# BLACK BEAN MILLING

A Thesis
Submitted to the Graduate Faculty
Of the
North Dakota State University
Of Agriculture and Applied Sciences

By

Claudia Elizabeth Carter

In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE

Major Program:
Cereal Science

April 2014

Fargo, North Dakota

# North Dakota State University
## Graduate School

**Title**

Black Bean Milling

**By**

Claudia Elizabeth Carter

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

SUPERVISORY COMMITTEE:

Dr. Frank Manthey
Chair

Dr. Senay Simsek

Dr. Clifford Hall III

Dr. Juan Osorno

Approved:

April 25, 2014
Date

Dr. Richard Horsley
Department Chair

## ABSTRACT

Black bean milling to produce whole-bean flour and cotyledon flour by using a centrifugal mill and a burr mill/roller milling system, respectively, were investigated. The effect of black bean seed pretreatment (cooked-dried, soaked-dried, and tempered) on flour physical, chemical, and pasting characteristics were investigated.

Whole flour milling was done with a centrifugal mill using mesh size of 500 µm, rotor speed of 12,000 rpm, and mill feed of 267$\pm$18 g/min. Cooked-dried, soaked-dried, and tempered black bean milling yields for whole flour reached 58, 59, and 66%, respectively. Roller mill was used with durum wheat settings. Cooked-dried, soaked-dried, and tempered black bean milling yields for cotyledon flour reached 75, 73, and 75%, respectively.

Black bean seed changed physically and internally by cooking or soaking. Differences in moisture content were reflected to change milling-ability and physical quality of flour. Cooked-dried affected the most starch damage and pasting properties and for flour color.

## ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my advisor, Dr. Frank Manthey, his supervision and support provided me opportunities to develop skills and gain knowledge during this process. I would also like to thank Dr. Clifford Hall and Dr. Senay Simsek for their valuable information and resources, including laboratory and technical support. I also thank Dr. Juan Osorno for his availability and assistance to complete this work.

I would like to express my appreciation for the laboratory assistance provided by DeLane Olson, Kristin Whitney, Mary Niehaus, and Hiroshi Ando. I also thank Maribel Ovando-Martínez for providing me with valuable information from time to time. I would like to thank fellow graduate students and staff for their assistance and friendship. Special thanks to my friends Adriana Rodriguez, Etsehiwot Gebreselassie, Johanna Villamiza, and Elena de la Peña for their words of support and being the cheerleaders during this process.

To my father Julio Hidalgo, you taught me the invaluable significance of education and motivated me to study and pursue my dreams. To my mother Clara Navia de Hidalgo, thank you for all the years you have willingly and happily given your precious time to our family. You have always shown me the unconditional love of a mother. To my brothers, thank you for always believing in me and providing me with supportive words when I needed them. I am equally thankful to my parents-in-law, Roger and Shirley Carter for your limitless support and love.

With immense love, respect, and appreciation I would like to thank my loving and caring husband Jason Carter for your never-ending and encouraging support, hugs, and love. I am blessed to have two wonderful children, Lucas and Lily Carter, who are here to bring laugher and remind me that simple events are the most precious moments to cherish in life. To God, thank you for blessing me with such a wonderful family and life.

# DEDICATION

I would like to dedicate this thesis to my loving children, Lucas and Lily Carter, who are my strength and inspiration. I hope one day I become your inspiration and you remember that the most valuable tool I can give you is education.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDIX TABLES

Table Page

## FORMAT OF THESIS

This thesis has an overall Abstract, General Introduction, and Literature Review. The literature cited in the Introduction and Literature Review is given at the end of each section. The thesis is written as four separate papers. Each paper has an abstract, introduction, materials and methods, results and discussion, and conclusion followed by literature cited. At the end of the four papers, there is an Overall Conclusion and a brief discussion called Future Research and Applications. Due to the format of the thesis, there is redundancy in some places.

# GENERAL INTRODUCTION

For years, dry beans (*Phaseolus vulgaris* L.) have been used in the human diet as whole beans. Dry beans are part of a family called leguminosae and are globally consumed, mainly in low-income areas in the world (Tiwari et al 2011). Black beans are a market class within the dry bean category. Black beans, along with the other market classes of dry beans (pinto, navy, kidney beans) were cultivated about 7,000 years ago in Southwestern Mexico. Early chroniclers indicated cultivations of dry beans by the Aztec and Incan empires. The people of Axocopan used dry beans to pay tributes at the early colonial period in North America (Wu 2002). North Dakota and Michigan lead the US dry bean production (USDA-AMS 2012).

Black beans are a good source of protein, carbohydrate, dietary fiber, and important minerals and vitamins essential for the human diet (Sathe 2002). Dry beans also contain antinutritional factors (ANF) (i.e. trypsin inhibitors, tannins, phytic acid, phenolic compounds, and lectins), which have been extensively studied (Valdebouze et al 1980; Gueguen 1983; Champ 2002). These ANFs factors together with indigestible bean proteins hinder the absorption of the nutrients like calcium, iron, and zinc in the human gut. The removal of ANFs can be challenging and requires treatment methods to reduce or remove the level of antinutrients in beans before consumption. Examples of treatment methods to reduce ANFs include fermentation, germination, thermal treatments (cooking) and soaking procedures (Abd El-Hady and Habiba 2003; Martin-Cabrejas et al 2004). These ANF could be responsible for the underutilization of black beans in food products.

Black bean and other dry bean utilization studies have been concerned mostly with whole seed because for years this was the only consumption practice. However, there is an increasing interest in using dry bean flour to produce novel and low-cost food products (Siddiq and

Uebersax 2013). The high nutritional value of black bean is another reason for the interest in using it as flour in other products. Spaghetti, bread and snacks are examples of food products to which bean flour can be added. Hence, the inclusion of bean flour in these products will improve their nutritional value (Aguilera et al 1982; Chillo et al 2008; Han et al 2010) The consumption of beans and bean products can reduce and prevent some metabolic diseases such as diabetes, heart disease, and colon cancer (Siddiq et al 2010). Also, the new trend of incorporating non-wheat flours into food products is driven by the increasing gluten free demand. Dry bean flours have attracted attention as an ingredient (Otto et al 1997).

The growing competition in the global market, combined with an increase in the scale of operations, forces producers to use raw materials and final products in granular form that is relatively easy to store, handle and process (Tiwari et al 2011). Black bean flour can further be processed by fractionation to isolate its components (i.e. protein, starch, and dietary fiber) and use them in food applications.

Before dry milling, black beans could be subjected to pretreatment of one or more of the following: soaking, cooking, tempering, and drying. The pretreatments as well as the milling procedures used impact the quality of flour obtained (Kerr et al 2000). Soaking or cooking before milling could aid in the reduction/elimination of some of the mentioned ANFs in black beans. One of the biggest variables in flour quality is particle size which could impact the functional and physiochemical properties of black bean flour and the subsequent end product quality (Kerr et al 2001).

Black beans are mostly milled using hammer mills. The hammer mill is an impact type mill where particle size reduction depends on hammer design (Koch 2002). A disadvantage of hammer milling is that it is less energy efficient if compared to roller milling. Particle size distribution is less uniform with hammer mills compared to a roller mill (Koch 2002).

Other available and not well studied mills used to mill black beans are centrifugal and roller mills. Centrifugal mills are used to grind entire seed without, in the case of dicotyledonous seeds, separation of seed coat and embryo from the cotyledon. Centrifugal mill uses the particle size reduction principles of impact and shearing forces. Centrifugal mill can grind any soft, medium-hard, brittle, and fibrous materials (Anonymous 2014). Therefore, centrifugal mill can be used and studied as a potential mill for producing black bean flour.

The roller mill offers potential to mechanically remove seed coat from the cotyledon. This could become advantageous to improve black bean seed economical utilization. The break section and reduction section are two sections of a roller mill. Roller mills have a set of paired rolls that can be corrugated or smooth. Each roll in a pair can rotate at the same speed or can rotate at different speed (Posner and Hibbs 2005). Differential in roll speed results in shear action, which is used to remove bran from the wheat kernel.

Black bean milling or dry beans milling in general, has not been studied extensively. Wheat milling has been studied for years, and its advances in knowledge have led to the understanding of the different wheat milling procedures used today. However, dry bean milling has attracted an interest due to the increasing need of non-wheat food ingredients available for food applications. In general, limited information is available concerning with dry bean milling on different mills. No literature was found that reported black bean seed pretreatment and the effect on flour physical quality, chemical composition, and pasting properties. In the research reported in this thesis, black beans were selected over other market classes due to the dark seed coat, which could be used to visually assess the removal of seed coat from the cotyledon during milling.

The present study was undertaken with the flowing objectives:

1. To determine the effect of pretreatments (cooked-dried, soaked-dried, and tempered) on the black bean seed physical and chemical composition.

2. To determine the best condition for black bean milling using a centrifugal mill for whole bean flour.

3. To determine the effect of black bean seed pretreatments (cooked-dried, soaked-dried, and tempered) on the physical quality, chemical composition, and pasting properties of flour obtained by centrifugal milling.

4. To determine the effect of black bean seed pretreatments (cooked-dried, soaked-dried, and tempered) on physical quality, chemical composition, and pasting properties of cotyledon flour obtained by a burr mill/roller mill system. Also, the seed coat removal and cotyledon flour extraction was determined.

## Literature Cited

Abd El-Hady, E. A., and Habiba, R. A. 2003. Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. LWT – Food Sci. Technol. 36:285-293.

Aguilera, J. M., Lusas, E. W., Uebersax, M. A., Zabik, M. E. 1982. Development of food ingredients from navy beans (*Phaseolus vulgaris*) by roasting, pin milling, and air classification. J. Food Sci. 47:1151-1154.

Anonymous. 2014. Ultra Centrifugal Mill ZM 200. [Web Page]. Located at: http://www.retsch.com/products/milling/rotor-mills/zm-200/ (Accessed 4/9/14).

Champ, M. J. 2002. Non-nutrient bioactive substances of pulses. Br. J. Nutr. 88:S307-S319.

Chillo, S., Laverse, J., Falcone, P. M., Protopapa, A., and Del Nobile, M. A. 2008. Influence of the addition of buckwheat flour and durum wheat bran on spaghetti quality. J. Cereal Sci. 47:144-152.

Gueguen, J. 1983. Legume seed protein extraction, processing and end product characteristics. Plant Food Human Nutr. 32:267-303.

Han, J., Janz, J., and Gerlat, M. 2010. Development of gluten-free cracker snacks using pulse flours and fractions. Food Res. Int. 43:627-633.

Kerr, W. L., Ward, C. D. W., McWatters, K. H., and Resurreccion, A. V. A. 2000. Effect of milling and particle size on functionality and physiochemical properties of cowpea flour. Cereal Chem. 77:213-219.

Kerr, W. L., Ward, C. D. W., McWatters, K. H., and Resurreccion, A. V. A. 2001. Milling and particle size of cowpea flour and snack chip quality. Food Res. Int. 34:39-45.

Koch, K. 2002. Hammermills and roller mills. Kansas State University. Manhattan, KS. [Web Page]. Located at: http://www.ksre.ksu.edu/bookstore/pubs/mf2048.pdf. (Accessed 4/9/14).

Martin-Cabrejas, M. A., Sanfiz, B., Vidal, A., Molla, E., Esteban, R. M., and Lopez-Andréu, F. J. 2004. Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem. 52:261-266.

Otto, T., Baik, B., and Czuchajowska, Z. 1997. Microstructure of seeds, flours, and starches of legumes. Cereal Chem. 74:445–451.

Posner, E. S., and Hibbs, A. N. 2005. The grinding process. Pages 185-219 in: Wheat Flour Milling 2nd Ed. American Assoc. of Cereal Chem.: St. Paul, MN.

Sathe, S. K. 2002. Dry bean protein functionality. Crit. Rev. Biotechnol. 22:175-223.

Siddiq, M., and Uebersax, M. A. 2013. Dry Beans and Pulses Production and consumption—An Overview. Pages 3-22 in: Dry Beans and Pulses: Production, Processing and Nutrition. First Ed. John Wiley & Sons, Inc.: Oxford, UK.

Siddiq, M., Ravi, R., Harte, J. B., and Dolan, K. D. 2010. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.) flour. LWT – Food Sci. Technol. 43:232-237.

Tiwari, B. K., Gowen, A., and McKenna, B. 2011. Introduction. Pages 1-4 in: Pulse Foods: Processing, Quality and Nutraceutical Applications First Ed. Academic Press: San Diego, CA.

USDA Agricultural Marketing Service. 2012. Bean Market News 2011 Summary. [Web Page]. Located at: www.ams.usda.gov/mnreports/lsaba.pdf (Accessed on 4/9/14).

Valdebouze, P., Bergeron, E., Gaborit, T., and Delort-Laval, J. 1980. Content and distribution of trypsin inhibitors and haemagglutinins in some legume seeds. Can. J. Plant Sci. 60:695-701.

Wu, X. 2002. Correlation of physio-chemical characteristics in the seed coat and canning quality in different dark red kidney bean (*Phaseolus vulgaris* L.) cultivars (Masters Dissertation, University of Wisconsin-Stout). Available from Thesis database, UW-Stout.

# LITERATURE REVIEW

## Black Bean Seed Structure

### Seed Coat

Black bean seed has three major components: seed coat (testa), cotyledons, and embryonic axis. Seed coat represents 8.5% of the black bean seed weight (Rahman 2007). The seed coat main function is to protect the cotyledons and embryo from insects and disease. It helps regulate the movement of moisture into and out of the seed. The seed coat microstructure is mainly composed of four layers: waxy cuticle layer, epidermis, hypodermis, and interior parenchyma layer. These layers have been identified by several authors in navy, pinto, and adzuki beans (Sefa-Dedeh and Stanley 1979; Swanson et al 1985). The waxy cuticle layer (i.e. the outer most layer of the seed coat) restricts movement of water into the seed (Bukovac et al 1981). The epidermal layer is made up of palisade cells that are perpendicularly oriented to the surface. In black beans, the hypodermis layer is made up of hourglass cells oriented parallel to the surface.  Not all legume seeds have hourglass cells. The interior parenchyma cells are protoplast free and are elongated cells parallel to the surface of cotyledon (Tiwari and Singh 2012). 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).

### Cotyledon

Cotyledons are the major part of the black bean seed structure, accounting for 89.5% of the black bean seed weight. Siddiq and Uebersax (2013) stated cotyledons from dry beans are botanically a “segment of the embryo and are thus differentiated from the endosperm of common cereal grains”. Cotyledons are the storage organs and photosynthetic structures. The cotyledons are living tissue and function as leaf tissue and as a source for the starch and protein needed during seed germination. Dry cotyledons in navy beans have 39.3% starch, 27.5% protein, 1.7% lipids, and 3.5% ash content (Powrie et al 1960). The cotyledons have parenchyma cells without nuclei and are packed with starch granules that are embedded in a matrix of storage proteins (Tiwari and Singh 2012). The parenchyma cells are surrounded by cell walls and middle lamellae. The middle lamella is a pectin layer, which acts as a cement for cell walls of adjacent cells. It was reported that within the cotyledon structure there are differences between the periphery and the inner portion of the cotyledon cell structure (Otto et al 1997). The cell in the center is loosely packed with large intercellular spaces; whereas, cotyledon cells of the periphery are tightly packed (Kosson et al 1994; Otto et al 1997).

**Embryonic Axis**

The embryo is a minor portion of the black bean seed, representing only 2% or less of the seed weight (Tiwari and Singh 2012). In dicotyledenous seed, the embryo and the cotyledons are living tissue. The embryonic axis has the radicle and plumule. The function of the embryonic axis is to serve as nutrition organs to the embryo during germination. They also have an important role during water imbibition by the seed coat (Tiwari and Singh 2012) as the parts of the embryo (raphe, micropyle and hilum) are the entry points for water diffusion into the seeds. Water permeability is greatest in the hilum or micropyle areas. The embryo is rich in lipids, vitamins, and enzymes required for the growth and development of the plant during germination (Siddiq and Uebersax 2013).

## Major Chemical Constituents

### Carbohydrate

Carbohydrates (60-65%) are the major chemical component in dry beans (Siddiq and Uebersax 2013). The carbohydrate portion contains starch (main storage carbohydrate) and non-starch polysaccharides (dietary fiber) and oligosaccharides (Bravo et al 1998).

<u>Starch</u>

Starch is the main nutrient in black bean, accounting for approximately 16-22% of total carbohydrates present in the seed (Hoover et al 2010). Raw dry bean starches appear to be oval, smooth, and elliptical (Gujska et al 1994; Hoover and Ratnayake 2002). Granule size is important in determining many functional properties of starch. Larger starch granules tend to be more crystalline than smaller ones.

Starch is mainly composed of amylose and amylopectin. The total amylose in black beans was 27.2-39.3% (Hoover et al 2010). Total amylose in dry beans is higher than amylose content ≈ 20% in cereal grains in (Hu et al 2010). Amylopectin chain length was intermediate and could possibly yield any type of crystalline structure depending on the environmental temperature (Hizukuri 1985). Amylopectin is ascribed to generate the ordered crystalline structure of starch granules while amylose is considered to disrupt this structural order. Starch granules within the cells of black bean can be stained with acid Fuchsin and Toluidine Blue (Figure 1). The starch granules are embedded in a protein matrix.

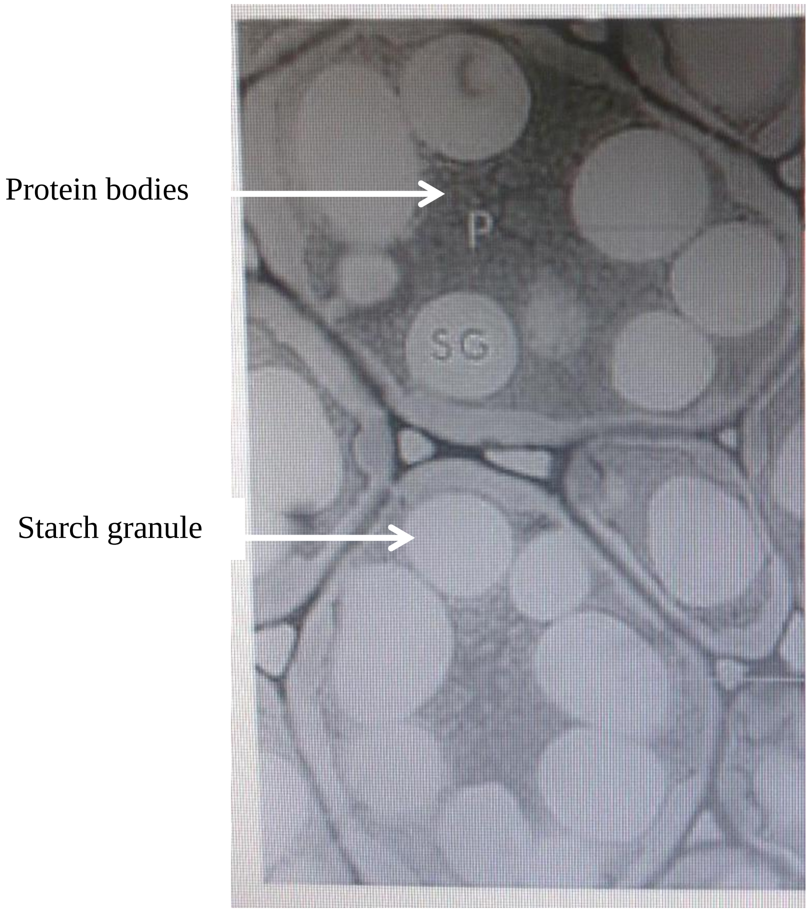


Figure 1. Bright field micrograph of raw black bean stained with acid Fuchsin and Toluidine Blue O to differentiate protein bodies from starch granule within cells (Adapted from Wood et al 1998).

*Starch Functionality*

Bean starches from different growing conditions and genera are distinct in swelling factors, gelatinization temperatures, and other functionalities (Hoover and Ratnayake 2002). Black bean starch could provide unique properties to food systems, such as high gelation temperature, resistance to shear thinning, fast retrogradation, high resistant starch and high elasticity of gel, due to their higher amylose content compared to cereal starches (Ambigaipalan et al 2011). Thus, the utilization of black bean starch as a new ingredient in the food industry has drawn the attention of researchers.

Dry beans starches are known to show higher viscosity than cereal starches (Lineback and Ke 1975). Thus, dry bean starches are more resistant to swelling and rupture towards shear than cereal starches. Several factors have been proposed that could influence this property, such as starch granules size and shape, starch ionic charge, degree and type of degree of granules crystallinity, the protein and fat present, and probably degree and molecular size of starch fractions branching (Schoch and Maywald 1968).

Non-Starch Polysaccharides (NSP)

The NSPs are the principal components of the structural plant cell wall which provide structural features (Selvendran and Robertson 1990). They are complex carbohydrates which include cellulose, hemicellulose, pectin, and mucilage (Cummings and Englyst 1995). The NSPs are the major source of the dietary fiber (DF), which can be further classified as soluble or insoluble dietary fiber. NSPs in dry beans can range from 5-20% with a high portion being insoluble (Tiwari et al 2011). Higher levels of insoluble NSPs than soluble were found in white and pinto beans (Bravo 1999). Findings by Gooneratne et al (1994) showed NSP contents in the embryo, hull, and cotyledon of mung beans of 0.4%, 2.5%, and 10.4%, respectively, and total fiber content of 13.3% as NSP.

Dicots tend to have less hemicellulose and more pectin than monocots (Caffall and Mohnen 2009). Cellulose was reported to be the major component in navy beans seed coat, which was found to be more than 60%, followed by hemicellulose (20%), and small amount of lignin (2%) (Srisuma et al 1991). The pectin layer is found in the plant primary cell wall and the middle lamellae. The pectin content degrades during boiling or other thermal treatment where the possible dissolution of the middle lamellae occurs and the breakdown of pectin through βelimination (Aguilera et al 2009). Mucilage is in the outer layer of the seed (epidermis layer) and is a sticky compound (Harris and Ferguson 1999), which has potential to be used as food ingredient (Brennan et al 2006).

During cooking, the high temperature affects the polysaccharides structure in many ways, mostly breaking linkages and promoting depolymerization (Mattson 1946; Jones and Boulter 1983; Ilker and Szczesniak 1990; Del Valle and Stanley 1995). 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. This process also promotes polysaccharides depolymerization and changes the nutritional properties of the dietary fiber by increasing its water-solubility (Brett and Waldron 1996).

*NSP Functionality*

Soluble NSPs do not completely dissolve in water; however, they have the ability to swell and form a gel or gummy solution. Other functions of NSPs in foods are defined as swelling capacity, water-holding capacity, oil-binding capacity, and cation exchange capacity. There is an increasing interest in the use of dry beans in novel food products due to their significant amounts of dietary fiber (Bressani 1993).

**Protein**

Proteins are one of the main nutrients in black bean and are primarily located in the cotyledon and embryonic axis, with little present in the seed coat. Since the cotyledons are the major part of the seed, they contribute the most to protein content (Tiwari and Singh 2012). Within the cotyledons, the parenchyma cells store carbohydrates as well as the proteins (Tiwari and Singh 2012). The protein bodies (Figure 1) are in the cell matrix between the starch granules and appear as small rounded structures (Wood et al 1998). Berrios et al (1998) reported that black bean protein matrix is composed of individual protein bodies that are small (1-10 μm) and spherical to oval.

Major proteins in dry beans are albumin and globulin (storage protein). Albumins are water soluble whereas globulins are salt soluble (Boye et al 2010). Dry beans are rich in amino acid lysine but lack methionine, cysteine and tryptophan (Deshpande and Nielsen 1987).

Protein Functionality

Black bean proteins provide functional properties such as water holding capacity, fat binding, foaming and gelation. However, there is little information on dry bean protein functionality in food products and its relation with performance. Proteins in bean flour are believed to restrict starch granules swelling and reduce the amylogram viscosity. Hamaker and Griffin (1993) and Yang and Chang (1999) indicated that proteins in rice flour restrict starch granules swelling and reduce the amylogram viscosity. Protein removal was reported to increase the paste viscosity of starch (Yang and Chang, 1999). But Liang and King (2003) observed the opposite effect.

**Lipids**

Lipids in black beans are mostly found in the embryo axis. Lipids in black beans are made up of triacylglycerides and small portion of free fatty acids, sterols, and sterols esters, phospholipids and glycolipids (Sutivisedsak et al 2010). Black bean fat content was reported to be 2.2% (Sutivisedsak et al 2010). Polyunsaturated fatty acids (PUFAs) represent an important lipid class present in dry beans. The most important PUFAs in black beans are linolenic (C18:3) and linoleic (C18:2) acids. Palmitic (C16:0) and oleic (C18:1) acids were detected in significant quantities in black beans (Sutivisedsak et al 2010). Grela and Günter (1994) reported that fat content of common bean (*Phaseolus vulgaris*, cultivar Longina) was 2.2%. Fat content for navy, pinto, and red kidney beans was found to be 1.5, 1.2, and 1.1%, respectively (Meiners et al 1976). Lipid content depends on different factors such as dry bean variety, environmental growing conditions and other growing factors. Lipids in black beans are not a function of storage compound as in soybeans which contain high fat content of ≈20% (Coelho and Benedito 2008). Lipid fraction contains the essential vitamins, E and K of dry beans (Campos-Vega et al 2010). No literature was found that addressed the importance of black bean lipids in functionality; therefore, no lipid functionality is provided in this section.

## Minor Chemical Constituents

### Minerals

Metal ions regulate a range of physiological mechanisms in the plant. Black beans are rich in minerals such as calcium, iron, copper, zinc, potassium, phosphorus, and magnesium (Deshpande and Damodaran 1990). In particular, dry beans contain high amounts of iron, calcium, and zinc. Most of the phosphorus and iron are found in the cotyledons. Calcium, copper, zinc, potassium, phosphorus, and magnesium are mainly found 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). Minerals play an important role in the genetic material including transcription of DNA, translation of RNA and then cell division (Tiwari and Singh 2012).

## Vitamins

Black beans are good source of water soluble vitamins especially B vitamins (thiamine, riboflavin, niacin, pyridoxamine, pyridoxal, and pyridoxine), which are found mostly in the cotyledons. Also, black beans are rich source of folates, which are between 400-600 µg/100g. (Kadam and Salunkhe 1989).

## Other Phytochemicals

Black bean has other phytochemicals such as enzyme inhibitors, lectins, phytates, oligosaccharides and phenolic compounds. The seed coat is rich in phytic acid, tannins, and phenolic compounds (Adebooye and Singh 2007). These compounds exhibit antioxidant activities and protect the seed from oxidative damage. Seed coat pigmentation is due to the presence of phenolic compounds such as phenolic acids, and polyphenols such as tannins, flavonoids and anthocyanins. There is a relationship between tannins and seed coat color, where black beans with a dark purple color have more tannins than white or light colored beans seed coat (Diaz et al 2010). Flavonoids are present in the seed coat, whereas non-flavonoid compounds are present in the cotyledons. Phytic acid is a major storage form for phosphorus and inositol in dry bean seeds. Phytic acid is also known as phytates if in salt form. Dry beans have a high amount of phytates (Sandberg 2002).

Other important bioactive constituents are enzyme inhibitors. Protein digestibility could be affected by enzyme inhibitors in the dry bean seed such as trypsin and chymotrypsin inhibitors. Proteins with high digestibility are desired owing to their positive contribution to nutritional value (Boye et al 2010). These enzyme inhibitors are inactivated during processing such as soaking, cooking, and germination (Jood et al 1989).

Most of the described phytochemicals are considered antinutritional factors (ANFs) in black beans. The inactivation of ANFs is important to ensure nutrient absorption and proper contribution of health benefits by consuming beans (Uebersax 2006). The best pretreatment applied to eliminate/reduce these ANFs would be the one that improves protein and carbohydrate digestibility and does not alter protein content in the bean flour (Maskan and Altan 2011).

## Black Bean Processing

### Whole Bean

Traditionally, black bean processing has involved canning and roasting where the bean remains intact and is eaten as whole cooked seeds. Black bean processing and use by the food industry have increased due to the health benefits offered by dry beans. Due to their low digestion rate, edible dry beans are considered as low glycemic food (Jenkins et al 1983). During cooking, starch digestibility increases due to loss of granular structure during phase transition. A high degree of gelatinization allows starch to be rapidly digested. Accordingly, the processing conditions of bean-based foods influence their glycemic responses (Wang 2013).

The inclusion of black bean food ingredients (flours and fractions-protein, starch, and fiber) into processed food products has increased in recent years (Siddiq and Uebersax 2013). Dry beans can suffer from post-harvest losses during storage due to inappropriate storage conditions. In particular, too high a temperature or insufficient drying increases losses (Tiwari et al 2011). Also, difficulties are encountered due to deficient supply of high quality and reasonably priced raw material, and not adequate internationally recognized quality standard and common nomenclature (Tiwari et al 2011). These factors should be considered when designing a processing system to ensure the final desired product is profitable.

For processing black beans, water inclusion has always been necessary. In fact, soaking of beans with water before cooking has been used for several decades (Buckle and Sambudi 1990). In traditional dry bean cooking, beans are soaked overnight to reduce cooking times. This facilitates starch gelatinization and protein denaturation during cooking (Bellido et al 2003). Cooking, soaking, and drying are pretreatments used in the preparation of whole beans.

**Drying**

Dry beans are dried after harvesting. This step is essential due to the high moisture content in the seed at harvest (18 to 25%). This drying process will reduce the moisture content of the bean seed to 9 to 12%, which is considered an optimum range for safe storage (Tiwari et al 2011). Drying can be done by using different techniques and instruments. The most common method in developing countries for drying is sun drying. Other artificial and commercial methods can be utilized, such as thin-layer drying and fluidized-bed drying. Regardless of the method used, drying beans follow a general concept of removal of free moisture until the seed reaches equilibrium internally. Temperature and moisture content of the seed are factors that will affect drying. Also, the relative humidity and air velocity used to dry the seed will influence drying but to a lesser extend (Kundu et al 2005).

**Soaking**

Soaking is a process whereby water is used to soften the hard cotyledons of beans to reduce cooking time. This process consists of hydrating the seeds with water. During soaking, and initial rapid water uptake occurs due to the filling of capillaries on the surface of the seed coats and at the hilum (Siddiq and Uebersax 2013). The results obtained from soaking will depend on the legume’s genus, species, and cultivar (Tiwari et al 2011). Also, soaking time, temperature, pH, and salinity of the water as well as the storage conditions of the seed before processing can all affect the soaked seeds (Prodanov et al 2004; Carmona-Garcia et al 2007). Black beans contain bioactive compounds, which include ANF, which can be reduce/eliminated by soaking (Tiwari and Singh 2012).

### Cooking

Cooking brings a number of changes not only for physical characteristics, but also in chemical composition of dry beans. Beans are cooked by a boiling process until they become soft. Pressure boiling and steaming can also be used. Cooking will cause changes in beans such as reduction in nitrogen solubility. Starch gelatinization, swelling, leaching of amylose and loses of the crystalline structure can occur during cooking (Ovando-Martinez et al 2011). Also, reduction in phytic acid, inactivation of trypsin inhibitors, and α-galactoside reduction occurs when cooking beans (El-Adawy 2002; Prodanov et al 2004). Cooking also improves their organoleptic properties such as improvement in texture as it is processed and changes from hard to soft and reduction of the beany flavor occurs (El-Adawy 2002; Prodanov et al 2004).

When dry beans are subjected to thermal processing, changes in their structure occurs. The addition of heat can cause changes in protein structure and functionality, also alterations in nutritional, physicochemical, and functional properties of the seeds. Ovando-Martinez et al (2011) stated that the amount of protein associated with insoluble dietary fiber could explain the reduction of protein available for digestion in the cooked beans.

## Milling

### Particle Size Reduction

Particle size reduction involves the application of a force to reduce the average size of the particles. Size reduction can use combination of forces (i.e. abrasion, shearing, compression, and impact). A pearler uses abrasion as a primary force. Mills such as roller and centrifugal mill use shearing as one of their primary forces. Ball mills utilize compression while hammer mills utilize impact forces. Most plant materials possess fibrous structure, and they are not well processed by compression but are well ground under impact, tensile or shear conditions (Laskowski et al 1993).

The moving action of the grinding parts transfers energy to the material being milled. The absorbed energy is stored as stress energy. When a threshold level of stress energy is exceeded, a fracture is formed and energy is released primarily as heat. The material encounters mechanical stress first and then ruptures by excess in stored energy, which depends upon material hardness and ease to crack or its friability (Earle and Earle 2004).

Particle size of milled dry beans is an important variable of flour quality. The particle size influences the functional and physiochemical properties of the flour (Kerr et al 2000). Kerr et al (2000; 2001) reported that the particle size affected the end product attributes, where chips had a higher snapping force when made from fine cowpea flour than with coarse flour. McWatters (1983) and Ngoddy et al (1986) also reported that particle size will affect flour properties, hence the end-product quality. 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.

Limsangouan and Isobe (2009) demonstrated that particle size impacts the functional properties of bean samples when using different milling processes (screw crushing, hammer milling and jet milling). The results obtained were based on the particle size of the flour and the volume of air flushing in the process. The screw crusher produced coarser particles when compared to the hammer mill and jet mill. Hammer mill produced fine particles and jet mill even finer particles (Sander and Schonert 1999; Picot and Lacroix, 2003; Sowbhagya et al 2007; Limsangouan and Isobe 2009). Antioxidant capacity, phenolic content, and resistant starch were higher when milled using hammer milling compared to the other mills used.

Limsangouan and Isobe (2009) explained that since particle size is an important parameter in choosing an extraction procedure (Franco et al 2007), and higher performance is associated with a smaller particle size, it was considered that the hammer mill was more suitable than the screw crusher. They also explained that the jet mill showed a slightly different efficiency with respect to the functional properties compared with the hammer mill, because the jet mill process flushed a large quantity of air (nozzle pressure = 7.0 $kg/cm^3$; air volume = 2.6 $m^3/min$) that produced higher oxidation during the grinding process.

**Physical Seed Properties**

The size, shape, weight, volume, porosity, density, and coefficient of friction are factors that influence the performance and quality of milling dry beans (Altuntas and Demirtola 2007). There are seed parameters relevant to milling yields of beans: 1) when the seed coat fraction is low, the milling yield will increase; assuming other seed attributes are the same, 2) seed size should be uniform to obtain higher milling yields, 3) seed shape should be uniform to reduce broken seeds, and 4) moisture content should also have to be considered for quality of milling (Wang 2005; Wood et al 2008; Goyal et al 2009; Wood 2010).

**Moisture Conditioning**

Moisture conditioning, sometimes referred to as tempering, is used to bring the grain/seed to desired moisture content before milling. Tempering is done by adding water depending on the seed moisture level. In dry beans studies, tempering has been used as a pretreatment for micronization—an infrared heating process used in plant materials—which reduces cooking times in dry beans (Bellido et al 2003). Tempering beans can result in smaller nutritional losses when compared to soaking/drying or cooking/drying; however, studies in this area of only tempering dry beans before milling are scarce.

**Seed Hardness**

Seed hardness is a concept that provides understanding of the mechanical properties of the seeds. From a milling point of view, seed hardness is important because it affects the milling time and energy expenditure as well as the final ground product properties and appearance (De Francisco et al 1982). If the milling step is used as a pretreatment for further dry fractionation to separate starch from protein fraction, seed hardness also have a significant impact. This is because the yields of starch and protein concentrates were found to be related to seed hardness (Tyler and Panchuk 1982).

Most studies have concluded that moisture of the seed is one of the most important factor affects hardness (Gasiorowski and Kolodziejczyk 1990; Morris 2002; Tranquilli et al 2002). The seed coat of black beans, which possess several layers of cells, absorbs water slower than does the endosperm or the germ (Frączek et al 2005). It is also important to explain that seed hardness also encompasses not only the hardness of the seed coat but also the hardness of the interior of the seed. This hardness measurement is influenced by the combination of those two components.

Studies have been based on compressive strength of seeds, and on seed hardness and elasticity (Liu et al 1990; Foutz et al 1993; Haman et al 1994; Zayas et al 1996; Frączek et al 2005). For example, when moisture content of wheat grain is increased, the wheat outer layer (bran) gains elasticity (Glenn an Johnston 1992). In this case seed hardness and elasticity of the grain can be determined. This is important because the bran becomes resistant and tough and endosperm more susceptible to size reduction so the bran is separated from the endosperm during milling. Frączek et al (2005) studied seed hardness on cereal grains and dry seeds and suggested that mechanical properties changed more for the seed coat of dry seeds than cereal grains, specifically those of the endosperm and germ. They also 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".

Mechanical properties of seeds such as hardness are affected by anatomical compositions and moisture content. Seed hardness is an important parameter for the miller. Seed hardness could predict the grinding time and energy expenses of the milling process. Milling affects properties and appearance of the final ground product, i.e. flour. Seed hardness determination will differ depending upon the seed specific applications.

**Black Bean Milling**

Milling is a process where reduction of grains to meal or flour occurs. Milling includes grinding, sieving, and purifying (Limsangouan and Isobe 2009). Some legumes (i.e. peas and lentils) are subjected to a dehulling and splitting process before milling (Tiwari et al 2011). Dehulling is the process whereby the seed coat is removed and splitting is when the seed is divided into the two cotyledons. In general, dehulling dry beans is a very difficult process and it is not done due to highly attached seed coat to the cotyledons.

Dry bean flour can be obtained by using high speed mills (i.e. hammer mill and pin mill). The milling operation is based on the principle of impact and shear forces, or a combination of both. In an impact mill the energy of a rupture is mainly concentrated at a single point, whereas in a roller mill the energy is more evenly dispersed (Dijkink and Langelaan 2002). The fineness of the particles of impact mills is controlled by the peripheral speed of the rotors and the feed rate. When more fineness of particles is required, the number of particles and number of surface contacts (collision with hammers/pins) is increased (Patil et al 2005).

## Mill Description

### Centrifugal Mill

Centrifugal mills are used to grind entire seed to produce whole flour. Centrifugal mill consists of three parts: 1. vibratory feeder; 2. rotor and screen; and 3. vacuum system (Figure 2). Vibratory feeder controls feed rate of seeds into the milling chamber. The vacuum system cools the mill and milled product by drawing air through the milling chamber. Rotor and screen are found in the milling chamber. The center of the rotor is flat open area where the seeds are first deposited by the feeder. Wedged shaped blades are located at the end of the rotor. As the rotor spins, the seeds are impacted by the blades and thrown centrifugally against a grated screen. Thus, centrifugal mill uses the particle size reduction principle of impact and shearing forces. Centrifugal mill can grind any soft, medium-hard, brittle, and fibrous materials (Anonymous 2014).

Figure 2. Centrifugal mill (left) and the rotor with the screen (right).

**Burr Mill**

Burr mill consists of a set of knives which uses a cutting, shearing, and crushing actions for particle size reduction (Haque 1991). Burr mills, also known as plate mills, have two circular plates (Figure 3) where material is fed between them. One of the circular plates is fixed and the other rotates. The material come in contact with the two plates where is sheared and crushed and exists through the edge of the plates. Burr mills have the plates horizontally mounted (Earle and Earle 2004). Particle size principle of the burr mill is mainly due to cutting and shearing forces.

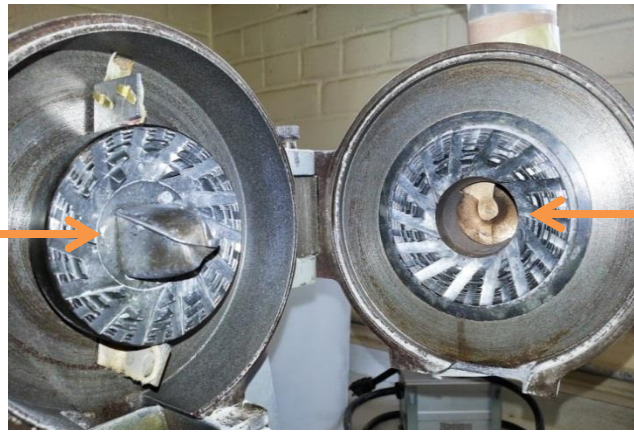

Figure 3. Open burr mill and its plates.

**Roller Mill**

Roller mills (Figure 4) are commonly used in the grain milling industry to mill wheat into bran and flour. Roller mill utilizes a multiple stage approach in series of rolls for fine particle size reduction. Roller mill includes two sections—break section and reduction section. Roll configurations include roll surface, roll-speed differential, and roll gap. Roll surface 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 which 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).

The basic designed roller mill has two rolls positioned together and separated by small gap. The paired rolls can rotate at the same speed or can rotate at different speed (Posner and Hibbs 2005). Differential in speed results in shear action which is used to remove bran from the wheat kernel. Compression force is present when feed material is drawn between the rolls, whereas, shearing forces result when roll-speed differential and roll corrugation are used

(Schorno 2006). Roller mills are energy efficient, particle size distribution obtained is uniform, and in general, there create less noise and dust. (Koch 2002).

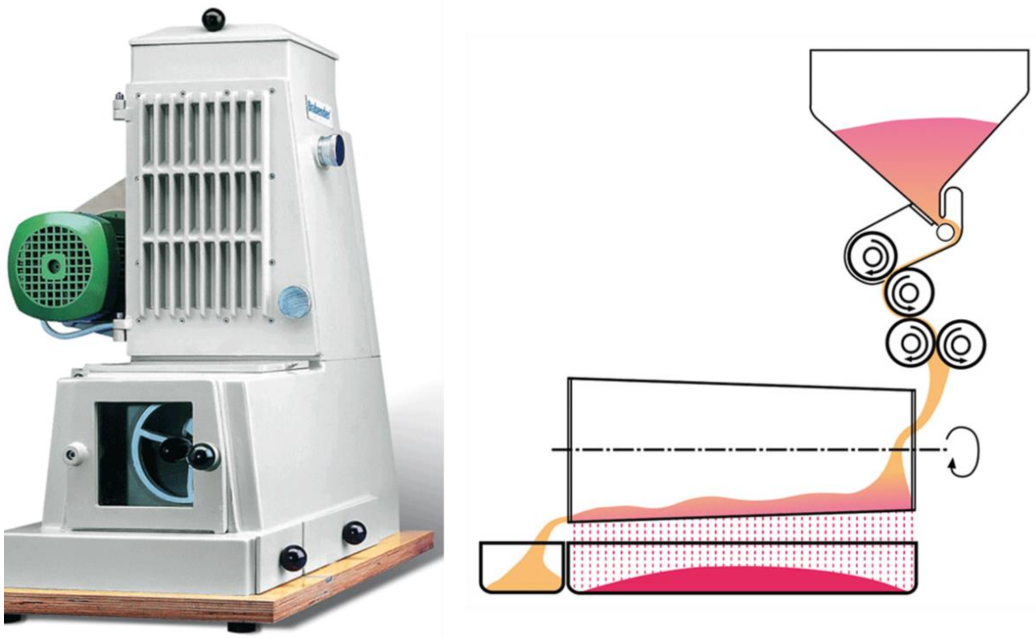

Figure 4. Photograph of a roller mill and scheme showing the set of paired rolls. Photograph from www.brabender.com.

## Literature Cited


Adebooye, O. C., and Singh, V. 2007. Effect of cooking on the profile and phenolics, tannins, phytate, amino acid, fatty acid and mineral nutrients of whole-grain and decorticated vegetable cowpea (*Vigna unguiculata* L. Walp). J. Food Qual. 30(6):1101-1120.

Aguilera, J. M., Lusas, E. W., Uebersax, M. A., and Zabik, M. E. 1982. Development of food ingredients from navy beans (*Phaseolus vulgaris*) by roasting, pin milling, and air classification. J. Food Sci. 47:1151-1154.

Aguilera, Y., Martin, O., Cabrejas, M. A. Benitez, V, Molla, E., Lopez-Andreu, F. J., estaban, R. M. 2009. Changes in carbohydrates fraction during dehydration process of common legumes. J. Food Compos. Anal. 22:678-683.

Altuntas, E., and Demirtola, H. 2007. Effect of moisture content on physical properties of some grain legume seeds. NZ J. Crop Hort. Sci. 35(4):423-433.

Ambigaipalan, P., Hoover, R., Donner, E., Liu, W., Jaiswal, S., Chibbar, R., Nantanga, K. K. M., and Seetharaman, K. 2011. Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physicochemical properties. Food Res. Int. 44:2962–2974.

Anonymous. 2014. Ultra Centrifugal Mill ZM 200. [Web Page]. Located at: http://www.retsch.com/products/milling/rotor-mills/zm-200/ (Accessed on 4/10/14).

Bellido, G. G., Arntfield, S. D., Scanlon, M. G., and Cenkowski, S. 2003. The effect of micronization operational conditions on the physicochemical properties of navy beans (*Phaseolus vulgaris* L.). J. Food Sci. 68:1731-1735.

Berrios, J. De J., Swanson, B. G., and Cheong, W. A. 1998. Structural characteristics of stored black beans (*Phaseolus Vulgaris* L.). Scanning. 20:410–417.

Boye, J., Zare, F., and Pletch, A. 2010. Review: Pulse proteins: Processing, characterization, functional properties and applications in food and feed. Food Res. Int. 43:414–431.

Bravo, L. 1999. Effect of processing on the non-starch polysaccharides and in vitro starch digestibility of legumes. Food Sci. Technol. Int. 5:415-423.

Bravo, L., Siddhuraju, P., and Saura-Calixto, F. 1998. Effect of various processing methods on the in vitro starch digestibility and resistant starch content of Indian pulses. J. Agric. Food Chem. 46: 4667-4674.

Brennan, C. S., Suter, M., Matia-Merino, L., Luethi, T., Ravindran, G., Goh, K., Ovortrup, J. 2006. Gel and pasting behavior of fenugreek-wheat starch and fenugreek-wheat flour combinations. Starch/Staerke. 58:527-535.

Bressani, R. 1993. Grain quality of common beans. Food Rev. Int. 9:237–297.

Brett, C., and Waldron, K. 1996. Bioactive Cell Wall Components. Pages 235-255 in: Physiology And Biochemistry Of Plant Cell Walls 2nd Ed. Chapman and Hall. London, UK.

Buckle, K. A., and Sambudi, H. 1990. Effect of soaking and boiling treatments on the quality of winged bean seed. J. Sci. Food Agric. 53:379–383.

Bukovac, M. J., Rasmussen, H. P., and Shull, V. E. 1981. The cuticle: Surface structure and function. Scan. Electr. Microscopy 3:213-223.

Caffall, K. H. and Mohnen, D. 2009. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbodydr. Res. 344:1879-1900.

Campos-Vega, R., Loarca-Pina, G., Oomah, B. D. 2010. Minor components of pulses and their potential impact on human health. Food Res. Int. 43:461-482.

Carmona-Garcia, R., Osorio-Diaz, P., Agama-Acevedo, E., Tovar, J., and Bello-Perez, L. A. 2007. Composition and effect of soaking on starch digestibility of *Phaseolus vulgaris* (L.) cv. ‘Mayocoba’. Int. J. Food Sci. Technol. 42:296-302.

Coelho, C. M. M., and Benedito, V. A. 2008. Seed development and reserve compound accumulation in common bean (*Phaseolus vulgaris* L.). Seed Sci. Biotechnol. 2:42-52.

Creason, H. 1975. Grinding and corrugating rolls. Bull. Assoc. Oper. Millers, November, p. 3569.

Cummings, J. H., and Englyst, H. N. 1995. Gastrointestinal effects of food carbohydrate. Am. J. Clin. Nutr. 61:938S-945S.

De Francisco, A., Shepherd, A. D., Hosney, R. C., Varriano-Marston, E. 1982. Decorticating pearl millet and grain sorghum in a laboratory abrasive mill. Cereal Chem. 59:1-5.

Del Valle, J. M., and Stanley, D. W. 1995. Reversible and irreversible components of bean hardening. Food Res. Int. 28:455–463.

Deshpande, S. S. and Damodaran, S. 1990. Food legumes: Chemistry and Technology. Pages 147-241 in: Advances in Cereal Science and Technology. Vol. X.Y. Pomeranz, Ed. American Assoc. of Cereal Chem.: St. Paul, MN.

Deshpande, S. S., and Nielsen, S. S. 1987. In vitro enzymatic hydrolysis of phaseolin, the major storage protein of *Phaseolus vulgaris* L. J. Food Sci. 52:1326-1329.

Diaz, A. M., Caldas G. V., and Blair M. W. 2010. Concentrations of condensed tannins and anthocyanins in common bean seed coats. Food Res. Int. 43:595–601.

Dijkink, B. H., and Langelaan, H. C. 2002. Milling properties of peas in relation to texture analysis. Part 1. Effect moisture content. J. Food Eng. 51(2):99-104.

Earle, R. L., and Earle, M. D. 2004. Unit Operations in Food Processing. Web Ed. New Zealand: The New Zealand Institute of Food Science and Technology Inc. [Web Page]. Located at: http://www.nzifst.org.nz/unitoperations/sizereduction1.htm#plate (Accessed on 4/7/14).

El-Adawy, T. A. 2002. Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods Human Nutr. 57:83–97.

Foutz, T.L., Thompson, S. A., and Evans, M. 1993. Comparison of grain loading response of packed grain and individual kernels. Transactions ASAE. 36:568-576.

Franco, D., Pinelo, M., Sineiro J., and Nonez, M. J. 2007. Processing of *Rosa rubiginosa*: Extraction of oil and antioxidant substances. Bioresour. Technol. 98:3506-3512.

Frączek, J., Hebda, T., Đlipek, Z., and Kurpaska, S. 2005. Effect of seed coat thickness on seed hardness. Can. Biosyst. Eng. 47:4.1-4.5.

Gasiorowski, H., and Kolodziejczyk, P. 1990. Microhardness rye seeds. Przeglad Zbozowo – Mlynarski. English Abstract. 5(6):9-12.

Glenn, G., and Johnston, R. 1992. Mechanical properties of starch, protein and endosperm and their relationship to hardness in wheat. Food Struct. 11:187–199.

Gooneratne, J., Majsak-Newman, G., Robertson, J. A., and Selvendran, R. R., 1994. Investigation of factors that affect the solubility of dietary fiber, as nonstarch polysaccharides, in seed tissues of mung bean (*Vigna radiata)* and black gram (*Vigna mungo*). J. Agri. Food Chem. 42:605-611.

Goyal, R., Vishwakarma, R., and Wanjari, O. 2009. Effect of moisture content on pitting and milling efficiency of pigeon pea grain. Food Bioprocess. Technol. 3:146-149.

Grela, E. R., and Günter, K. D. 1994. Fatty acid composition and tocopherol content of some legume seeds. Anim. Feed Sci. Technol. 52:325-331.

Gujska, E., Reinhard, W. D. and Khan, K. 1994. Physico-chemical properties of field pea, pinto and navy bean starches. J. Food Sci. 59:634–637.

Haman, J., Dobrzanski, B., Szot, B., and Stepniewski, A. 1994. Strength of shell in compression test of rape seed. Int. Agrophysics 8:245-250.

Hamaker, B. R., and Griffin, V. K. 1993. Effect of disulfide bond-containing protein on rice starch gelatinization and pasting. Cereal Chem. 70:377-380.

Haque, E. 1991. Application of size reduction theory to roller mill design and operation. Cereal Foods World 36:368-375.

Harris, P. J., and Fergunson, L. R. 1999. Dietary fibres may protect or enhance carcinogenesis. Mutual Res. 443:95-110.

Hizukuri, S. 1985. Relationship between the distribution of the chain-length of amylopectin and the crystalline-structure of starch granules. Carbohydr. Res. 141:295-306.

Hoover, R., and Ratnayake, W. S. 2002. Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. Food Chem. 78:489-498.

Hoover, R., Hughes, T., Chung, H. J., and Liu, Q. 2010. Composition, molecular structure, properties, and modification of pulse starch: a review. Food Res. Int. 43:399-413.

Hu, G., Burton, C., and Yang, C. 2010. Efficient measurement of amylose content in cereal grains. J. Cereal Sci. 51:35-40.

Ilker, R., and Szczesniak, A. 1990. Structural and chemical bases for texture of plant based foodstuffs. J. Texture Stud. 21:1–36.

Jenkins, D. J. A., Wolever, T. M. S., Jenkins, A. L., Thorne, M. J., Lee, R., Kalmusky, J., Reichert, R., and Wong, G. S. 1983. The glycemic index of foods tested in diabetic-patients-a new basis for carbohydrate exchange favoring the use of legumes. Diabetologia 24:257-264.

Jones, P. M. B., and Boulter, D. J. 1983. The cause of reduced cooking rate in Phaseolus vulgaris following adverse storage conditions. J. Food Sci. 48:623–649.

Jood, S., Chauhan, B. M., and Kapoor, A. C. 1989. Protein digestibility (in vitro) of chickpea and blackgram seeds as affected by domestic processing and cooking. Plant Foods Hum. Nutr. 39:149–154.

Kadam, S. S., and Salunkhe, D. K. 1989. Minerals and vitamins: Pages 117-121 in: Handbook of World Food Legumes. Salunke, D. K. Ed. CRC Press: Boca Raton, Fl.

Kerr, W. L., Ward, C. D. W., McWatters, K. H., and Resurreccion, A. V. A. 2000. Effect of milling and particle size on functionality and physiochemical properties of cowpea flour. Cereal Chem. 77:213-219.

Kerr, W. L., Ward, C.D.W., McWatters, K. H., and Resurreccion, A. V. A. 2001. Milling and particle size of cowpea flour and anck chip quality. Food Res. Int. 34:39-45.

Koch, K. May 2002. Hammermills and roller mills. Kansas State University. Manhattan, KS. [Web Page]. Located at: http://www.ksre.ksu.edu/bookstore/pubs/mf2048.pdf. (Accessed on 4/9/14).

Kosson, R., Czuchajowska, Z., Pomeranz, Y. 1994. Smooth and wrinkled peas. II. Distribution of protein, lipid, and fatty acids in seed and milling fractions. J. Agric. Food Chem. 42:96-99.

Kundu, K. M., Das, R., Datta, A. B., and Chatterjee, P. K. 2005. On the analysis of drying process. Dry. Technol. 23:1093-1105.

Laskowski J., Skonecki S., and Melcion J.-P. 1993. The ability to compression of ground plant materials (Abstract in English). Proceedings of the VI Konf. Nauk.- Tech. Machinery Design and Maintenance in Food Industry. Politechnika Gdanska, SIiTMP, Warszawa. 1:87-92.

Liang, X., and King, J. M. 2003. Pasting and crystalline property differences of commercial and isolated rice starch with added amino acids. J. Food Sci. 68:832–838.

Limsangouan, N., and Isobe, S. 2009. Effect of milling process on functional properties of legumes. Kasetsart. J. Nat. Sci. 43:745-751.

Lineback, D. R., and Ke, C. H. 1975. Starches and low-molecular -weight carbohydrates from chick pea and horse bean flours. Cereal Chem. 52:334-347.

Liu, M., Haghighi, K., Stroshine, R. L., and Ting, E. C. 1990. Mechanical properties of the soybean cotyledon and failure of soybean kernels. Transaction ASAE. 32:559- 566.

Maskan, M., and Altan, A. 2011. Nutritional changes During Extrusion Cooking: Pages 87-102 in: Advances in Food Extrusion Technology. Mary Ellen Camire Ed. CRC Press. Boca Raton, Fl.

Mattson, S. 1946. The cookability of yellow pear: A colloid-chemical and biochemical study. Acta Agriculturae Suecana II. 2:185–231.

McWatters, K. H. 1983. Compositional, physical, and sensory characteristics of akara processed from cowpea pasta and Nigerian cowpea flour. Cereal Chem. 60:333-336.

Meiners, C. R., Derise, N. L., Lau, H. C., Ritchey, S. J., and Murphy, E. W. 1976. Proximate composition and yield of raw and cooked mature dry legumes. J. Agric. Food Chem. 24:1122-1126.

Morris, C. F. 2002. Puroindolines: The molecular basis of wheat grain hardness. Plant Molec. Biol. 48:633-647.

Ngoddy, P. O., Enwere, N. J., and Onurah, V. I. 1986. Cowpea flour performance in akara and moin-moin preparations. Trop. Sci. 26:101-119.

Otto, T., Baik, B., and Czuchajowska, Z. 1997. Microstructure of seeds, flours, and starches of legumes. Cereal Chem. 74:445-451.

Ovando-Martinez, M., Osorio-Diaz, P., Whitney, K., Bello-Perez, L.A., and Simsek, S. 2011. Effect of the cooking on physicochemical and starch digestibility properties of two varieties of common bean *(Phaseolus vulgaris* L.*)* grown under different water regimes. Food Chem. 129:358-365.

Patil, R. T., Berrios, J. De J., Tang, J., Pan J., and Swanson B. G. 2005. Milling and particle size characterization of pin milled legumes. ASAE Paper No. 056077. St. Joseph, Mich.

Picot, A., and Lacroix, C. 2003. Effect of micronization on viability and thermotolerance of probiotic freeze-dried cultures. Int. Dairy J. 13:455-462.

Posner, E. S., and Hibbs, A. N. 2005. The grinding process. Pages 185-219 in: Wheat Flour Milling 2nd Ed. American Assoc. of Cereal Chem.: St. Paul, MN.

Powrie, W. D., Adams, M. W., Pflug, I. J. 1960. Chemical, anatomical, and histochemical studies of the Navy bean seed. Agronom. J. 52: 163-167.

Prodanov, M., Sierra, I., and Valverde-Vidal, C. 2004. Influence of soaking and cooking on the thiamin, riboflavin and niacin contents of legumes. Food Chem. 84:271-277.

Rahman, M.S. 2007. Postharvest Handling of Grains and Pulses: Pages 74-119 in: Handbook of Food Preservation. 2nd Ed. Ajit K. Mahapatra and Yubin Lan Eds. CRC Press. Boca Raton, FL.

Sandberg, A. S. 2002. Bioavailability of minerals in legumes. Br. J. Nutr. 88:281-285.

Sander, U., and Schonert, K. 1999. Operational conditions of a screw-feeder-equipped high-pressure roller mill. Powder Technol. 105:282-287.

Schoch, T. J., and Maywald, E. C. 1968. Preparation and properties of various legume starches. Cereal Chem. 45:564-573.

Schorno, A. L. 2006. Flaxseed milling and the stability of milled flaxseed. Ph.D. Dissertation, North Dakota State University, Fargo.

Selvendran, R. R., and Robertson, J. A. 1990. The Chemistry of Dietary Fibre – An Holistic View of Cell Wall Matrix. Pages 40-43 in: Dietary Fibre: Chemical and Biological Aspects. Southgate, D.A.T. Ed. Woodhead Publishing, Cambridged, UK.

Sefa-Dedeh, S., and Stanley, D. W. 1979. Textural implications of the microstructure of legumes. Food Technol. 33:77-83.

Siddiq, M., and Uebersax, M. A. 2013. Dry Beans and Pulses Production and consumption—An Overview; Market Classes and Physical and Physiological Characteristics of Dry Beans; Composition of Processed Dry Bean and Pulses: Pages 3-22; 55-74; 103-128 in: Dry Beans and Pulses: Production, Processing and Nutrition. First Ed. John Wiley & Sons, Inc.: Oxford, UK.

Sowbhagya, H. B., Sampathu, S. R. and Krishnamurthy, N. 2007. Evaluation of size reduction on the yield and quality of celery seed oil. J. Food Eng. 80:1255-1260.

Srisuma, N. Ruengsakulrach, S., Uebersax, M. A., Bennick, M. R., and Hammerschmidt, R. 1991. Cell-wall polysaccharides of navy beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem. 39:855-858.

Sutivisedsak, N., Bryan R. Moser, B. R., Sharma, B. K., Evangelista, R. L., Cheng, H. N., Lesch, W. C., Tangsrud, R. R., and Biswas, A. 2010. Physical properties and fatty acid profiles of oils from black, kidney, great northern, and pinto beans. J. Am. Oil Chem. Soc. 88(2):193-200. DOI 10.1007/s11746-010-1669-8.

Swanson, B. G., Hughes, J. S., and Rasmussen, P. H. 1985. Seed microstructure: a review of water imbibitions in legumes. Food Microstuct. 4:115-124.

Tiwari, B. K., and Singh, N. 2012. Chapters 1-5, 7. Pages 1-82, 107-129 in: Pulse Chemistry and Technology. First Ed.: Cambridge: The Royal Society of Chemistry. Cambridge, UK.

Tiwari, B. K., Gowen, A., and McKenna, B. 2011. Introduction; Chapters 6-8. Pages 157-222 in: Pulse Foods: Processing, Quality and Nutraceutical Applications. First Ed.: Academic Press. San Diego, CA.

Tranquilli, G., Heaton, J., Chicaiza, O., and Dubcovsky, J. 2002. Substitutions and deletions of genes related to grain hardness in wheat and their effect on grain texture. Crop Sci. 42:1812-1817.

Tyler, R. T., and Panchuk, R. D. 1982. Effect of seed moisture content on the air classification of field peas and faba beans. Cereal Chem. 59:31-33.

Uebersax, M. A. 2006. Dry Edible Beans: Indigenous Staple and Healthy Cuisine. Professor, Department of Food Science & Human Nutrition, Michigan State University. [Web Page]. Located at: http://forumonpublicpolicy.com/archive06/uebersax.pdf. (Accessed on 4/10/14).

Wang, N. 2005. Optimization of a laboratory dehulling process for lentil *(Lens culinaris).* Cereal Chem. 82:671-676.

Wang, H. 2013. Studies on *Phaseolus Vulgaris* L. Var. Great Northern bean for utilization in food processing. M.S. Thesis, University of Nebraska, Lincoln).

Wood, J. A. 2010. The influence of seed morphology, ultra-structure and chemistry on the milling quality of chickpea. Ph.D. Dissertation, School of Environment and Rural Science, University of New England, Armidale.

Wood, D. F., Venet, C., and Berrios, J. De J. 1998. Microstructure of the processing of black beans (*Phaseolus vulgaris* L.) from purée to drum-dried flakes. Scanning. 20: 335–344.

Wood, J. A., Knights, E. J., and Harden, S. 2008. Milling performance in desi-type chickpea (*Cicer arietinum* L.): efects of genotype, environment and seed size. J. Sci. Food Agric. 88:108-115.

Yang, C., and Chang, W. 1999. Effects of protein and lipid binding to starch on the physicochemical and pasting properties of rice flour. Food Sci. Agric. Chem. 1:277-285.

Zayas, I., Martin, C. R., Steele, J. L., and Katsevich, A. 1996. Wheat classification image analysis and crush-force parameters. Transaction of the ASAE. 39:2199-2204.

# PAPER 1. PHYSICAL AND CHEMICAL COMPOSITION OF BLACK BEANS (*Phaseolus vulgaris* L.) AND EFFECT OF SEED PRETREATMENT

## Abstract

Black bean seed structure is composed of cotyledon, seed coat, and embryo. The cotyledon had the highest protein (26%), lipid (4.4%), total starch (35.3%), and ash (4.2%) contents. Black bean seed are often pretreated by cooking or soaking in order to reduce antinutritional factors commonly found in the seed. The effect of pretreatment on the seed appearance was recorded. Changes in seed appearance were recorded for pretreated seed during soaking, cooking, followed by drying as well as tempered. For wet physical appearance, seed weight gain and cooking or soaking loss were studied. Seeds soaked for 24 h gained 213% of their weight. Leaching of soluble compounds from the seed was greater with soaking 24 h than with cooking in boiling water for 20 min. After drying the cooked or soaked seeds, the overall seed dimensions returned to the original non-treated seed dimensions 9.6, 5.9, and 4.9 mm for length, width, and thickness, respectively. The 100-seed weight ranged from 19 to 23 g for all pretreated samples. Seed test weight decreased with increased time of pretreatment. The lowest test weight was for soaked-dried and cooked-dried seeds. Moisture conditioning and seed hardness were other parameters studied for pretreated seeds. Seed coats held more moisture than cotyledon for all pretreatments. Fracture force of the cooked-dried or soaked-dried seeds decreased with increased pretreatment level, whereas, fracture force of tempered seeds increased as moisture content of the seed increased.

## Introduction

Black beans are mostly consumed as intact cooked seeds; however, a significant raise in black bean flour production had been observed (Siddiq and Uebersax 2013) due to its utilization in novel foods to increase nutritional value or in gluten-free based foods. For years, the microstructure, morphology, and characteristics of cereal grains have been studied and well understood, which allowed advances in technology for wet and dry milling of wheat and corn (Otto et al 1997). However, little is known of black bean seed structure and the effect of cooking or soaking followed by drying or tempering on black bean structure. In order to further understand black bean flour composition, black bean seed structure and effect of pretreatments needs to be studied.

Changes in seed structure during soaking or cooking are well-studied. Cooking brings a number of changes not only on physical characteristics, but also in chemical composition of black beans. Cooking is done as a boiling process whereby the seed becomes soft. Starch gelatinization, swelling, leaching of amylose and loses of the crystalline structure occurs during cooking (Ovando-Martinez et al 2011). Soaking is a process whereby water is used to soften the hard cotyledons of beans to reduce cooking time. This process consists of hydrating the seeds with water. Soaking time, temperature, pH, and salinity of the water as well as the storage conditions of the seed before processing can all affect the soaked seeds (Prodanov et al 2004; Carmona-Garcia et al 2007). During cooking or soaking, seed weight gain and cooking or soaking loss can be studied to determine the effect of both pretreatments. Tempering is not widely used for black beans; however, it has been used as a pretreatment before micronization - an infrared heating process used in plant materials, which reduces cooking times in dry beans (Bellido et al 2003).

Seed characteristics such as seed dimension, 100-seed weight, and test weight are examples of physical appearance of the seeds. Also the study of seed physical structure based on seed hardness can be studied. The hardness value is the maximum force of compression of a food material (Bourne 2013). Fracturability is recorded when a compression curve shows its first significant peak (where the force falls off) during the probe's first compression of the food material (Bourne 2013). Seed hardness provides understanding of the mechanical properties of the seeds which is important for milling processes. Most researchers have concluded that seed moisture is one of the most important factors that affect hardness (Gasiorowski and Kolodziejczyk 1990; Morris 2002; Tranquilli at al 2002).

The objective of this experiment was to evaluate the differences in black bean seed physical structure after pretreatments. This paper provides general information that will be used as a reference for the following papers based on seed studied parameters.

## Materials and Methods

### Pretreatments

Black beans were obtained from Kelley Bean Company (Scottsbluff, NE). Schematic representation of black bean seed pretreatments used is shown in Figure 5. Clean black beans were cooked in distilled water for 5, 10, 15, and 20 min. Black beans were soaked in distilled water for 6, 12, 18, and 24 h. Both cooked and soaked samples were drained and placed on baking sheets and dried to 10% moisture content using a forced-air oven at 50 ºC for 24 h.

Black beans were tempered with distilled water to 6, 8, 10, 12, and 14% moisture. Moisture conditioning was allowed to equilibrate throughout the seed at room temperature for 72 h before milling. Cooked-dried, soaked-dried, and tempered pretreatments were considered as three separate experiments.

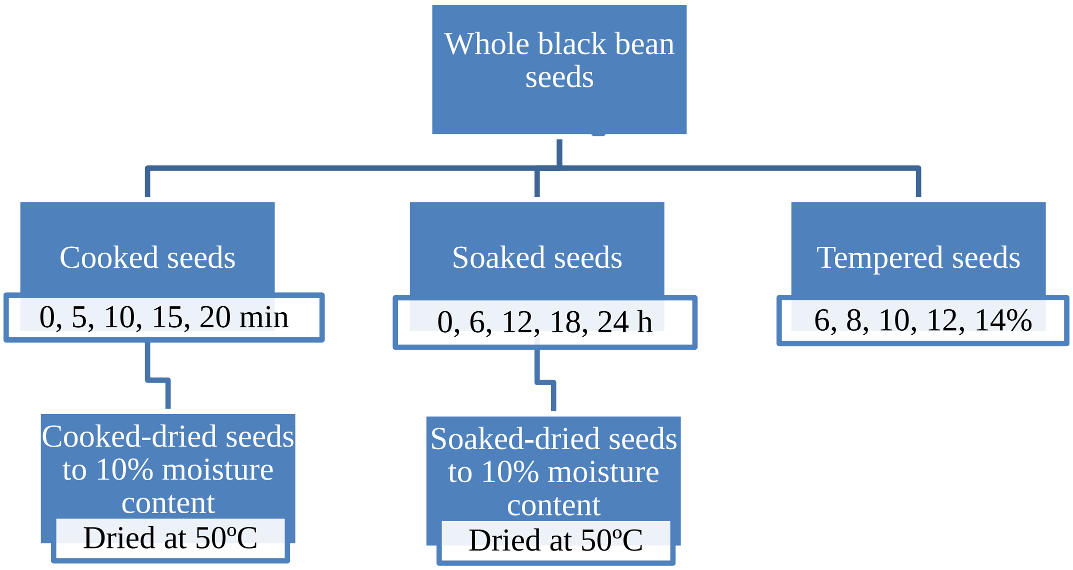


Figure 5. Schematic representation of black bean seed pretreatments.

**Proximate Chemical Composition**

Proximate chemical composition of raw black bean seed, cotyledon, and seed coat fractions were determined. 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 was analyzed using Leco combustion nitrogen analyzer (LECO Corp. St. Joseph, MI, USA). Protein content was calculated as %N × 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. Cotyledon and seed coat were hand separated and moisture content measured according to AACC International Approved Method 44-15.02.

## Scanning Electronic Microscopy (SEM)

Treated black bean seeds, as well as flour, were mounted on cylindrical aluminum mounts with silver paint (SPI Supplies, Structure Probe Inc., West Chester, Pennsylvania 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. For the seed, four parts were analyzed: seed coat surface, seed coat interior, cotyledon top surface and cotyledon interior. Flour samples were from non-treated, cooked-dried (20 min), soaked-dried (24 h), and tempered (14% moisture content) seeds.

## Physical Seed Properties

Wet seed appearance for both cooked and soaked seeds were determined. Seed weight gain after cooking or soaking the seed was recorded. Also, cooking and soaking loss was determined. Seed dimension was determined for seeds dried after soaking or cooking. Seed dimension was calculated using a caliper and measured the seed length, width, and thickness as shown in Figure 6. Mass of 100 black bean seeds was determined by taking 100 seeds randomly and measuring the mass of seeds for all the different treatment using an electric balance. Test weight was determined before and after all seed treatments. Test weight of whole black bean seeds was measured using the hectoliter weight (Ohaus, Des Plaines, IL, USA) procedure approved by GIPSA.

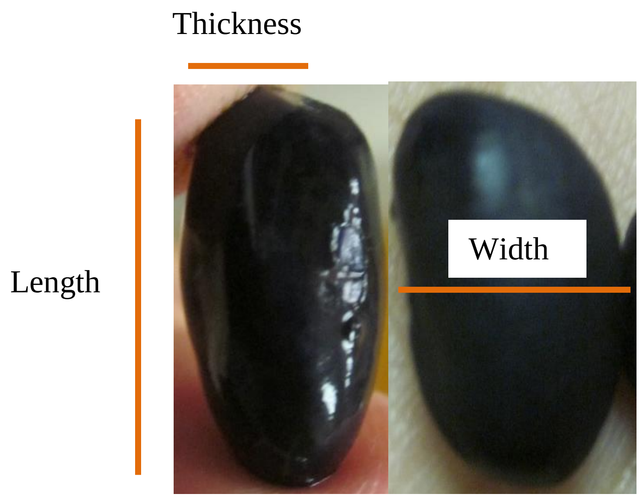

Figure 6. Length, width, and thickness measurements of black bean seeds.

**Seed Hardness**

Seed hardness was determined before and after all seed treatments. A compression test was conducted using a Texture Analyzer (TA) to measure black bean seed hardness. The fracture force of the seed in terms of force (Newtons) required breaking the seed along its principal axis was measured. The TA had a TPA probe fitted onto a 50 kg load cell, which moved downwards with a crosshead speed of 1 mm/sec. The seed sample was placed on the fixed base with the hilum on the left side (Figure 7). The seed samples were compressed with a 40% strain and a trigger force of 4 g. The parameters calculated from the force-distance curve were fracture force and maximum compression force. 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 when reached at 40% strain of seed. These determinations were done on 10 seeds per black bean pretreatment.

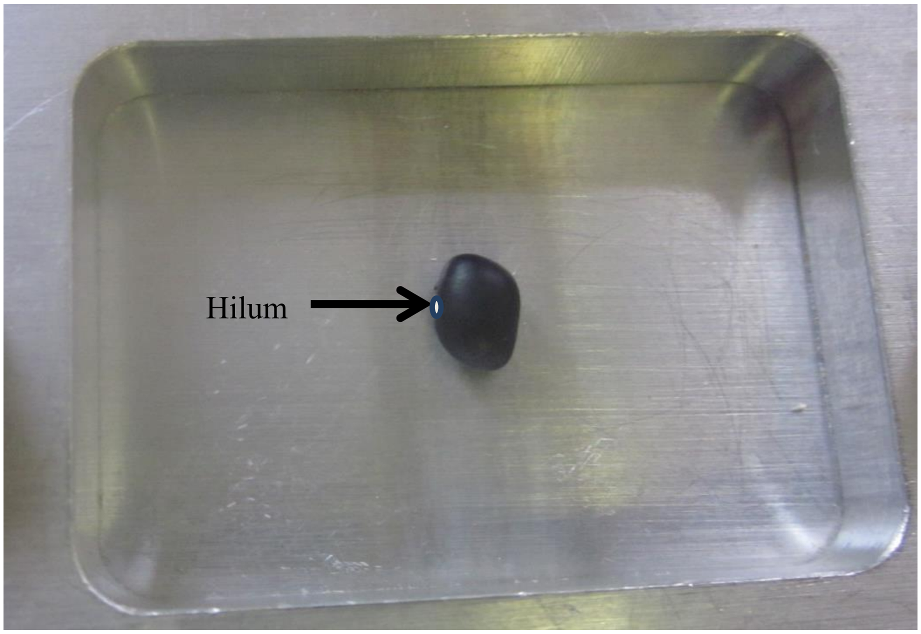

Figure 7. Position of black bean seed for compression test, hilum of seed on the left side.

## Experimental Design and Data Analysis

The experimental design was a randomized complete block. 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 bean seed is composed of two cotyledons, seed coat, and embryo. Black bean seed and its parts without treatment are presented in Figure 8. Each seed component physiologically has its own and unique structure and composition. The first noticeable physical characteristic of a black bean seed is the outer portion, which is the seed coat. The black appearance of black bean is due to high concentration of anthocyanins in the seed coat (Siddiq and Uebersax 2013).

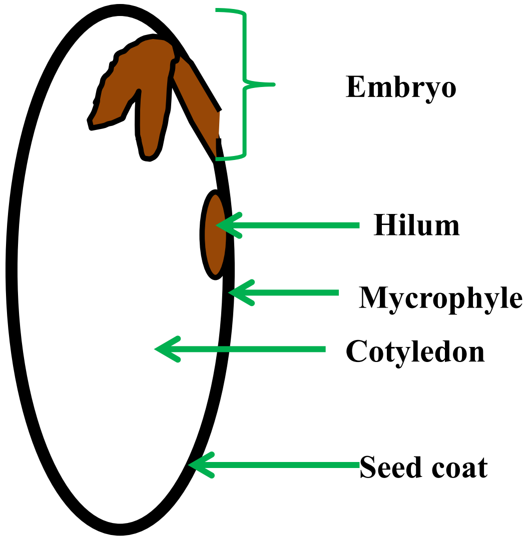


Figure 8. General structure of black bean seed.

The seed coat accounts for 8% of the seed weight. The seed coat has two major functions. First, the seed coat protects the entire seed from external factors that might damage it such as insect and microbial attack. Second, during seed development the seed coat supplies nutrients that are imported through the phloem (Boesewinkel and Bouman 1995; Van Dongen et al 2003). The seed coat has an extensive vascular network that is important since during seed development, the main function of the seed coat is to release nutrients for the embryo (Ammerlaan et al 2001).

The seed coat microstructure is mainly composed of four layers: waxy cuticle layer, epidermis, hypodermis, and interior parenchyma layer (Tiwari and Singh 2012). The waxy cuticle layer is the outer most layers and restricts movement of water into the seed. The epidermal layer is made up of palisade cells that are perpendicularly oriented to the surface (Figure 9). In black beans, the hypodermis layer is made up of hourglass cells oriented parallel to the surface.  Not all legume seeds have hourglass cells. The inter most layer include the interior parenchyma cells, which are protoplast free and are elongated cells parallel to the surface of cotyledon (Tiwari and Singh 2012).

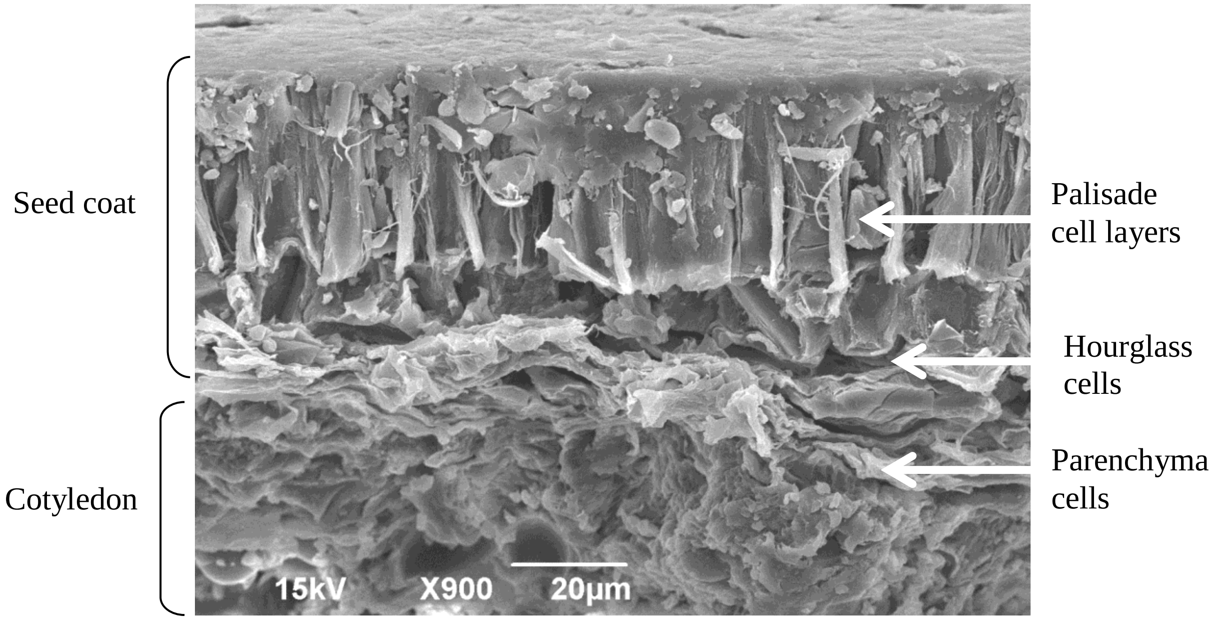

Figure 9. Scanning electron microscopy micrograph of seed coat structure.

Cotyledons (Figure 10) are the major part of the black bean seed structure accounting for 90% of the seed weight. Cotyledon is living tissue that consists of parenchyma cells without nuclei and is packed with starch granules that are embedded in a matrix of storage proteins (Tiwari and Singh 2012) as shown in Figure 11. Otto et al (1997) suggested that pea cotyledon have structural differences between the outer and inner layers. The differences are that cotyledon cells in the center of the cotyledon are loosely packed with large intercellular spaces; whereas, cotyledon cells of the outer layer are tightly packed (Otto et al 1997).

Figure 10. Dissected split cotyledon of black bean.

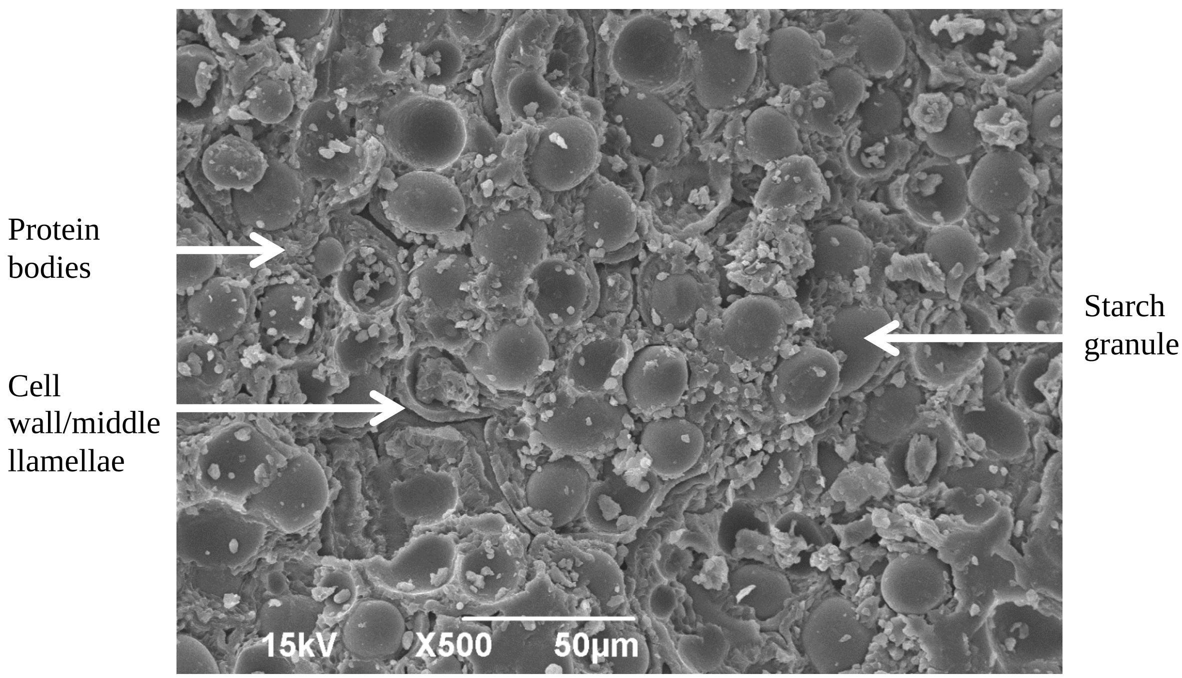


Figure 11. Scanning electron microscopy micrograph of flour showing cotyledon starch granules/protein bodies, and cell wall/middle lamellae.

The embryo is the third component of black bean seed, representing only 2% or less of the seed weight. In dicotyledenous seed, the embryo and the cotyledons are living, whereas in monocotyledonous grains embryo and aleuronic layer are the living tissue.  The embryonic axis (Figure 12) has the radicle and plumule, and the plumule is composed of the hypocotyl and epicotyl. The function of the embryonic axis is to serve as nutrition organs to the embryo during germination. They also have an important role during water imbibition by the seed coat (Tiwari and Singh 2012), because parts of the embryo (micropyle and hilum) are the entry points for water diffusion into the seeds. Water permeability is greatest in the hilum or micropyle areas.

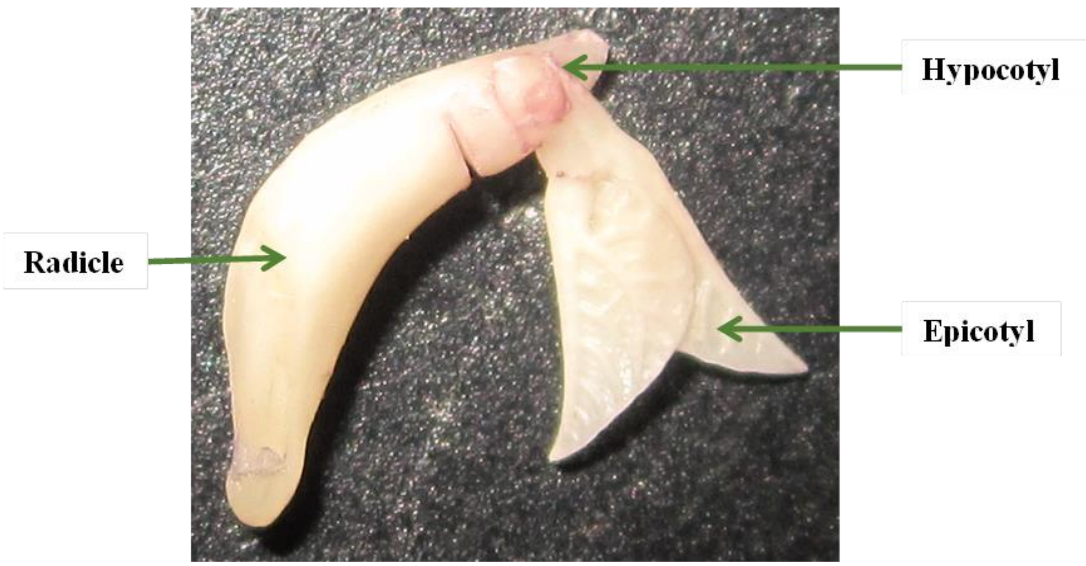

Figure 12. Dissected embryo from a black bean seeds and its parts.

**Proximate Chemical Composition**

Percentages on a dry weight basis of ash, protein, total lipids, and total starch contents for whole black bean flour, cotyledon, and seed coat fractions are presented in Table 1. Clean separation of the cotyledon from the seed coat and embryo was not accomplished. The embryo mostly was removed from the seed coat and cotyledon; however, some embryo contamination occurred in seed coat and cotyledon fractions. In general, for all proximate composition (except for total starch) of flour, protein, ash, and lipid content values were greater for cotyledon flour than for whole bean flour (Table 1).

Table 1. Proximate composition[a] of black bean fractions.

| Black bean fraction | Moisture content (%) | Ash | Protein | Total lipids | Total Starch |
|---|---|---|---|---|---|
| | | (%) | | | |
| Whole seed | 10.2 | 3.52 | 24.0 | 2.0 | 34.1 |
| Cotyledon | 9.7 | 4.23 | 25.5 | 4.4 | 35.3 |
| Seed coat | 8.2 | 4.05 | 19.0 | 1.1 | ND[b] |

[a]Dry weight basis.
[b]ND: Not detectable.

Mugendi et al (2010) found that dehulled mucuna seeds contained higher crude protein, crude fat and ash content than the whole bean. They suggested that the increase was due to the seed coat being comprised mainly of fiber; whereas the protein, fat and ash are concentrated mainly in the cotyledon fraction. Similar results were found for black bean flour after removing the seed coat. Deshpande et al (1982) reported 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. Whole flour proximate composition values (Table 1) are in agreement with values reported by Wang et al (2010) for black beans.

Ash content of the cotyledon fraction was found to be 4.23% (Table 1), similar to the value reported by Snyder (1936). Ash content for whole black bean flour was less than for cotyledon flour. Similar outcome was reported by Eknayake et al (1999) reported that ash content in whole bean flour was 3.9% while in cotyledon flour the ash content increased to 4.3%. They also reported an increase in protein and lipid content for cotyledon flour. The high ash values for cotyledon flour is indicative of high minerals content such as calcium, iron, phosphorus, magnesium, and potassium (Wang et al 2010).

Whole black bean flour protein content was found to be 24% (Table 1), which is in agreement with black bean protein content found by Siddiq et al (2010). Protein content for the cotyledon fraction (without seed coat) was 25.5%, higher than what was found in the whole black bean fraction. The removal of the seed coat caused an increase in the relative protein content of the cotyledon fraction. Increased protein content by removing seed coat have been reported by Deshpande et al (1982) and Alonso et al (1998).

Lipid content of cotyledon flour was 4.4%, higher than lipid content of whole flour and seed coat fraction of 2 and 1.1%, respectively (Table 1). Whole black bean flour lipid content is in agreement with values reported by Dzudie and Hardy (1996), whereas cotyledon flour lipid content was slightly higher than value reported by Akinjayeju and Ajayi (2011), who reported dehulled black bean flour lipid content of 3.1%. Lipids in black beans are mostly found in the embryo axis. Lipid content depends on different factors such as dry bean variety, environment conditions and other growing factors.

The major carbohydrate component of the cotyledon fraction is starch. Whole black bean flour and cotyledon fraction total starch was 34.1% and 35.3%, respectively (Table 1). 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; total starch was not detectable for seed coat fraction. As stated by Deshpande et al (1982) and Alonso et al (2000) reported that removal of the seed coat increased in the concentrations of dry bean nutrients that are predominantly in the cotyledon fraction on a unit weight basis. Similarly, removal of the seed coat increased percent protein in cotyledon fraction due to removal of the seed coat.

## Physical Seed Properties

Cooked Seeds

*Seed Appearance*

Cooked seeds looked bigger in size than non-treated seeds and did not swell equally (figure 13). This indicates that individual seeds differed in their absorption of water during cooking. The seed coat of some remained intact even after 20 min cooking. However, most seed coats ruptured and some cotyledon splitting occurred. After cooking, the seed coat looked lighter in color and felt rubbery when felt with fingers. The seed coat came off easily after seeds were cooked and cooled.

Figure 13. Seed coat and cotyledon appearance after cooking. In the second seed from the left seed coat rupturing is shown.

Bean splitting during cooking resulted in more exudation of starch into the cooking water. The cotyledons splitting is related to the high hydration capacity and swelling capacity of the seeds (Tiwari and Singh 2012). Also, the tendency of the seed coat to split during cooking or soaking is related to the chemical constituents of the seed coat specifically pectin content and calcium content in the seed coat and starch gelatinization behavior (Lu and Chang 1996). The difference in swelling and cooking could be related to the hard-to-cook phenomenon. It has been described that hard-to-cook is when seeds do not hydrated completely and remain hard even after extensive cooking (Stanley and Aguilera 1985). The hard-to-cook phenomenon is developed during storage conditions such as high temperature and high humidity environments. The factors influencing the hard-to-cook phenomenon had been related to several reasons such as the formation of insoluble pectate, polymerization and cross-linking of phenolic compounds, lignification of middle lamellae, and protein-starch interactions (Jones and Boulter 1983; Liu 1997). All these factors and other not well known mechanisms are thought to make strong adhesion of the seed coat to the cotyledon surface (Sefa-Dedeh et al 1978).

*Cooked Weight Gain and Cooking Loss*

Cooking impacted cooked weight gain and cooking loss of black bean seeds (Table 2). In general, the cooked weight gain increased 25% as cooking time increased from 5 to 20 min. Cooked weight gain was due to the water absorbed by the seeds during cooking.

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

| Cooked Seeds (min) | Cooked Weight Gain (%) | Cooking Loss (g) |
|---|---|---|
| 5 | 150c | 1.2b |
| 10 | 159c | 3.6a |
| 15 | 175b | 3.6a |
| 20 | 176a | 3.6a |

[a]Values followed by same letter are not significantly different at $P$=0.05.

Abu-Ghannam and Mckenna (1997) stated that the principle factor of rate of water absorption in whole red kidney beans was the seed coat. Their results suggested that at high temperatures (i.e., ≥40 ºC), there was a plasticizing effect on the seed coat of red kidney beans. They showed that the plasticity effect was manifested by the rubbery texture of seed coats as soaking temperature increased. They also showed that as the plasticity of the seed coat was enhanced, the water absorption rates increased and subsequently lead to low levels of saturation moisture content due to the high extraction rates of soluble material.

Cooking also had an impact on cooking loss (Table 2). Similar values were observed after cooking for 10, 15, or 20 min while a third less cooking loss (1.2 g) was observed at 5 min. The highest cooked weight gain (176%) and solids lost (3.6 g) was in black beans cooked at 15 or 20 min. This suggests that optimal cook time is approximately 15 minutes.

The cooking water left in the beaker was very dark due to high amounts of anthocyanins. Anthocyanins are water soluble natural pigments. They are responsible for the natural purple and blue colors of flowers, fruits, vegetables as well as for black beans (Jung and Bae 2014). Pigments found in the seed coat are phenolic compounds such as polyphenols and more particular tannins and flavonoids (anthocyanins) (Siddiq and Uebersax 2013).

Cooking loss probably contained solids lost from disruption of starch granules during gelatinization and from soluble oligosaccharides such as raffinose (Siddiq and Uebersax 2013). The raffinose family of oligosaccharides (i.e., α-galacto-oligosaccharides raffinose, stachyose and verbascose) are soluble carbohydrates found in appreciable concentrations in dry beans (Tosh and Yada 2010), which could have been responsible for the high cooking loss as time increased (Table 2).

## Cooked-Dried Seeds

### *Seed Appearance*

After cooked seeds were dried, the seeds shrank and looked similar to their original non-treated dry seed. Furthermore, some splitting and seeds with ruptured seed coats were also observed (Figure 14). As the moisture inside the seed increased, seeds became plastic and forces associated with drying deformed the shape of the seed. The cooked-dried cotyledons looked mostly dark due to the seed coat colors compounds leaching into the cooking water and onto the cotyledons (Figure 15).

Figure 14. Cooked-dried seeds split cotyledons and seed coat rupture.


Figure 15. Seed coat (A) and cotyledon (B) fractions from cooked-dried seeds.

Structural changes in the seed coat surface and interior caused by cooking-drying can be seen in SEM micrograph (Figure 16). The seed coat of the surface of cooked-dried seeds had open space suggesting that the seed coat began to disintegrate during cooking. The interior of the seed coat after cooked-dried pretreatment showed erosion of structure. The seed coat is rich in pectin substances that are water soluble (Tiwari et al 2011). The presence of seed coat components that are water soluble such as pectin and anthocyanins and the prolonged cooking times might have caused the change in seed coat structure.

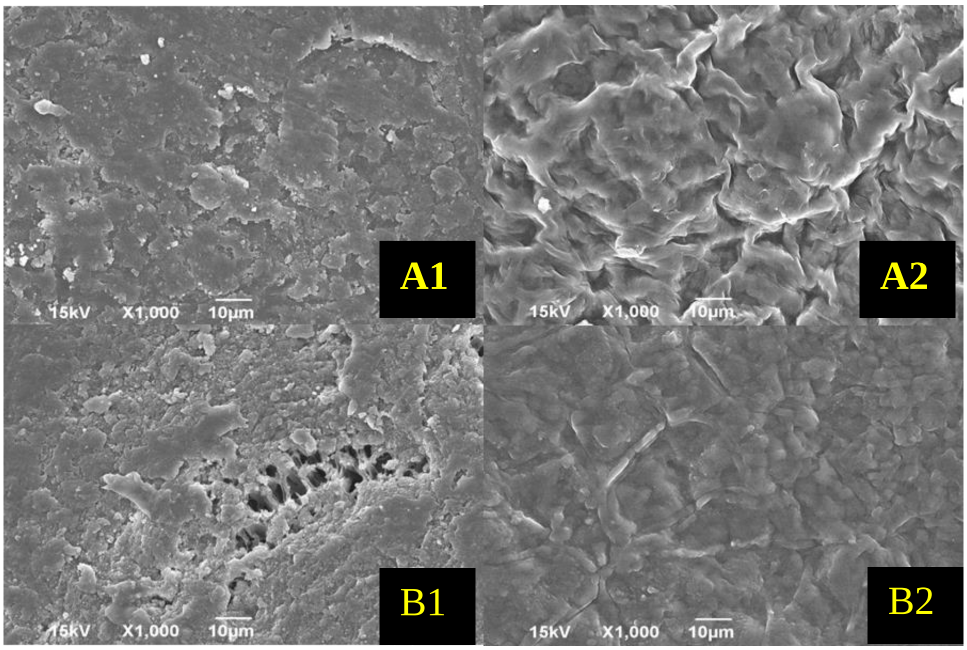

Figure 16. Scanning electron microscopy micrograph of non-treated (A) and cooked-dried (B) of seed coat surface (1) and interior (2).

Structural changes in the cotyledon surface and interior caused by cooked-dried pretreatment can be seen in SEM micrograph (Figure 17). The cotyledon of the interior of non-treated cotyledon displayed the division of the cells and the cellular spaces intact. In addition, a nicely packed and organized structure can be seen. However, due to cooking, the spaces between cells were not seen possibly due to the middle lamellae pectic compounds' solubility in water (Tiwari et al 2011).

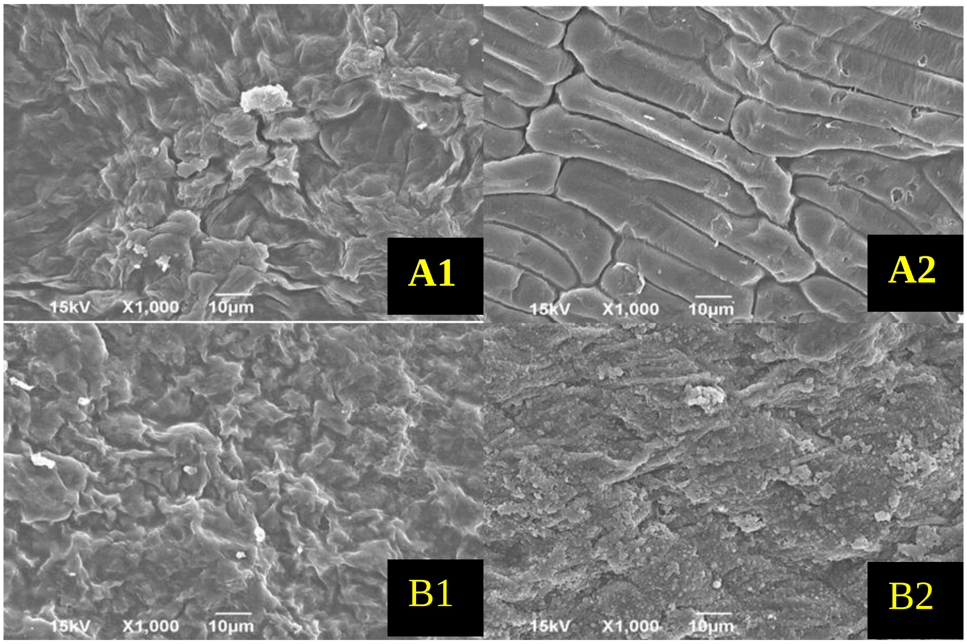

Figure 17. Scanning electron microscopy micrograph of non-treated (A) and cooked-dried (B) of cotyledon cut longitudinal surface (1) and interior (2).

*Seed Physical Tests*

Cooked-dried pretreatment significantly affected seed dimension (i.e., length, width, and thickness), 100-seed weight and seed test weight (Table 3). In general, as cooking time increased, seed dimension values, length and width, increased 0.9 and 0.3 mm, respectively. The 100-seed weight for black bean seeds ranged from 18.9 to 21.3 g. The 100-seed weight decreased as pretreatment time increased. This might have been because of the loose in shape and size integrity after cooking and drying. There was no literature found on 100-seed weight of cooked-dried seeds. However, values were similar within the range of accepted non-treated 100-seed weight of black beans. The 100-seed weight for black bean seed (non-treated) ranged from 19 to 23 g (Kelly et al 2006).

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

| Cooked-Dried (min) | Length | Seed Dimension Width (mm) | Thickness | 100-Seed Weight (g) | Test Weight (kg/hL) |
|---|---|---|---|---|---|
| 0 | 9.6c | 5.9d | 4.9b | 20.4b | 79.2a |
| 5 | 9.2d | 6.1bc | 4.7c | 21.3a | 72.5b |
| 10 | 9.6c | 6.1cd | 5.0a | 19.6c | 71.2c |
| 15 | 10.1b | 6.2ab | 4.9ab | 19.2d | 69.8d |
| 20 | 10.5a | 6.2a | 4.9ab | 18.9e | 68.1e |

[a]Values followed by same letter are not significantly different at *P*=0.05.

Test weight of black bean seeds decreased with increasing cooking times. Test weight of black bean seed cooked for 20 min decreased by ≈16% when compared to non-treated seeds, which reflects the increase in seed size and loss in weight associated with cooking-drying pretreatment. The cooked-dried seeds lost their integrity in size and shape (Figure 14) and thus weight loss likely was caused by leaching of cell material.

<u>Soaked Seeds</u>

*Seed Appearance*

Soaked seeds looked bigger than non-treated seeds. Increase in seed size was not uniform across all soaked seeds (Figure 18). Some seeds for all soaked treatments remained small and intact while others swelled and their seed coat ruptured and cotyledons split, which suggests that seeds treated the same absorbed different amounts of water. The high dark intensity color of the seed coat faded away after 24 h soaking. The seed coat came off easily after seeds were soaked. The seed coat looked glossy. Similar observations were made by Uebersax et al (1991), who described the seed coat as glossy after soaking.

Figure 18. Seed coat and cotyledon appearance after soaking. In the second seed from the left seed coat rupturing is shown.

The hydration of the seeds by soaking is a diffusion-driven mass transfer process. Soaking uniformly assists the expansion of the seed coat and cotyledons (Tiwari and Singh 2012). The seed coat thickness as well as the hilum size was reported to influence the water absorption of cowpeas during the initial stage of soaking, whereas protein content was more important in affecting water absorption at later soaking stages (Sefa-Dedeh and Stanley 1979).

*Soaked Weight Gain and Soaking Loss*

Soaking effect was significant for soaked weight gain and solids loss of black bean seeds (Table 4). In general, soaked weight gain increased as soaking time increased, thus showing significant difference in soaking time. Seed weight gain was similar after 12 h of soaking indicating that seeds had become saturated with water. The seed weight gain is related to the water absorbed. Soaking effect was significant for solids loss in soaking water. As soaking time increased, the residue obtained in the water increased. The soaking loss was not statistically different. Most of soaking loss had occurred by 6 h soaking. The soaking water of the solids left in the beaker for black beans was very dark due to the high amounts of anthocyanins which are responsible for the dark color.

Table 4. Mean soaked weight gain and soaking loss values[a] of soaked seeds.

| Soaked Seeds (h) | Soaked Weight Gain (%) | Soaking Loss (g) |
|---|---|---|
| 6 | 191c | 6.0a |
| 12 | 198b | 5.6b |
| 18 | 199b | 6.4a |
| 24 | 213a | 6.2a |

[a]Values followed by same letter are not significantly different at *P*=0.05.

Soaking samples continued to absorb water slowly with time and a constant seed weight gain was observed. Phlak et al (1989) reported that the rate of water absorption related to the seed weight gain was reduced with prolonged soaking time and this was attributed to the filling of free capillary and intermicellar spaces with water and the swelling of hydrocolloids. As soaking proceeds, the rate of water uptake by the seeds decreases due to the extraction of soluble material (Siddiq and Uebersax 2013) such as soluble amylose fraction and proteins as well as oligosaccharides (i.e., raffinose and stachyose).

Seed weight gain and water absorption results agree with observations of Anton et al (2008). Soaking process led to high water absorption that increased swelling and made the cotyledons expand and break the seed coat integrity (Figure 18). After the initial high rate of water absorption, a slow absorption in later stages was suggested (Ituen et al 1985). The initial rapid water absorption attributed possibly to the filling of capillaries on the surface of the seed coats and at the hilium (Hsu et al 1983).

Soaked-Dried Seeds

*Seed Appearance*

During soaking, the seed swelled and absorbed water, and as the seeds dried water was removed and the seed shrank. Drying the seeds with hot air caused splitting of seed coat and partially split of the cotyledon (figure 19). The splitting possibly was caused by changes in both the moisture content and temperature gradients due to drying temperatures and the internal moisture content during drying (Liu et al 1989). During drying, the rapid changes in seed moisture caused the shrinkage of seed coat leading to its stress. Thus, the splitting of the seed coat, caused by shrinkage, was considered an important mechanism that caused seed coat cracking and cotyledons splitting as the seed coat did not prevent the separation of the cotyledons for both cooked-dried and soaked-dried seeds. Discoloration of seed coat and stained cotyledons during soaking was observed (Figure 20).

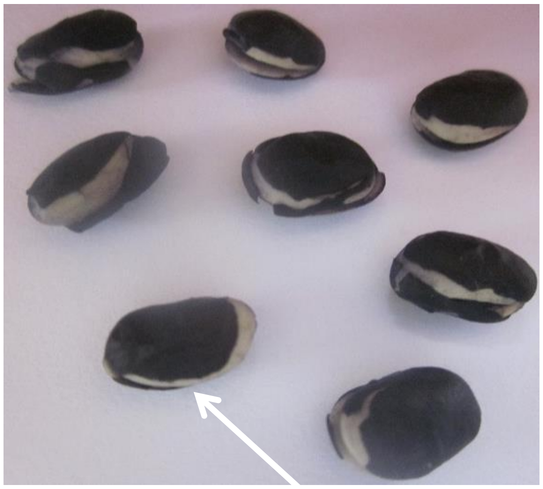

Figure 19. Seed coat rupture and splitting effect of soaked-dried seeds.


Figure 20. Seed coat (A) and cotyledon (B) fractions from soaked-dried seeds.

The outer surface of the seed coat shows some pitting and the inner surface from the soaked-dried pretreatment had some pitting and inner surface shows erosion of structure similar to that found for cooked-dried seed (Figure 21). The seed coat is rich in soluble pectin substances that can be disintegrated during soaking. The solubility of other compounds, such as anthocyanins, and prolonged soaking times might have changed the seed coat structure.

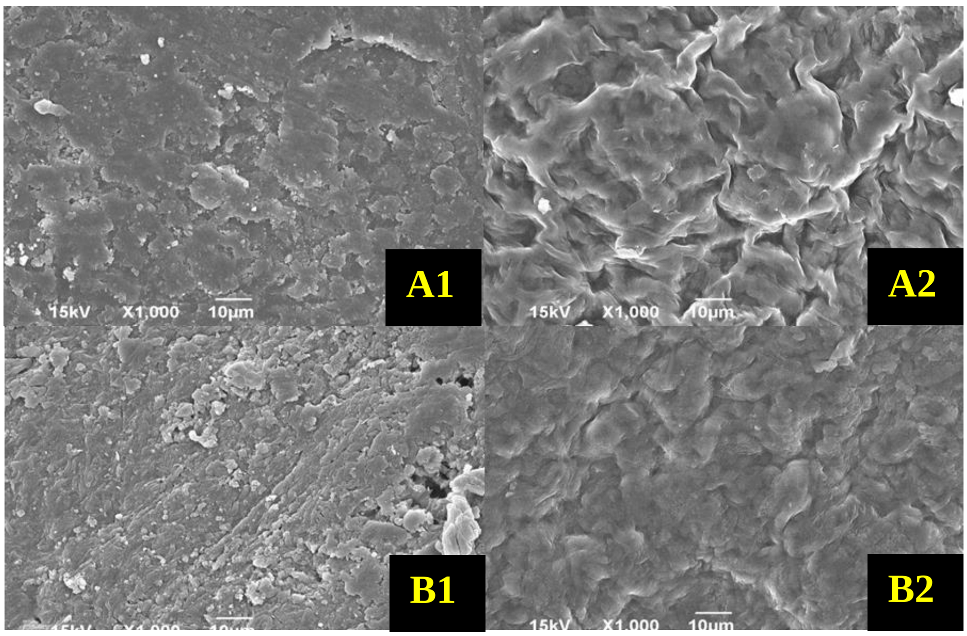


Figure 21. Scanning electron microscopy micrograph of non-treated (A) and soaked-dried (B) of seed coat surface (1) and interior (2).

Structural changes in the cotyledon surface and interior caused by soaking-drying pretreatment can be seen in SEM micrograph (Figure 22). The cotyledon of the surface is seen as more loosely than the soaked-dried cotyledon with some corrugation. The cotyledon of the interior of non-treated cotyledon displayed the division of the cells. However, due to soaking, the spaces between cells almost disappeared due to water penetration and possible pectin solubility, which might appear as the fraction in the outer part of the cotyledon (Tiwari et al 2011).

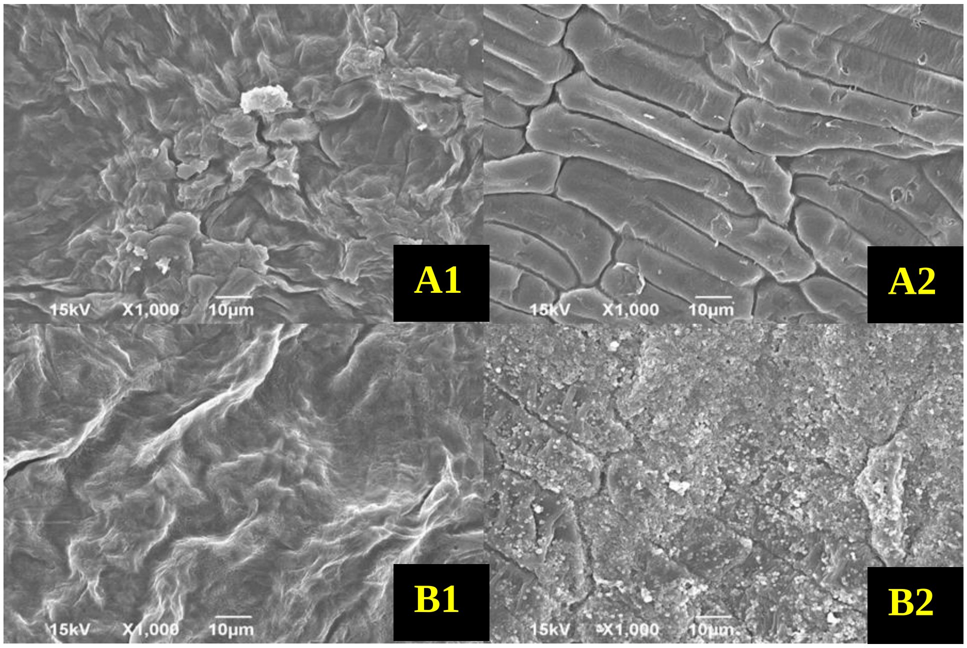

Figure 22. Scanning electron microscopy micrograph of non-treated (A) and soaked-dried (B) of cotyledon cut longitudinal surface (1) and interior (2).

*Seed Physical Tests*

Soaked-dried pretreatment had significantly different seed dimension (i.e., length, width, and thickness), 100-seed weight and seed test weight (Table 5). Soaked-dried pretreatment caused a 0.9, 1.0, and 0.3 mm increase in seed length, width, and thickness, respectively. The increase in length, width, and thickness were small, but still significant. The swelling of the seeds during soaking was greater than the contraction.

Table 5. Mean physical properties values[a] of soaked-dried seeds.

| Soaked-Dried (h) | Seed Dimension Length | Width (mm) | Thickness | 100-Seed Weight (g) | Test Weight (kg/hL) |
|---|---|---|---|---|---|
| 0 | 9.6c | 5.9d | 4.9c | 20.4d | 79.2a |
| 6 | 9.2d | 5.9d | 4.7d | 21.3a | 69.9b |
| 12 | 9.6c | 6.1c | 4.7d | 20.8b | 68.3c |
| 18 | 9.9b | 6.5b | 5.0b | 20.4c | 64.2d |
| 24 | 10.5a | 6.9a | 5.2a | 19.6e | 62.9e |

[a]Values followed by same letter are not significantly different at *P*=0.05.

The 100-seed weight decreased as the time of the soaked-dried pretreatment increased. The 100-seed weight for black bean ranged from 19.6 to 21.3 g. There was no literature found on 100-seed weight of soaked-dried seeds. However, values were within the range (19 to 23 g) of accepted non-treated 100-seed weight of black beans (Kelly et al 2006). Test weight of black bean seeds decreased with increasing pretreatment times. Test weight of black bean seed soaked-dried for 24 h min decreased by ≈21% compared to non-treated black bean seeds. Black bean seeds absorbed water during soaking resulting in an increased seed size. During the subsequent drying, the seed size decreased (Table 5). The soaked-dried seeds lost their integrity and size and shape (Figure 13). This can be reflected in the decrease of test weight found for soaked-dried seeds.

Tempered Seeds

*Seeds Appearance*

Tempering did not change the physical appearance of black bean seed. The seed coat and cotyledon remained intact without splitting or seed coat ruptured. The seed coat remained similar to non-treated seeds and cotyledon retained its color (Figure 23).

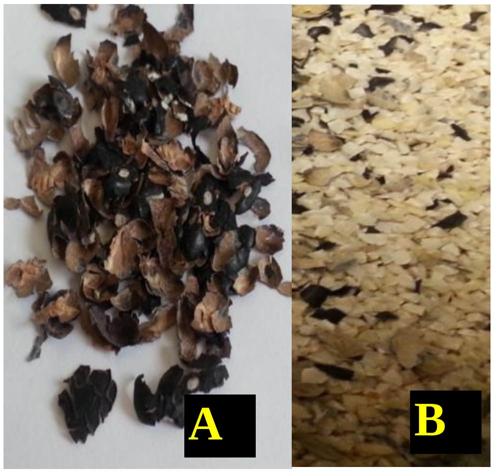


Figure 23. Seed coat (A) and cotyledon (B) fractions from 14% moisture content beans.

For all tempering pretreatments, the seed coat after removal and the seed looked similar inside and outside. SEM micrographs (Figure 24) show that outer surface of seed coat was not noticeably affected by tempering but the inner surface did show some structural erosion. The seed coat in the inside looked brownish in color and the cotyledon remained white in color.

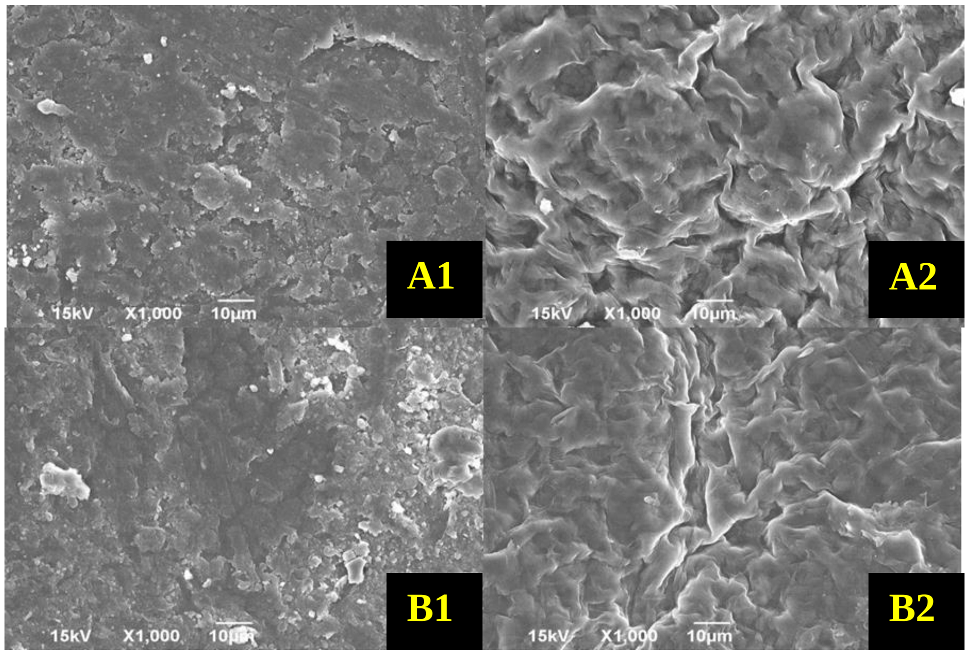

Figure 24. Scanning electron microscopy micrograph of non-treated (A) and tempered (B) seed coat surface (1) and interior (2).

Structural changes in the cotyledon surface and interior caused by tempering can be seen in SEM micrograph (Figure 25). The surface of the tempered cotyledon had a smoother appearance than did the non-treated cotyledon. The cotyledon cells of the interior of non-treated when compared to tempered cotyledon cells showed the cells divided by extracellular spaces. However, some wrinkling of the cells can be seen, possibly due to some water penetration into the cotyledon fraction. Besides the observation of the cotyledon, no major changes occurred to tempered seeds on the surface and interior of the cotyledon.

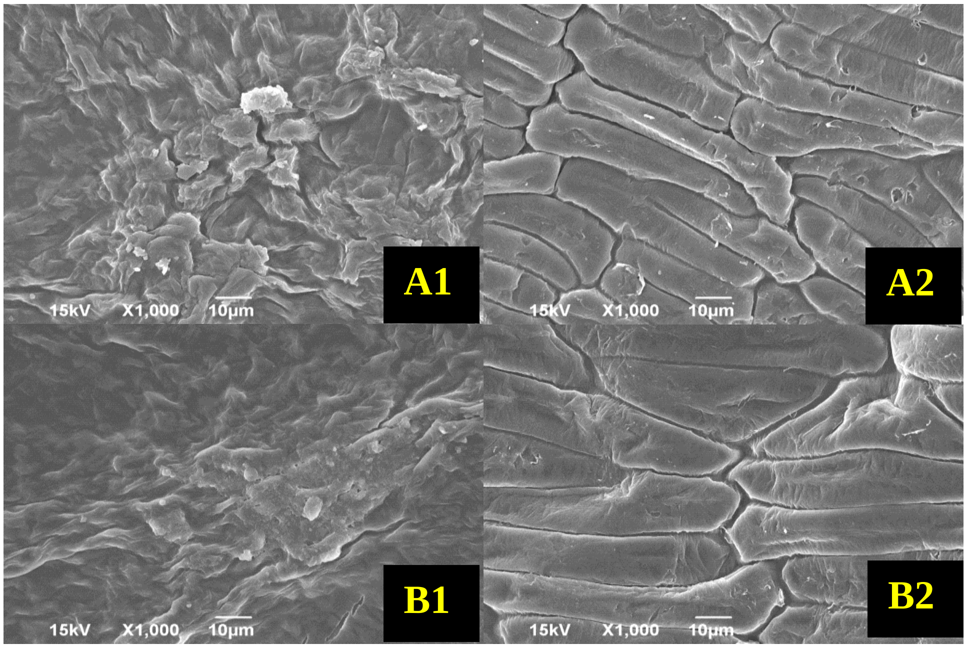

Figure 25. Scanning electron microscopy micrograph of non-treated (A) and tempered (14%) (B) seed. Cotyledon cut longitudinal surface (1) and interior (2).

*Seed Physical Tests*

Tempered pretreatment was significant for seed dimension (i.e., length, width, and thickness), 100-seed weight and seed test weight (Table 6). As tempering level increased, seed dimension values slightly increased. This small increase in size was reflected by decline in test weight. Altuntas and Demirtola (2007) reported that as moisture content increased in kidney bean seeds length, width, and thickness slightly increased. The 100-seed weight for tempered seed ranged from 19.2 to 22.7 g. Amin et al (2004) reported increasing lentil seed moisture content lead to increased 1000-seed weight and showed a linear relationship. Altuntas and Demirtola (2007) reported a similar outcome as moisture content increased for pea, kidney beans, and black-eyed peas. This positive linear relationship of 1000-seed weight, in the case of those studies, and moisture content of the seed were also reported by Aviara et al (1999) and Vilche et al (2003) for guna seeds and quinoa seeds, respectively.

Table 6. Mean physical properties values[a] of tempered seeds.

| Tempered Seeds (%) | Seed Dimension Length | Width (mm) | Thickness | 100-Seed Weight (g) | Test Weight (kg/hL) |
|---|---|---|---|---|---|
| 6 | 9.4c | 5.8c | 4.9b | 19.2e | 79.8a |
| 8 | 9.5bc | 5.8c | 4.9b | 20.0d | 79.4ab |
| 10 | 9.6b | 5.9b | 4.9b | 20.4c | 79.2b |
| 12 | 9.6b | 5.9b | 5.0a | 21.7b | 78.3c |
| 14 | 9.7a | 6.0a | 5.0a | 22.7a | 77.6d |

[a]Values followed by same letter are not significantly different at $P$=0.05.

As moisture content increased, a decrease in test weight was observed (Table 6). The decrease in test weight with an increase in moisture could be attributed to the weight gained due to absorption of moisture being relatively lower than the corresponding volumetric expansion (Tiwari and Singh 2012). Other studies used bulk density as a measurement of seed density and its changes due to moisture content variation (Bhattacharya et al 2005). These authors found that the bulk density of lentil seeds decreased with increased moisture content. They suggested that the decrease in seeds bulk density might have been due to the absorption of water by the outer coating and the filling of the gap between it and the cotyledon, leading to a marginal increase in volume.

**Moisture Conditioning**

Moisture content of the cotyledon and seed coat of some pretreated seed were measured (Table 7). Pretreatments selected were cooked-dried 5 and 20 min, soaked-dried 6 and 24 h, and tempered to 6, 10, and 14%. In general, for all pretreatments, cotyledon had lower moisture content than did the seed coat. For all cooked-dried and soaked-dried pretreatments, cotyledon moisture content was similar as non-treated cotyledon moisture content.

Table 7. Cotyledon and seed coat moisture content for selected pretreatments.

| Pretreatment | Cotyledon | Seed coat |
|---|---|---|
| Cooked-dried (min) | % | |
| 0 | 9.2 | 10.4 |
| 5 | 8.5 | 9.2 |
| 20 | 8.8 | 9.4 |
| Soaked-dried (h) | | |
| 0 | 9.2 | 10.1 |
| 6 | 9.4 | 10.7 |
| 24 | 9.4 | 10.5 |
| Tempered (%) | | |
| 6 | 6.7 | 7.3 |
| 10 | 9.2 | 10.5 |
| 14 | 13.6 | 14.0 |

Different cotyledon moisture content was seen and expected for the different tempering levels. Seed coat had higher moisture content than cotyledons. Seeds tempered to 14% had seed coat moisture content of 14% and a cotyledon moisture content of 13.6%. Similarly, seeds cooked 20 min and dried had seed coat moisture content of 9.4% and cotyledon moisture content of 8.8%. It is important to mention that during drying the seeds were spread in drying sheets and they were in contact to other seeds, which could have limited the air flow in some parts of the seeds, hence, uneven drying occurred.

The results indicated that the two seed components (seed coat and cotyledon) dry at different rates and showed different moisture contents (Table 7). Possibly, the seed coat is the first to be wetted and the last to lose moisture as moisture migrates from cotyledon to seed coat to the atmosphere. Temperature and moisture gradients possibly caused stress in the seed coat as well as in the cotyledons during drying. Overhults et al. (1973) observed the physical damage in soybeans during drying and stated similar observations of differences in seed coat and cotyledon moisture content.

## Seed Hardness

### Cooked-Dried Seeds

Cooked-dried pretreatment significantly affected bean fracture and peak force (Table 8). For cooked samples, fracture and peak force decreased as cooking time increased. The lowest fracture and peak force observed was for seed cooked 15 and 20 min. The fracture force decreased ≈48% for seeds cooked for 20 min compared to the non-treated seeds. Peak force for seed cooked for 20 min decreased ≈17% compared to non-treated seeds.

Table 8. Mean seed fracture force and peak values[a] for cooked-dried seeds.

| Cooked-Dried (min) | Fracture Force (N) | Peak Force (N) |
|---|---|---|
| 0 | 107a | 269a |
| 5 | 64b | 272a |
| 10 | 61b | 260a |
| 15 | 51c | 218b |
| 20 | 56c | 224b |

[a]Values followed by same letter are not significantly different at *P*=0.05.

### Soaked-Dried Seeds

Soaked-dried pretreatment significantly affected bean fracture and peak force (Table 9). Soaking black bean seeds resulted in a decreased in fracture and peak force compared to non-treated seeds. However, compared to different soaking times, similar values were obtained. Compression curves for cooked-dried and soaked-dried seeds for the extreme treatments are presented in Figure 26. The linear force was considered an elastic zone where the sample was deformed elastically in a linear fashion and that can be observed at the first portion of the graph for both pretreatments. Fracture force was a non-linear region where a major fracture occurred at the first cracking point. Peak force was the highest peak and was related to fracture and when the two cotyledons split. For either cooked-dried or soaked-dried seeds, the fracture force was low which implied the seed fractured easily.

Table 9. Mean seed fracture force and peak values[a] for soaked-dried seeds.

| Soaked-Dried (h) | Fracture Force (N) | Peak Force (N) |
|---|---|---|
| 0 | 110a | 270a |
| 6 | 57b | 184b |
| 12 | 46b | 185b |
| 18 | 53b | 205b |
| 24 | 54b | 181b |

[a]Values followed by same letter are not significantly different at *P*=0.05.

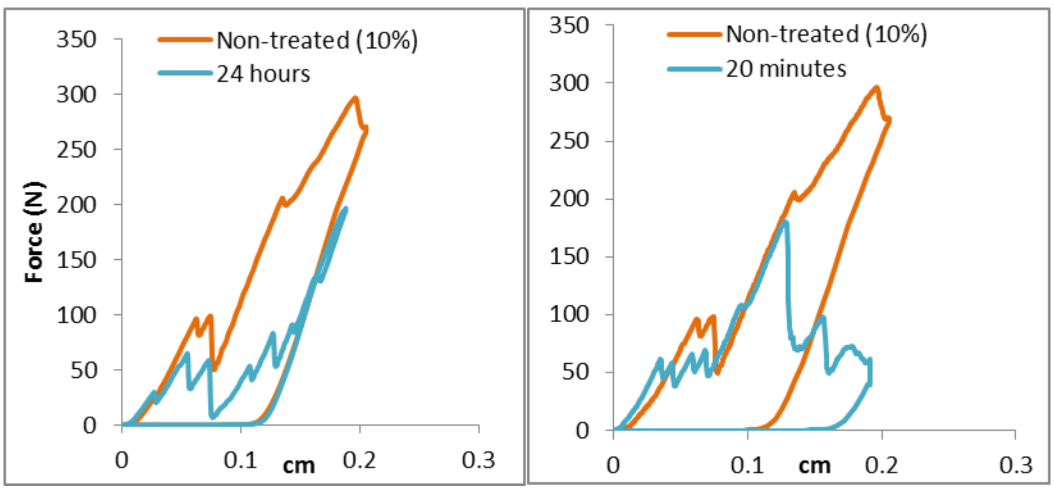

Figure 26. Compression curves for non-treated (10%) and soaked-dried (24 h) (left) and cooked-dried (20 min) (right) black bean seeds.

Cooked-dried or soaked-dried beans whose middle lamella has been dissolved during the cooking or soaking process become soft. With hard beans, the cell walls do not dissolve and they might become tougher, resulting in fracturing across the cotyledons. The seed hardness could be considered to be measured for seeds with seed coat or seeds without the seed coat. Aguilera and Lillford (2008) stated that food properties are related to how the elements forming the food relates to one another and not the composition itself, hence the different layers in the seed coat and cotyledons could be measured separately to detect differences in them.

Frączek et al (2005) suggested that dry bean seed coat was classified as water permeable. When the water penetrated, the seed increased in volume during moistening. Depending upon the rate of this process, the seed coat might split. The increase in volume probably caused a pressure increase for the seed coat which resulted in linear strains for the seed coat and a subsequent decreased of seed coat thickness. Thus, a possible decrease in the seed coat thickness due to moistening and drying could have contributed to cause the decrease in seed hardness. Further studies will need to be done to tests this.

### Tempered Seeds

Tempered seed pretreatment significantly affected bean fracture and peak force (Table 10). The fracture force was the first major rupture point where the seed cracks for first time. As moisture content increased, the fracture force increased. The increasing trend of fracture force when moisture content increased was observed. Similar pattern was exhibited for peak force (Table 10).

Table 10. Mean for seed fracture force and peak values[a] for tempered seeds.

| Tempered (%) | Fracture Force (N) | Peak Force (N) |
|---|---|---|
| 6 | 83d | 194e |
| 8 | 86d | 242d |
| 10 | 108c | 271c |
| 12 | 203b | 296b |
| 14 | 254a | 310a |

[a]Values followed by same letter are not significantly different at *P*=0.05.

Compression curve for black bean seed hardness is presented in Figure 27 for seeds tempered to 6 and 10% moisture content. Linear force was considered an elastic zone where the sample was deformed elastically in a linear fashion. Fracture force was a non-linear region where a major fracture occurred. Peak force was the highest peak and was related to fracture and when the two cotyledons split. For black bean samples with 6% moisture content, multiple fracture regions were observed during several fractures, which depended upon seed moisture, structure and speed of the compression.

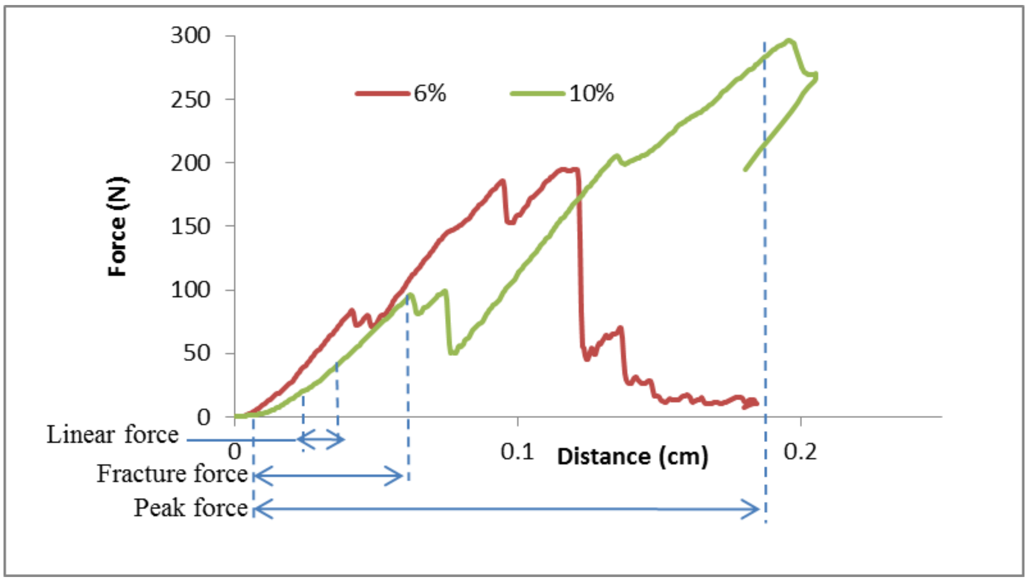

Figure 27. Compression curves for 6% and 10% tempered black bean seeds.

Compression curves were studied and interpreted based on similar findings by Bhattacharya et al (2005). They divided the compression curve into six different regions, which was adapted to interpret black bean hardness compression curves obtained from different moisture content. The number of fracture points after the first fracture decreased at very high moisture content. These results agree with the findings on lentils compression (Bhattacharya et al 2005). Peak force or firmness of the seeds significantly increased with moisture content increased. Paulson (1978) studied the firmness of soybeans and found similar outcome. He explained that the highest firmness values for soybeans were between 11 to 14% moisture content. The water probably diffused differently into the seed coat and cotyledon as suggested by Bhatty (1988). Thus, it was suggested by Bhattacharya et al (2005) that only the seed coat became soft and not the cotyledon. Moisture content of black bean seeds was significantly correlated ($R^2 = 0.88$) with the hardness of the seeds studied. Black bean seed hardness increased rapidly at levels of moisture content from 10 to 14%.

Frączek et al (2005) compared seed hardness of beans (i.e., Wiejska, Atena, Jubilatka and Augustynka) at different moisture contents. They 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).

## Conclusions

Cooked-dried and soaked-dried pretreatments caused more changes physically and internally in the black bean seeds when compared to tempering pretreatment. The changes in seed shape and size was mainly affected by cooked-dried and soaked-dried pretreatments. Internal changes also were more affected by those pretreatment due to the high water and for cooking the high temperature used. The pretreatment changes the seed hardness. For cooked-dried or soaked-dried decreased in the fracture and peak force was observed, which suggested high brittleness. In contrast, tempering samples increased both seed fracture and peak force as moisture content increased which suggested the seed become though and more difficult to rupture.

## Literature Cited

AACC International. Approved methods of the American Association of Cereal Chemists. 2000. 10th Ed. Methods (44-15.02), (08-01.01), (46-30.01), (76-13.01). AACC, St. Paul, MN.

Abu-Ghannam, N., and Mckenna, B. 1997. Hydration kinetics of red kidney beans (*Phaseolus vulgaris* L.). J. Food Sci. 62:520-523.

Aguilera, J. M., and Lillford, P. J. 2008. Structure-properties relationships in foods. Pages 229-237 in: Food Material and Science Principles and Practice. J. M. Aguilera and P. J. Lillford, Eds. Springer: New York, NY.

Akinjayeju, O., and Ajayi, O. F. 2011. Effects of dehulling on functional and sensory properties of flours from black beans (*Phaseolus vulgaris*) - properties of black beans flours. Food Nutr. Sci. 2:344-349.

Alonso, R., Orue, A., and Marzo, F. 1998. Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. Food Chem. 63:505-512.

Alonso, R., Aguirre, A., and Marzo, F. 2000. Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chem. 68:159-165.

Altuntas, E., and Demirtola, H. 2007: Effect of moisture content on physical properties of some grain legume seeds. New Zealand J. Crop Hort. Sci. 35:423-433.

American Oil Chemists Society (AOCS). 1998. Official methods of analysis, 16th Ed. Method Ba3-38. American Oil Chemist Society. Arlington, VA Amin, M. N., Hossain, M. A., and Roy, K. C. 2004. Effects of moisture content on some physical properties of lentil seeds. J. Food Eng. 65:83-87.

Ammerlaan, A. M. H., Wouterlood, M., Van Aelst, A. C., and Borstlap, A. C. 2001. Structure of the developing pea seed coat and the post-phloem transport pathway of nutrients. Located at: file:///C:/Users/Administrator.plantsci-181415/Downloads/c2.pdf. (Accessed on 4/25/14).

Anton, A. A., Ross, K. A., Beta, T., Fulcher, R. G., and Arntfield, S. D. 2008. Effect of pre-dehulling treatments on some nutritional and physical properties of navy and pinto beans (*Phaseolus vulgaris* L.). LWT - Food Sci. Technol. 41:771-778.

Aviara, N. A., Gwandzang, M. I., and Haque, M. A. 1999. Physical properties of guna seeds. J. Agric. Eng. Res. 73:105-111.

Bellido, G. G., Arntfield, S. D., Scanlon, M. G., and Cenkowski, S. 2003. The effect of micronization operational conditions on the physicochemical properties of navy beans (*Phaseolus vulgaris* L.). J. Food Sci. 68:1731-1735.

Bhattacharya, S., Narasimha, H. V., and Bhattacharya, S. 2005. The moisture dependent physical and mechanical properties of whole lentil pulse and split cotyledon. Int. J. Food Sci. Technol. 40:213-221.

Bhatty, R. S. 1988. Composition and quality of lentil (*Lens culinaris* Medik): A review. Can. Inst. Food Sci. Technol. J. 21:144–160.

Boesewinkel, F. D., and Bouman, F. 1995. The seed: structure and function. Pages 1-24 in: Seed Development and Germination. Kigel J., Galili, G., Eds. Marcel Dekker: New York, NY.

Bourne, M. 2013. [Web Page]. Located at: http://128.121.92.221/texture_profile_analysis.html. (Accessed on 4/10/14).

Carmona-Garcia, R., Osorio-Diaz, P., Agama-Acevedo, E., Tovar, J., and Bello-Perez, L. A. 2007. Composition and effect of soaking on starch digestibility of *Phaseolus vulgaris* (L.) cv. ‘Mayocoba’. Int. J. Food Sci. Technol. 42:296-302.

Deshpande, S. S., Sathe, S. K., Cornforth, D., and Salunkhe, D. K. 1982. Effects of dehulling on functional properties of dry bean (*Phaseolus vulgaris* L.) flours. Cereal Chem. 59:396 -401.

Dzudie, T., and Hardy, J. 1996. Physicochemical and functional properties of flours prepared from common beans and green mung beans. J. Agric. Food Chem. 44:3029-3032.

Eknayake, S., Jansz, E. R., and Naira, B. M. 1999. Proximate composition, mineral and amino acid content of mature *Canavalia gladiata* seeds. Food Chem. 66:115-119.

Frączek, J., Hebda1, T., Đlipek, Z., and Kurpaska, S. 2005. Effect of seed coat thickness on seed hardness. Can. Biosyst. Eng. 47:4.1-4.5.

Gasiorowski, H., and Kolodziejczyk, P. 1990. Microhardness rye seeds. Abstract: Przeglad Zbozowo – Mlynarski. 5(6):9-12, 19.

[GIPSA] Grain Inspection, Packers and Stockyards Administration. 1997. Book II: Grain grading procedures. Located at: http://www.usda.gov/gipsa/reference-library/handbooks/grain insp/grbook2/gihbk2.htm. (Accessed on 4/22/14).

Hsu, K. H., Kim, C. J., and Wilson, L. A. 1983. Factors affecting water uptake of soybeans during soaking. Cereal Chem. 60:208-211.

Ituen, E. U., Mittal, J. P., and Adeoti, J. S. 1985. Water absorption in cereal grains and its effect on their rupture stress. J. Food Process. Eng. 8:147–158.

Jung, Y. S., and Bae, D. G. 2014. Natural dyeing with black cowpea seed coat. I. Dyeing properties of cotton and silk fabrics. Fibers and Polym. 15:138-144-138.

Jones, P. M. B., and Boulter, D. 1983. The cause of reduced cook rate in *Phaseolus vulgaris* following adverse storage conditions. J. Food. Sci. 48:623-626.

Kelly, J. D., Varner, G. V., and Sprague, C. L. 2006. Condor, a new black bean for Michigan. Michigan State University Extension. Exten. Bull. E-2962.

Liu, K. 1997. Storage proteins and hard-to-cook phenomenon in legume seeds. Food Technol. 51(5):58,60-61.

Liu, M, Haghighi, K, and Stroshine, R. L. 1989. Viscoelastic characterization of the soybean seedcoat. Trans. ASAE. 32:946-952.

Lu, W., and Chang, K. C. 1996. Correlations between chemical composition and canning quality attributes of navy bean (*Phaseolus vulgaris* L.). Cereal Chem. 73:785-787.

Morris, C. F. 2002. Puroindolines: The molecular basis of wheat grain hardness. Plant Molecul. Biol. 48:633-647.

Mugendi, J. B., Njagi, E. N. M., Kuria, E. N., Mwasaru, M. A., Mureithi, J. G., and Apostolides, Z. 2010. Effects of processing technique on the nutritional composition and anti-nutrient content of mucuna bean (*Mucuna pruriens* L.). African J. Food Sci. 4:156-166.

Otto, T., Baik, B. K., and Czuchajowska, Z. 1997. Wet fractionation of garbanzo bean and pea flours. Cereal Chem. 74:445-451.

Ovando-Martinez, M., Osorio-Diaz, P., Whitney, K., Bello-Perez, L.A., and Simsek, S. 2011. Effect of the cooking on physicochemical and starch digestibility properties of two varieties of common bean *(Phaseolus vulgaris* L.*)* grown under different water regimes. Food Chem. 129:358-365.

Overhults, D. G, White, H. E., Hamilton, H. E., and Ross, I. J. 1973. Drying soybeans with heated air. Trans. ASAE. 16:112-113.

Paulson, R. M. 1978. Fracture resistance of soybean to compressive loading. Trans. Am. Soc. Agric. Eng. 21:1210-1216.

Phlak, L. C., Caldwell, K. B., and Stanley D. W. 1989. Comparison of methods used to characterise water imbibition in hard-to-cook beans. J. Food Sci. 54:326-336.

Prodanov, M., Sierra, I., and Valverde-Vidal, C. 2004. Influence of soaking and cooking on the thiamin, riboflavin and niacin contents of legumes. Food Chem. 84:271-277.

Sefa-Dedeh, S., and Stanley, D. W. 1979. Textural implications of the microstructure of legumes. Food Technol. 33:77-83.

Sefa-Dedeh, S., Stanley, D. W., and Voisy, P. W. 1978. Effect of storage time and cooking conditions on the hard-to-cook defect of cowpeas (*Vigna unguiculata*). J. Food Sci. 44:790-793.

Siddiq, M., and Uebersax, M. A. 2013. Composition of Processed Dry Bean and Pulses: Pages; 103-128 in: Dry Beans and Pulses: Production, Processing and Nutrition. First Ed. John Wiley & Sons, Inc.: Oxford, UK.

Siddiq, M., Ravi, R., Harte, J. B., and Dola, K. D. 2010. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris L.)* flours. Food Sci. Tech. 43:232-237.

Snyder. E. B. 1936. Some factors affecting the cooking quality of the pea and great northern types of dry beans. Nebraska Agr. Exp. Sta. Res. Bull. 85: 31.

Stanley, D. W., and Aguilera, J. M. 1985. A review of texture defects in cooked reconstituted legumes—The influence of structure composition. J. Food Biochem. 9:277-323.

Tiwari, B. K., Gowen, A., and McKenna, B. 2011. Pulse-based Food Product. Pages 249-282 in: Pulse Foods: Processing, Quality and Nutraceutical Applications. First Ed.: Academic Press. San Diego, CA.

Tiwari, B. K., and Singh, N. 2012. Pulse Grain Characteristic. Pages 1-33 in: Pulse Chemistry and Technology. First Ed.: Cambridge: The Royal Society of Chemistry. Cambridge, UK.

Tosh, S. M., and Yada, S. 2010. Dietary fibres in pulse seeds and fractions: Characterization, functional attributes, and applications. Food Res. Intl.43:450–460.

Tranquilli, G., Heaton, J., Chicaiza, O., and Dubcovsky, J. 2002. Substitutions and deletions of genes related to grain hardness in wheat and their effect on grain texture. Crop Sci. 42:1812-1817.

Uebersax, M. A., Ruengsakulrach, S., and Occena, L. G. 1991. Strategies and procedures for processing dry bean. Food Technol. 45:104-111.

Van Dongen, J. T., Ammerlaan, A. M. H., Wouterlood, M., Van Aelst, A. C., and Borstlap, A. C. 2003. Structure of the developing pea seed coat and post-phloem transport pathway of nutrients. Ann. Bot. 91:729-737.

Vilche, C., Gely, M., and Santalla, E. 2003. Physical properties of quinoa seeds. Biosystems Eng. 86:59-65.

Wang, N., Hatcher D. W., Toews, R., and Gawalko, E. J. 2009. Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). LWT - Food Sci. Technol. 42:842–848.

Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., and Gawalko, E. J. 2010. Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chicpeas (*Cicer arietinum* L.). Food Res. Int. 43:589-594.

## PAPER 2. MILLING BLACK BEANS (*Phaseolus vulgaris* L.) WITH A CENTRIFUGAL MILL

### Abstract

There is an increase in dry bean flour utilization in food products. Hence, understanding dry bean milling and its processing effect on final flour quality is important. Black beans were milled using a centrifugal mill. The effects of feed rate (46±2, 133±11, and 246±18 g/min), rotor speed (10,000, 12,000, and 14,000 rpm), and screen mesh opening size (250, 500, and 1,000 µm) on flour quality were determined. These milling parameters affected black bean flour temperature, moisture content, particle size, and yield. Screen mesh size had the greatest effect on flour quality. Screen mesh opening of 250 µm resulted in the greatest fine particle yield and also the greatest loss of flour moisture content. Conversely, milling with a screen mesh opening of 1,000 µm had the greatest coarse particle yield. Rotor speed impacted flour properties in a greater degree than mill feed rate. Milling black beans with a rotor speed of 14,000 rpm and a screen mesh size of 250 or 500 µm caused overheating and screen plugging. Milling problems did not occur with rotor speeds of 10,000 or 12,000 rpm. The best settings for milling black beans using a centrifugal mill to obtain the highest fine particles yields were screen mesh opening of 500 µm, rotor speed of 12,000 rpm, and mill feed of 267 g/min.

### Introduction

Black beans have a rich nutrient profile including high protein and fiber contents, as well as, vitamins and minerals. In addition, black beans have micronutrients important to humans including iron, zinc and folic acid (Uebersax 2006). The rich nutrient profile of black beans creates an opportunity to develop and introduce healthier food products by a portion of wheat flour with black bean flour. The use of black bean flour as an ingredient in novel and low-cost food products is growing. Spaghetti, bread and snacks are examples of food products to which bean flour can be added (Tiwari et al 2011).

Centrifugal mills are used to grind entire seed without, in the case of monocotyledonous seeds, separation of bran and germ from the endosperm or in the case of dicotyledonous seeds, seed coat and germ from the cotyledon. Centrifugal mill consists of three parts: 1. vibratory feeder; 2. rotor and mesh screen, and 3. vacuum system. Vibratory feeder controlled feed rate of seeds into the milling chamber. The vacuum system cools the mill and milled product by drawing air through the milling chamber. Rotor and mesh screen are found in the milling chamber. The center of the rotor is flat open area where the seeds are first deposited by the feeder. Wedged shaped blades are located at the end of the rotor. As the rotor spins, the seeds are impacted by the blades and thrown centrifugally against a grated mesh screen. Thus, centrifugal mill uses the particle size reduction principles of impact and shearing forces. Centrifugal mill can grind any soft, medium-hard, brittle, and fibrous materials (Anonymous 2014).

Milling converts whole grain/seed into smaller particles collectively called flour. Particle size reduction occurs due to the application of a force. When the seed or any material is subjected to a force, it absorbs the force as strain energy (Schorno 2006). Earle and Earle (2004) reported that when the local strain energy in a material exceeds a critical level, fractures occur along lines of weakness in the material and the stored energy is released. Most energy expended during milling is released as heat.

The range of particle size distribution is dependent on the mill used for grinding (Nishita and Bean 1982). Milling efficiency is related to uniformity of the particle size distribution. The differences in particle sizes can impact the bioavailability of macronutrients (carbohydrate, proteins) and their digestion (Wondra et al 1995). Fine particles provide greater surface area per volume and give more bioavailability than coarse particles. For example, in barley and sorghum flour, the kinetic of starch digestion by α-amylase was dependent on the particle size of the flours (Al-Rabadi et al 2009).

Particle size reduction affects damage to starch granules by the disruption of the granular structure of starch (Tran et al 2011). Heat exposure of the material during milling could change flour characteristics, such damage in starch, protein denaturation and lipid oxidation. Milling procedures can be adapted depending upon flour type and granulation desired. A search of the literature failed to find any information on the use of a centrifugal mill in milling black beans. Therefore, research was conducted to determine the mill settings or conditions for black bean milling. Settings such as feed rate, rotor speed (rpm), and screen mesh opening size used were studied. The best settings were selected based on:

1. The ease of milling without milling problems.
2. Favorable high fine particle yields.
3. Milling feed rate.

## Materials and Methods

### Mill Conditions

Black beans were obtained from Kelley Bean Company (Scottsbluff, NE). The moisture content of seeds when first received was at 10%. Centrifugal Mill (model ZM 200, Retsch GmbH, Haan, Germany) was evaluated for its ability to mill black beans.  The mill was operated using a vibratory feeder (model DR100, Retsch GmbH, Haan, Germany) and a vacuum (Nilfisk

GM 80, Hungary) attachment that air cooled the mill and mill product. Key variables tested were screen mesh size, rotor speed, and feed rate (Table 11). Each milling treatment consisted of 250 g beans.

Table 11. Centrifugal mill configurations.

| Screen Mesh Size (µm) | Rotor Speed (rpm) | Angular Velocity (rad/sec) | Mill Feed Rates (g/min) |
|---|---|---|---|
| 250 | 10,000 | 1,047 | 46±2 |
| 500 | 12,000 | 1,257 | 133±11 |
| 1,000 | 14,000 | 1,466 | 246±18 |

Data recorded for each mill treatment was: air temperature and relative humidity, initial sample weight, milling time, flour temperature, and flour weight. Seed and flour temperatures were measured before and after milling, respectively, using an infrared thermometer (VWR). Flour moisture content was measured immediately after milling using the forced-air oven method using approved method 44-15.02 (AACC International 2000). Milled samples were sieved using a vibratory sieve shaker (Retsch GmbH, Haan, Germany) with mesh sizes of 250, 150, 100 and 50 µm. Sieved fractions were recorded as flour on top of 250, 150, 100, 50 and particles that passed through 50 µm. Where portion of top of 250 µm was considered coarse and what passed through 50 µm was considered fine. Each sieve contained 10 sieving balls to aid in the passage of bean flour through the sieves. Without the balls, a coarse flour layer formed on the screen mesh surface and blocked fine particles from passing through the screen mesh (Figure 28).

Figure 28. Fine flour on top blocked by the layer on the bottom.

**Physical Seed Properties**

Mass of 100 black bean seeds was determined by taking 100 seeds randomly and measuring the mass of seeds for all the different treatment using an electric balance. Test weight was determined before and after all seed treatments. Test weight of whole black bean seeds was measured using the hectoliter weight (Ohaus, Des Plaines, IL, USA) procedure approved by GIPSA.

**Experimental Design and Data Analysis**

The experimental design was a randomized complete block design (RCBD) with a factorial arrangement of rotor speed (3) and mill rate (3). Each screen mesh size was considered a separate experiment. For each experiment, treatments were replicated three times. Each replicate was milled on a separate day. Data were analyzed with 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

Test weight, 100-kernel weight, and moisture content of black beans were 79.2 kg/hL, 20.4 g, and 10%, respectively. These results are similar to those reported by Kelly et al (2006). Feed rate was controlled using the vibratory feeder settings of 30, 40, and 50 which corresponded to $46 \pm 2$, $133 \pm 11$, and $246 \pm 18$ g/min, respectively. The air temperature was 22 °C and relative humidity 27% during milling.

While cleaning the mill between samples, it was noted that cleaning was noticeably more difficult due to finer particles and more static electricity associated with milling with the 250 µm screen. Cleaning this mill was time consuming, due to the several parts that needed to be cleaned to obtain most of the mill material and also to reduce contamination. The setting selected was feed rate of $246 \pm 18$, g/min, screen mesh opening size of 500 µm and rotor speed of 12,000 rpm.

The vibratory feeder setting of 60 was not used because it fed too many seeds into the mill causing the mill to overheat and stop. When the mill overheated, the rotor head temperature was 84 °C and the flour temperature was 47 °C. Milling many samples using screen mesh size of 250 µm caused overheating, particularly at the high feed rate, i.e. $246 \pm 18$ g/min, which resulted in flour temperature of 40 °C. The 250 µm screen mesh tended to plug at the high feed rate. When the mill overheated and stopped, it was allowed to cool down for about 15 min. However, during milling with 500 and 1,000 µm mesh screen, overheating of the mill and plugging of screens were not an issue. To reduce overheating, a vacuum was attached to the mill to draw air through the mill. A fan also was used to keep the mill cooled. Vacuum and fan were used at all times.

Rotor speed x feed rate interaction was significant for temperature gain of milled black bean flour when using 250, 500, and 1,000 μm screen mesh size (Tables A1-A3 and Table 12). For 250 μm screen mesh, changing feed rate caused greater effect on temperature gain than did changing the rotor speed. Flour temperature gain was greatest with the high feed rate for each rotor speed when the 250 μm screen mesh was used. The gain in temperature was greatest with high rotor speed at each feed rate. The interaction of rotor speed x feed rate, varied in differences in temperature gain between low and intermediate feed rate and rotor speed.

Table 12. Mean temperature gained values[a] during milling black bean[b] into flour as affected by feed rate setting and rotor speed for 250, 500, and 1,000 μm screen mesh size.

| Screen Mesh Size | Rotor Speed | | Feed Rate Setting | | |
|---|---|---|---|---|---|
| | | | 30 | 40 | 50 |
| (μm) | (rpm) | (rad/sec) | Temperature Gain (°C) | | |
| 250 | 10,000 | 1,047 | 13.5b,B | 13.7b,B | 16.0c,A |
| | 12,000 | 1,257 | 13.6b,C | 15.1a,B | 16.7b,A |
| | 14,000 | 1,466 | 14.4a,B | 14.5a,B | 17.5a,A |
| 500 | 10,000 | 1,047 | 6.1c,B | 6.2c,B | 8.4b,A |
| | 12,000 | 1,257 | 9.2b,A | 9.1b,A | 10.2b,A |
| | 14,000 | 1,466 | 10.3a,B | 10.3a,B | 12.9a,A |
| 1,000 | 10,000 | 1,047 | 1.0b,B | 2.1b,A | 2.5b,A |
| | 12,000 | 1,257 | 3.0a,A | 2.9b,A | 2.9b,A |
| | 14,000 | 1,466 | 3.6a,A | 4.2a,A | 4.3a,A |

[a]Different lowercase later across rows indicates significant differences (*P*=0.05). Different uppercase later across column indicates significant differences (*P*<0.05).
[b]Seed temperature was 21 °C and initial seed moisture content was 10%.

Changing rotor speed caused greater effect on temperature gain of flour than did changing feed rate when the 500 μm screen mesh was used (Table 12). Gain in temperature was greatest with rotor speed of 14,000, intermediate with 12,000 and least with 10,000 rpm. Low and medium feed rate caused similar gains in flour temperature. The greatest temperature gain occurred with fastest feed rate. The interaction occurred with feed rate not affecting the gain in flour temperature with 12,000 rpm rotor speed.

Changing rotor speed caused greater effect on temperature gain than did changing feed rate when the 1,000 µm screen mesh was used (Table 12). Gain in flour temperature was greatest with 14,000 rpm rotor speed. Feed rate did not affect the gain in flour temperature except for low feed rate, i.e. 10,000 rpm, which caused the least temperature gain. Average flour temperature gain was greatest with 250 µm mesh screen (15°C), intermediate with 500 µm screen mesh (9.2°C) and least with 1,000 µm screen mesh (3°C).

Rotor speed x feed rate interaction was significant for moisture content of milled black bean flour after milling using 250, 500, and 1,000 µm screen mesh (Tables A1-A3 and Table 13). Rotor speed did not affect moisture content of flour when milled at the high feed rate setting and 250 µm mesh screen. Beans milled on 14,000 rpm rotor speed produced flour with lower moisture contents than with 10,000 rpm when milling using the low or intermediate feed rate setting.

Table 13. Mean moisture content values[a] of milled black bean[b] flour as affected by feed rate setting and rotor speed for 250, 500, and 1,000 μm screen mesh size.

| Screen Mesh Size | | Rotor Speed | | Feed Rate Setting | | | |
|---|---|---|---|---|---|---|---|
| | | | | 30 | 40 | 50 | |
| (μm) | | (rpm) | (rad/sec) | Moisture Content (%) | | | Gain/loss |
| 250 | | 10,000 | 1,047 | 8.47a,A | 8.40a,AB | 8.37a,C | -0.10 |
| | | 12,000 | 1,257 | 8.40b,A | 8.53ab,A | 8.60a,A | 0.20 |
| | | 14,000 | 1,466 | 8.33b,A | 8.33b,C | 8.57a,B | 0.24 |
| | Gain/loss | | | 0.14 | 0.07 | -0.20 | |
| 500 | | 10,000 | 1,047 | 10.10a,A | 9.97b,A | 10.03a,A | -0.07 |
| | | 12,000 | 1,257 | 9.63b,B | 9.73b,B | 9.97a,A | 0.34 |
| | | 14,000 | 1,466 | 9.17b,B | 9.63b,A | 9.73a,A | 0.56 |
| | Gain/loss | | | 0.93 | 0.34 | 0.30 | |
| 1,000 | | 10,000 | 1,047 | 10.90a,A | 10.83a,A | 10.87a,A | -0.03 |
| | | 12,000 | 1,257 | 10.40b,B | 10.80a,A | 10.40b,B | 0.00 |
| | | 14,000 | 1,466 | 10.47b,B | 10.53b,B | 10.60a,A | 0.13 |
| | Gain/loss | | | 0.43 | 0.30 | 0.27 | |

[a]Different lowercase later across rows indicates significant differences (*P*=0.05). Different uppercase later across column indicates significant differences (*P*<0.05).
[b]Seed temperature was 21 °C and initial seed moisture content was 10%.

With the 500 μm screen mesh, flour moisture content was lower with rotor speed of 14,000 than of 10,000 rpm, at each feed rate. Feed rate did not affect moisture content when milled at 10,000 rpm. At 12,000 and 14,000 rpm, moisture content was lower with the low feed rate than with the high feed rate. With the 1,000 μm screen mesh, flour moisture was greater at 10,000 rpm than with 14,000 rpm. Feed rate did not affect moisture content when milled at 10,000 rpm. With 12,000 rpm moisture was lower at 46$\pm$2 and 246$\pm$18 g/min than with 133$\pm$11 g/min feed rate. At 14,000 rpm the moisture content was lowest with 46$\pm$2 g/min feed rate.

The starting moisture content of the black bean was 10%. Average flour moisture content was greatest with 1,000 μm screen mesh (10.6%), intermediate with 500 μm screen mesh (9.8%) and least with 250 μm screen mesh (8.4%). These results suggest that there was small increase in flour moisture when milled with 1,000 µm mesh screen, little or no effect on moisture when milled with 500 µm mesh screen, and a decrease in moisture when milled with 250 µm mesh screen. Loss of moisture reflects the increase in heat generated during milling and the high yield of fine particles (discussed below) associated with milling with the 250 µm screen. Higher surface area of flour with fine particles, such as seeds milled with screen mesh size of 250 µm, allowed the samples to dry more than using screen mesh size of 500 and 1,000 µm. Kerr et al (2000) also reported a decrease in moisture content of flour as screen mesh size decreased. Differences in moisture content obtained from the different screen mesh sizes could be important. Changes in flour temperature based on screen mesh sizes can be important for functional properties of the flour and its uses.

Rotor speed and feed rate main effects were significant for coarse particle formation when milling with the 250 µm screen mesh size (Tables A1-A3 and Table 14). Coarse particle content was 6.5, 4.8 and 3.6 % (LSD 0.05 = 0.3) for flour obtained by milling at 10,000, 12,000 and 14,000 rpm, respectively. Coarse particle content was 4.5, 4.9 and 5.6% (LSD 0.05 =0.3) in flour obtained at low, intermediate, and high feed rates, respectively. Fewer beans and particles in the milling chamber with low feed rate might result in higher number of impacts with rotor blades than what would occur at high feed rate. As a result, more fine particles were obtained.

Table 14. Mean coarse particle size % values[a] of milled black bean[b] flour as affected by feed rate setting and rotor speed for 500 µm screen mesh size.

| Screen Mesh Size | Rotor Speed | | Feed Rate Setting | | |
|---|---|---|---|---|---|
| | | | 30 | 40 | 50 |
| (µm) | (rpm) | (rad/sec) | Coarse Particle Size (%) | | |
| 500 | 10,000 | 1,047 | 30.8a,A | 27.9a,B | 31.2a,A |
| | 12,000 | 1,257 | 22.8b,C | 23.6b,B | 24.9b,A |
| | 14,000 | 1,466 | 18.3c,B | 19.5c,A | 20.0c,A |

[a]Different lowercase later across rows indicates significant differences ($P$=0.05). Different uppercase later across column indicates significant differences ($P$<0.05).
[b]Seed temperature was 21 °C and initial seed moisture content was 10%.

Rotor speed by feed rate interaction was significant for coarse particle content when milling with 500 µm screen (Table 14). Yield of coarse particles was greater when milling at 10,000 rpm than at 14,000 rpm. In addition, yield of coarse particles was greatest for flour obtained using the highest feed rate. The interaction occurred by variable response with intermediate speed and feed rate. Rotor speed main effect was significant for coarse particle content when milling with 1,000 µm screen. Yield of coarse particles was greatest (56.7%) with 10,000 rpm, intermediate (49.1%) with 12,000 rpm and least (42.2%) with 14,000 rpm (LSD 0.05=1.5).

Although considered separate experiments, coarse particle content was least with 250 µm screen mesh (5%), intermediate with 500 µm screen mesh (24%) and greatest with 1,000 µm screen mesh (49%). These data indicate that the 250 um screen would not be suitable for producing coarse particle size flour. The best setting for producing coarse particle size would be to mill using a rotor speed of 10,000 rpm and coarse mesh (1,000 µm) screen.

Rotor speed x feed rate interaction was significant for fine particle content when milling with 250 µm mesh screen (Tables A1-A3 and Table 15). Milling with feed rate setting 46$\pm$2 g/min, fine particle size content was greater with 12,000 and 14,000 than with 10,000 rpm. At feed rate setting 133±11 g/min, fine particle size content was greater with 10,000 and 14,000 rpm than with 12,000 rpm, while at feed rate setting 246±18 g/min the greatest fine particle size content occurred with 14,000 rpm. Greatest yield of fine particles occurred when milling was completed using a rotor speed of 12,000 or 14,000 rpm and a slow feed rate.

Table 15. Mean fine particle size % values[a] of milled black bean[b] flour as affected by feed rate setting and rotor speed for 250 µm screen mesh size.

| Screen Mesh Size | Rotor Speed | | Feed Rate Setting | | |
|---|---|---|---|---|---|
| | | | 30 | 40 | 50 |
| (µm) | (rpm) | (rad/sec) | Fine Particle Yield (%) | | |
| 250 | 10,000 | 1,047 | 71.3a,B | 69.5a,A | 57.3b,B |
| | 12,000 | 1,257 | 75.3a,A | 63.5b,B | 58.4c,B |
| | 14,000 | 1,466 | 73.9a,AB | 72.8a,A | 67.7b,A |

[a]Different lowercase later across rows indicates significant differences (*P*=0.05). Different uppercase later across column indicates significant differences (*P*<0.05).
[b]Seed temperature was 21 °C and initial seed moisture content was 10%.

Rotor speed main effect and feed rate main effect were significant for fine particle content with the 500 µm screen. Fine particle yield was 53.6% with low feed rate, 55.0% with intermediate feed rate, and 54.4% with high feed rate (LSD 0.05=0.08). Although statistically significant, the effect of feed rate was quite small and probably of no practical implications. Greatest fine particle yield (58.4%) occurred with 14,000 rpm rotor speed, intermediate (54.5%) with 12,000 rpm and least (50.2%) with 10,000 rpm (LSD 0.05 =0.8).

Rotor speed main effect was significant for fine particle size content with 1,000 µm mesh screen. Fine particle size content was greatest (39.5%) when milling at 14,000 rpm, intermediate (34.4%) at 12,000 rpm, and least (29.4%) at 10,000 rpm. Although considered separate experiments, fine particle size content was greatest with 250 µm screen mesh (68%), intermediate with 500 µm screen mesh (54%) and least with 1,000 µm screen mesh (34%).

Although, screen mesh size of 250 µm yielded the greatest of fine particles, it was difficult to mill black bean seeds, where overheating and overloading being the two major problems. Screen mesh size of 500 µm yielded fine particles without encountering milling difficulties. Moisture loss was not impacted by using screen mesh size of 500 µm.

## Conclusions

The results from this study indicate that the best settings for a centrifugal mill of similar size were: rotor speed of 12,000 rpm, screen mesh size of 500 µm, and a mill feed rate of high 246±18, g/min for black bean milling. The best setting was selected because flour was obtained without major problems during milling. Favorable fine particle yields, minimal temperature gain, and moisture content of 10.2%, 10 °C, and 54.4%, respectively. If coarse particles were desired for future research this study indicated that could be using rotor speed of 10,000, screen mesh size of 1,000 µm, and a mill feed rate of low 46±2 g/min.

## Literature Cited

AACC International. Approved methods of the American Association of Cereal Chemists. 2000. 10th Ed. Methods (44-15.02) AACC, St. Paul, MN.

Al-Rabadi, G. J., Gilbert, R. G., and Gidley, M. J. 2009. Effect of particle size on kinetics of starch digestion in milled barley and sorghum grains by porcine alpha-amylase. J. Cereal Sci. 50:198-204.

Anonymous. 2014. Ultra Centrifugal Mill ZM 200. [Web Page]. Located at http://www.retsch.com/products/milling/rotor-mills/zm-200/ (Accessed on 4/10/14).

Earle, R. L., and Earle, M. D. 2004. Unit Operations in Food Processing. Web Ed. New Zealand: The New Zealand Institute of Food Science and Technology Inc. [Web Page]. Located at: http://www.nzifst.org.nz/unitoperations/sizereduction1.htm#plate (Accessed 4/7/14).

Grain Inspection, Packers and Stockyards Administration. [GIPSA]. 1997. Book II: Grain grading procedures. Located at: http://www.usda.gov/gipsa/reference-library/handbooks/grain insp/grbook2/gihbk2.htm. (Accessed on 4/22/14).

Kelly, J. D., Varner, G. V., and Sprague, C. L. 2006. Condor, a new black bean for Michigan. Michigan State Univ. Ext. Ext. Bull. E-2962.

Kerr, W. L., Ward, C. D. W., McWatters, K. H., and Resurreccion, A. V. A. 2000. Effect of milling and particle size on functionality and physiochemical properties of cowpea flour. Cereal Chem. 77:213-219.

Nishita, K. D., and Bean, M. M. 1982. Grinding methods: their impact on rice flour properties. Cereal Chem. 59:46-49.

Tiwari, B. K., Gowen, A. and McKenna, B. 2011. Intoduction. Pages 1-7 in: Pulse Foods: Processing, Quality and Nutraceutical Applications. First Ed.: Academic Press. San Diego, CA.

Tran, T. B., Shelat, K. J., Tang, D., Li, E., Gilbert, R. G., and Hasjim, J. 2011. Milling of rice grains. The degradation on three structural levels of starch in rice flour can be independently controlled during grinding. J. Agric. Food Chem. 59:3964-3973.

Schorno, A. L. 2006. Flaxseed milling and the stability of milled flaxseed. Ph.D. Dissertation, North Dakota State University, Fargo.

Uebersax, M. A. 2006. Dry edible beans: indigenous staple and healthy cuisine. Department of Food Science and Human Nutrition, Michigan State University. [Web Page]. Located at: http://forumonpublicpolicy.com/archive06/uebersax.pdf. (Accessed on 4/10/14).

Wondra, K. J., Hancock, J. D., Behnke, K. C., and Stark, C. R. 1995. Effects of mill type and particle-size uniformity on growth-performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Animal Sci. 73:2564-2573.

# PAPER 3. EFFECT OF PREMILLING SEED TREATMENTS ON FLOUR QUALITY OF BLACK BEANS (*Phaseolus vulgaris* L.) MILLED WITH A CENTRIFUGAL MILL


## Abstract

Pretreated black bean seeds were milled using a centrifugal mill to produce black bean flour. Black bean seeds were pretreated by cooking the bean followed by drying (cooked-dried), soaking the beans and then drying (soaked-dried), or tempered. Black bean flour temperature, bulk density, particle size distribution, and color were analyzed, along with ash, protein, starch damage, and total starch contents. Pasting properties of black bean flour were also evaluated. Pretreatments affected flour temperature during milling, bulk density and particle size distribution, particularly for cooked-dried and soaked-dried samples. Flour color was greatly affected by the cooked-dried and soaked-dried pretreatments. Pretreatments had the greatest impact on black bean flour starch damage; whereas they had little or no effect on total starch, protein, and ash contents. Cooked-dried pretreatment was found to have the greatest impact on flour characteristics such as in pasting properties due to high starch damage.


## Introduction

Uncooked black bean seed market has been affected by the increasing popularity of fast and ready-to-eat foods. Uncooked black beans, require long processing times and preparations (Siddiq and Uebersax 2013). They are usually soaked overnight and then cook until soft. This is a tedious process.

Today, black bean flour is being used as an ingredient in food products, which has resulted in an interest in black bean milling.  The importance of adding black bean flour comes from their rich nutrient profile. Black beans are a good source of protein, carbohydrate, dietary fiber, and important minerals and vitamins essential for the human diet (Sathe 2002). However, black beans contain antinutritional factors (ANF) (i.e. trypsin inhibitors, tannins, phytic acid, phenolic compounds, and lectins). Antinutritional factors have been extensively studied (Valdebouze et al 1980; Champ 2002), as they could limit black beans and other dry beans utilization. Fortunately, black bean ANF can be eliminated by pretreatments of the seeds such as soaking and cooking (Hosfield and Uebersax 1980; Uebersax et al 1991).

Soaking is a pretreatment traditionally used to reduce cooking time of dry beans (Jackson and Varriano-Martson 1981). Yasmin et al (2008) reported that during soaking there was leaching of tannins, which almost eliminate tannin content. Reductions of other ANF such as trypsin inhibitors and phytic content have been reported (Alonso et al 1998; Abd and Habiba 2003).

Cooking is a process whereby black bean seeds are cooked in boiling water at 100 °C, which increases seed plasticity and water absorption (Abu-Ghannam and McKenna 1997). Cooking of dry beans improves color and texture of cooked beans and inactivates undesirable enzymes. Cooking inactivates ANF such as protease inhibitors and lectins. Some ANFs are somewhat heat resistant (i.e. phytic acids), however, by cooking the majority of ANFs are reduced to acceptable levels, which also improve organoleptic quality of dry beans (Rehman et al 2004).

Milling converts whole seeds into flour. Particle size reduction occurs due to the application of a force. Materials being milled absorb the force as strain energy (Schorno 2006). Earle and Earle (2004) described that when the local strain energy in a material exceeds a critical level, the material will fracture along a line of weakness and stored energy is released. Energy expended during milling is released mostly as heat. Desired black bean flour particle size is determined by the end use. For example, for chips coarse particles could be used, whereas, for a cake the consumer will find the visible coarse black bean into the baked product not appealing. Color of the flour will affect the final product color. Hence, color will influence the consumer's preference (Pomeranz and Meloan 1987).

Research was conducted to determine the effect of pretreated seeds (cooked-dried, soaked-dried, and tempered black beans) on milling and flour properties. Seeds were milled on a centrifugal mill using mill configuration determined to be the best for producing fine particles (Paper 2, page 95).

## Materials and Methods

### Pretreatments

Black beans were obtained from Kelley Bean Company (Scottsbluff, NE). The moisture content of seeds was kept constant at 10%. Each milling sample consisted of 150 g of seeds.

Clean black beans were cooked in distilled water for 5, 10, 15, and 20 min or soaked in distilled water for 6, 12, 18, and 24 h. Both cooked and soaked bean samples were drained, placed on baking sheets, and dried to 10% moisture content using a forced-air oven at 40-50 ºC.

Black beans were tempered with distilled water to 6, 8, 10, 12, and 14% moisture. Seed moisture was allowed to equilibrate at room temperature for 72 h before milling. Cooked-dried, soaked-dried, and tempered pretreatments were considered as three separate experiments (Table 16).

Table 16. Black bean seed pretreatments.

| Cooked-Dried[a] (min) | Soaked-Dried* (h) | Tempered (%) |
|---|---|---|
| 0 | 0 | 6 |
| 5 | 6 | 8 |
| 10 | 12 | 10 |
| 15 | 18 | 12 |
| 20 | 24 | 14 |

[a]Cooked and soaked seeds were dried to 10% moisture content at 50 ºC after being cooked or soaked, respectively.

**Milling Procedure**

Black beans were milled on a centrifugal mill (model ZM 200, Retsch GmbH, Haan, Germany). The mill was operated using a vibratory feeder (model DR100, Retsch GmbH, Haan, Germany) and a vacuum (Nilfisk GM 80, Hungary) attachment that air cooled the mill and mill product. Centrifugal milling configuration selected was screen mesh size of 500 µm with rotor speed of 12,000 rpm and a vibratory feeder setting of 50. All milled samples were stored at 4 ºC for later chemical analysis.

<u>Particle Size Determination</u>

Particle size determination was done using a vibratory sieve shaker (Retsch GmbH, Haan, Germany) set up with 250, 150, 100, and 50 µm mesh sieves. Each sieve contained 10 polyurethane ball sieve cleaners that aided in the sifting process. Milling yield was based on the weight of the fine particles. Fine particles were considered flour that went through the 50 µm sieve. Coarse particles were considered flour that did not passed through the 50 µm sieve. All milled samples were stored at 4 ºC for later chemical analysis.

**Physical Quality of Flour**

Black bean flour moisture content, milling time, milled product temperature, and final flour weight were recorded. Flour temperature was measured using an infrared thermometer (VWR). Flour moisture content was measured right after milling using a forced-air oven set at 130 °C for 1 hour according to approved method 44-15.02 of the AACC International (2000).

Whole, coarse and fine fractions were evaluated for color measurement (CIE L-value) using Minolta 310 colorimeter (Minolta Corp., Ramsey, NJ, U.S.A.). Color difference was also determined, which is defined as "the magnitude and character of the difference between two colors under specified conditions". Color difference ($\Delta E^*_{ab}$) was calculated using the equation:

$$\Delta E^*ab = \sqrt{(L2 - L1)^2 + (a2 - a1)^2 + (b2 - b1)^2} \qquad \text{(X-Rite Inc, 2007)}$$

Where *L1, a1*, and *b1* were CIE L, a, b values for no-treated sample, while *L2*, *a2*, and *b2* were CIE L, a, b values of a pretreated sample.

Bulk density was determined following the procedure of Okaka and Potter (1979), where 50 g of bean flour was put into a 100 mL measuring cylinder by pouring through a funnel and tapped to a constant volume. Bulk density (g/cm$^3$) was calculated by dividing the weight of flour (g) by flour volume (cm$^3$).

**Chemical Composition of Flour**

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. The amount of starch damage was determined using an enzymatic starch damage assay kit (Megazyme International, Co. Wicklow, Ireland) according to AACC International Approved Method 76-31.01. 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 was analyzed using Leco combustion nitrogen analyzer (LECO Corp. St. Joseph, MI, USA). Protein content was calculated as %N × 6.25.

### Pasting Properties of Flour

Pasting properties of cooked-dried, soaked-dried, and tempered samples were determined using a Rapid Visco-Analyzer (Newport Scientific (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 it 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.

### Experimental Design and Data Analysis

The experimental design was a randomized complete block. 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

### Cooked-Dried Seeds

#### Physical Quality of Flour

Black beans cooked for 5, 10, and 15 min gained less temperature during milling than did the non-treated (Table 17). The air temperature was 21 °C and the relative humidity was 20%. Black beans cooked for 20 min had similar temperature gain as non-treated seeds. Feed rate declined from 248.2 g/min to 171.8 g/min when comparing non-treated beans to those cooked for 20 min, respectively (Table 17). Decrease in feed rate as cook time increased probably reflects the change in seed shape and increased size and decreased weight that occurred with cooking (Paper 1, Table 3). The cooked-dried pretreatment influenced the feed rate possibly difference of the packing and moving through the feeder to the mill which can be due to the differences in shape of cooked-dried seeds as seen in Figure 14 in Paper 1. In addition, this could have contributed to the differences obtained in milling and further flour and chemical quality of flour from cooked-dried seeds.

In general, an increased in bulk density was observed as seed cooking time increased (Table 17). Bulk density of flour from non-treated beans was 0.77 $g/cm^3$ and from beans cooked 20 min was 0.85 $g/cm^3$. This was ≈ 10% increase in bulk density for flour from seed cooked for 20 min. Bulk density results suggested that the flour particles from different cooking times differed in how they packed.

Table 17. Mean physical quality values[a] of flour from cooked-dried seeds.

| Cooked-Dried[b] (min) | Temperature Gain (ºC) | Feed Rate (g/min) | Bulk Density ($g/cm^3$) | Fine Particles Yield (≤50 μm) (%) |
|---|---|---|---|---|
| 0 | 5.2a | 248.2a | 0.77c | 64.8a |
| 5 | 2.2c | 212.9b | 0.83b | 58.4b |
| 10 | 3.8b | 195.9c | 0.82b | 58.0b |
| 15 | 3.0bc | 180.0d | 0.85a | 57.3bc |
| 20 | 5.5a | 171.8d | 0.85a | 55.4c |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 20 °C and initial seed moisture content was 10%.

Packing efficiency could be affected by particle size and shape. Possibly, when black bean seed was cooked a change in its components by the high temperature and water uptake made the flour physical properties changed. Fine particle yield was approximately ≈ 15% lower for black bean flour obtained from the 20 min cooked-dried beans compared to the non-treated beans (Table 17).

Cooked-dried seed pretreatments significantly affected L-value and color difference (Table 18). As cooked-dried times increased, a decreased flour lightness or L-value for whole, coarse, and fine flours was observed. The L-value of whole, coarse and fine flours decreased from 81.4 to 71, 66.1 to 53.9, and 86.3 to 79, respectively. The most significant color difference ($\Delta E^*_{ab}$) of whole flour was between the flours of the 20 min cooking (12.1) and 5 min cooking (6.9). As expected based on the reduction in lightness, color difference increased as cooking seed times increased and was greatest in 20 min cooked seeds.

Table 18. Mean L-values and color difference values[a] of flour fractions from cooked-dried seeds.

| Cooked-Dried | Whole Flour | | Coarse Flour | | Fine Flour | |
|---|---|---|---|---|---|---|
| (min) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) |
| 0 | 81.4a | 0.0d | 66.1a | 0.0d | 86.4a | 0.0d |
| 5 | 76.2b | 6.9c | 56.4bc | 10.7bc | 83.3b | 4.6c |
| 10 | 74.6c | 8.6b | 57.5b | 9.8c | 81.2c | 6.7b |
| 15 | 73.4c | 9.8b | 54.6cd | 12.5ab | 80.5c | 7.2b |
| 20 | 71.0c | 12.1a | 53.9d | 13.2a | 78.9d | 8.6a |

[a]Values followed by same letter are not significantly different at $P$=0.05.

The L-value for flour color is commonly used to measure the degree of lightness (Siddiq et al, 2013). This suggested that flour fractions decreased in lightness as cooking times increased. Color from fine particle had the highest L-value or lightness due to finer particle size. The color differences for coarse and fine fractions followed similar patterns where longer cooking times produced greater color differences. It was observed that during cooking of black beans, soluble color compounds, anthocyanins, leached into the cooking water medium and stained the seeds

(Figure 15 in Paper 1). Flour color is an important quality factor because it transfers to the final product and defines its acceptability, marketability and freshness.

### Chemical Composition of Flour

Cooked-dried seed pretreatment affected total starch, starch damage, and ash content (Table 19). Cooking followed by drying of the seeds resulted in a significant ($P$=0.05) increase in total starch and starch damage, but ash content was reduced significantly ($P$=0.05) as cooking time increased (Table 19). Total starch varied from 37.3 to 42.3%. The lowest total starch was observed in non-treated seeds, whereas 5 min cooked had the highest. The increase in total starch for cooked black beans is in agreement with results obtained by other researchers (Hoover and Zhou 2003; Wang et al 2010). Hoover and Zhou 2003 reported that cooked/steamed black bean samples had higher total starch than raw. Wang et al (2010) reported an 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 makes them more susceptible and accessible to starch hydrolyzing enzymes attack; hence higher total starch is reported. The increase in total starch can be related to the loss of soluble solids during cooking, which would increase the proportion of starch in cooked seeds. Also, when comparing the lower values of total starch in non-treated seeds to the higher total starch for the cooked-dried seeds, it can be suggested that the presence of amylase inhibitors contributed to the lower value. This inhibitor prevents the amylase from degrading the starch and thereby the starch hydrolysis in the test assay (MacCarty 2005).

Table 19. Mean chemical composition[a] of flour from cooked-dried seeds.

| Cooked-Dried[b] (min) | Total Starch | Starch Damaged | Protein Content (%) | Ash Content |
|---|---|---|---|---|
| 0 | 37.8c | 0.5e | 24.1a | 3.48b |
| 5 | 42.4a | 2.5d | 23.8b | 3.77a |
| 10 | 39.7b | 3.5c | 23.8ab | 3.59ab |
| 15 | 40.3b | 4.4b | 23.8b | 3.13c |
| 20 | 39.2bc | 4.7a | 24.1a | 2.92d |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Values are dry weight based.

Starch damage (Table 19) increased for all cooked-dried pretreatments. Starch damage varied from 0.5 to 4.7%. The non-treated seeds had the lowest starch damage and cooked-dried for 20 min the highest. 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 showed increased starch damage more than raw beans from 1.0 to 4.0%, which is in agreement with this study starch damage values after cooking.

Ash content significantly decreased as cooking times increased (Table 19). Flour from 20 min cooked-dried seed pretreatment showed the lowest ash content which decreased ≈ 20% when compared to flour from non-treated seeds. Ash content and changes after cooking dry bean seeds are related to mineral and vitamins as well as oligosaccharides present in the seed. It has been reported, cell membrane permeability increases during thermal processing which allows ions, vitamins, minerals, and small molecules to diffuse from seeds into the cooking water (Siddiq and Uebersax 2013). Leaching of minerals as well as oligosaccharides was observed in white beans (Kon 1979). The decrease in ash content possibly resulted from leaching of certain minerals into the cooking water. Similar results were also reported for field peas and black beans by Wang et al (2008) and Wang et al (2010), respectively.

Protein content was not affected by cooking pretreatments (Table 19). Protein content values ranged from 23.7 to 24.1%. 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.

Pasting Properties of Flour

Pasting properties were affected by cooked-dried seed pretreatment of black bean (Table 20). With increasing cooking times, peak, trough, final viscosity, and setback decreased, and breakdown values were low when compared to wheat flour pasting values. The peak values decreased from 158.1 to 35.9 RVU as cooking time increased from 0 to 20 min. Similarly, trough, final viscosity, and setback values decreased from 181.7 to 32.6 RVU, 229.6 to 67.6 RVU, and 78.3 to 35.1 RVU, respectively.

Table 20. Mean pasting properties[a] of flour from cooked-dried seeds.

| Cooked-Dried (min) | Peak | Trough | Breakdown (RVU) | Final Viscosity | Setback |
|---|---|---|---|---|---|
| 0 | 158.1a | 151.7a | 4.4bc | 229.6a | 78.3b |
| 5 | 111.2b | 106.6b | 4.7b | 189.7b | 83.2a |
| 10 | 67.5c | 61.6c | 5.8a | 138.1c | 76.5b |
| 15 | 43.1d | 38.6d | 4.5bc | 89.6d | 51.1c |
| 20 | 35.9e | 32.6e | 3.4c | 67.6e | 35.1d |

[a]Values followed by same letter are not significantly different at $P$=0.05.

Pasting properties are useful as 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). Breakdown values were low which indicated the presence of restriction in starch granules swelling and high amylose content. High setback values suggests high tendency for retrogradation to occur (Kim et al 1997) as was for 0 and 5 min cooked beans. Final viscosities large decrease from 229.6 to 67.6 RVU for 0 and 20 min cooked beans, respectively, suggest a decreasing capacity of the flour to retrograde and form a strong gel. The low peak viscosity and no 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.

## Soaked-Dried Seeds

### Physical Quality of Flour

Soaked-dried seed pretreatments affected the physical quality of flour compared to non-treated seed (Table 21). The air temperature was 26 °C and the relative humidity was 20%. Soaked-dried treated seed caused an increase in flour temperature gained during milling and in bulk density and a decrease in feed rate and in fine particle content. This suggested that soaked-dried pretreatments affected and caused changes in the seed physical structure. Interestingly, among the soaked-dried pretreatments there were little or no differences detected in flour temperature gained during milling, bulk density, feed rate, or fine particle yield. The water penetration and changes in the cotyledon structure (Figure 22 in Paper 1) could explain the differences in particle size distribution.

Table 21. Mean physical quality values[a] of flour from soaked-dried seeds.

| Soaked-Dried[b] (h) | Temperature Gained (ºC) | Feed Rate (g/min) | Bulk Density ($g/cm^3$) | Fine Particles Yield (≤50 μm) (%) |
|---|---|---|---|---|
| 0 | 5.5c | 243.9a | 0.77b | 64.3a |
| 6 | 7.6ab | 176.7c | 0.86a | 57.4c |
| 12 | 6.4bc | 181.9bc | 0.86a | 59.4b |
| 18 | 7.7ab | 182.7bc | 0.87a | 59.4b |
| 24 | 8.3a | 186.3b | 0.86a | 57.9bc |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Seed temperature was 24 °C and initial seed moisture content was 10%.

Soaked-dried seed pretreatment reduced L-values for whole flour, coarse flour and fine flour when compared to flour from non-treated black bean seed (Table 22). Similar to physical properties discussed above, L-values were similar regardless of soaking time. As explained for cooking pretreatments, the finer the particle size, the higher the L-value or the lighter the flour. However, the whole and coarse flour fractions had lower lightness values and dark color was observed (Figure 20 in Paper 1). The dark color and leaching out of the pigment came from the presence of anthocyanins in the seed coat of black beans, which are water soluble (Jung and Bae 2014).

Table 22. Mean L-values and color difference values[a] of flour fractions from soaked-dried seeds.

| Soaked-Dried | Whole Flour | | Coarse Flour | | Fine Flour | |
|---|---|---|---|---|---|---|
| (h) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) |
| 0 | 81.3a | 0.0b | 65.9a | 0.0b | 86.2a | 0.0b |
| 6 | 76.9b | 5.7a | 59.4b | 7.1a | 83.9b | 3.1a |
| 12 | 77.1b | 4.7a | 59.5b | 6.8a | 83.6b | 3.1a |
| 18 | 77.4b | 4.3a | 58.9b | 7.4a | 83.5b | 3.0a |
| 24 | 76.9b | 4.7a | 59.2b | 7.1a | 83.8b | 2.9a |

[a]Values followed by same letter are not significantly different at *P*=0.05.

## Chemical Composition of Flour

Soaking black bean seed had little or no effect on total starch and protein content (Table 23). In general, starch damage of flour from soaked-dried seeds increased when compared to non-treated sample. Starch damaged increased from 0.6 in non-treated sample to 1.9% for the 6 h soaked-dried sample.

Table 23. Mean chemical composition[a] of flour from soaked-dried seeds.

| Soaked-Dried[b] (h) | Total Starch | Starch Damaged (%) | Protein Content | Ash Content |
|---|---|---|---|---|
| 0 | 37.9a | 0.6b | 24.3a | 3.45a |
| 6 | 34.3b | 1.9a | 24.0b | 3.32ab |
| 12 | 35.6ab | 1.1ab | 24.4a | 3.22b |
| 18 | 33.6b | 1.9a | 24.6a | 3.22ab |
| 24 | 36.1ab | 1.1ab | 24.3a | 2.81c |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Values are dry weight based.

Another possible contributing factor of reduction in total starch was seed germination (Figure 29). Onset of seed germination would result in release of amylase that would cause damage to the starch granule. Soaked beans were high in water content and dried at low temperatures (50 °C). Conditions such as high moisture and temperatures in that range possibly triggered amylase activity resulting from germinating seeds (Figure 29). Labaneiah and Luh (1981) reported that total starch content decreased in germinated black beans.

Figure 29. Germinating black bean seeds.

Soaked-dried seed pretreatment effect was significant for ash content. The non-treated sample had the greatest ash content. Beans soaked for 24 h and dried had reduced ash content. Ash content was reduced by ≈ 20% when compared flour from non-treated sample to soaked-dried sample of 24 h. As explained in cooking sections, the decrease in ash content can be attributed to leaching of minerals into the soaking water. These results have been suggested for cooking as well as for soaking. Labaneiah and Luh (1981) reported lower values in ash content of raw compared to soaked black bean seeds.

Pasting Properties of Flour

Soaked-dried seed pretreatments affected flour pasting properties (Table 24). Flour peak viscosity and trough viscosity were not affected by soaked-dried pretreatments. Breakdown viscosity was decreased 3.7 to 6.4 RVU, which is probably of no practical importance. The peak viscosity for soaked-dried pretreatment increased from 143.6 to 150.9 RVU, trough viscosity increased from 135.9 to 144.2 RVU, final viscosity increased from 191.2 to 238.1 RVU, and setback increased from 73.4 to 93.9 RVU when comparing non-treated sample to the soaked-dried 24 h sample. In general, black bean pasting viscosities tend to be lower than those reported for other starches (Shuey and Tipples (1980). Su et al (1998) worked with six different dry beans and suggested that the low pasting viscosities might be due to relaxation of the secondary bonds such as hydrogen bonds, or the interaction between starch and proteins during heating.

Table 24. Mean pasting properties[a] of flour from soaked-dried seeds.

| Soaked-Dried (h) | Peak | Trough | Breakdown | Final Viscosity | Setback |
|---|---|---|---|---|---|
| | | | (RVU) | | |
| 0 | 143.6a | 135.9a | 10.1a | 191.2b | 73.4c |
| 6 | 146.1a | 142.4a | 3.7d | 222.8ab | 80.4bc |
| 12 | 148.4a | 143.1a | 5.3c | 227.3a | 84.3abc |
| 18 | 150.3a | 143.1a | 7.2b | 233.8a | 90.7ab |
| 24 | 150.9a | 144.2a | 6.7b | 238.1a | 93.9a |

[a]Values followed by same letter are not significantly different at *P*=0.05.

**Tempered Seeds**

<u>Physical Quality of Flour</u>

Tempered seed pretreatment affected physical quality of flour (Table 25). The air temperature was 26 °C and the relative humidity was 20%. Flour temperature gain was lowest for milled black beans with a moisture content of 6%.  Flour temperature gained in bean flours with 8% moisture or more (9.1 °C) was about twice as high as that for beans with 6% moisture (4.5 °C). Feed rate during milling declined from 293 to 267 g/min as seed moisture content increased from 6 to 14%, respectively. Similarly, bulk density of flour declined from 0.81 to 0.68 g/cm$^3$ as seed moisture content increased from 6 to 14%, respectively. Tempered seed pretreatments effect was significant for fine particle yield (Table 25). Fine particle yield was greater with seeds that were tempered to 8 to14% compared to yield from seeds with 6% moisture content.

Tempering compared to cooking or soaking is a mild seed pretreatment where excess of water is not used, only wetting of the seeds to a certain level is achieved. Feed rate were not affected by the different moisture content levels used. However, bulk density significantly decreased as moisture content increased. Irvine et al (1992) reported similar results where it showed declined bulk densities, with increased in moisture content for flaxseed, lentils, and faba bean flour samples. This could have been attributed to the high content of fiber in the seed coat and the probability that the seed coat absorbed more moisture, which caused the seed to retain more water and subsequently gave less dense packing flour.

Table 25. Mean physical quality values[a] of flour from tempered seeds.

| Tempered[b] (%) | Seed Moisture (%) | Temperature Gain (°C) | Feed Rate (g/min) | Bulk Density (g/cm$^3$) | Fine Particles Yield (≤50 µm) (%) |
|---|---|---|---|---|---|
| 6 | 6.5 | 4.5b | 292.6a | 0.81a | 58.5b |
| 8 | 8.2 | 8.9a | 286.1b | 0.80b | 64.4a |
| 10 | 10.0 | 9.1a | 266.7d | 0.76c | 65.8a |
| 12 | 11.7 | 9.0a | 273.6c | 0.74d | 64.1a |
| 14 | 13.8 | 9.4a | 267.1d | 0.68e | 63.8a |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 25 °C and initial seed moisture content was 10%.

Tempered pretreatment significantly affected L-value and color difference (Table 26). L-values slightly decreased for all fractions (whole, coarse, and fine) when moisture content increased from 6 to 14%. Color difference was more significant for whole flour than fine and coarse fractions. L-values for flour obtained from tempered seeds decreased as moisture content increased. This suggested that at high moisture content, leaching of pigments occur and flour darken in color. Similar results were observed in tempered pretreated beans (Bellido et al 2003). As stated previously, fine particle were lighter in color than whole and coarse fractions.

Table 26. Mean L-values and color difference values[a] of flour fractions from tempered seeds.

| Tempered (%) | Seed Moisture (%) | Whole Flour (L) | Whole Flour ($\Delta E^*_{ab}$) | Coarse Flour (L) | Coarse Flour ($\Delta E^*_{ab}$) | Fine Flour (L) | Fine Flour ($\Delta E^*_{ab}$) |
|---|---|---|---|---|---|---|---|
| 6 | 6.5 | 82.2a | 0.9c | 67.1a | 0.7b | 86.9a | 0.5b |
| 8 | 8.2 | 82.5a | 1.2b | 66.6a | 0.4b | 87.1a | 0.7b |
| 10 | 10.0 | 82.0a | 0.0e | 66.9a | 0.0b | 86.1b | 0.0b |
| 12 | 11.7 | 81.4b | 0.8d | 64.5b | 2.1a | 86.2b | 0.56b |
| 14 | 13.8 | 80.1c | 1.8a | 65.0b | 1.6a | 84.9c | 1.7a |

[a]Values followed by same letter are not significantly different at *P*=0.05.

Chemical Composition of Flour

Tempered seed pretreatments had little or no effect on total starch, protein, and ash contents (Table 27). Starch damaged showed no significant differences for samples tempered 6 to 10% moisture content. However, the starch damage increased to 1.2 and 2.4 % for samples at 12 and 14% moisture content, respectively.

As it was a mild seed pretreatment, changes in chemical composition were not expected and the results showed no significant changes for totals starch, protein, and ash contents. However, for seeds tempered at 12 and 14% moisture content starch damaged increased, which suggested that the seed was more affected by this high moisture content and seed structure toughened, which made the milling harsher on the seed during grinding. No changes were observed for protein and ash contents.

Table 27. Mean chemical composition[a] of flour from tempered seeds.

| Tempered[b] (%) | Seed Moisture (%) | Total Starch | Starch Damaged | Protein Content | Ash Content |
|---|---|---|---|---|---|
| | | | (%) | | |
| 6 | 6.5 | 41.6a | 0.6b | 23.8a | 3.44a |
| 8 | 8.2 | 38.7b | 0.6b | 24.0a | 3.44a |
| 10 | 10.0 | 41.3a | 0.6b | 24.1a | 3.47a |
| 12 | 11.7 | 39.2b | 1.2b | 24.2a | 3.36a |
| 14 | 13.8 | 40.5a | 2.4a | 24.1a | 3.35a |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Values are dry weight based.

Pasting Properties of Flour

Tempered pretreatments did not affect pasting properties of black bean (Table 28). Tempering black bean seeds was not expected to significantly impact on the flour pasting properties.

Table 28. Mean pasting properties[a] of flour from tempered seeds.

| Tempered (%) | Peak | Trough | Breakdown | Final Viscosity | Setback |
|---|---|---|---|---|---|
| | | | (RVU) | | |
| 6 | 148.6a | 139.4a | 9.1ab | 225.0a | 85.6a |
| 8 | 147.8a | 140.5a | 7.2bc | 229.6a | 89.1a |
| 10 | 153.2a | 142.5a | 10.7a | 233.7a | 91.1a |
| 12 | 149.6a | 142.4a | 7.1bc | 222.6a | 80.1a |
| 14 | 144.3a | 138.8a | 5.4c | 217.8a | 79.0a |

[a]Values followed by same letter are not significantly different at *P*=0.05.

The differences observed for physical quality of flour such as flour temperature and feed rate can be related to the seed hardness (Table 10 in Paper 1). The fracture force required to first crack the seed increased with increasing the seed moisture content. This is related that the seed becomes tougher and more malleable than dry seed such as 6 to 10% moisture content seeds.

Paulson (1978) reported the highest toughness or most firm values for soybean samples was between 11 to 14% moisture content.

## Conclusions

Pretreated seeds (cooked-dried, soaked-dried, and tempered black beans) affected milling and flour properties. Fine particle yields decreased with cooked-dried or soaked-dried pretreatment. Tempered pretreatment showed no significant changes for fine particle yield. The effect of cooked-dried pretreatment on flour chemical properties was the greatest due to utilization of high temperatures, which caused changes internally. Both soaking and cooking impacted the final flour color by decreasing the lightness due to high leaching of seed coat pigments into the cooking water. Pasting properties of black bean flour was greatly influenced for cooked-dried pretreatment. In general, breakdown values were low which suggested a weakening effect by the high protein, some lipid and fiber present in black bean seeds.

## Literature Cited


AACC International. Approved methods of the American Association of Cereal Chemists. 2000. 10$^{th}$ Ed. Methods (44-15.02), (08-01.01), (46-30.01), (76-13.01). AACC, St. Paul, MN.

Abd, E., and Habiba, R. 2003. Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. LWT-Food Sci. Technol. 36:285-293.

Abu-Ghannam, N., and McKenna, B. 1997. Hydration kinetics of red kidney beans (*Phaseolus vulgaris* L.). J. Food Sci. 62:520-523.

Alonso, R., Orue, A., and Marzo, F. 1998. Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. Food Chem. 63:505-512.

Bellido, G. G., Arntfield, S. D., Scanlon, M. G., and Cenkowski, S. 2003. The effect of micronization operational conditions on the physicochemical properties of navy beans (*Phaseolus vulgaris* L.). J. Food Sci. 68:1731-1735.

Bello-Pérez, L. A., and Paredes-López, O. 2009. Starches of some food crops, changes during processing and their nutraceutical potential. Food Eng. Rev. 1:50-65.

Champ, M. J. 2002. Non-nutrient bioactive substances of pulses. Br. J. Nutr. 88:S307-S319.

Chung, H. J., Liu, Q., Donner, E., Hoover, R., Warkentin, T., and Vandenberg, B. 2008. Composition, molecular structure, properties, and in vitro digestibility of starches from newly released Canadian pulse cultivars. Cereal Chem. 85:471–475.

Earle, R. L., and Earle, M. D. 2004. Unit Operations in Food Processing. Web Ed. New Zealand: The New Zealand Institute of Food Science and Technology Inc. [Web Page]. Located at: http://www.nzifst.org.nz/unitoperations/sizereduction1.htm#plate (Accessed on 4/7/14).

Eyarua, R., Shrestha, A. K., and Arcot, J. 2009. Effect of various processing techniques on digestibility of starch in red kidney bean (*Phaseolus vulgaris*) and two varieties of peas (*Pisum sativum*). Food Res. Int. 42:956-962.

Hoover, R., and Zhou, Y. 2003. In vitro and in vivo hydrolysis of legume starches by a-amylase and resistant starch formation in legumes-a review. Carbohydr. Polym. 54:401-417.

Hosfield, G. L., and Uebersax, M. A. 1980. Variability in physio-chemical properties and nutritional components of tropical and domestic dry bean germplasm. J. Amer. Soc. Hort. Sci. 105:246-252.

Irvine, D. A., Jayas, S. S., White, N. D. G., and Britton, M. G. 1992. Physical properties of flaxseed, lentils, and fababeans. Can. Agric. Eng. 34:75–81.

Jackson, G. M., and Varriano-Martson, E. 1981. Hard-to-cook phenomenon in beans: Effects of accelerated storage on water absorption and cooking time. J. Food Sci. 46:799-803.

Jung, Y. S., and Bae, D. G. 2014. Natural dyeing with black cowpea seed coat. I. Dyeing properties of cotton and silk fabrics. Fibers and Polym. 15:138-144-138.

Kim, Y. S., Wiesenborn, D. P., and Grant, L. A. 1997. Pasting and thermal properties of potato and bean starches. Starch/Starke. 49:97–102.

Kon, S. 1979. Effects of soaking temperature on cooking and nutritional quality of beans. J. Food Sci. 44:1329-1340.

Labaneiah, M. E. O., and Luh, B. S. 1981. Changes of starch, crude fiber, and oligosaccharides in germinating dry beans. Cereal Chem. 58:135-138.

MacCarty, M. F. 2005. Nutraceutical resources for diabetes prevention. Med. Hypotheses. 64:151-158.

Okaka, J. C., and Potter, N. N. 1979. Physico-chemical and functional properties of cowpea powders processed to reduce beany flavor. J. Food Sci. 44(4):1235-1240.

Ovando-Martínez, M., Osorio-Díaz, P., Whitney, K., Bello-Pérez, L., and Simsek, S. 2011. Effect of the cooking on physicochemical and starch digestibility properties of two varieties of common bean (*Phaseolus vulgaris* L.) grown under different water regimes. Food Chem. 129:358-365.

Paulson, R. M. 1978. Fracture resistance of soybean to compressive loading. Trans. Am. Soc. Agric. Eng. 21:1210-1216.

Pomeranz, Y., and Meloan, C. E. 1987. Food analysis: Theory and Practice. 2nd Ed. AVI: New York, NY.

Rehman, Z. U., Rashid, M., and Shah, W. H. 2004. Insoluble dietary fiber components of food legumes as affected by soaking and cooking processes. Food Chem. 85:245-249.

Sathe, S. K. 2002. Dry bean protein functionality. Crit. Rev. Biotechnol. 22:175-223.

Schorno, A. L. 2006. Flaxseed milling and the stability of milled flaxseed. Ph.D. Dissertation, North Dakota State University, Fargo.

Shuey, W. C., and Tipples, K. H. 1980. The amylograph handbook. American Assoc. of Cereal Chem.: St. Paul, MN.

Siddiq, M., Ravi, R., Harte, J. B., and Dola, K. D. 2010. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.) flours. Food Sci. Technol. 43:232-237.

Siddiq, M., Kelkar, S., Harte, J. B., Dolana, K. D., and Nyombaire, G. 2013. Functional properties of flour from low-temperature extruded navy and pinto beans (*Phaseolus vulgaris* L.). Food Sci. Technol. 50:215–219.

Siddiq, M., and Uebersax, M. A. 2013. Composition of Processed Dry Bean and Pulses: Pages; 103-128 in: Dry Beans and Pulses: Production, Processing and Nutrition. First Ed. John Wiley & Sons, Inc.: Oxford, UK.

Su, H.S., Lu. W., and Chang, K.C. 1998. Microstructure and physicochemical characteristics of starches in six bean varieties and their bean paste products. J. Food Sci. Technol. (LWT). 31:265-273.

Uebersax, M. A., Ruengsakulrach, S., and Occena, L. G. 1991. Strategies and procedures for processing dry bean. Food Technol. 45:104-111.

Valdebouze, P., Bergeron, E., Gaborit, T., and Delort-Laval, J. 1980. Content and distribution of trypsin inhibitors and haemagglutinins in some legume seeds. Can. J. Plant Sci. 60:695-701.

Wang, N., Hatcher, D. W., and Gawalko, E. J. 2008 Effect of variety and processing on nutrients and certain antinutrients in field peas (*Pisum sativum*). Food Chem. 111:132-138.

Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., and Gawalko, E. J. 2010. Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). Food Res. Int. 43:589-594.

Yasmin, A., Zeb, A., Khalil, A., Paracha, G., and Khattak, A. 2008. Effect of processing on anti-nutritional factors of red kidney bean (*Phaseolus vulgaris*) grains. Food Bioprocess. Technol. 1:415-419.

# PAPER 4. MILLING BLACK BEANS (*Phaseolus vulgaris* L.) WITH A BURR MILL/ROLLER MILL SYSTEM


## Abstract

Pretreated black bean seeds were milled using a burr mill/ roller mill system, which produced two products-cotyledon flour and a seed coat fraction. Black bean seeds were pretreated by cooking the bean followed by drying (cooked-dried), soaking the beans and then drying (soaked-dried), or tempered. Seed coat recovery and flour extraction were improved ≈5% for both cooked-dried and soaked-dried seeds than for non-treated seeds. Flour extraction decreased ≈50% for seeds tempered to 14% moisture content. Seeds cooked for 15 min followed by drying produced the highest flour extraction (≈74%) and highest seed coat recovery (≈13%) of all treatments. Tempering pretreatment showed a negative linear relationship between bulk density and moisture content; where bulk density decreased as moisture content increased. Cotyledon flour color was affected most by cooked-dried or soaked-dried seed treatments. Coarse and fine particle yields were affected more by tempering pretreatment than by either the cooked-dried or soaked-dried pretreatments. At high moisture content, fine particle yield decreased due to particles blocking the sieve. In general, all pretreatments impacted for starch damage while only the cooked-dried pretreatment reduced all pasting values. The cooked-dried and soaked-dried pretreatment caused a decreased ash content, which was considered important as it represents the importance of solute leaching.


## Introduction

Black bean seed has three main parts: cotyledon, seed coat, and embryo. A milling system was developed that resulted in mechanical separation of seed coat from the cotyledon, followed by further size reduction of the cotyledon to flour. This system used a prebreak step utilizing a burr mill. Burr mill consists of a set of blades which uses a cutting, shearing, and crushing actions for particle size reduction (Haque 1991). Burr mills, also known as plate mills, have two circular plates where material is fed between them. One of the circular plates is fixed and the other rotates. The material come in contact with the two plates where it is sheared and crushed and exists through the edge of the plates. Burr mills have the plates horizontally mounted (Earle and Earle 2004).

After the pre-break step, the bean pieces were loaded onto a roller mill. The milling action of a roller mill offers potential to further mechanically remove seed coat from cotyledon. Roller mill utilizes multiple stage approach to size reduction. Roller mill is a common mill used to mill wheat into bran and flour. Roller mill includes two sections: break section and reduction section. It has a set of paired rolls that can be corrugated or smooth. Each roll in a pair can rotate at the same speed or can rotate at different speed (Posner and Hibbs 2005). Differential in speed results in a shearing action that is used to remove bran from the wheat kernel. Compression force is present when feed material is drawn between the rolls, whereas, shearing forces result when roll-speed differential and roll corrugation are used (Schorno 2006).

The utilization of a roller mill can aid in the removal of seed coat. Separation of seed coat from the cotyledon can be advantageous by expanding bean flour uses in food product applications. The advantage of being able to separate the seed parts rely on the additional economic advantages as well as other attributed such as improve cotyledon flour digestibility and palatability as some of the ANFs such as tannin are reduced by removing the seed coat (Towo et al 2003).

Literature failed to provide information on the use of roller milling black beans. Research was conducted using a burr mill/roller mill system to determine the effect of pretreated seeds (i.e., cooked-dried, soaked-dried, and tempered black beans) on cotyledon flour milling yield, flour physical quality, chemical composition, and pasting properties.

## Materials and Methods

### Pretreatments

Black beans were obtained from Kelley Bean Company (Scottsbluff, NE). The moisture content of seeds was kept constant at 10%. Each milling sample consisted of 150 g of seeds.

Clean black beans were cooked in distilled water for 5, 10, 15, and 20 min or soaked in distilled water for 6, 12, 18, and 24 h. Both cooked and soaked bean samples were drained, placed on baking sheets, and dried to 10% moisture content using a forced-air oven at 40-50 ºC.

Black beans were tempered with distilled water to 6, 8, 10, 12, and 14% moisture. Seed moisture was allowed to equilibrate at room temperature for 72 h before milling. Cooked-dried, soaked-dried, and tempered pretreatments were considered as three separate experiments (Table 29).

Table 29. Black bean seed pretreatments.

| Cooked-Dried[a] (min) | Soaked-Dried[b] (h) | Tempered (%) |
|---|---|---|
| 0 | 0 | 6 |
| 5 | 6 | 8 |
| 10 | 12 | 10 |
| 15 | 18 | 12 |
| 20 | 24 | 14 |

[a,b]Cooked and soaked seeds were dried to 10% moisture content at 50 ºC.

### Milling System

A milling system was integrated to obtain cotyledon flour and seed coat fraction. A schematic diagram is shown in Figure 30 and the description process is below.

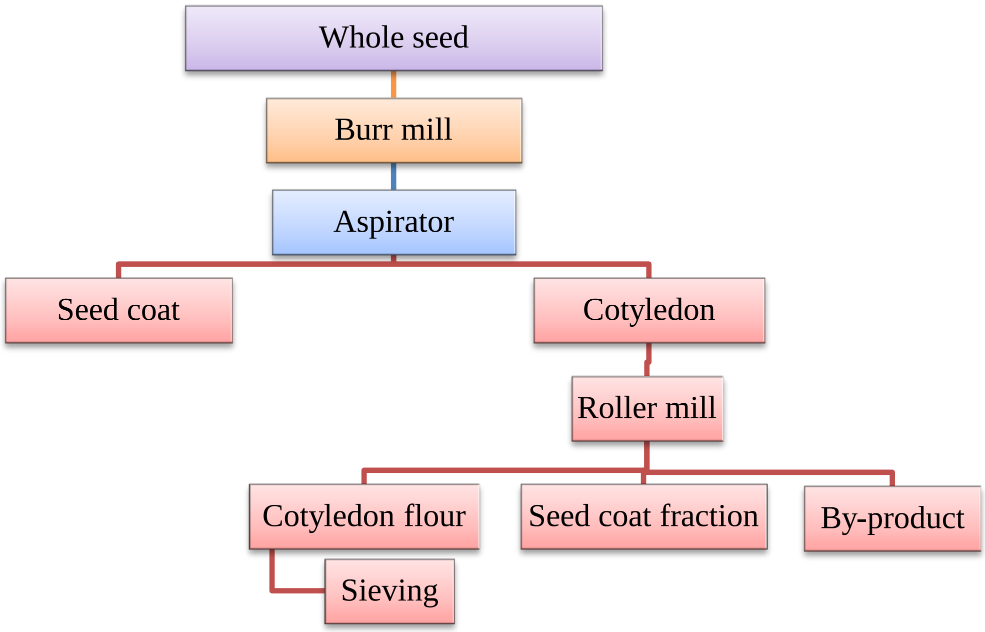


Figure 30. Schematic diagram of roller milling system for black beans.

Burr Mill

A laboratory-type burr mill (model 308, Labconco, Kansas City, MO, USA) was used as a prebreak system before milling with a roller mill (Quadrumat Jr., Brabender Instruments, South Hackensack, NJ, USA) configured for milling durum wheat into semolina and bran/embryo . The settings such as gap and feed rate were fixed.

Aspirator

A commercial aspirator (Agriculex Inc, Guelph, Ont., Canada) was used to remove the seed coat from the prebreak seed fraction. The air flow used was set on setting 3 of the aspirator. Samples without seed coat were milled in the roller mill. Seed coat rich percent was the portion recovered by the aspirator.

Roller Mill

Roller mill was evaluated with fixed durum wheat milling settings. A black bean warm up sample was used before the bean samples. Sample size used was 150 g. Three fractions were recovered by milling with the roller mill: cotyledon flour, bran (seed coat), and shorts. Two step milling was used. The flour fraction was milled twice. A sieve with screen size opening of 425 µm was used. The top fraction was shorts and bottom was cotyledon flour. Flour was sieved for 30 seconds.

Particle Size Determination

Particle size determination was done using a vibratory sieve shaker (Retsch GmbH, Haan, Germany) set up with 250, 150, 100, and 50 µm mesh sieves. Each sieve contained 10 polyurethane ball sieve cleaners that aided in the sifting process. Milling yield was based on the weight of the fine particles. Fine particles were considered flour that went through the 50 µm sieve. Coarse particles were considered flour that did not passed through the 50 µm sieve. All milled samples were stored at 4 ºC for later chemical analysis.

**Physical Quality of Flour**

Black bean flour moisture content, milling time, milled product temperature, and final flour weight were recorded. Flour temperature was measured using an infrared thermometer (VWR). Flour moisture content was measured right after milling using a forced-air oven set at 130 °C for 1 hour according to approved method 44-15.02 of the AACC International (2000).

Whole, coarse and fine fractions were evaluated for color measurement (CIE L-value) using Minolta 310 colorimeter (Minolta Corp., Ramsey, NJ, U.S.A.). Color difference was also determined, which is defined as “the magnitude and character of the difference between two colors under specified conditions”. Color difference ($\Delta E^*_{ab}$) was calculated using the equation:

$$\Delta E^*ab = \sqrt{(L2 - L1)^2 + (a2 - a1)^2 + (b2 - b1)^2} \qquad \text{(X-Rite Inc, 2007)}$$

Where *L1, a1*, and *b1* were CIE L, a, b values for no-treated sample, while *L2, a2*, and *b2* were CIE L, a, b values of a pretreated sample.

Bulk density was determined following the procedure of Okaka and Potter (1979), where 50 g of bean flour was put into a 100 mL measuring cylinder by pouring through a funnel and tapped to a constant volume. Bulk density ($g/cm^3$) was calculated by dividing the weight of flour (g) by flour volume ($cm^3$).

**Chemical Composition of Flour**

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. The amount of starch damage was determined using an enzymatic starch damage assay kit (Megazyme International, Co. Wicklow, Ireland) according to AACC International Approved Method 76-31.01. 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 was analyzed using Leco combustion nitrogen analyzer (LECO Corp. St. Joseph, MI, USA). Protein content was calculated as %N × 6.25.

**Pasting Properties of Flour**

Pasting properties of cooked-dried, soaked-dried, and tempered samples were determined using a Rapid Visco-Analyzer (Newport Scientific (Perten Instruments, Springfield, IL, USA). Cotyledon 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 it 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.

**Experimental Design and Data Analysis**

The experimental design was a randomized complete block. 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

Cotyledon flour, seed coat fraction, and by-product fraction were obtained after running whole black beans through the burr mill/roller mill system (Figure 31). By-product is a mixture of cotyledon, seed coat and embryo. This fraction could be further processed to further separate those fractions; however, this was not done in this study.

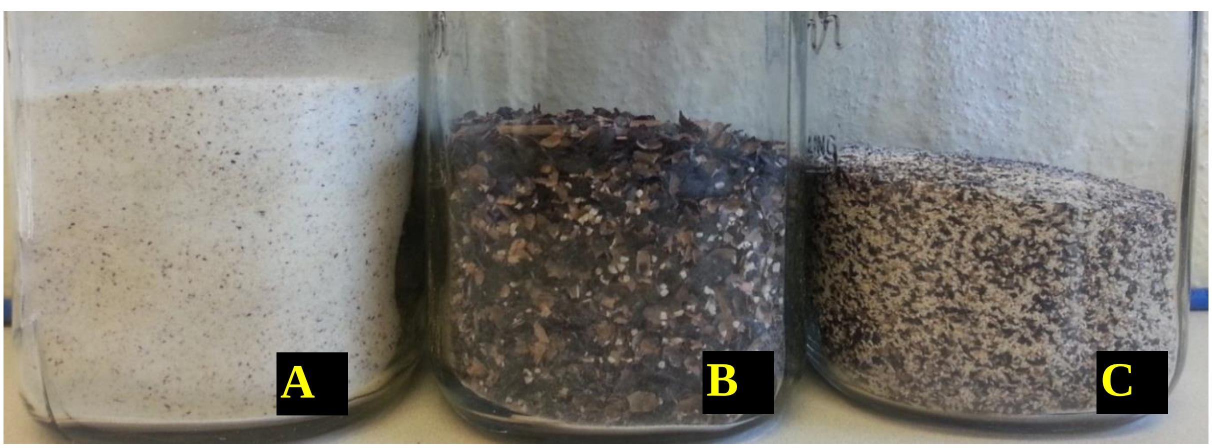


Figure 31. Cotyledon flour (A), seed coat fraction (B), and by-product (C) after burr mill/roller milling system.

### Cooked-Dried Seeds

Burr Mill

Burr mill resulted in two fractions: cotyledon and seed coat. Cooked-dried pretreatment affected cotyledon and seed coat recovery after milling with burr mill (Table 30). Both were inversely proportional since as cotyledon rich fraction decreases, the seed coat rich fraction increases. The 20 min cooked-dried treatment, compared to non-treated, result in a decrease in cotyledon rich fraction from 91.5 to 86.3% and an increased 8.8 to 12.4% seed coat rich fraction. Seed coat fraction was close to the 8% of the seed weight (Paper 1) for non-treated treatment. Whereas, for any cooked-dried treatment the seed coat fraction was higher than 8%. The step where the aspirator was used to separate the seed coat from the cotyledon was not very efficient in that separation process since the air blowing could have separated the embryo part into the seed coat fraction.

Table 30. Mean fractions (%) and cotyledon fraction particle size distribution values[a] from cooked-dried seeds following burr milling.

| Cooked-Dried[b] (min) | Cotyledon Rich Fraction | Seed Coat Rich Fraction (%) | >2.8 (mm) |
|---|---|---|---|
| 0 | 91.5a | 8.8c | 87.4d |
| 5 | 87.8b | 10.3b | 88.8b |
| 10 | 86.3c | 12.7a | 88.2c |
| 15 | 86.5c | 12.8a | 88.8b |
| 20 | 86.3c | 12.4a | 89.8a |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 20 °C and initial seed moisture content was 10%.

Cotyledon fraction and seed coat recovery with a burr mill were not significantly different for samples cooked for 10, 15, and 20 min. However, by visual evaluation, the ease of the seed coat removal was apparent for the cooked-dried pretreatment. This also can be related to the changes of the seed coat interior surface changes (Paper 1, Figure 16). Seed coat rich fraction was greatest for samples cooked for 10 and 15 min. Cooked-dried pretreatment effect was significant for particle size distribution for split cotyledons (Table 30). The split cotyledons had highest fraction of >2.8 mm particle, ranging from 87.4 to 89.8%.

Roller Mill

Cooked-dried pretreatment was significant for cotyledon flour extraction and by-product (Table 31). Air temperature was 21 °C and relative humidity was 20%. Flour extraction was improved ≈ 5% for any cooked-dried treatment when compared to non-treated. The by-product obtained was reduced by any cooked-dried treatment. The further use or processing of the by-product could be important to increase economic advantage of the black bean milling operation. In addition, seed coat removal efficiency is important to ensure the economic potential as to obtain intact seed coat and cotyledon fractions. In particular, seed coat removal efficiency opens the opportunity to obtain 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).

The overall extraction rate for cotyledon flour using the burr mill/roller mill system ranged from 72.1 to 74.9%. Extraction was greatest with beans cooked 5 min then dried, intermediate with non-treated and beans cooked 10 and 15 min then dried, and least with beans cooked 20 min and dried.

Table 31. Mean cotyledon flour extraction (%), by-product (%), and total cotyledon flour extraction (%) values[a] from cooked-dried seeds obtained from roller milling.

| Cooked-Dried[b] (min) | Cotyledon Flour Extraction | By-Product | Total Cotyledon Flour Extraction[c] |
|---|---|---|---|
| | | (%) | |
| 0 | 80.7c | 19.3a | 73.8ab |
| 5 | 85.3a | 14.7c | 74.9a |
| 10 | 84.9a | 15.1c | 73.3bc |
| 15 | 85.3a | 14.7c | 73.8ab |
| 20 | 83.6b | 16.4b | 72.1c |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 20 °C and initial seed moisture content was 10%.
[c]Total cotyledon flour extraction was calculated as (cotyledon rich fraction x cotyledon flour extraction)/100.

*Physical Quality of Flour*

Cooked-dried pretreatment affected the physical quality of flour (Table 32). Air temperature was 23 °C and relative humidity was 18%. Within cooked-dried pretreatments, feed rate decreased when as cooked times increased. Feed rate was greatest for cooked-dried treatment of 5 and 10 min (≈ 60 g/min). Change in feed rate seems to reflect the increase in the amount of cotyledons >2.8 mm found after milling on the burr mill. During roller milling, temperature gain (2.8 ºC) of the product was greater for 20 min cook-dried treatment than for other cooked-dried treatment, but was similar to the temperature gain of non-treated which 3.2 ºC.

Cooked-dried pretreatment affected coarse and fine particle yield (Table 32). Coarse particle yield was much greater than that for fine particles, which was expected since the roller mill used in this research was configured to mill durum wheat into semolina, coarsely ground endosperm of durum wheat. Coarse particle yield increased, whereas, fine particle yield decreased as cooking time increased. The coarse particle yield increased ≈5% when compared non-treated to 20 min cooking. Fine particle yield decreased from 15 to 3% of non-treated to 20 min cooking. Changes during cooking affected black bean seed granulation during milling.

Table 32. Mean physical quality and particle size distribution values[a] of cotyledon flour from cooked-dried seeds.

| Cooked-Dried[b] (min) | Feed Rate (g/min) | Temperature Gain (ºC) | Bulk Density ($g/cm^3$) | Coarse Particles Yield (%) | Fine Particles Yield (%) |
|---|---|---|---|---|---|
| 0 | 51.9b | 3.2a | 0.79a | 81.7c | 18.7a |
| 5 | 59.7a | 1.8b | 0.76b | 84.9b | 15.0b |
| 10 | 59.8a | 2.0b | 0.74c | 84.8b | 15.1b |
| 15 | 47.5c | 1.9b | 0.74c | 85.8a | 14.1c |
| 20 | 42.1d | 2.8a | 0.73c | 85.9a | 15.1c |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 20 °C and initial seed moisture content was 10%.

Bulk density of the cotyledon flour decreased from 0.79 $g/cm^3$ to 0.73 $g/ cm^3$ from non-treated to 20 min cooked-dried pretreatment. The decline in bulk density is attributed in part to the decline in fine particles found in the cotyledon flour (Table 32). Bulk density is affected by particle size and shape. Smaller particles generally have better packing efficiency than do large particles. Cooked-dried pretreatment affected particles compaction. Regardless the treatment, bulk density values obtained in this study were slightly higher than those reported by Dzudie and Hardy (1996) and Siddiq et al (2010), who reported bulk density values for bean flour of 0.50 and 0.52 g/ml, respectively. The differences in bulk density might be attributed to bean source and pretreatment used as well as difference in particle size and packing behavior. Additionally, the high temperature treated during cooking might had impact the seed composition and the flour structure, which resulted in less dense packing particles as cooking time increased.

During the process of cooking and subsequent drying of the seed, the seed internally and externally changed. The structural changes of the seed due to the cooked-dried pretreatment could be responsible for all the changes in flour characteristics (Figure 14 in Paper 1). It was observed the cotyledon splitting and changes in the seed coat possibly which could have affected the obtained flour and its particle size distribution. Furthermore, the splitting can be reflected in the differences of coarse and fine yields. Also, seed hardness and the fracture differences could have caused the particles to differ. Seed hardness for cooked-dried seeds was lower than non-treated seeds (Table 8 in Paper 1).

Cooked-dried pretreatment was significantly different for flour color (L-value and color difference) (Table 33). The color difference increased as cooking time increased when compared to the non-treated sample. In general, L-values (lightness) for whole, coarse, and fine flours decreased as cooking time increased. The darkening of the flour (or reduced in lightness) can be attributed to discoloration of the seed coat into the cooking water which stained the cotyledon (Figure 15 in Paper 1).

Table 33. Mean L-values and color difference values[a] for cotyledon flour from cooked-dried seeds.

| Cooked-Dried | Whole Flour | | Coarse Flour | | Fine Flour | |
|---|---|---|---|---|---|---|
| (min) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) |
| 0 | 83a | 0.0e | 82.8a | 0.0e | 85.8a | 0.0d |
| 5 | 78b | 7.2d | 76.4b | 8.4d | 80.7b | 6.9c |
| 10 | 75c | 10.5c | 73.6c | 11.7c | 78.9bc | 9.4ab |
| 15 | 73d | 12.9b | 71.0d | 14.3b | 77.8cd | 10.8b |
| 20 | 71e | 14.7a | 69.3e | 16.0a | 76.3d | 12.3a |

[a]Values followed by same letter are not significantly different at $P$=0.05.

Cooking treatment greatly affected the seed coloration due to leaching the seed coat color. Seed coat pigmentation is due to the presence of anthocyanins located in the cells arranged in layers (Culver and Cain 1952; Burns and Winzer 1962). In this case, the color change of the flour is mainly attributed to the color pigments from the seed coat into the cotyledon to the final flour.

### *Chemical Composition of Flour*

Cooked-dried pretreatment was only significantly different for starch damage and ash content (Table 34). Protein and total starch content remained similar among samples. Starch damaged of flour from cooked-dried seeds increased as cooked time increased. Starch damage increased from 0.3 for the non-treated seed to 3.1% for the seed cooked 20 min. Cooking would cause starch damage due to gelatinization process whereby starch granules swell and disrupt. The premature disruption of the starch granules showed a tendency to contribute to high damaged starch.

Table 34. Mean chemical composition[a] of cotyledon flour from cooked-dried seeds.

| Cooked-Dried[b] (min) | Total Starch | Starch Damaged | Protein Content | Ash Content |
|---|---|---|---|---|
| | | (%) | | |
| 0 | 34.0ab | 0.3e | 25.6a | 4.31a |
| 5 | 31.4b | 1.5d | 25.7a | 3.58b |
| 10 | 34.5ab | 2.1c | 25.5a | 3.43b |
| 15 | 32.3b | 2.7d | 25.6a | 3.11c |
| 20 | 35.6a | 3.1a | 25.7a | 3.16c |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Values are dry weight based.

The SEM micrograph (Figure 32) reinforces that starch granules were damaged by thermal treatment. Also, starch granules appeared oval in shape and some more round and spherical can be seen as well. The intact starch granules are seen for non-treated flour and appeared to be smooth without the presence of fissures or holes. However, extensively damaged starch granules are seen, with splitting and are more openly exposed, as well as gelatinized starch is present in the cooked-dried flour (Chung et al 2008). The granules surface influences the reactivity towards enzymes and the rate and extends of hydration (Hoover and Ratnayake 2002).

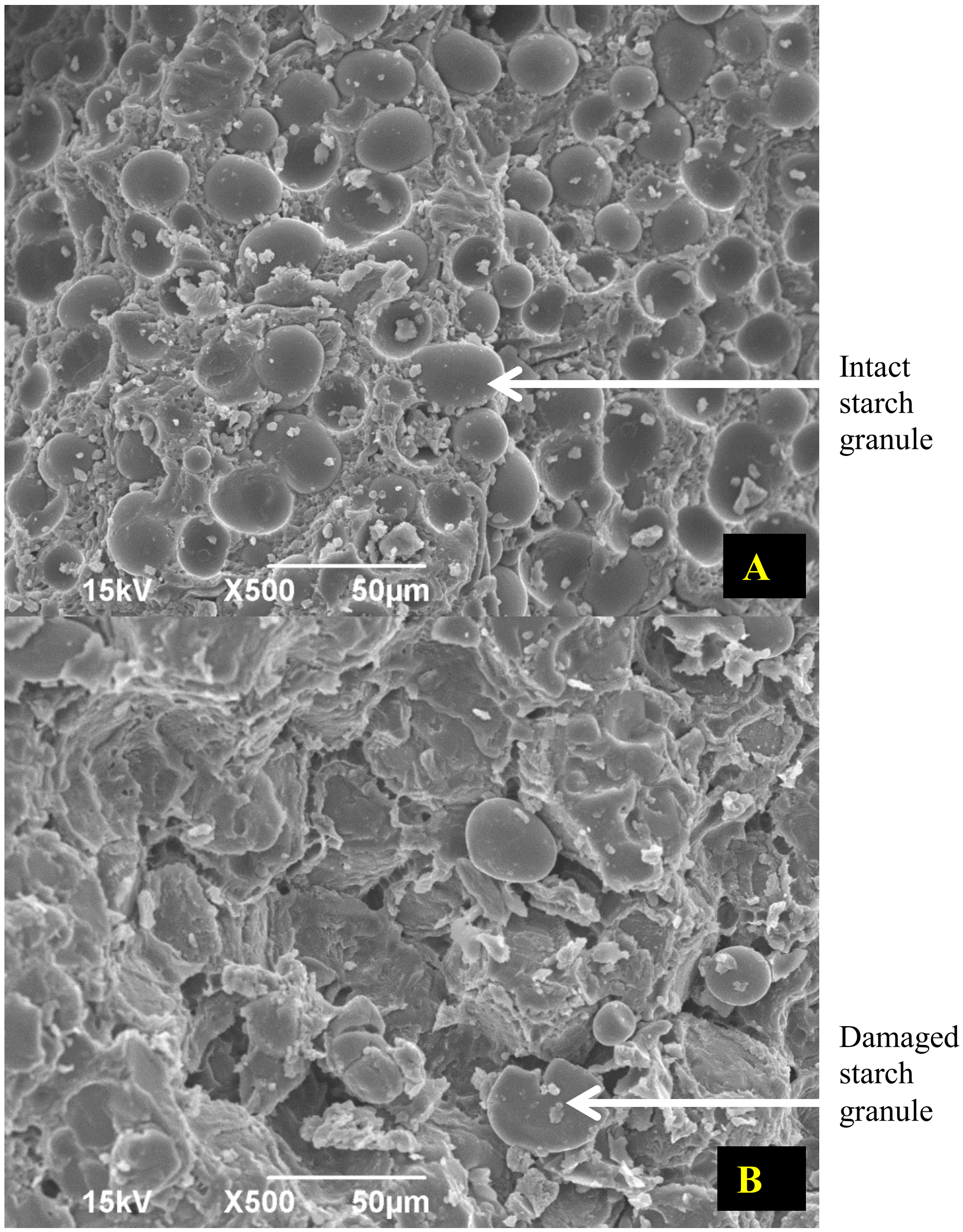


Figure 32. Scanning electron microscopy micrograph comparing flour appearance with visible starch granules for cotyledon flour from non-treated (A) and cooked (20 min)-dried (B) black bean seeds.

Gowen et al (2006) reported that after drying chickpeas the cells shrivel and shrank and became more amorphous and leached solutes, which were no longer visible in extracellular spaces due to having adhered to the cell surface during the drying process.

In general, for cooked-dried pretreatment the flour ash content decreased ≈ 27% compared to non-treated sample. The decreased ash content was due to leaching of minerals into the cooking water. Cell membrane permeability increases during thermal processing, which allows ions, vitamins, minerals, and small molecules to diffuse from seeds into the cooking water (Siddiq and Uebersax 2013). Similar results were also reported for field peas and black beans by Wang et al (2008) and Wang et al (2010), respectively.

*Pasting Properties of Flour*

In general, cooked-dried pretreatment decreased all pasting properties (Table 35). All parameters significantly decreased as cooked times increased. Peak viscosity decreased from 51.3 to 10.3 RVU as cooking went from non-treated to 20 min cooked. Breakdown viscosity slightly decreased as cooked times increased from 2.4 to 1.0 RVU for non-treated and 20 min cooked, respectively. 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. Low breakdown values were attributed possibly to high amylose content and starch granule restriction to swelling. Final viscosities decreased significantly from 98.7 to 19.5 RVU, which indicated low tendency to form a strong gel after cooling. Trough viscosity decreased from 49.6 to 9.3 RVU is influenced by the rate of amylose exudation, granule swelling and amylose-lipid complex formation (Wani et al 2012). Setback viscosity decreased from 50.3 to 10.1 RVU and it has been reported that low setback values at high cooking times suggested low retrogradation, hence low tendency of flour to form a gel during cooling (Wani et al 2012).

Table 35. Mean pasting properties[a] for cotyledon flour from cooked-dried seeds.

| Cooked-Dried (min) | Peak | Trough | Breakdown (RVU) | Final Viscosity | Setback |
|---|---|---|---|---|---|
| 0 | 51.3a | 49.6a | 2.4a | 98.7a | 50.3a |
| 5 | 27.8b | 25.1b | 2.7a | 55.8b | 30.8b |
| 10 | 14.6c | 12.9c | 1.7b | 28.3c | 15.4c |
| 15 | 13.3c | 12.2c | 1.2bc | 24.3c | 12.1d |
| 20 | 10.3d | 9.3d | 1.0c | 19.5d | 10.1d |

[a]Values followed by same letter are not significantly different at $P$=0.05.

**Soaked-Dried Seeds**

Burr Mill

Soaked-dried pretreatment affected cotyledon and seed coat recovery after milling with the burr mill (Table 36). The effect was seen as inversely proportional. Cotyledon rich fraction decreased from 90.7 in non-treated beans to 87.1% in the 24 h soaked-dried treatment. Seed coat rich fraction was low 9.2% for non-treated seeds, whereas higher values (12.7 to 13.2%) were obtained for all soaked-dried treatments. For soaked-dried pretreatments, the seed coat was easily removed than for non-treated, which can be attributed to the seed hardness (Table 9 in Paper 1).

The increase in seed coat rich fraction might be attributed to some contamination of cotyledon and embryo into the seed coat fraction during the air separation. Also, the seed coat tended to absorbed water, which could have impacted the weight of the seed coat removed. The particle distribution of the obtained cotyledon rich fraction was significant. As soaked time increased, a decrease in >2.8 mm particles were obtained.

Table 36. Mean fractions (%) and cotyledon fraction particle size distribution values[a] from soaked-dried seeds following burr milling.

| Soaked-Dried[b] (h) | Cotyledon Rich Fraction | Seed Coat Rich Fraction (%) | >2.8 (mm) |
|---|---|---|---|
| 0 | 90.7a | 9.2b | 87.4a |
| 6 | 86.6b | 13.2a | 85.8c |
| 12 | 87.4b | 12.4a | 86.4b |
| 18 | 87.8b | 12.0a | 86.7b |
| 24 | 87.1b | 12.7a | 85.4c |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 20 °C and initial seed moisture content was 10%.

Similar results were obtained by Anton et al (2008), who observed an increase of ≈29% in seed coat yield by soaking for 16 h and heat drying for 60 min. Soaking and high water absorption resulted in swelling, cotyledon expansion, and seed coat integrity breakage. This eased the process of seed coat detachment from cotyledons due to shrinkage of cotyledons during drying. Also, by the use of high water amount, the seed coat detached in its totality producing heavy particles which is explained by studies from Ehiwe and Reichert (1987) and Anderson et al (1994).

When seed were soaked and then dried the seed structural changes as well as further processing were observed. Oomah et al (2010) demonstrated that as soaking time increased, hull yield increased for both studied market classes-great northern and pink beans. It was also stated that water absorption was inversely correlated with hull thickness which in turn is associated with seed hardness (González et al 2006). The successful seed coat recovery before milling allowed a better flour extraction.

Roller Mill

Flour extraction was improved ≈ 3% for seed soaked 24 h and dried when compared to non-treated (Table 37). The by-product obtained was slightly reduced by any soaked-dried treatment. Again, the separation could be improved by further using the by-product to obtain improved rich cotyledon and seed coat fractions. The overall extraction rate for cotyledon flour using the burr mill/roller mill system ranged from 71.8 to 73.3%. Extraction was greatest with non-treated beans and beans soaked for 6 h. Soaking 12, 18, and 24 h and drying resulted in similar total cotyledon flour extraction.

Table 37. Mean cotyledon flour extraction (%), by-product (%), and total cotyledon flour extraction (%) values[a] from soaked-dried seeds obtained from roller milling.

| Soaked-Dried[b] (h) | Cotyledon Flour Extraction | By-Product | Total Cotyledon Flour Extraction[c] |
|---|---|---|---|
| | | (%) | |
| 0 | 80.7d | 19.7a | 73.2a |
| 6 | 84.6a | 15.4d | 73.3ab |
| 12 | 82.8b | 17.2c | 72.4abc |
| 18 | 81.8c | 18.2b | 71.8c |
| 24 | 83.1b | 16.9c | 72.4bc |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Seed temperature was 20 °C and initial seed moisture content was 10%.
[c]Total cotyledon flour extraction was calculated as (cotyledon rich fraction x cotyledon flour extraction)/100.

*Physical Quality of Flour*

Soaked-dried pretreatment affected the physical quality of flour except for bulk density (Table 38). Feed rate was reduced by soaked-dried seed pretreatment. Feed rate tended to decrease with soaking time. During roller milling, flour temperature gain was variable but relatively small, ranging from 2.7 to 3.7 ºC. In general, increasing soaked-dried pretreatment time increased coarse particle yield and decreased fine particle yield (Table 38). The non-treated sample yielded 82.2% of coarse particles. Pretreatment with the initial 6 h caused a change in coarse and fine particle yields that was also affected by longer treatments. Coarse particles increased from 82.2 to 87% and fine particles decreased from 19 to 12.9%, when comparing non-treated to cooked-dried 6 h sample. This can be related to also the seed hardness (Paper 1). Seed hardness for soaked-dried seed were found to be less than non-treated seed (Table 9 in Paper 1). Also, a curve with more initial peaks was observed, which could be related to that the seed have more ease to fracture. The differences in seed hardness could have contributed to the differences in particle size distribution.

Table 38. Mean physical quality and particle size distribution values[a] of cotyledon flour from soaked-dried seeds.

| Soaked-Dried[b] (h) | Feed Rate (g/min) | Temperature Gain (ºC) | Bulk Density ($g/cm^3$) | Coarse Particles Yield (%) | Fine Particles Yield (%) |
|---|---|---|---|---|---|
| 0 | 52.5a | 3.2ab | 0.79a | 82.2b | 19.0a |
| 6 | 47.8ab | 2.7b | 0.78a | 87.1a | 12.9b |
| 12 | 42.3bc | 3.7a | 0.78a | 87.1a | 13.1b |
| 18 | 41.6c | 3.0b | 0.78a | 87.2a | 13.2b |
| 24 | 42.7bc | 3.7a | 0.78a | 87.0a | 13.1b |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Seed temperature was 20 °C and initial seed moisture content was 10%.

Soaked-dried pretreatment was significant for flour color (L-value and color difference) (Table 39). In general, L-values (lightness) for whole, coarse, and fine flours decreased for all soaked-dried treatments. Color differences were less for fine flour than whole or coarse flour. The flour became darker (reduced in lightness) for soaked-dried treatment due to the seed coat color which leached out into the soaking water. The rich dark seed coat color is the high presence of anthocyanins, which are reported to be water soluble (Jung and Bae 2014). The darkening of the cotyledon fraction after soaked-dried pretreatment can be observed in Figure 20 from Paper 1.

Table 39. Mean L-values and color difference values[a] for cotyledon flour from soaked-dried seeds.

| Soaked-Dried | Whole Flour | | Coarse Flour | | Fine Flour | |
|---|---|---|---|---|---|---|
| (h) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) |
| 0 | 83.1a | 0.0c | 82.7a | 0.0c | 85.9a | 0.0c |
| 6 | 77.6b | 5.9b | 77.5b | 5.5b | 84.4b | 3.0b |
| 12 | 76.5c | 6.9a | 76.3c | 6.4a | 83.4c | 3.5b |
| 18 | 77.3b | 6.0b | 77.0bc | 5.7b | 83.5c | 3.3b |
| 24 | 77.4b | 5.9b | 77.0bc | 5.6b | 82.3d | 4.2a |

[a]Values followed by same letter are not significantly different at *P*=0.05.

*Chemical Composition of Flour*

Total starch, starch damage and protein content were not affected by soaked-dried pretreatments (Table 40). Soaked-dried pretreatment only affected ash content. Ash content decreased as soaked times increased probably due to mineral diffusion into the soaking water. The flour from non-treated beans appeared as tightly packed cells with starch granules embedded in a protein matrix (Figure 33).

Table 40. Mean chemical composition[a] of cotyledon flour from soaked-dried seeds.

| Soaked-Dried[b] (h) | Total Starch | Starch Damaged | Protein Content | Ash Content |
|---|---|---|---|---|
| | | | (%) | |
| 0 | 35.2a | 0.3a | 25.6a | 4.17a |
| 6 | 30.8b | 0.3a | 25.7a | 3.56b |
| 12 | 32.3ab | 0.3a | 25.8a | 3.49b |
| 18 | 32.5ab | 0.3a | 25.6a | 3.37c |
| 24 | 34.6a | 0.4a | 25.8a | 3.33c |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Values are dry weight based.

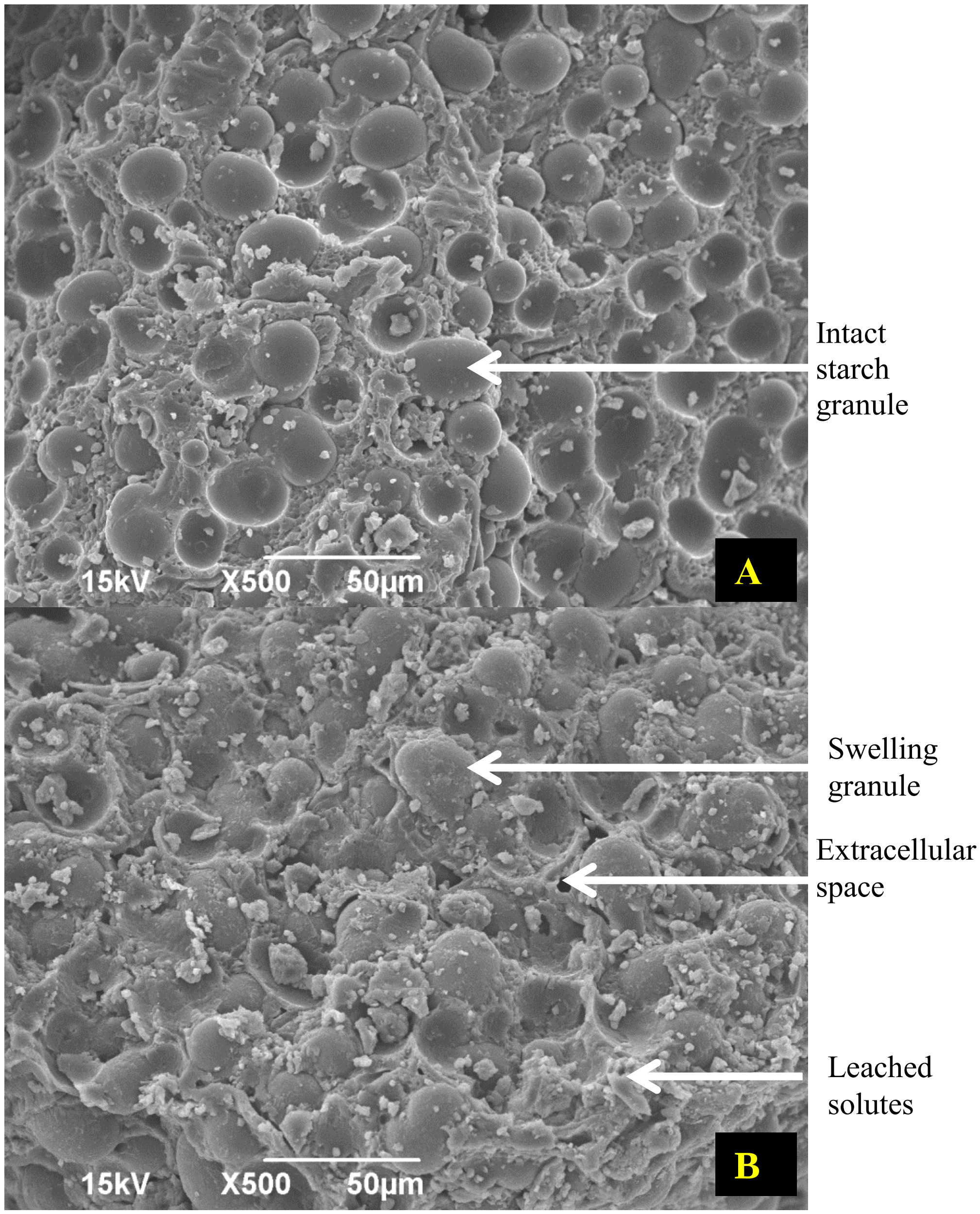

Figure 33. Scanning electron microscopy micrograph comparing flour appearance with visible starch granules for cotyledon flour from non-treated (A) and soaked (24 h)-dried (B) black bean seeds.

By soaking, some spaces between cells are observed due to imbibing of water during soaking. The starch granules remained in a non-gelatinized state and cell walls were still visibly intact. Similar observations were found by Gowen et al (2006) for chickpea flour. They also stated that by drying the cooked chickpeas the cells shrivel and shrank and became more amorphous, where some leached solutes were visible in extracellular spaces. Similar observations were seen for this soaked-cooked flour sample (Figure 33).

*Pasting Properties of Flour*

Soaked-dried pretreatment affected all pasting properties except for final and setback viscosities (Table 41). The magnitude of effect of soak-dried pretreatment was small when compared to the effect of cooked-dried pretreatments (Tables 35 and 41). Slight changes were observed for peak viscosities, where values ranged from 44.0 to 59.4 RVU. For trough viscosities, values ranged from 41.4 to 57.2 RVU. Sample with the greatest peak and trough viscosities was flour sample from soaked-dried treatment of 18 h, with values of 59.4 RVU and 57.2 RVU, respectively. The low breakdown viscosity has been attributed to the high amylose content and starch granule restriction to swelling for black bean flour (Wani et al 2012).

Table 41. Mean pasting properties[a] for cotyledon flour from cooked-dried seeds.

| Soaked-Dried (h) | Peak | Trough | Breakdown (RVU) | Final Viscosity | Setback |
|---|---|---|---|---|---|
| 0 | 53.6a | 49.7b | 2.5b | 99.8ab | 49.8a |
| 6 | 44.0b | 41.4c | 2.6b | 86.5b | 45.1ab |
| 12 | 55.0a | 51.3ab | 3.7a | 101.8a | 50.4a |
| 18 | 59.4a | 57.2a | 2.2b | 96.7ab | 39.7b |
| 24 | 54.1a | 49.8b | 4.3a | 93.5ab | 43.7ab |

[a]Values followed by same letter are not significantly different at $P$=0.05.

**Tempered Seeds**

Burr Mill

Black bean seed tempered to 12 and 14% moisture reduced the cotyledon rich fraction and increased the seed coat rich fraction obtained after milling with the burr mill (Table 42). Large cotyledon fraction (>2.8 mm) tended to decrease with seed tempered to 12 and 14% moisture, which corresponds to their decline in cotyledon rich fraction.

Table 42. Mean fractions (%) and cotyledon fraction particle size distribution values[a] from tempered seeds following burr milling.

| Tempered[b] (%) | Cotyledon Rich Fraction | Seed Coat Rich Fraction | >2.8 mm |
|---|---|---|---|
| | | (%) | |
| 6 | 91.4a | 8.3a | 86.2a |
| 8 | 91.3ab | 8.5a | 85.9a |
| 10 | 91.0ab | 8.7a | 87.4a |
| 12 | 90.3bc | 9.3a | 83.4a |
| 14 | 89.8c | 9.4a | 83.2a |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Seed temperature was 20 °C.

Roller Mill

Tempered pretreatment affected cotyledon flour extraction and by-product (Table 43). Cotyledon flour extraction decreased as seed moisture content increased, and oppositely, by-products increased. Cotyledon flour extraction was greatest for samples at 6% moisture content with 82% extraction yield, and by-product was the lowest at 18%.

Table 43. Mean cotyledon flour extraction (%), by-product (%), and total cotyledon flour extraction values[a] from tempered seeds obtained from roller milling.

| Tempered[b] (%) | Cotyledon Flour Extraction | By-Product (%) | Total Cotyledon Flour Extraction[c] |
|---|---|---|---|
| 6 | 82.0a | 18.0c | 74.9a |
| 8 | 81.1a | 19.0c | 74.0a |
| 10 | 80.7a | 19.3c | 73.4a |
| 12 | 75.0b | 25.0b | 67.7b |
| 14 | 55.0c | 45.1a | 49.4c |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 20 °C.
[c]Total cotyledon flour extraction was calculated as (cotyledon rich fraction x cotyledon flour extraction)/100.

Changes in the seed structure occur by changing the moisture content. For example, Altuntas and Demirtola (2007) explained that by increasing moisture content in kidney beans (*Phaseolus vulgaris* L.), seed dimension and seed volume also increases (Paper 1 in Table 6). These seed structural changes might have contributed to the milling-ability of the seed. Also, it is important to consider the change in seed hardness. Hardness of black bean seed increased with increased amounts of seed moisture content (Table 10 Paper 1). The number of fracture points after the first fracture force decreased at very high moisture content, which was shown in Figure 20 with a smooth curve. These results agree with the findings of Bhattacharya et al (2005) on lentils compression. Peak force or firmness of the seeds significantly increased with increased moisture content. Paulson (1978) studied the firmness of soybeans and found similar outcome. He reported that soybeans showed highest toughness or most firm values for samples between 11 to 14% moisture content. In addition, the mill made a different noise, which seemed to indicate that the mill was working harder to mill beans tempered to 12 and 14% moisture content. The overall extraction rate for cotyledon flour using the burr mill/roller mill system ranged from 49.4

to 74.9%. Extraction was greatest with black bean seeds having 6% moisture content. Overall cotyledon flour extraction declined with increased seed moisture content, which resulted in lowest extraction in seed tempered to 14% moisture.

*Physical Quality of Flour*

Tempered pretreatment affected all physical quality of flour except for feed rate and coarse particle yield (Table 44). Feed rate remained in the range of 50.4 to 53.9 g/min. During roller milling, flour temperature gain was greatest (4 °C) with seed at 6% moisture and least (2.2 °C) with seed at 14% moisture content. The effect of temper pretreatment on flour temperature gain and on feed rate is relatively small and might not be of practical importance.

Table 44. Mean physical quality and particle size distribution values[a] of cotyledon flour from tempered seeds.

| Tempered[b] (%) | Moisture Content (%) | Feed Rate (g/min) | Temperature Gain (ºC) | Bulk Density (g/cm$^3$) | Coarse Particles Yield (%) | Fine Particles Yield (%) |
|---|---|---|---|---|---|---|
| 6 | 7.60e | 53.9ab | 4.0a | 0.81a | 83.5a | 16.6c |
| 8 | 8.83d | 53.7ab | 3.8ab | 0.81a | 82.8a | 17.0bc |
| 10 | 10.73c | 52.5ab | 3.2bc | 0.79b | 81.4a | 18.6b |
| 12 | 12.13b | 52.9ab | 2.7cd | 0.76c | 78.7a | 21.1a |
| 14 | 14.13a | 50.4b | 2.2d | 0.68d | 75.8a | 17.1bc |

[a]Values followed by same letter are not significantly different at $P$=0.05.
[b]Seed temperature was 20 °C.

Moisture content of the seed greatly affected bulk density of the flours (Table 44). Bulk density decreased from 0.81 to 0.68 g/cm$^3$ as moisture content increased from 6 to 14%, respectively. The relationship between moisture content (Mc) and bulk density ($\rho_b$) appeared linear and can be represented by the regression equation:

$$\rho_b = 0.925 - 0.0155Mc \qquad (R^2=0.81)$$

This negative linear relationship between bulk density and moisture content has been reported by several authors (Desphande et al 1993 for soybean; Aviara et al 1999 for guna seed; Altuntaş and Yildiz 2007 for faba beans). The higher bulk density for drier seeds (6-10%) could be attributed to higher fine particles, which increases packing efficiency (Sahoo and Srivastava 2002; Sacilik et al 2003). The packing depended upon particle size distribution. Zhang and Brusewitz (1994) reported the decrease in bulk density of milled mustard seeds with increased moisture content.

Tempering pretreatment showed no significant effect for coarse particle yield. Coarse particles decreased from 83 to 76% when moisture content of the seed increased from 6 to 14% moisture content (Table 44). In contrast, fine particle yields were significantly affected by tempering pretreatments. The fine particles yields fluctuated from 17 to 21% without any pattern. The fluctuation in results is probably related to the difficulty in sieving flour as moisture content increased. Most of the fine flour stayed on top of sieve of 50 µm mesh size due to fine particles aggregation. The bean flour particle was observed to form agglomerates, which prevented the flour to pass through the sieve of 50 µm mesh sieve. At high moisture content, flour particles form agglomerates. Palzer (2005) stated that agglomerates are particles joined together and bigger porous secondary particles-conglomerates are formed. Dhanalakshmi et al (2011) stated that agglomerates form due to the sticking of particles produced by physical or chemical forces as a result of changes in the particle surface. This can also be triggered by environmental changes (e.g. moisture). Therefore, agglomerated particles likely were more prevalent for milled seed at 12 and 14% moisture content than others.

In general, there was an apparent increase in L-values for flours of all fractions at 12 and 14% moisture content (Table 45). These interesting results might be attributed to uneven sieving of bean flours at 12 and 14% moisture content due to the agglomeration of particles. Possibly due to slight wet particles, lighter color could have shown. Fine particles could have remained in the coarse particles leading to a lighter flour appearance, hence the L-values was high for 12 and 14% moisture content samples. There was no obvious leaching out of the seed coat pigment into the cotyledon as in the previous pretreatment.

Table 45. Mean L-values and color difference values[a] for cotyledon flour from tempered seeds.

| Tempered | Whole flour | | Coarse flour | | Fine flour | |
|---|---|---|---|---|---|---|
| (%) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) | (L) | ($\Delta E^*_{ab}$) |
| 6 | 82.5c | 0.0d | 81.5d | 0.0d | 86.8b | 0.0c |
| 8 | 82.8bc | 0.5c | 82.0c | 0.8c | 86.3bc | 0.5b |
| 10 | 82.8bc | 0.8bc | 82.8b | 1.5b | 85.8c | 0.8b |
| 12 | 83.4ab | 1.1ab | 83.1b | 1.8b | 88.0a | 1.5a |
| 14 | 83.7a | 1.3a | 83.5a | 2.3a | 87.0b | 0.8b |

[a]Values followed by same letter are not significantly different at $P$=0.05.

*Chemical Composition of Flour*

Tempering pretreatment affected total starch content and starch damage but did not affect protein content or ash content (Table 46). Total starch content seemed to increase, from 32.3 to 38.2%, as seed moisture content increased from 6 to 14%. This apparent increase in starch content is attributed to the solubilization of pectin from the cell walls/middle lamellae (Figure 34), which has been proposed to enhance the accessibility of amylase to starch used in the assay to determine total starch content (Wang et al 2010).

Table 46. Mean chemical composition[a] of cotyledon flour from tempered seeds.

| Tempered[b] (%) | Total Starch | Starch Damaged | Protein Content (%) | Ash Content |
|---|---|---|---|---|
| 6 | 32.3c | 0.3c | 25.4a | 3.89b |
| 8 | 34.0c | 0.3c | 25.7a | 4.36a |
| 10 | 34.3bc | 0.3c | 25.5a | 4.18ab |
| 12 | 36.9ab | 0.4b | 25.5a | 4.05ab |
| 14 | 38.2a | 0.5a | 25.7a | 4.07ab |

[a]Values followed by same letter are not significantly different at *P*=0.05.
[b]Values are dry weight based.

Starch damage was greatest for sample tempered to 14% moisture content with value of 0.5%. Sample with lowest starch damage was tempered to 6% moisture content with value of 0.3%. When seeds tempered to 12 and 14% moisture content were milled, a high-pitched noise was made by the mill rolls. The high tempered seeds had difficulty passing through the rolls during milling. In addition, the higher starch damage values for these high moisture content samples were possibly related to the seed hardness. Seed hardness values for both 12 and 14% tempered seed were 203 and 254 N which was higher than non-treated value of 83 N. In this case, beans at lower moisture content presented less starch damage due to its brittleness properties and required less force than higher moisture content seeds; this is in agreement with a study done in cowpeas by Ige (1977), who reported that the cowpeas became brittle at low moisture content hence they required less force to rupture.

The SEM micrograph (Figure 34) for tempered treatment of 14% seem to show that tempering caused the dissolution of cell wall/middle lamellae material as the extracellular space is more obvious and the cellular division is clear. The middle lamellae/cell wall is more apparent than the non-treated flour.

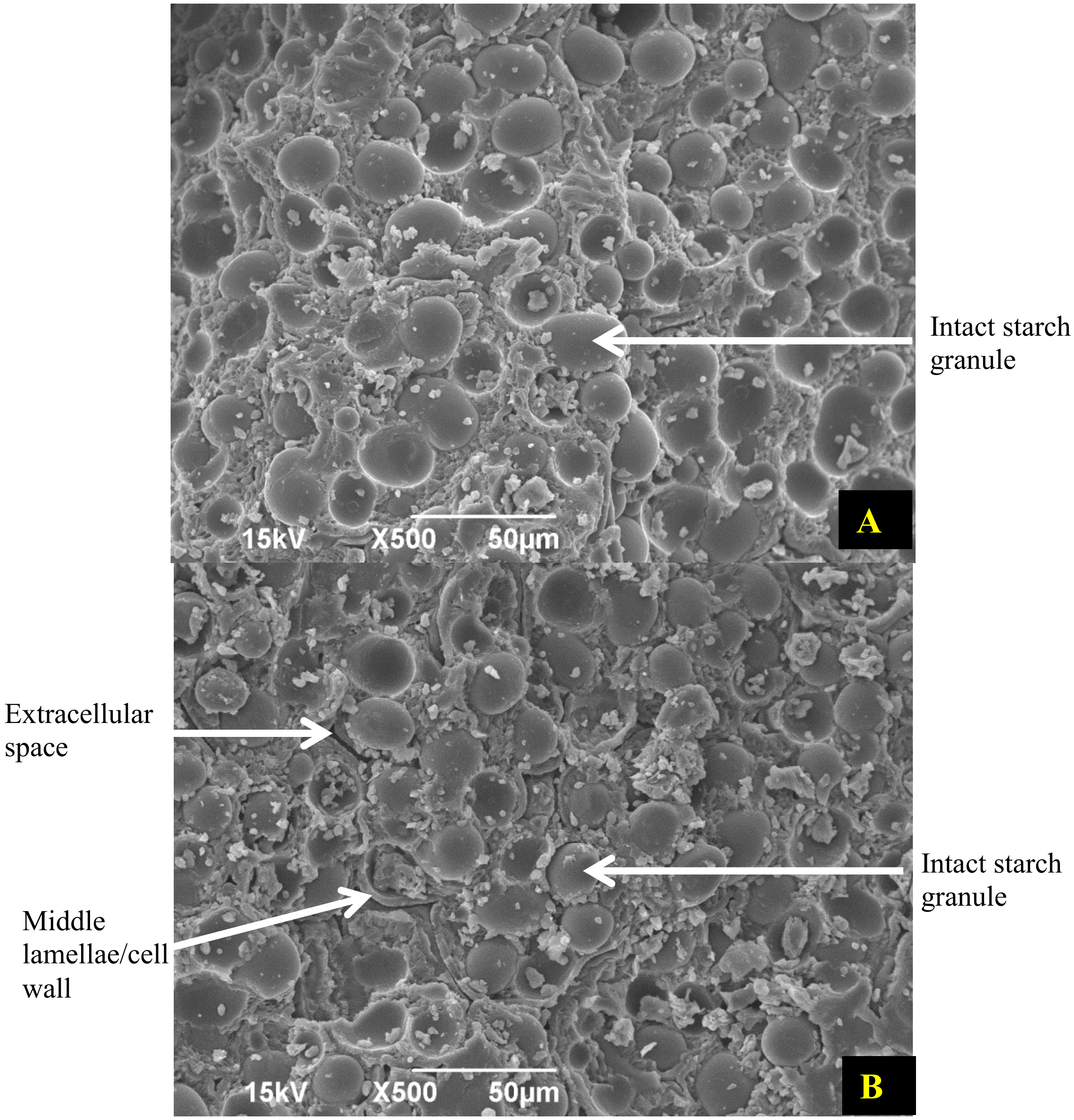


Figure 34. Scanning electron microscopy micrograph comparing flour appearance with visible starch granules for cotyledon flour from non-treated (A) and tempered (14%) (B) black bean seeds.

*Pasting Properties of Flour*

Tempering pretreatment was significant for all pasting properties except for breakdown viscosity (Table 47). For peak, trough, final viscosity, and setback greatest values were observed when tempered to 12 and 14% moisture content. The high pasting values for seeds tempered to 12 and 14% might be due to greater starch damage than other treatments (Table 46). Flour sample from seed tempered to 14% moisture content indicated greatest peak viscosity, which is indicative of high water binding capacity of starch. Low breakdown values obtained for all flours suggested low tendency to form a gel after cooling. Final viscosity values were greatest for flour from seed tempered to14% moisture content (119.3 RVU), which indicates good stability of the cooked paste. A study reported by 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.

Table 47. Mean pasting properties[a] for cotyledon flour from tempered seeds.

| Tempered (%) | Peak | Trough | Breakdown (RVU) | Final Viscosity | Setback |
|---|---|---|---|---|---|
| 6 | 46.7b | 44.2b | 2.5a | 93.2c | 49.0b |
| 8 | 47.4b | 44.8b | 2.7a | 94.6c | 49.9b |
| 10 | 51.0b | 48.3b | 2.6a | 98.2c | 49.9b |
| 12 | 58.3a | 55.8a | 2.5a | 110.0b | 54.2a |
| 14 | 61.8a | 58.5a | 2.3a | 119.3a | 55.0a |

[a]Values followed by same letter are not significantly different at $P$=0.05.

Low setback values have a low tendency to retrograde. While flour from black bean seeds tempered to 14% moisture content recorded the highest setback value (55.0 RVU) while flour from seeds at 6% moisture content had the lowest (49.0 RVU) setback value (Table 47). These results indicated that foods prepared and consumed from flour originating from seed of 6% moisture content will produce less retrogradation. This could be advantageous since retrogradation produces adverse effects on the properties of food products, especially for sensory properties (Miyazaki et al 2005, Sandhu and Singh 2007).

## Conclusions

The utilization of pretreatments impacted the outcome of milling black beans with the burr mill/roller mill system, especially for the removal of seed coat from the cotyledon. Either soaking or cooking improved the toughness of the seed coat which remained more intact than the seed coat from tempered pretreatment. This could be important to obtain a cleaner seed coat removal. However, the aspirator step possibly removed parts of light cotyledon and embryo into the seed coat fraction contaminating it. An improve air-classification system should be used to increase seed coat removal efficiency. Milling was possible with the roller mill when the seeds were pre-broken using the burr mill. More difficulties were observed when using high tempered black bean seed than other pretreatments. Hence, cotyledon flour extraction and seed coat removal were mostly influenced by tempered pretreatment at high moisture contents (12% and 14%). Fine particle yields were greatest adversely impacted by high moisture content where agglomerates and sieving problems were encountered. Flour color (L-value and color difference) was greatest affected by cooked-dried and soaked-dried seed pretreatments. However, L-values for tempered seeds were as high as 88 for fine fraction. Cooked-dried pretreatments had the highest impact in flour chemical composition as well as in pasting properties. Pasting properties were more affected by cooked-dried than others pretreatments. Pasting values were reduced for cotyledon flour.

## Literature Cited


AACC International. Approved methods of the American Association of Cereal Chemists. 2000. 10th Ed. Methods (44-15.02), (08-01.01), (46-30.01), (76-13.01). AACC, St. Paul, MN.

Anton, A., Ross, K. A., Beta, T., Fulcher, R. G., and Arntfield, S. D. 2008. Effect of pre-dehulling treatments on some nutritional and physical properties of navy and pinto beans (*Phaseolus vulgaris* L.). LWT- Food Sci. Technol. 41:771-778.

Altuntas, E., and Demirtola, H. 2007: Effect of moisture content on physical properties of some grain legume seeds. New Zealand J. Crop Hort. Sci. 35:423-433.

Altuntas. E., and Yildiz, M. 2007. Effect of moisture content on some physical and mechanical properties of faba bean (*Vicia faba* L.) grains. J. Food Eng. 78:174-183.

Anderson, J. C., Idowu, A. O., Singh, U., and Singh, B. 1994. Physicochemical characteristics of flours of faba bean as influenced by processing methods. Plant Foods Human. Nutr. 45:371–379.

Aviara, N. A., Gwandzang, M. I., and Haque, M. A. 1999. Physical properties of guna seeds. J. Agric. Eng. Res. 73:105-111.

Bhattacharya, S., Narasimha, H. V., and Bhattacharya, S. 2005. The moisture dependent physical and mechanical properties of whole lentil pulse and split cotyledon. Int. J. Food Sci. Technol. 40:213-221.

Burns, E. E., and Winzer, J. W. 1962. Pigment characteristics of the southern pea, *Vigna sinensis*. Proc. Am. Soc. Hort. Sci. 80:449-456.

Chung, H. J., Liu, Q., Donner, E., Hoover, R., Warkentin, T., and Vandenberg, B. 2008. Composition, molecular structure, properties, and in vitro digestibility of starches from newly released Canadian pulse cultivars. Cereal Chem. 85:471–475.

Culver, W. H., and Cain, R. F. 1952. Nature, causes and correction of discoloration of canned blackeye and purple hull peas. Tex. Agri. Exp. Stn. Bulletin no. 748 p. 23.

Deshpande, S. D., S. Bal, and T. P. Ojha. 1993. Physical properties of soybean. J. Agri. Eng. Res. 56: 89-98.

Deshpande, S. S., Sathe, S. K., Cornforth, D., and Salunkhe, D. K. 1982. Effects of dehulling on functional properties of dry bean (Phaseolus vulgaris L.) flours. Cereal Chem. 59:396–401.

Dhanalakshmi, K., Ghosal, S., and Bhattacharya, S. 2011. Agglomeration of food powder and applications. Crit. Rev. Food Sci. Nutr. 51:432–441.

Dzudie, T., and Hardy, J. 1996. Physicochemical and functional properties of flours prepared from common beans and green beans. J. Agric. Food Chem. 44:3029-3032.

Earle, R. L., and Earle, M. D. 2004. Unit Operations in Food Processing. Web Ed. New Zealand: The New Zealand Institute of Food Science and Technology Inc. [Web Page]. Located at: http://www.nzifst.org.nz/unitoperations/sizereduction1.htm#plate (Accessed on 4/7/14).

Ehiwe, A. O. F., and Reichert, R. D. 1987. Variability in dehulling quality of cowpea, pigeon pea, and mung bean cultivars determined with the tangential abrasive dehulling device. Cereal Chem. 64:86–90.

González, A. M., Monteagudoa, A. B., Casquerob, P. A., De Rona, A. M., and Santallaa, M. 2006. Genetic variation and environmental effect on agronomical and commercial quality trait in the main European market classes of dry bean. Field Crops Res. 95:336-347.

Gowen, A., Abu-Ghannam, N., Frias, J., and Oliviera, J. 2006. Optimisation of dehydration and rehydration properties of cooked chickpeas (*Cicer arietinum* L.) undergoing microwave-hot air combination drying. Trend Food Sci. Technol. 17:177-183.

Han, K. H., Fukushima, M., Ohba, K., Shimada, K., Sekikawa, M., Chiji, H., Lee, C.H., and Nakano, M. 2004. Hepatoprotective effects of the water extract from adzuki bean hulls on acetaminophen induced damage in rat liver. J. Nutr. Sci. Vitaminol. (Tokyo). 50:380-383.

Hangen, L., and Bennink, M. R. 2002. Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. Nutr. Cancer. 44:60–65.

Haque, E. 1991. Application of size reduction theory to roller mill design and operation. Cereal Foods World 36:368-375.

Hoover, R., and Ratnayake, W. S. 2002. Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. Food Chem. 78:489-498.

Jung, Y. S., and Bae, D. G. 2014. Natural dyeing with black cowpea seed coat. I. Dyeing properties of cotton and silk fabrics. Fibers and Polym. 15:138-144.

Ige, M. T. 1977. Measurement of some parameters affecting the handling losses of some varieties of cowpea. J. Agric. Eng. Res. 22:127-133.

Miyazaki, M., Maeda, T., and Morita, N. 2005. Starch retrogradation and firming of bread containing hydroxyl-propylated, acetylated and phosphorylated cross-linked tapioca starches for wheat flour. Cereal Chem. 82:639-644.

Okaka, J. C., and Potter, N. N. 1979. Physico-chemical and functional properties of cowpea powders processed to reduce beany flavor. J. Food Sci. 44(4):1235-1240.

Oomah B. D., Ward, S., and Balasubramanian, P. 2010. Dehulling and selected physical characteristics of Canadian dry bean (*Phaseolus vulgaris* L.) cultivars. Food Res. Int. 43:1410–1415.

Palzer, S. 2005. The effect of glass transition on the desired and undesired agglomeration of amorphous food powders. Chem. Eng. Sci. 60:3959–3968.

Paulson, R. M. 1978. Fracture resistance of soybean to compressive loading. Trans. Am. Soc. Agric. Eng. 21:1210-1216.

Posner, E. S., and Hibbs, A. N. 2005. The grinding process. Pages 125-153 in: Wheat Flour Milling 2nd Ed. American Assoc. of Cereal Chem.: St. Paul, MN.

Sacilik, K., Öztürk, R., and Keskin, R. 2003. Some physical properties of hemp seed. Biosystems Eng. 86:213-215.

Sahoo, P. K., and Srivastava, A. P. 2002. Physical properties of okra seed. Biosystems Eng. 83:441-444.

Sandhu, K. S., and Singh, N. 2007. Some properties of corn starches II: physicochemical, retrogradation, pasting and gel textural properties. Food Chem. 10:1499-1507.

Schoch, T. J., and Maywald, E. C. 1968. Preparation and properties of various legume starches. Cereal Chem. 45:564-573.

Schorno, A. L. 2006. Flaxseed milling and the stability of milled flaxseed. Ph.D. Dissertation, North Dakota State University, Fargo.

Siddiq, M., Ravi, R., Harte, J. B., and Dolan, K. D. 2010. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* L.) flours. LWT - Food Sci. Technol. 43:232-237.

Towo, E. E., Svanberg, U., and Ndossi, G. D. 2003. Effect of grain pre-treatment on different extractable phenolic groups in cereals and legumes commonly consumed in Tanzania. J. Sci. Food Agric. 83:980–986.

Wang, N., Hatcher, D. W., and Gawalko, E. J. 2008 Effect of variety and processing on nutrients and certain antinutrients in field peas (*Pisum sativum*). Food Chem. 111:132-138.

Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., and Gawalko, E. J. 2010. Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). Food Res. Int. 43:589-594.

Wani, A. A., Singh, P., Shah, M. A., Weisz, U. S., Gul, K., and Wani, I. A. 2012. Rice starch diversity: effects on structural, morphological, thermal, and physicochemical properties: a review. Comprehen. Rev. Food Sci. Safety. 11:417-436.

Zhang, X. and Brusewitz, G. H. 1994. Moisture effect on roller milling mustard seed. Trans ASAE 37:491-493.

## OVERALL CONCLUSION

Two milling approaches were studied for the production of black bean flour. By using a centrifugal milling approach, whole black bean flour was obtained. The removal of seed coat from the cotyledons was possible by using burr mill/roller milling system. The cotyledon flour was obtained by milling the cotyledons with the roller mill. In addition, the use of different seed pretreatments (i.e., cooked-dried, soaked-dried, and tempered) altered seed structure, milling-ability, physical quality, chemical composition, and pasting properties of both whole and cotyledon flour.

An intact black bean seed is composed of the protective layer seed coat, the cotyledons and embryo which proximate analysis differed to each other. Both cooking and soaking black bean seeds caused a changed in the seed physical appearance such as seed coat expansion and rupture as well as cotyledon splitting. During drying, both cooked and soaked pretreated seed went back to the original non-treated seed appearance including similar seed dimension in length, width, and thickness, 100-seed weight, and test weight. SEM micrograph showed differences in seed coat and cotyledon structure with more changes seen for cooked-dried and soaked-dried seeds. Due to the physical changes of the seeds, seed hardness decreased for both cooked-dried and soaked-dried seed which showed a high ability of the seed to fracture, whereas tempered seeds hardness increased as moisture content increased. The seed became tough and malleable.

The best setting for centrifugal milling black beans was using a screen with mesh size of 500 µm, rotor speed of 12,000 rpm, and mill feed of $267 \pm 18$ g/min. This setting was selected to minimize milling problems and maximize the fine particle yields and was utilized to compare the effect of seed pretreatment. By using a roller mill with durum wheat setting, milling yields for cooked-dried, soaked-dried, and tempered pretreatments were approximately 75, 73, and 74%, respectively. Milling yields probably will vary by using a roller mill designed for bread wheat milling. In general, for both milling approaches all pretreatments had a major impact on feed rate as well for the flour bulk density. Flour bulk density had inverse relationship with moisture content, as one increased the other decreased. High moisture content (12 % and 14 %) impacted the particle size determination, specifically affected the fine particle yield due to problems of flour aggregation. Flour color was highly influenced by the cooking or soaking pretreatment where the seed coat dark pigment (anthocyanins) leached and stained the cotyledon, hence both whole flour and cotyledon flour L-values (lightness) significantly decreased.

Chemical composition of the whole and cotyledon flour indicated that the cooking or soaking caused a reduction in ash content due to leaching solutes into the water. However, for all pretreatments total starch and protein content did not changed at any practical level. Starch damaged was more affect by cooked-dried pretreatment that the other two due to starch gelatinization. Also, pasting properties of both obtained flours were majorly affected by the cooked-dried pretreatment as starch gelatinized at boiling water temperature and more accessible surface area was there available for enzyme to hydrolyze the starch.

From this study it can be concluded that the type of mill used, as well as the conditions used during pretreatment and milling, will influence the physical, chemical, and pasting properties of the flour.

## FUTURE RESEARCH AND APPLICATION

Future research needs to be done to develop a fast and efficient way for the appropriate milling optimization of black beans. A standard milling procedure needs to be developed for not only black bean milling but for other dry bean market classes. Research can also concentrate in further learn changes in starch/protein matrix of the cotyledon fraction during milling. Also, studies need to be done on the effect of bean flour particle size distribution and flour physical quality, chemical composition, and functionality (for example for fine, medium, and coarse particles). The future research should include an integrated process from milling to flour to final product utilization and the differences for each step.

For application, black bean or other market classes can be seen as an additional strategy proposed for increasing the use and production and to contemplate the opportunity for the seeds to be used as raw materials for further processing in the industry, rather than a vegetable to be eaten as a whole. Protein, starch, and fiber are the three major components which have useful functional properties still to be further studied and used in food products.

As mentioned in the paper when discussing pasting properties, black bean had a stable development and high end-point viscosity when compared to cereal and tuber flours. Black bean and other market classes possess good gelling properties, but more studies need to be done to take preventative steps to prevent or eliminates their high level of syneresis, which will not be desired in some food products such as sauces. The further studies in black bean and other market classes' fractions could open doors for the development of its selections which could be produced to suit a wide range of food and non-food applications.

## APPENDIX

Table A1. ANOVA of milling characteristics obtained by centrifugal mill and 250 µm screen mesh size.

| Dependent variable | Source of variation | Df | MS | F-value |
|---|---|---|---|---|
| Temperature gain (ºC) | | | | |
| | Rep | 2 | 0.324 | 3.50 |
| | Rotor Speed (RS) | 2 | 2.714 | 29.26* |
| | Feed Rate (FR) | 2 | 21.090 | 227.32* |
| | RS * FR | 4 | 0.556 | 5.99* |
| | Error | 16 | 0.092 | |
| Flour Moisture (%) | | | | |
| | Rep | 2 | 0.021 | 2.63 |
| | Rotor Speed (RS) | 2 | 0.038 | 4.75* |
| | Feed Rate (FR) | 2 | 0.044 | 5.53* |
| | RS * FR | 4 | 0.030 | 3.74* |
| | Error | 16 | 0.008 | |
| Particle Size – Coarse (%) | | | | |
| | Rep | 2 | 0.046 | 0.55 |
| | Rotor Speed (RS) | 2 | 19.256 | 226.10* |
| | Feed Rate (FR) | 2 | 2.870 | 33.71* |
| | RS * FR | 4 | 0.056 | 0.67 |
| | Error | 16 | 0.085 | |
| Particle Size – Fine (%) | | | | |
| | Rep | 2 | 8.967 | 0.93 |
| | Rotor Speed (RS) | 2 | 92.992 | 9.63* |
| | Feed Rate (FR) | 2 | 350.984 | 36.34* |
| | RS * FR | 4 | 41.803 | 4.33* |
| | Error | 16 | 9.658 | |

*Significant at *P*=0.05; Df=degrees of freedom; and MS=mean square.

Table A2. ANOVA of milling characteristics ofbtained by centrifugal mill and 500 µm screen mesh size.

| Dependent variable | Source of variation | Df | MS | F-value |
|---|---|---|---|---|
| Temperature gain (ºC) | | | | |
| | Rep | 2 | 1.082 | 3.69 |
| | Rotor Speed (RS) | 2 | 40.864 | 139.45* |
| | Feed Rate (FR) | 2 | 1.255 | 4.29 |
| | RS * FR | 4 | 3.741 | 12.77* |
| | Error | 16 | 0.293 | |
| Flour Moisture (%) | | | | |
| | Rep | 2 | 0.060 | 5.02 |
| | Rotor Speed (RS) | 2 | 0.613 | 50.98* |
| | Feed Rate (FR) | 2 | 0.173 | 14.43* |
| | RS * FR | 4 | 0.100 | 8.38* |
| | Error | 16 | 0.012 | |
| Particle Size – Coarse (%) | | | | |
| | Rep | 2 | 0.445 | 0.52 |
| | Rotor Speed (RS) | 2 | 259.481 | 301.10* |
| | Feed Rate (FR) | 2 | 7.422 | 8.61* |
| | RS * FR | 4 | 3.886 | 4.51* |
| | Error | 16 | 0.861 | |
| Particle Size – Fine (%) | | | | |
| | Rep | 2 | 0.440 | 0.61 |
| | Rotor Speed (RS) | 2 | 151.417 | 211.01* |
| | Feed Rate (FR) | 2 | 4.864 | 6.78* |
| | RS * FR | 4 | 1.447 | 2.02 |
| | Error | 16 | 0.717 | |

*Significant at $P$=0.05; Df=degrees of freedom; and MS=mean square.

Table A3. ANOVA of milling characteristics obtained by centrifugal mill and 1,000 μm screen mesh size.

| Dependent variable | Source of variation | Df | MS | F-value |
|---|---|---|---|---|
| Temperature gain (ºC) | | | | |
| | Rep | 2 | 0.043 | 0.31 |
| | Rotor Speed (RS) | 2 | 7.453 | 53.72* |
| | Feed Rate (FR) | 2 | 2.733 | 19.63* |
| | RS * FR | 4 | 1.576 | 11.36* |
| | Error | 16 | 0.138 | |
| Flour Moisture (%) | | | | |
| | Rep | 2 | 0.014 | 2.97 |
| | Rotor Speed (RS) | 2 | 0.333 | 68.57* |
| | Feed Rate (FR) | 2 | 0.043 | 8.91* |
| | RS * FR | 4 | 0.066 | 13.71* |
| | Error | 16 | 0.004 | |
| Particle Size – Coarse (%) | | | | |
| | Rep | 2 | 0.495 | 0.18 |
| | Rotor Speed (RS) | 2 | 474.729 | 174.41* |
| | Feed Rate (FR) | 2 | 3.305 | 1.21 |
| | RS * FR | 4 | 1.193 | 0.44 |
| | Error | 16 | 2.721 | |
| Particle Size – Fine (%) | | | | |
| | Rep | 2 | 0.852 | 0.43 |
| | Rotor Speed (RS) | 2 | 228.798 | 114.38* |
| | Feed Rate (FR) | 2 | 0.512 | 0.26 |
| | RS * FR | 4 | 0.197 | 0.10 |
| | Error | 16 | 1.999 | |

*Significant at $P$=0.05; Df=degrees of freedom; and MS=mean square.

Table A4. Mean actual feed rate of milled black bean flour as affected by feed rate setting and rotor speed for 250, 500, and 1,000 μm screen mesh size.

| Screen mesh size | Rotor speed* | | Feed rate setting | | |
|---|---|---|---|---|---|
| | | | 30 | 40 | 50 |
| μm | rpm | rad/sec | Actual feed rate (g/min) | | |
| 250 | 10,000 | 1,047 | 46.2cA | 122.7bA | 221.7aA |
| | 12,000 | 1,257 | 45.3cA | 120.0bA | 232.1aA |
| | 14,000 | 1,466 | 43.1cA | 119.5bA | 226.2aA |
| 500 | 10,000 | 1,047 | 46.4cA | 136.1bA | 255.8aA |
| | 12,000 | 1,257 | 47.8cA | 128.6bA | 242.9aAB |
| | 14,000 | 1,466 | 47.9cA | 125.7bA | 236.9aB |
| 1,000 | 10,000 | 1,047 | 44.8cA | 144.4bA | 249.8aB |
| | 12,000 | 1,257 | 48.7cB | 146.8bA | 275.8aA |
| | 14,000 | 1,466 | 50.8cB | 150.0bA | 272.9aA |

*Different lowercase later across rows indicates significant differences ($P$=0.05). Different uppercase later across column indicates significant differences ($P$<0.05)
*Air temperature was 22 °C. Relative humidity was 27%. Initial seed moisture content was 10%.