | Whole bean flour | | Cotyledon flour | |
|--------------------------------------|------------|---------|------------------|---------|-----------------|---------|
| | L | ΔE*ab | L | ΔE*ab | L | ΔE*ab |
| Tempered moisture (%) ^b | | | | | | |
| 7.7 | 24.65a | 0.00d | 79.65a | 0.00d | 83.07b | 0.00d |
| 10 | 22.00b | 2.87c | 79.59a | 0.53d | 83.48b | 0.64cd |
| 20 | 20.76cd | 3.96ab | 78.18b | 1.66c | 83.50b | 0.82c |
| 30 | 21.21bc | 3.54bc | 77.86b | 2.02c | 84.56a | 2.02b |
| 40 | 20.93cd | 3.80ab | 76.45c | 3.57b | 83.44b | 3.26a |
| 50 | 20.26d | 4.47a | 75.39d | 4.70a | 81.78c | 2.42b |
| Drying temperature (°C) ^c | | | | | | |
| 20 | 21.46 ns | 3.13 ns | 78.34 ns | 2.14 ns | 82.76 ns | 1.12 ns |
| 90 | 21.81 ns | 3.08 ns | 77.37 ns | 2.03 ns | 83.83 ns | 1.93 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over temper moisture

^cValues averaged over drying temperature

ns – Not Significant

Effect of Cooking and Tempering Pretreatments on Flour Chemical and Functional Properties

Chemical Composition of Flour

Cooked-Dried Whole Bean Flour

Cooked-dried seed pretreatment affected starch damage and ash content (Table 16). Cooking followed by drying of the seeds resulted in a significant ($P=0.05$) increase in starch damage and a decrease in ash content ($P=0.05$) as cooking time increased (Table 16). Cooked-dried pretreatment did not affect total starch, lipid and protein contents. Wang et al. (2010) reported a small increase in total starch from 38.8 to 39.1 g/g dry matter for raw and cooked black beans. Eyarua et al. (2009) reported total starch of nonsoaked-cooked red kidney beans to

be higher than total starch of raw beans. They suggested that starch gelatinization and dispersion of starch molecules made them more susceptible and accessible to starch hydrolyzing enzymes attack; hence, higher total starch was reported. As there is no significant different among cooking times in total starch values, it suggests that cooking loss is due to the soluble oligosaccharides (Table 2).

Table 16. Mean chemical composition^a of whole bean flour from cooked-dried seeds.

| | Moisture | Ash | Protein | Lipid | Starch | Starch Damage |
|--------------------------------------|----------|---------|---------|--------|---------|---------------|
| | (%) | | | | | |
| Cooking time (min) ^b | | | | | | |
| 0 | 6.4 ns | 4.08a | 21.8 ns | 1.9 ns | 40.6 ns | 0.4d |
| 5 | 5.9 ns | 3.90b | 21.3 ns | 1.7 ns | 42.3 ns | 1.8c |
| 10 | 6.3 ns | 3.74c | 21.5ns | ND | 40.7 ns | 2.9b |
| 20 | 6.3 ns | 3.35d | 21.5 ns | 1.6 ns | 41.6 ns | 6.2a |
| Drying temperature (°C) ^c | | | | | | |
| 20 | 6.3 ns | 3.77 ns | 21.4 ns | 1.7 ns | 41.5 ns | 3.2 ns |
| 90 | 6.1 ns | 3.76 ns | 21.7 ns | 1.6 ns | 41.1 ns | 2.4 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over cooking time

^cValues averaged over drying temperature

ns – Not Significant

ND- Not Determined

Starch damage (Table 16) increased with cooking time from 0.4 to 6.2 %. During cooking, starch granules gelatinized and lost their crystalline structure. Starch granules also swelled, which would allow amylose to leach into the cooking water. These factors might have contributed to an increase in starch damaged for all cooked beans. Similar results were reported by Ovando-Martinez et al. (2011) where cooked black beans had 1-4% higher starch damage than raw beans, which is in agreement with this study starch damage values after cooking. Cooking would cause starch damage due to gelatinization process whereby starch granules swell and disrupt.

Ash content significantly decreased as cooking times increased (Table 16). Flour from 20 min cooked-dried seed pretreatment had the lowest ash content, which decreased ca. 18% when compared to flour from non-treated seeds. Ash content after cooking dry bean seeds are related to loss of minerals during cooking. Kon (1979), Wang et al. (2008 and 2010), and Siddiq and Uebersax (2013) reported that cell membrane permeability increased during thermal processing, which allowed minerals and other small molecules to diffuse from seeds into the cooking water.

Cooking pretreatments did not affect protein content (Table 16). Protein content values ranged from 21.3 to 21.8%. Similar protein content for raw black bean was reported by Siddiq et al. (2010). Kon (1979) also suggested that by thermal processing, proteins were denatured and rendered insoluble so that leaching could not occur. Similarly, lipid content was not affected significantly by cooking pretreatments (Table 16). Lipid content values ranged from 1.6 to 1.9%. Drying temperature did not significantly affect the chemical composition of black beans.

Cooked-Dried Cotyledon Flour

Cooked-dried seed pretreatment affected ash and protein of cotyledon flour (Table 17). Cooked-dried pretreatment resulted in a ($P=0.05$) decrease in ash content as cooking time was increased (Table 17). Total starch and lipid content was not significantly affected by the cooked-dried pretreatment. Cooking time by drying temperature interaction was significant for starch damage (Table 18). Starch damage increased with cooking time. With 5 min cooking time, drying temperature did not affect starch damage; however, with 10 and 20 min of cooking, starch damage was greater when cooked beans were dried at 20 than 90°C. The lower apparent starch damage with 90°C than 20°C is attributed to the formation of amylase resistant starch associated with high temperature drying. Resistant starch can be determined according to AACC method 32-40.01 by using the Megazyme assay kit.

Table 17. Mean chemical composition^a of cotyledon flour from cooked-dried seeds.

| | Moisture | Ash | Protein (%) | Lipid | Starch |
|---|----------|---------|----------------|--------|---------|
| Cooking time (min) ^b | | | | | |
| 0 | 6.7b | 4.03a | 22.2b | 3.1 ns | 41.1 ns |
| 5 | 6.6b | 3.83b | 23.2a | 2.9 ns | 43.7 ns |
| 10 | 6.7b | 3.66c | 22.9a | ND | 43.2 ns |
| 20 | 7.0a | 3.32d | 23.2a | 2.7 ns | 43.8 ns |
| Drying temperature (°C) ^c | | | | | |
| 20 | 7.0 ns | 3.73 ns | 22.9 ns | 2.9 ns | 43.0 ns |
| 90 | 6.5 ns | 3.69 ns | 22.9 ns | 2.6 ns | 42.9 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over cooking time

^cValues averaged over drying temperature

ns – Not Significant

ND- Not Determined

Table 18. Mean starch damage (%)^a of cotyledon flour from cooked-dried seeds.

| Cooking time | Starch Damage (%) | |
|--------------|-------------------|--------|
| | 20 °C | 90 °C |
| 0 | 0.5d,A | 0.5c,A |
| 5 | 2.1c,A | 1.9b,A |
| 10 | 3.7b,A | 2.5b,B |
| 20 | 8.1a,A | 5.0a,B |

^aDifferent uppercase letters across rows indicates significant differences between drying temperature ($P=0.05$). Different lowercase letters across column indicates significant differences between cooking time ($P=0.05$)

Tempered-Dried Whole Bean Flour

Tempered-dried pretreatments had little or no effect on total starch content, starch damage, protein content, and ash content (Table 19). Drying temperature did not significantly affect the chemical composition of black beans.

Table 19. Mean chemical composition^a of whole bean flour from tempered-dried seeds.

| | Ash | Protein | Lipid | Starch | Starch Damage |
|--------------------------------------|---------|---------|--------|---------|---------------|
| | (%) | | | | |
| Tempered moisture (%) ^b | | | | | |
| 7.7 | 4.00 ns | 21.5 ns | 1.9 ns | 39.0c | 0.4b |
| 10 | 4.06 ns | 21.5 ns | ND | 39.3bc | 0.4b |
| 20 | 4.08 ns | 21.5 ns | ND | 40.9a | 0.4b |
| 30 | 4.06 ns | 21.6 ns | 1.8 ns | 41.6a | 0.6a |
| 40 | 4.43 ns | 21.4 ns | ND | 41.7a | 0.6a |
| 50 | 4.19 ns | 21.4 ns | 1.8 ns | 40.6ab | 0.6a |
| Drying temperature (°C) ^c | | | | | |
| 20 | 4.09 ns | 21.6 ns | 1.8 ns | 40.2 ns | 0.5 ns |
| 90 | 4.19 ns | 21.4 ns | 1.7 ns | 40.8 ns | 0.5 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over temper moisture

^cValues averaged over drying temperature

ns – Not Significant

ND – Not Determined

Tempered-Dried Cotyledon Flour

Temper moisture content and drying temperature pretreatments had little or no effect on starch, lipid, protein and ash contents (Table 20). Starch values were greater in 30-50% temper moisture levels on weight basis as they have given the highest seed coat extraction. Tempered moisture level by drying temperature interaction was significant for starch damage (Table 21). Except for 7.7% moisture, starch damage was greater when dried at 90 than 20°C. Starch damage did increase as tempered moisture level increased.

Table 20. Mean chemical composition^a of cotyledon flour from tempered-dried seeds.

| | Ash | Protein | Lipid | Starch |
|--------------------------------------|---------|---------|--------|---------|
| | (%) | | | |
| Tempered moisture (%) ^b | | | | |
| 7.7 | 4.02 ns | 22.3 ns | 3.1 ns | 41.6b |
| 10 | 4.05 ns | 22.5 ns | ND | 41.0b |
| 20 | 4.03 ns | 22.6 ns | ND | 41.8b |
| 30 | 4.00 ns | 22.5 ns | 3.0 ns | 42.2b |
| 40 | 4.05 ns | 22.7 ns | ND | 44.0a |
| 50 | 4.15 ns | 23.2 ns | 3.0 ns | 42.7ab |
| Drying temperature (°C) ^c | | | | |
| 20 | 4.05 ns | 22.5 ns | 3.1 ns | 41.9 ns |
| 90 | 4.06 ns | 22.7 ns | 3.0 ns | 42.5 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over temper moisture

^cValues averaged over drying temperature

ns – Not Significant

ND – Not Determined

Table 21. Mean starch damage^a of cotyledon flour from tempered-dried seeds.

| Temper moisture level | Starch Damage | |
|-----------------------|---------------|--------|
| | 20 °C | 90 °C |
| 7.7 | 0.5b,A | 0.5a,A |
| 10 | 0.4a,B | 0.5a,A |
| 20 | 0.5b,B | 0.6b,A |
| 30 | 0.6c,B | 0.8c,A |
| 40 | 0.6c,B | 1.0e,A |
| 50 | 0.7d,B | 0.9d,A |

^aDifferent uppercase letters across rows indicates significant differences between drying temperature ($P=0.05$). Different lowercase letters across column indicates significant differences between temper moisture ($P=0.05$)

Pasting Properties of Flour

Cooked-Dried Whole Bean Flour

Pasting properties are useful to predict functional behavior of starch during heating and cooling while processing (Bello-Pérez and Paredes-López 2009). Granule swelling, amylose

leaching, starch crystallinity, amylose content and amylopectin chain influence pasting properties (Chung et al. 2008).

Table 22. Mean pasting properties^a of whole bean flour from cooked-dried seeds.

| | Peak | Trough | Breakdown | Final Viscosity | Setback |
|--------------------------------------|-------|--------|-----------|--------------------|---------|
| | (RVU) | | | | |
| Cooking time (min) ^b | | | | | |
| 0 | 90a | 89a | 1.9b | 138a | 49c |
| 5 | 63b | 60b | 3.4a | 130a | 70a |
| 10 | 49c | 45c | 4.1a | 107b | 61b |
| 20 | 13d | 11d | 1.9b | 33c | 22d |
| Drying temperature (°C) ^c | | | | | |
| 20 | 53 ns | 51 ns | 2.6 ns | 98 ns | 48 ns |
| 90 | 55 ns | 52 ns | 3.0 ns | 105 ns | 53 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^bValues averaged over cooking time

^cValues averaged over drying temperature

ns – Not Significant

Drying temperature did not affect pasting properties of cooked dry beans (Table 22).

Pasting properties of whole bean flour were affected by cooked-dried seed pretreatment of black bean (Table 22). In general, cooked-dried pretreatment decreased all pasting properties as cooking times increased. The peak viscosity values decreased from 90 to 13RVU as cooking time increased from 0 to 20 min. Similarly, trough and final viscosity values decreased from 89 to 11 RVU and 138 to 33 RVU, respectively over the 20 minute cook. Set back value was highest with 5 min cooking time.

Final viscosities decreased significantly from 138 to 33 RVU, which indicates a tendency to form a weak gel after cooling. Trough viscosity decreased from 89 to 11 RVU. Trough viscosity is influenced by the rate of amylose exudation, granule swelling and amylose-lipid complex formation (Wani et al. 2012). Breakdown viscosity is a measure of the ease with which the swollen starch granules can be disintegrated. As starch granules were disintegrated by high

temperature cooking, the breakdown viscosity was lowest for 20 min cooking treatment and since some starch granules were already broken as seen by starch damage data. Setback viscosity decreased and it has been reported that low setback values at high cooking times reflect low retrogradation, hence low tendency of flour to form a gel during cooling (Wani et al. 2012).

Cooked-Dried Cotyledon Flour

Pasting properties of black bean cotyledon flour were affected by cooked-dried seed pretreatment (Table 23). Peak viscosity and trough values decreased as cooking time increased. The peak values decreased from 95 to 24 RVU as cooking time increased from 0 to 20 min. Similarly, trough values decreased from 91 to 21 RVU. Set back value was highest with 5 min cooking time. Breakdown values were not affected by the cooking time. The drying temperature did not affect any of the pasting properties.

Table 23. Mean pasting properties^a of cotyledon flour from cooked-dried seeds.

| | Peak | Trough | Breakdown | Final Viscosity | Setback |
|--------------------------------------|-------|--------|-----------|-----------------|---------|
| | (RVU) | | | | |
| Cooking time (min) ^b | | | | | |
| 0 | 95a | 91a | 3.8 ns | 146ab | 55c |
| 5 | 82a | 78a | 4.3 ns | 164a | 86a |
| 10 | 64b | 59b | 4.5 ns | 131b | 71b |
| 20 | 24c | 21c | 3.0 ns | 50c | 29d |
| Drying temperature (°C) ^c | | | | | |
| 20 | 67 ns | 63 ns | 4.2 ns | 121 ns | 58 ns |
| 90 | 66 ns | 62 ns | 3.6 ns | 124 ns | 62 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

ns – Not Significant

^b Values averaged over cooking time

^c Values averaged over drying temperature

Breakdown values were low, which indicated the presence of restriction in starch granules swelling and high amylose content (27-39%) (Hoover et al. 2010). High setback values

reflect a high level of retrogradation (Kim et al. 1997) as was for 5 and 10 min cooked beans. Final viscosities decreased as cooking time increased from 0 to 20 min, and suggest a decreasing capacity of the flour to retrograde and form a strong gel. The low peak viscosity and low breakdown could be due to weakening of the starch by protein-lipid-fiber interactions (Chung et al. 2008). Peak viscosity has been reported to be influenced by amylose content, properties of amylopectin chain length, and phosphorous content (Chung et al. 2008). Long cooking times attributed to probably high amylose leaching out. Thus, declined in peak viscosities were possibly influenced by differences in amylose content.

Black bean starch is found in protein matrix inside the cotyledon. Cooking denatures protein, which can restrict starch granule swelling. Cooking can gelatinize some starch which will retrograde upon cooling. Pasting properties of pregelatinized starch would have lower viscosity and lower breakdown values.

Tempered-Dried Whole Bean Flour

Temper moisture content main effect was significant for the pasting properties of whole bean flour (Table 24). For peak, trough and final viscosity lowest values were observed when tempered to 40% moisture content. Flour sample from seed tempered to 10% moisture content indicated greatest peak viscosity, which is indicative of high water binding capacity of starch. Low breakdown values obtained for all flours suggests tendency to form a weak gel after cooling. Final viscosity values were greatest for flour from seed tempered to 30% moisture content (146 RVU), which indicates good stability of the cooked paste. Schoch and Maywald (1968) suggested that bean starch presented restricted swelling power. They showed a decrease in swelling and solubilization, and stabilization of swollen granule against mechanical shearing. They also showed curves with no pasting peak rather with very high viscosity, which remained

constant or else increased during cooking. Low setback values have a low tendency to retrograde. While flour from black bean seeds tempered to 30% moisture content recorded the highest setback value (65 RVU) while flour from seeds at 10% moisture content had the lowest (48 RVU) setback value (Table 24). Drying temperature did not significantly affect the pasting properties of whole black bean flour (Table 24).

Table 24. Mean pasting properties^a of whole bean flour from tempered-dried seeds.

| | Peak | Trough | Breakdown | Final Viscosity | Setback |
|--------------------------------------|-------|--------|-----------|-----------------|---------|
| | (RVU) | | | | |
| Tempered moisture (%) ^b | | | | | |
| 7.7 | 91a | 89a | 2.0b | 138ab | 49c |
| 10 | 96a | 94a | 1.4b | 143a | 48c |
| 20 | 92a | 89a | 2.7ab | 145a | 56b |
| 30 | 85b | 81b | 4.3a | 146a | 65a |
| 40 | 77c | 73c | 4.3a | 131b | 59b |
| 50 | 85b | 81b | 4.4a | 142a | 61ab |
| Drying temperature (°C) ^c | | | | | |
| 20 | 89ns | 86 ns | 3.5 ns | 138 ns | 52 ns |
| 90 | 86ns | 83ns | 2.9 ns | 144 ns | 60 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^b Values averaged over temper moisture

^c Values averaged over drying temperature

ns – Not Significant

Tempered-Dried Cotyledon Flour

Temper moisture content main effect was significant for some pasting properties of cotyledon bean flour (Table 25). Temper moisture content had little or no effect on pasting properties of cotyledon black bean flour (Table 25). Similarly, drying temperature did not affect pasting properties of cotyledon flour. Cotyledon flour pasting values were slightly higher than whole bean flour pasting values.

Table 25. Mean pasting properties^a of cotyledon flour from tempered-dried seeds.

| | Peak | Trough | Breakdown (RVU) | Final Viscosity | Setback |
|--------------------------------------|--------|----------|--------------------|--------------------|---------|
| Tempered moisture (%) ^b | | | | | |
| 7.7 | 99 ns | 94ab | 4.7bc | 151d | 57c |
| 10 | 102 ns | 99a | 2.9c | 155cd | 56c |
| 20 | 95ns | 90b | 5.1ab | 163bc | 73b |
| 30 | 94 ns | 89b | 5.0b | 171a | 82a |
| 40 | 96 ns | 92b | 4.5bc | 166ab | 74b |
| 50 | 97 ns | 90b | 7.0a | 162bc | 73b |
| Drying temperature (°C) ^c | | | | | |
| 20 | 97 ns | 90.91 ns | 5.8 ns | 154 ns | 63 ns |
| 90 | 98 ns | 93.70 ns | 3.9 ns | 168 ns | 75 ns |

^aValues followed by same letter are not significantly different at $P=0.05$.

^b Values averaged over temper moisture

^c Values averaged over drying temperature

ns – Not Significant

CONCLUSIONS

Dry beans are divided into eleven market classes in the US according to their color, size and shape. They are a low cost source of protein and important source of carbohydrates, dietary fiber, certain minerals and vitamins in the human diet. Dry bean utilization by the food industry can be increased by developing value-added processing applications. Separation of seed coat from the cotyledon can expand the use of bean flour in food product applications by creating different food ingredients such as cotyledon flour and seed coat flour.

In this research, the chemical and physical changes in the whole bean flour and cotyledon flour were less for tempered-dried pretreatment than for cooked-dried pretreatments, which greatly impacted flour chemical composition as well as pasting properties. Pretreatments impacted the seed coat removal of black beans with the burr mill/roller mill system. Tempered-dried pretreatment made the seed coat removal easier, compared to cooked-dried pretreatment. Tempered to 30%, 40%, or 50% and dried at 90°C resulted in the cleanest and greatest seed coat yield. Aspiration system used was not effective enough to separate light cotyledon particles from the embryo particles, which were removed along with the seed coat fraction. An improve air-classification system should be used to increase seed coat removal efficiency. The burr mill only generated high yields of the clean seed coat fraction, when the seeds were subjected to appropriate pretreatment. Separated seed coat can be used to fulfill the higher dietary fiber requirement in food applications while cotyledon flour can be used with improved functional qualities and health benefits.

FUTURE RESEARCH AND APPLICATIONS

Future research needs to be done to develop a standard milling procedure for other dry bean market classes. Research can be conducted on different mill settings and on the effect of bean flour particle size distribution and flour physical quality, chemical composition, and functionality. It is necessary to evaluate the antinutrient levels after different pretreatments. The future research should include an integrated process from milling to final product utilization. More studies need to prevent some of the negative qualities of dry bean flour in food applications. Further studies can be done in black bean and other market classes' milled fractions to produce wide range of food and non-food applications.

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APPENDIX

Table A1. Analysis of variance for effect of boiling time and drying temperature on black bean grain quality.

| Variable | Source | df | MS | F value | Pr>F |
|----------|-------------------------|----|--------|---------|--------|
| L | Rep | 2 | 0.728 | 0.27 | 0.7658 |
| | Drying Temperature (DT) | 1 | 29.526 | 57.64 | 0.0169 |
| | Error a (DT*Rep) | 2 | 0.512 | 0.19 | 0.8277 |
| | Boiling Time (BT) | 3 | 13.666 | 5.13 | 0.0164 |
| | DT*BT | 3 | 1.246 | 0.47 | 0.7106 |
| | Error b | 12 | 2.666 | | |
| a | Rep | 2 | 0.559 | 2.03 | 0.1744 |
| | Drying Temperature (DT) | 1 | 0.499 | 3.66 | 0.1957 |
| | Error a (DT*Rep) | 2 | 0.136 | 0.49 | 0.6221 |
| | Boiling Time (BT) | 3 | 4.333 | 15.72 | 0.0002 |
| | DT*BT | 3 | 0.132 | 0.48 | 0.7027 |
| | Error b | 12 | 0.276 | | |
| b | Rep | 2 | 1.972 | 8.28 | 0.0055 |
| | Drying Temperature (DT) | 1 | 1.238 | 1.17 | 0.3925 |
| | Error a (DT*Rep) | 2 | 1.058 | 4.44 | 0.0359 |
| | Boiling Time (BT) | 3 | 0.558 | 2.34 | 0.1247 |
| | DT*BT | 3 | 0.126 | 0.53 | 0.6702 |
| | Error b | 12 | 0.238 | | |
| 100-KWT | Rep | 2 | 0.287 | 1.66 | 0.2309 |
| | Drying Temperature (DT) | 1 | 0.238 | 0.46 | 0.5659 |
| | Error a (DT*Rep) | 2 | 0.513 | 2.96 | 0.0901 |
| | Boiling Time (BT) | 3 | 0.419 | 2.42 | 0.1168 |
| | DT*BT | 3 | 0.141 | 0.81 | 0.5116 |
| | Error b | 12 | 0.173 | | |

Table A1. Analysis of variance for effect of boiling time and drying temperature on black bean grain quality (continued).

| Variable | Source | df | MS | F value | Pr>F |
|-----------------|-------------------------|-----------|-----------|----------------|----------------|
| Length | Rep | 2 | 0.741 | 8.95 | 0.0042 |
| | Drying Temperature (DT) | 1 | 1.893 | 23.00 | 0.0408 |
| | Error a (DT*Rep) | 2 | 0.082 | 0.99 | 0.3984 |
| | Boiling Time (BT) | 3 | 2.142 | 25.88 | <.0001 |
| | DT*BT | 3 | 0.248 | 2.99 | 0.0731 |
| | Error b | 12 | 0.083 | | |
| Width | Rep | 2 | 0.132 | 5.46 | 0.0206 |
| | Drying Temperature (DT) | 1 | 0.100 | 4.49 | 0.1684 |
| | Error a (DT*Rep) | 2 | 0.022 | 0.92 | 0.4241 |
| | Boiling Time (BT) | 3 | 0.138 | 5.70 | 0.0116 |
| | DT*BT | 3 | 0.024 | 1.00 | 0.4270 |
| | Error b | 12 | 0.024 | | |
| Thickness | Rep | 2 | 0.087 | 9.41 | 0.0035 |
| | Drying Temperature (DT) | 1 | 0.308 | 159.79 | 0.0062 |
| | Error a (DT*Rep) | 2 | 0.002 | 0.21 | 0.8146 |
| | Boiling Time (BT) | 3 | 0.106 | 11.49 | 0.0008 |
| | DT*BT | 3 | 0.018 | 1.90 | 0.1840 |
| | Error b | 12 | 0.009 | | |
| Volume | Rep | 2 | 2931.478 | 10.55 | 0.0023 |
| | Drying Temperature (DT) | 1 | 8024.555 | 77.51 | 0.0127 |
| | Error a (DT*Rep) | 2 | 103.531 | 0.37 | 0.6967 |
| | Boiling Time (BT) | 3 | 5927.037 | 21.33 | <.0001 |
| | DT*BT | 3 | 893.390 | 3.21 | 0.0616 |
| | Error b | 12 | 277.912 | | |

Table A2. Analysis of variance for effect of boiling time and drying temperature on black bean milling.

| Variable | Source | df | MS | F value | Pr>F |
|--------------------|-------------------------|----|-----------|---------|--------|
| Fracture point | Rep | 2 | 2741.542 | 7.73 | 0.0070 |
| | Drying Temperature (DT) | 1 | 287.042 | 0.46 | 0.5677 |
| | Error a (DT*Rep) | 2 | 624.542 | 1.76 | 0.2135 |
| | Boiling Time (BT) | 3 | 12072.153 | 34.03 | <.0001 |
| | DT*BT | 3 | 1100.931 | 3.10 | 0.0671 |
| | Error b | 12 | 354.708 | | |
| Peak force | Rep | 2 | 2043.375 | 10.97 | 0.0020 |
| | Drying Temperature (DT) | 1 | 3504.167 | 103.70 | 0.0095 |
| | Error a (DT*Rep) | 2 | 33.792 | 0.18 | 0.8364 |
| | Boiling Time (BT) | 3 | 3182.833 | 17.08 | 0.0001 |
| | DT*BT | 3 | 2193.944 | 11.78 | 0.0007 |
| | Error b | 12 | 186.306 | | |
| Whole bean flour L | Rep | 2 | 4.940 | 1.73 | 0.2193 |
| | Drying Temperature (DT) | 1 | 0.001 | 0.00 | 0.9864 |
| | Error a (DT*Rep) | 2 | 1.628 | 0.57 | 0.5806 |
| | Boiling Time (BT) | 3 | 12.597 | 4.40 | 0.0262 |
| | DT*BT | 3 | 3.216 | 1.12 | 0.3781 |
| | Error b | 12 | 2.861 | | |
| Whole bean flour a | Rep | 2 | 0.044 | 5.88 | 0.0166 |
| | Drying Temperature (DT) | 1 | 0.111 | 77.46 | 0.0127 |
| | Error a (DT*Rep) | 2 | 0.001 | 0.19 | 0.8281 |
| | Boiling Time (BT) | 3 | 0.105 | 14.12 | 0.0003 |
| | DT*BT | 3 | 0.031 | 4.12 | 0.0317 |
| | Error b | 12 | 0.007 | | |

Table A2. Analysis of variance for effect of boiling time and drying temperature on black bean milling (continued).

| Variable | Source | df | MS | F value | Pr>F |
|--------------------|-------------------------|----|-------|---------|--------|
| Whole bean flour b | Rep | 2 | 0.187 | 4.50 | 0.0348 |
| | Drying Temperature (DT) | 1 | 0.047 | 6.76 | 0.1216 |
| | Error a (DT*Rep) | 2 | 0.007 | 0.17 | 0.8485 |
| | Boiling Time (BT) | 3 | 1.158 | 27.82 | <.0001 |
| | DT*BT | 3 | 0.031 | 0.75 | 0.5442 |
| | Error b | 12 | 0.042 | | |
| Cotyledon flour L | Rep | 2 | 3.686 | 1.40 | 0.2849 |
| | Drying Temperature (DT) | 1 | 0.897 | 0.18 | 0.7095 |
| | Error a (DT*Rep) | 2 | 4.866 | 1.84 | 0.2003 |
| | Boiling Time (BT) | 3 | 1.411 | 0.53 | 0.6673 |
| | DT*BT | 3 | 2.867 | 1.09 | 0.3921 |
| | Error b | 12 | 2.639 | | |
| Cotyledon flour a | Rep | 2 | 0.124 | 3.97 | 0.0474 |
| | Drying Temperature (DT) | 1 | 0.051 | 0.95 | 0.4319 |
| | Error a (DT*Rep) | 2 | 0.054 | 1.73 | 0.2190 |
| | Boiling Time (BT) | 3 | 0.354 | 11.35 | 0.0008 |
| | DT*BT | 3 | 0.012 | 0.39 | 0.7640 |
| | Error b | 12 | 0.031 | | |
| Cotyledon flour b | Rep | 2 | 0.053 | 0.59 | 0.5695 |
| | Drying Temperature (DT) | 1 | 0.473 | 7.26 | 0.1146 |
| | Error a (DT*Rep) | 2 | 0.065 | 0.73 | 0.5019 |
| | Boiling Time (BT) | 3 | 0.064 | 0.72 | 0.5585 |
| | DT*BT | 3 | 0.270 | 3.03 | 0.0710 |
| | Error b | 12 | 0.089 | | |

Table A3. Analysis of variance for effect of boiling time and drying temperature on black bean chemistry.

| Variable | Source | df | MS | F value | Pr>F |
|---------------------------------|-------------------------|-----------|-----------|----------------|----------------|
| Whole bean flour moisture | Rep | 2 | 4.630 | 39.48 | <.0001 |
| | Drying Temperature (DT) | 1 | 0.403 | 11.09 | 0.0796 |
| | Error a (DT*Rep) | 2 | 0.036 | 0.31 | 0.7391 |
| | Boiling Time (BT) | 3 | 0.339 | 2.89 | 0.0796 |
| | DT*BT | 3 | 0.098 | 0.84 | 0.4990 |
| | Error b | 12 | 0.117 | | |
| Cotyledon flour moisture | Rep | 2 | 0.691 | 29.66 | <.0001 |
| | Drying Temperature (DT) | 1 | 1.038 | 9.90 | 0.0879 |
| | Error a (DT*Rep) | 2 | 0.105 | 4.49 | 0.0350 |
| | Boiling Time (BT) | 3 | 0.118 | 5.07 | 0.0170 |
| | DT*BT | 3 | 0.103 | 4.42 | 0.0259 |
| | Error b | 12 | 0.023 | | |
| Whole bean flour protein | Rep | 2 | 15.203 | 64.89 | <.0001 |
| | Drying Temperature (DT) | 1 | 0.667 | 3.36 | 0.2082 |
| | Error a (DT*Rep) | 2 | 0.198 | 0.85 | 0.4530 |
| | Boiling Time (BT) | 3 | 0.276 | 1.18 | 0.3586 |
| | DT*BT | 3 | 0.146 | 0.62 | 0.6132 |
| | Error b | 12 | 0.234 | | |
| Cotyledon flour protein | Rep | 2 | 21.065 | 169.97 | <.0001 |
| | Drying Temperature (DT) | 1 | 0.000 | 0.01 | 0.9228 |
| | Error a (DT*Rep) | 2 | 0.022 | 0.18 | 0.8378 |
| | Boiling Time (BT) | 3 | 1.310 | 10.57 | 0.0011 |
| | DT*BT | 3 | 0.118 | 0.95 | 0.4469 |
| | Error b | 12 | 0.124 | | |

Table A3. Analysis of variance for effect of boiling time and drying temperature on black bean chemistry (continued).

| Variable | Source | df | MS | F value | Pr>F |
|-------------------------------|-------------------------|----|--------|---------|--------|
| Seed coat flour protein | Rep | 2 | 9.460 | 181.00 | <.0001 |
| | Drying Temperature (DT) | 1 | 0.150 | 0.06 | 0.8317 |
| | Error a (DT*Rep) | 2 | 2.581 | 49.38 | <.0001 |
| | Boiling Time (BT) | 3 | 3.417 | 65.38 | <.0001 |
| | DT*BT | 3 | 0.474 | 9.06 | 0.0021 |
| | Error b | 12 | 0.052 | | |
| Whole bean flour ash | Rep | 2 | 0.007 | 0.64 | 0.5434 |
| | Drying Temperature (DT) | 1 | 0.000 | 0.04 | 0.8674 |
| | Error a (DT*Rep) | 2 | 0.007 | 0.67 | 0.5296 |
| | Boiling Time (BT) | 3 | 0.573 | 51.55 | <.0001 |
| | DT*BT | 3 | 0.000 | 0.03 | 0.9923 |
| | Error b | 12 | 0.011 | | |
| Cotyledon flour ash | Rep | 2 | 0.164 | 85.43 | <.0001 |
| | Drying Temperature (DT) | 1 | 0.009 | 3.60 | 0.1981 |
| | Error a (DT*Rep) | 2 | 0.003 | 1.33 | 0.3013 |
| | Boiling Time (BT) | 3 | 0.547 | 284.58 | <.0001 |
| | DT*BT | 3 | 0.002 | 0.92 | 0.4603 |
| | Error b | 12 | 0.002 | | |
| Whole bean flour starch | Rep | 2 | 26.384 | 8.87 | 0.0043 |
| | Drying Temperature (DT) | 1 | 0.592 | 0.29 | 0.6444 |
| | Error a (DT*Rep) | 2 | 2.046 | 0.69 | 0.5216 |
| | Boiling Time (BT) | 3 | 3.907 | 1.31 | 0.3156 |
| | DT*BT | 3 | 5.254 | 1.77 | 0.2071 |
| | Error b | 12 | 2.975 | | |
| Cotyledon flour starch | Rep | 2 | 7.674 | 2.55 | 0.1196 |
| | Drying Temperature (DT) | 1 | 0.115 | 0.02 | 0.9056 |
| | Error a (DT*Rep) | 2 | 6.384 | 2.12 | 0.1627 |
| | Boiling Time (BT) | 3 | 9.107 | 3.02 | 0.0714 |
| | DT*BT | 3 | 4.901 | 1.63 | 0.2350 |
| | Error b | 12 | 3.011 | | |

Table A3. Analysis of variance for effect of boiling time and drying temperature on black bean chemistry (continued).

| Variable | Source | df | MS | F value | Pr>F |
|---|-------------------------|-----------|-----------|----------------|----------------|
| Whole bean flour starch damage | Rep | 2 | 0.010 | 6.67 | 0.0113 |
| | Drying Temperature (DT) | 1 | 0.054 | 15.53 | 0.0588 |
| | Error a (DT*Rep) | 2 | 0.003 | 2.35 | 0.1378 |
| | Boiling Time (BT) | 3 | 0.417 | 280.87 | <.0001 |
| | DT*BT | 3 | 0.020 | 13.46 | 0.0004 |
| | Error b | 12 | 0.001 | | |
| Cotyledon flour starch damage | Rep | 2 | 0.006 | 1.64 | 0.2344 |
| | Drying Temperature (DT) | 1 | 0.073 | 19.30 | 0.0481 |
| | Error a (DT*Rep) | 2 | 0.004 | 1.02 | 0.3913 |
| | Boiling Time (BT) | 3 | 0.399 | 107.85 | <.0001 |
| | DT*BT | 3 | 0.032 | 8.69 | 0.0025 |
| | Error b | 12 | 0.004 | | |

Table A4. Analysis of variance for effect of boiling time and drying temperature on black bean pasting quality.

| Variable | Source | df | MS | F value | Pr>F |
|-------------------------------|-------------------------|-----------|-----------|----------------|----------------|
| Whole bean flour peak | Rep | 2 | 2389.486 | 29.37 | <.0001 |
| | Drying Temperature (DT) | 1 | 18.113 | 0.27 | 0.6570 |
| | Error a (DT*Rep) | 2 | 67.906 | 0.83 | 0.4577 |
| | Boiling Time (BT) | 3 | 6191.892 | 76.11 | <.0001 |
| | DT*BT | 3 | 40.400 | 0.50 | 0.6915 |
| | Error b | 12 | 81.356 | | |
| Whole bean flour trough | Rep | 2 | 2337.683 | 27.05 | <.0001 |
| | Drying Temperature (DT) | 1 | 10.895 | 0.15 | 0.7322 |
| | Error a (DT*Rep) | 2 | 70.505 | 0.82 | 0.4653 |
| | Boiling Time (BT) | 3 | 6183.461 | 71.55 | <.0001 |
| | DT*BT | 3 | 47.011 | 0.54 | 0.6614 |
| | Error b | 12 | 86.416 | | |
| Whole bean flour break | Rep | 2 | 0.682 | 0.88 | 0.4390 |
| | Drying Temperature (DT) | 1 | 0.905 | 6.50 | 0.1255 |
| | Error a (DT*Rep) | 2 | 0.139 | 0.18 | 0.8374 |
| | Boiling Time (BT) | 3 | 7.444 | 9.63 | 0.0016 |
| | DT*BT | 3 | 0.653 | 0.84 | 0.4954 |
| | Error b | 12 | 0.773 | | |
| Whole bean flour final | Rep | 2 | 5517.378 | 30.26 | <.0001 |
| | Drying Temperature (DT) | 1 | 289.259 | 1.71 | 0.3210 |
| | Error a (DT*Rep) | 2 | 169.066 | 0.93 | 0.4222 |
| | Boiling Time (BT) | 3 | 13700.121 | 75.15 | <.0001 |
| | DT*BT | 3 | 170.709 | 0.94 | 0.4533 |
| | Error b | 12 | 182.309 | | |

Table A4. Analysis of variance for effect of boiling time and drying temperature on black bean pasting quality (continued).

| Variable | Source | df | MS | F value | Pr>F |
|-------------------------------|-------------------------|----|----------|---------|--------|
| Whole bean flour set | Rep | 2 | 675.570 | 19.50 | 0.0002 |
| | Drying Temperature (DT) | 1 | 187.992 | 8.87 | 0.0967 |
| | Error a (DT*Rep) | 2 | 21.198 | 0.61 | 0.5584 |
| | Boiling Time (BT) | 3 | 2682.313 | 77.44 | <.0001 |
| | DT*BT | 3 | 45.099 | 1.30 | 0.3189 |
| | Error b | 12 | 34.638 | | |
| Cotyledon flour peak | Rep | 2 | 3206.591 | 23.53 | <.0001 |
| | Drying Temperature (DT) | 1 | 7.482 | 0.19 | 0.7080 |
| | Error a (DT*Rep) | 2 | 40.130 | 0.29 | 0.7501 |
| | Boiling Time (BT) | 3 | 5660.646 | 41.54 | <.0001 |
| | DT*BT | 3 | 10.390 | 0.08 | 0.9716 |
| | Error b | 12 | 136.270 | | |
| Cotyledon flour control | Rep | 2 | 2938.087 | 19.17 | 0.0002 |
| | Drying Temperature (DT) | 1 | 2.184 | 0.05 | 0.8451 |
| | Error a (DT*Rep) | 2 | 44.398 | 0.29 | 0.7536 |
| | Boiling Time (BT) | 3 | 5514.069 | 35.98 | <.0001 |
| | DT*BT | 3 | 5.643 | 0.04 | 0.9901 |
| | Error b | 12 | 153.261 | | |
| Cotyledon flour break | Rep | 2 | 6.244 | 5.36 | 0.0217 |
| | Drying Temperature (DT) | 1 | 1.576 | 5.45 | 0.1446 |
| | Error a (DT*Rep) | 2 | 0.289 | 0.25 | 0.7843 |
| | Boiling Time (BT) | 3 | 2.583 | 2.22 | 0.1389 |
| | DT*BT | 3 | 1.684 | 1.44 | 0.2786 |
| | Error b | 12 | 1.165 | | |

Table A4. Analysis of variance for effect of boiling time and drying temperature on black bean pasting quality (continued).

| Variable | Source | df | MS | F value | Pr>F |
|--------------------------|-------------------------|-----------|-----------|----------------|----------------|
| Cotyledon flour final | Rep | 2 | 7335.936 | 30.18 | <.0001 |
| | Drying Temperature (DT) | 1 | 61.248 | 0.33 | 0.6244 |
| | Error a (DT*Rep) | 2 | 186.459 | 0.77 | 0.4858 |
| | Boiling Time (BT) | 3 | 15085.626 | 62.06 | <.0001 |
| | DT*BT | 3 | 60.411 | 0.25 | 0.8608 |
| | Error b | 12 | 243.078 | | |
| Cotyledon flour set | Rep | 2 | 989.449 | 31.34 | <.0001 |
| | Drying Temperature (DT) | 1 | 86.716 | 1.76 | 0.3160 |
| | Error a (DT*Rep) | 2 | 49.327 | 1.56 | 0.2494 |
| | Boiling Time (BT) | 3 | 3567.425 | 112.99 | <.0001 |
| | DT*BT | 3 | 37.217 | 1.18 | 0.3587 |
| | Error b | 12 | 31.573 | | |

Table A5. Analysis of variance for effect of temper and drying temperature on black bean grain quality.

| Variable | Source | df | MS | F value | Pr>F |
|----------|-------------------------|----|--------|---------|---------|
| L | Rep | 2 | 2.052 | 3.33 | 0.0567 |
| | Drying Temperature (DT) | 1 | 1.117 | 8.60 | 0.0993 |
| | Error a (DT*Rep) | 2 | 0.130 | 0.21 | 0.8121 |
| | Temper Level (TL) | 5 | 15.085 | 24.44 | <0.0001 |
| | DT*TL | 5 | 0.204 | 0.33 | 0.8884 |
| | Error b | 20 | 0.617 | | |
| a | Rep | 2 | 0.146 | 8.80 | 0.0018 |
| | Drying Temperature (DT) | 1 | 0.046 | 168.91 | 0.0059 |
| | Error a (DT*Rep) | 2 | 0.000 | 0.02 | 0.9839 |
| | Temper Level (TL) | 5 | 0.393 | 23.72 | <.0001 |
| | DT*TL | 5 | 0.015 | 0.89 | 0.5064 |
| | Error b | 20 | 0.017 | | |
| b | Rep | 2 | 0.565 | 5.03 | 0.0170 |
| | Drying Temperature (DT) | 1 | 0.006 | 0.24 | 0.6724 |
| | Error a (DT*Rep) | 2 | 0.027 | 0.24 | 0.7914 |
| | Temper Level (TL) | 5 | 0.278 | 2.47 | 0.0671 |
| | DT*TL | 5 | 0.192 | 1.71 | 0.1779 |
| | Error b | 20 | 0.112 | | |
| 100-KWT | Rep | 2 | 1.382 | 5.90 | 0.0097 |
| | Drying Temperature (DT) | 1 | 2.285 | 11.37 | 0.0778 |
| | Error a (DT*Rep) | 2 | 0.201 | 0.86 | 0.4390 |
| | Temper Level (TL) | 5 | 0.516 | 2.20 | 0.0945 |
| | DT*TL | 5 | 0.235 | 1.00 | 0.4416 |
| | Error b | 20 | 0.234 | | |

Table A5. Analysis of variance for effect of temper and drying temperature on black bean grain quality (continued).

| Variable | Source | df | MS | F value | Pr>F |
|-----------|-------------------------|----|----------|---------|--------|
| Length | Rep | 2 | 0.433 | 8.10 | 0.0027 |
| | Drying Temperature (DT) | 1 | 0.096 | 5.32 | 0.1476 |
| | Error a (DT*Rep) | 2 | 0.018 | 0.34 | 0.7172 |
| | Temper Level (TL) | 5 | 1.839 | 34.38 | <.0001 |
| | DT*TL | 5 | 0.078 | 1.45 | 0.2492 |
| | Error b | 20 | 0.053 | | |
| Width | Rep | 2 | 0.023 | 0.89 | 0.4257 |
| | Drying Temperature (DT) | 1 | 0.092 | 13.04 | 0.0688 |
| | Error a (DT*Rep) | 2 | 0.007 | 0.28 | 0.7592 |
| | Temper Level (TL) | 5 | 0.283 | 11.16 | <.0001 |
| | DT*TL | 5 | 0.058 | 2.27 | 0.0866 |
| | Error b | 20 | 0.025 | | |
| Thickness | Rep | 2 | 0.001 | 0.06 | 0.9431 |
| | Drying Temperature (DT) | 1 | 0.007 | 0.25 | 0.6667 |
| | Error a (DT*Rep) | 2 | 0.026 | 1.33 | 0.2870 |
| | Temper Level (TL) | 5 | 0.161 | 8.17 | 0.0002 |
| | DT*TL | 5 | 0.035 | 1.79 | 0.1599 |
| | Error b | 20 | 0.020 | | |
| Volume | Rep | 2 | 373.406 | 1.02 | 0.3798 |
| | Drying Temperature (DT) | 1 | 1000.668 | 5.40 | 0.1458 |
| | Error a (DT*Rep) | 2 | 185.367 | 0.50 | 0.6112 |
| | Temper Level (TL) | 5 | 5927.141 | 16.14 | <.0001 |
| | DT*TL | 5 | 834.476 | 2.27 | 0.0865 |
| | Error b | 20 | 367.296 | | |

Table A6. Analysis of variance for effect of temper and drying temperature on black bean milling quality.

| Variable | Source | df | MS | F value | Pr>F |
|--------------------|-------------------------|----|-----------|---------|--------|
| Fracture point | Rep | 2 | 979.361 | 2.48 | 0.1091 |
| | Drying Temperature (DT) | 1 | 1722.250 | 7.02 | 0.1178 |
| | Error a (DT*Rep) | 2 | 245.250 | 0.62 | 0.5474 |
| | Temper Level (TL) | 5 | 20700.361 | 52.42 | <.0001 |
| | DT*TL | 5 | 595.117 | 1.51 | 0.2322 |
| | Error b | 20 | 394.906 | | |
| Peak force | Rep | 2 | 2056.361 | 4.89 | 0.0187 |
| | Drying Temperature (DT) | 1 | 12731.361 | 36.88 | 0.0261 |
| | Error a (DT*Rep) | 2 | 345.194 | 0.82 | 0.4546 |
| | Temper Level (TL) | 5 | 5104.894 | 12.13 | <.0001 |
| | DT*TL | 5 | 4631.294 | 11.00 | <.0001 |
| | Error b | 20 | 420.844 | | |
| Whole bean flour L | Rep | 2 | 0.764 | 2.82 | 0.0833 |
| | Drying Temperature (DT) | 1 | 8.362 | 7.67 | 0.1095 |
| | Error a (DT*Rep) | 2 | 1.091 | 4.03 | 0.0339 |
| | Temper Level (TL) | 5 | 17.242 | 63.68 | <.0001 |
| | DT*TL | 5 | 0.549 | 2.03 | 0.1183 |
| | Error b | 20 | 0.271 | | |
| Whole bean flour a | Rep | 2 | 0.083 | 5.48 | 0.0127 |
| | Drying Temperature (DT) | 1 | 0.028 | 0.85 | 0.4547 |
| | Error a (DT*Rep) | 2 | 0.033 | 2.18 | 0.1397 |
| | Temper Level (TL) | 5 | 0.058 | 3.87 | 0.0128 |
| | DT*TL | 5 | 0.027 | 1.78 | 0.1622 |
| | Error b | 20 | 0.015 | | |

Table A6. Analysis of variance for effect of temper and drying temperature on black bean milling quality (continued).

| Variable | Source | df | MS | F value | Pr>F |
|--------------------------|-------------------------|-----------|-----------|----------------|----------------|
| Whole bean flour b | Rep | 2 | 1.220 | 31.33 | <.0001 |
| | Drying Temperature (DT) | 1 | 2.045 | 14.13 | 0.0640 |
| | Error a (DT*Rep) | 2 | 0.145 | 3.72 | 0.0424 |
| | Temper Level (TL) | 5 | 2.970 | 76.28 | <.0001 |
| | DT*TL | 5 | 1.663 | 42.71 | <.0001 |
| | Error b | 20 | 0.039 | | |
| Cotyledon flour L | Rep | 2 | 0.119 | 0.18 | 0.8333 |
| | Drying Temperature (DT) | 1 | 10.433 | 3.87 | 0.1882 |
| | Error a (DT*Rep) | 2 | 2.699 | 4.16 | 0.0309 |
| | Temper Level (TL) | 5 | 4.854 | 7.48 | 0.0004 |
| | DT*TL | 5 | 2.472 | 3.81 | 0.0138 |
| | Error b | 20 | 0.649 | | |
| Cotyledon flour a | Rep | 2 | 0.047 | 14.20 | 0.0001 |
| | Drying Temperature (DT) | 1 | 1.240 | 19.01 | 0.0488 |
| | Error a (DT*Rep) | 2 | 0.065 | 19.87 | <.0001 |
| | Temper Level (TL) | 5 | 0.531 | 161.81 | <.0001 |
| | DT*TL | 5 | 0.215 | 65.43 | <.0001 |
| | Error b | 20 | 0.003 | | |
| Cotyledon flour b | Rep | 2 | 0.118 | 1.59 | 0.2278 |
| | Drying Temperature (DT) | 1 | 26.027 | 44.24 | 0.0219 |
| | Error a (DT*Rep) | 2 | 0.588 | 7.93 | 0.0029 |
| | Temper Level (TL) | 5 | 4.477 | 60.36 | <.0001 |
| | DT*TL | 5 | 7.524 | 101.43 | <.0001 |
| | Error b | 20 | 0.074 | | |

Table A7. Analysis of variance for effect of temper and drying temperature on black bean grain chemistry.

| Variable | Source | df | MS | F value | Pr>F |
|---------------------------------|-------------------------|----|--------|---------|--------|
| Whole bean flour moisture | Rep | 2 | 6.656 | 99.48 | <.0001 |
| | Drying Temperature (DT) | 1 | 9.507 | 27.63 | 0.0343 |
| | Error a (DT*Rep) | 2 | 0.344 | 5.14 | 0.0158 |
| | Temper Level (TL) | 5 | 0.480 | 7.17 | 0.0005 |
| | DT*TL | 5 | 0.525 | 7.84 | 0.0003 |
| | Error b | 20 | 0.067 | | |
| Cotyledon flour moisture | Rep | 2 | 0.235 | 8.19 | 0.0025 |
| | Drying Temperature (DT) | 1 | 8.742 | 40.71 | 0.0237 |
| | Error a (DT*Rep) | 2 | 0.215 | 7.49 | 0.0037 |
| | Temper Level (TL) | 5 | 0.535 | 18.66 | <.0001 |
| | DT*TL | 5 | 0.403 | 14.06 | <.0001 |
| | Error b | 20 | 0.029 | | |
| Whole bean flour protein | Rep | 2 | 19.822 | 112.65 | <.0001 |
| | Drying Temperature (DT) | 1 | 0.146 | 0.15 | 0.7345 |
| | Error a (DT*Rep) | 2 | 0.960 | 5.46 | 0.0128 |
| | Temper Level (TL) | 5 | 0.022 | 0.12 | 0.9853 |
| | DT*TL | 5 | 0.140 | 0.80 | 0.5644 |
| | Error b | 20 | 0.176 | | |
| Cotyledon flour protein | Rep | 2 | 27.368 | 86.15 | <.0001 |
| | Drying Temperature (DT) | 1 | 0.535 | 1.22 | 0.3846 |
| | Error a (DT*Rep) | 2 | 0.439 | 1.38 | 0.2740 |
| | Temper Level (TL) | 5 | 0.613 | 1.93 | 0.1340 |
| | DT*TL | 5 | 0.357 | 1.12 | 0.3800 |
| | Error b | 20 | 0.318 | | |

Table A7. Analysis of variance for effect of temper and drying temperature on black bean grain chemistry (continued).

| Variable | Source | df | MS | F value | Pr>F |
|-------------------------|-------------------------|----|--------|---------|--------|
| Seed coat flour protein | Rep | 2 | 7.379 | 11.02 | 0.0006 |
| | Drying Temperature (DT) | 1 | 29.322 | 13.94 | 0.0649 |
| | Error a (DT*Rep) | 2 | 2.104 | 3.14 | 0.0651 |
| | Temper Level (TL) | 5 | 4.209 | 6.28 | 0.0012 |
| | DT*TL | 5 | 1.527 | 2.28 | 0.0857 |
| | Error b | 20 | 0.670 | | |
| Whole bean flour ash | Rep | 2 | 0.380 | 3.64 | 0.0449 |
| | Drying Temperature (DT) | 1 | 0.093 | 5.69 | 0.1399 |
| | Error a (DT*Rep) | 2 | 0.016 | 0.16 | 0.8559 |
| | Temper Level (TL) | 5 | 0.149 | 1.43 | 0.2567 |
| | DT*TL | 5 | 0.108 | 1.03 | 0.4261 |
| | Error b | 20 | 0.104 | | |
| Cotyledon flour ash | Rep | 2 | 0.025 | 1.95 | 0.1686 |
| | Drying Temperature (DT) | 1 | 0.001 | 0.04 | 0.8645 |
| | Error a (DT*Rep) | 2 | 0.036 | 2.83 | 0.0830 |
| | Temper Level (TL) | 5 | 0.015 | 1.20 | 0.3453 |
| | DT*TL | 5 | 0.008 | 0.63 | 0.6815 |
| | Error b | 20 | 0.013 | | |
| Whole bean starch | Rep | 2 | 22.937 | 13.85 | 0.0002 |
| | Drying Temperature (DT) | 1 | 3.139 | 0.78 | 0.4699 |
| | Error a (DT*Rep) | 2 | 4.015 | 2.42 | 0.1141 |
| | Temper Level (TL) | 5 | 7.691 | 4.64 | 0.0056 |
| | DT*TL | 5 | 1.732 | 1.05 | 0.4185 |
| | Error b | 20 | 1.656 | | |
| Cotyledon flour starch | Rep | 2 | 13.780 | 5.94 | 0.0094 |
| | Drying Temperature (DT) | 1 | 3.560 | 3.96 | 0.1848 |
| | Error a (DT*Rep) | 2 | 0.898 | 0.39 | 0.6839 |
| | Temper Level (TL) | 5 | 6.831 | 2.94 | 0.0376 |
| | DT*TL | 5 | 2.323 | 1.00 | 0.4423 |
| | Error b | 20 | 2.320 | | |

Table A7. Analysis of variance for effect of temper and drying temperature on black bean grain chemistry (continued).

| Variable | Source | df | MS | F value | Pr>F |
|---|-------------------------|-----------|-----------|----------------|----------------|
| Whole bean flour starch damage | Rep | 2 | 0.000 | 0.75 | 0.4868 |
| | Drying Temperature (DT) | 1 | 0.000 | 6.26 | 0.1295 |
| | Error a (DT*Rep) | 2 | 0.000 | 1.07 | 0.3611 |
| | Temper Level (TL) | 5 | 0.001 | 9.42 | <.0001 |
| | DT*TL | 5 | 0.000 | 0.97 | 0.4580 |
| | Error b | 20 | 0.000 | | |
| Cotyledon flour starch damage | Rep | 2 | 0.000 | 0.81 | 0.4576 |
| | Drying Temperature (DT) | 1 | 0.002 | 38.60 | 0.0249 |
| | Error a (DT*Rep) | 2 | 0.000 | 1.96 | 0.1666 |
| | Temper Level (TL) | 5 | 0.001 | 44.82 | <.0001 |
| | DT*TL | 5 | 0.000 | 14.51 | <.0001 |
| | Error b | 20 | 0.000 | | |

Table A8. Analysis of variance for effect of temper and drying temperature on black bean pasting quality.

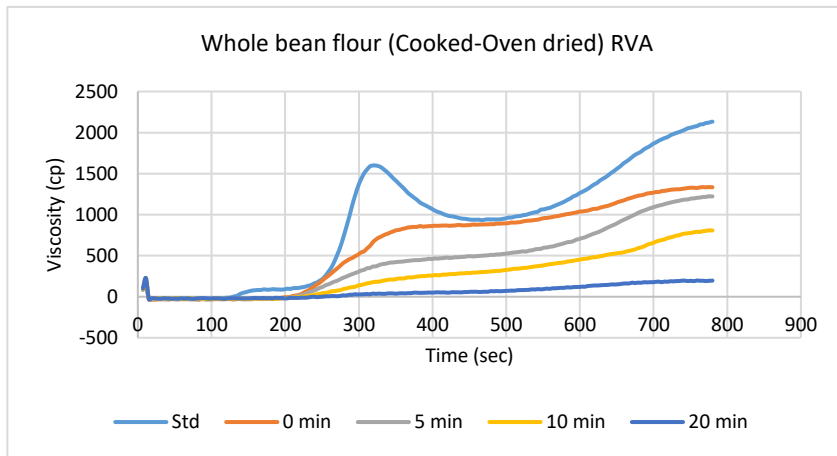
| Variable | Source | df | MS | F value | Pr>F |
|-------------------------------|-------------------------|-----------|-----------|----------------|----------------|
| Whole bean flour peak | Rep | 2 | 3306.420 | 164.06 | <.0001 |
| | Drying Temperature (DT) | 1 | 94.284 | 0.39 | 0.5941 |
| | Error a (DT*Rep) | 2 | 238.935 | 11.86 | 0.0004 |
| | Temper Level (TL) | 5 | 261.239 | 12.96 | <.0001 |
| | DT*TL | 5 | 78.186 | 3.88 | 0.0128 |
| | Error b | 20 | 20.154 | | |
| Whole bean flour trough | Rep | 2 | 3385.214 | 119.21 | <.0001 |
| | Drying Temperature (DT) | 1 | 62.068 | 0.31 | 0.6339 |
| | Error a (DT*Rep) | 2 | 200.523 | 7.06 | 0.0048 |
| | Temper Level (TL) | 5 | 361.336 | 12.72 | <.0001 |
| | DT*TL | 5 | 57.236 | 2.02 | 0.1201 |
| | Error b | 20 | 28.397 | | |
| Whole bean flour break | Rep | 2 | 1.388 | 0.41 | 0.6722 |
| | Drying Temperature (DT) | 1 | 3.331 | 1.89 | 0.3028 |
| | Error a (DT*Rep) | 2 | 1.760 | 0.51 | 0.6059 |
| | Temper Level (TL) | 5 | 10.161 | 2.97 | 0.0367 |
| | DT*TL | 5 | 1.789 | 0.52 | 0.7567 |
| | Error b | 20 | 3.426 | | |
| Whole bean flour final | Rep | 2 | 7362.973 | 159.14 | <.0001 |
| | Drying Temperature (DT) | 1 | 278.334 | 0.34 | 0.6186 |
| | Error a (DT*Rep) | 2 | 817.657 | 17.67 | <.0001 |
| | Temper Level (TL) | 5 | 173.691 | 3.75 | 0.0147 |
| | DT*TL | 5 | 258.351 | 5.58 | 0.0022 |
| | Error b | 20 | 46.266 | | |

Table A8. Analysis of variance for effect of temper and drying temperature on black bean pasting quality (continued).

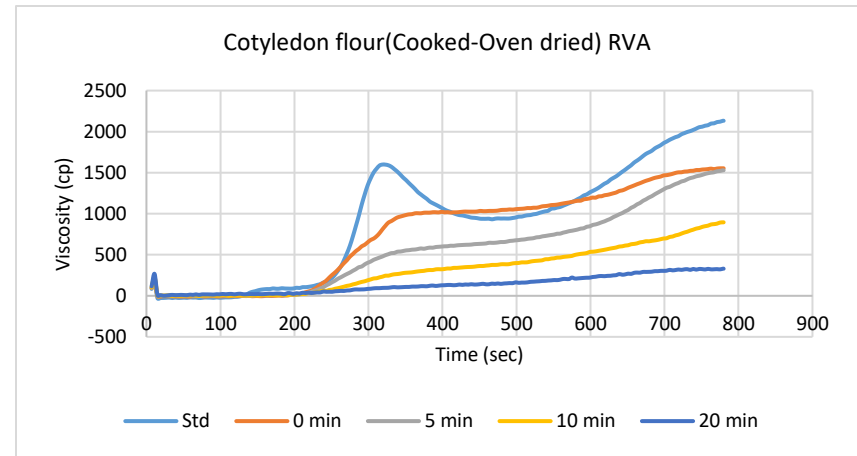
| Variable | Source | df | MS | F value | Pr>F |
|------------------------------|-------------------------|----|----------|---------|--------|
| Whole bean flour set | Rep | 2 | 807.685 | 37.12 | <.0001 |
| | Drying Temperature (DT) | 1 | 602.948 | 2.83 | 0.2346 |
| | Error a (DT*Rep) | 2 | 213.081 | 9.79 | 0.0011 |
| | Temper Level (TL) | 5 | 256.648 | 11.79 | <.0001 |
| | DT*TL | 5 | 181.328 | 8.33 | 0.0002 |
| | Error b | 20 | 21.761 | | |
| Cotyledon flour peak | Rep | 2 | 5319.664 | 264.17 | <.0001 |
| | Drying Temperature (DT) | 1 | 8.028 | 0.03 | 0.8820 |
| | Error a (DT*Rep) | 2 | 284.066 | 14.11 | 0.0002 |
| | Temper Level (TL) | 5 | 42.765 | 2.12 | 0.1045 |
| | DT*TL | 5 | 53.517 | 2.66 | 0.0534 |
| | Error b | 20 | 20.137 | | |
| Cotyledon flour trough | Rep | 2 | 4914.288 | 184.41 | <.0001 |
| | Drying Temperature (DT) | 1 | 69.945 | 0.35 | 0.6147 |
| | Error a (DT*Rep) | 2 | 200.546 | 7.53 | 0.0037 |
| | Temper Level (TL) | 5 | 79.557 | 2.99 | 0.0358 |
| | DT*TL | 5 | 43.722 | 1.64 | 0.1951 |
| | Error b | 20 | 26.649 | | |
| Cotyledon flour break | Rep | 2 | 10.023 | 3.87 | 0.0380 |
| | Drying Temperature (DT) | 1 | 30.618 | 4.19 | 0.1772 |
| | Error a (DT*Rep) | 2 | 7.304 | 2.82 | 0.0834 |
| | Temper Level (TL) | 5 | 10.459 | 4.04 | 0.0107 |
| | DT*TL | 5 | 7.789 | 3.01 | 0.0349 |
| | Error b | 20 | 2.591 | | |

Table A8. Analysis of variance for effect of temper and drying temperature on black bean pasting quality (continued).

| Variable | Source | df | MS | F value | Pr>F |
|--------------------------|-------------------------|-----------|-----------|----------------|----------------|
| Cotyledon flour final | Rep | 2 | 13746.901 | 259.70 | <.0001 |
| | Drying Temperature (DT) | 1 | 1832.411 | 1.27 | 0.3763 |
| | Error a (DT*Rep) | 2 | 1439.257 | 27.19 | <.0001 |
| | Temper Level (TL) | 5 | 327.375 | 6.18 | 0.0013 |
| | DT*TL | 5 | 350.732 | 6.63 | 0.0009 |
| | Error b | 20 | 52.935 | | |
| Cotyledon flour set | Rep | 2 | 2240.115 | 73.19 | <.0001 |
| | Drying Temperature (DT) | 1 | 1186.688 | 2.10 | 0.2845 |
| | Error a (DT*Rep) | 2 | 565.515 | 18.48 | <.0001 |
| | Temper Level (TL) | 5 | 641.622 | 20.96 | <.0001 |
| | DT*TL | 5 | 266.799 | 8.72 | 0.0002 |
| | Error b | 20 | 30.607 | | |

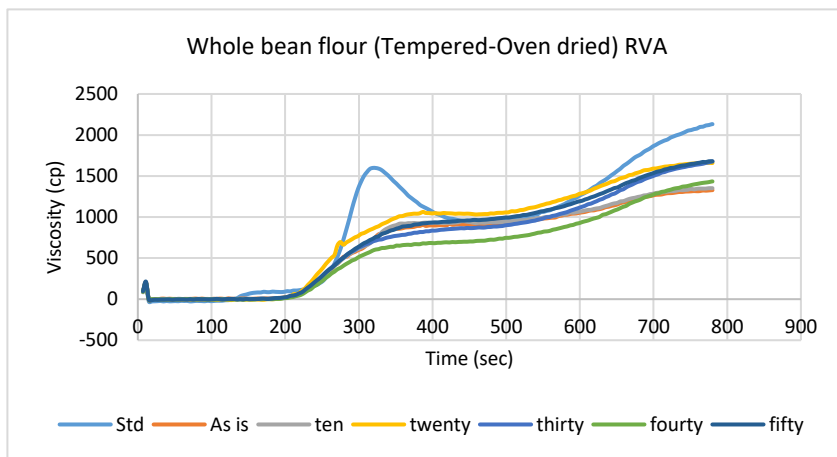


A

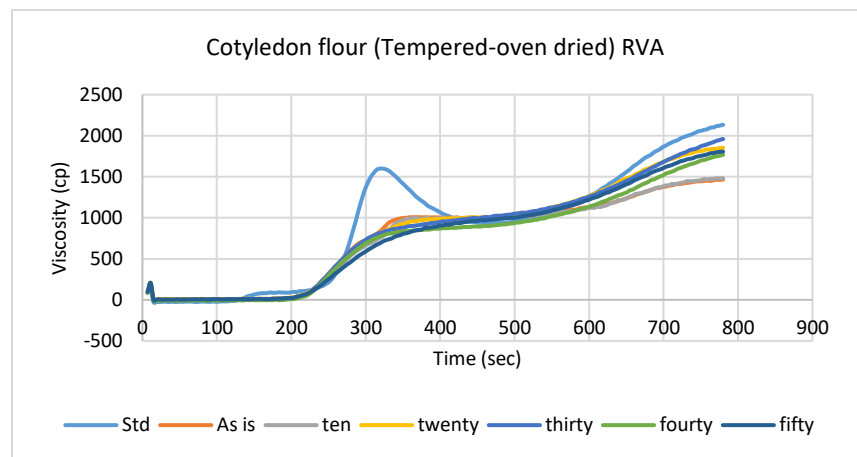


B

Figure A1. Cooked (0-20 min) oven dried (A) whole bean flour and (B) cotyledon flour pasting profiles



A



B

Figure A2. Tempered (as is to 50%) oven dried (A) whole bean flour and (B) cotyledon flour pasting profiles