BLACK BEAN MILLING AND FLOUR FUNCTIONALITY

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Dry bean utilization by the food industry can be increased by developing value-added processing applications. The goals of this research were to evaluate (1) the effect of milling method on the physical, chemical and functional properties of whole black bean flour and its fractions and (2) the effect of removing soluble phenolic compounds on the functional and rheological properties of black bean protein isolates. Black bean was milled with five laboratory mills [cyclone mill, hammer mill, stone mill (fine, medium, coarse), disc mill (fine, coarse), and centrifugal mill (10,000 or 12,000 rpm and 250, 500, 1000 μm aperture screen)] and the resulting flours were evaluated for their physical, chemical and flow properties of bulk samples and particle size fractions. Whole black bean flour and cotyledon flour were subjected to phenolic extraction and protein isolation, resulting in protein isolates with and without soluble phenolics. Solubility, wettability, dispersibility, water binding capacity, foam capacity and stability, emulsification capacity, and gelation properties of protein isolates were evaluated. Variation in milling method produced flours with significantly different flour characteristics. Geometric mean size of whole bean flour was negatively correlated with starch damage (r = -0.92), L* (r = -0.94), angle of repose (r = -0.94), and angle of slide (r = -0.80 to -0.90) and positively correlated with moisture (r = 0.72), and loose bulk density (r = 0.72). Milling method and particle size interaction was significant on characteristics of black bean flour fractions. Particle circularity of flour fractions had a negative correlation of r = -0.93, r = -0.81, $r \approx$ -0.95, and r = -0.94 with L*, angle of repose, angle of slide and compact density, respectively. Particle circularity had a positive correlation of r = 0.93 and r = 0.89 with average minimum particle size and loose bulk density, respectively. The removal of soluble phenolic compounds improved the brightness, solubility, wettability, dispersibility, foaming capacity, foaming stability, emulsion capacity,

emulsion stability and gelling properties of protein isolates. These findings will help food manufacturers to process black bean ingredients using different mill settings to achieve different functionalities depending on the consumer requirements.

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DEDICATION

To my daughter, Shanodya Brielle Christina Fernando, who brought pure happiness to my PhD.

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GENERAL INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are a type of leguminous crop that are harvested solely for the dry seed (FAO, 2015). Eleven main dry bean market classes are grown in the United States (US): black beans, kidney beans (light red and dark red), navy beans, pinto beans, great northern beans, small red beans, pink beans, small white beans, cranberry beans, and yellow-eye beans. Although dry beans of different market classes possess a similar seed structure, they vary widely among classes for color, size and shape (Siddiq & Uebersax, 2013). Black bean is the second leading class of dry beans produced in the US and it has had a tremendous production growth over the last three decades compared to other market classes (USDA-ERS, 2019).

Dry beans are used as a low-cost source of protein by people with low-income that live in developing countries where protein energy malnutrition is often prevalent. Dry beans are also a good source of carbohydrates, dietary fiber, certain minerals and vitamins in the human diet (Sathe, 2002; Van Heerden & Schonfeldt, 2004; Iqbal et al., 2006). Phenolic compounds present in dry beans possess antioxidant properties which are responsible for many health benefits (Madhujith & Shahidi, 2005).

Dry bean utilization by the food industry can be increased by developing value-added processing applications. The trend of incorporating non-wheat flours into food products is driven by an increased demand for nutrient dense foods. Several studies have explored the utilization of dry beans in traditional products, such as bread, pasta, and snacks (Aguilera et al., 1982; Han et al., 2010). The inclusion of dry bean flour in these products improved their nutritional value. In addition to their nutritional value, dry bean protein isolates or concentrates can be used in food systems, due to their ability to form gels, foams and emulsions (Sathe, 2002; Johnston et al., 2015; Ishaya & Aletor, 2019).

Limited literature is available specifically on dry bean milling. More research is available on general pulse milling (Kerr et al., 2000; Patil et al., 2005; Maskus et al., 2016). Pulses are seeds from plants in the legume family. Thus, dry beans are a subset of pulses. Milling is the first step in transforming intact seed into flour which then can be utilized as a value-added ingredient. Pulses can be milled into whole flour, cotyledon flour, starch/protein/fiber rich flours, protein isolates and protein concentrates. Overall, millabilty of pulses has been reported to be affected by grain physical properties (cleanliness, hardness, uniformity of kernel size and shape, test weight, thickness of seed coat), grain chemical components (starch, protein, fiber), grain pretreatments and mill type and settings (Wood et al., 2012; Carter, 2014; Pelgrom et al. 2015; Vishwakarma et al., 2018).

Pulse milling consists of all or a combination of the following steps: dehulling, splitting, grinding and separation (Vishwakarma et al., 2018). In Asia and Africa, dehulling is the most common pulse milling operation (Wood & Malcolmson, 2011). Removing the seed coat reduces cooking time and improves palatability and digestibility of pulses (Singh, 1995). Most food industries are now milling to produce whole pulse flour (Ardent Mills, 2019). Whole flour milling is straightforward and often requires a single pass through a mill, whereas producing dehulled flour and dry fractionated flour requires special pretreatments and/or further processing (sieving and air classification).

Milling is based on the application of impact, cutting, compression, abrasion, and shear forces, or a combination of them. Mills control the fineness of the particles by adjusting the peripheral speed of their rotors, the feed rate and sieve aperture size (Patil et al., 2005; Carter 2014). Mills have different properties based on the force(s) that is used. For example, an impact mill will concentrate its energy of rupture at a single point, whereas a compression mill will more evenly disperse energy across the seed (Dijkink & Langelaan, 2002; Carter, 2014).

Flour milled on different mills can have different particle characteristics (Kang et al., 2019). Particle size and particle shape govern the technological properties (flow properties, hydration properties, mixing) of powders and their application in food systems (Fu et al., 2012; Sun et al., 2019). The particle size of flour can be determined by different methods (sieve analysis, image analysis, laser analysis), but does not describe adequately the powder granulometry (Mikli et al., 2001). For a meaningful characterization of a flour sample, the shape of many individual particles needs to be analyzed. Therefore, automated image analysis with a specific software can be used to calculate the various shape descriptors of individual particles (Turchiuli et al., 2005).

Flow properties are important in storage, movement, and blending of flours. Flow properties are governed by the flour physical properties like particle size and shape (Abu-hardan & Hill, 2010; Jan et al., 2017). For example, flour flowability decreases as the particle size becomes smaller (Geldart et al., 2006; Landillon et al., 2008; Ambrose et al., 2015). But sometimes similar sized particles have been found to have contrasting flowability. The reason for this might be due to differences in other flour properties like particle shape and chemistry. Aspherical shape results in poor flour flowability due to intermittencies in flow rate (Fraige et al., 2008). Elongated or irregular particles tend to mechanically interlock or entangle with each other, thus reducing the flour flowability.

Physical stability, texture and mechanical characteristics can be affected by the interaction of dry bean protein with other food ingredients (Kiosseoglou & Paraskevopoulou, 2011). Phenolic compounds characteristically possess a significant binding affinity for proteins, which can lead to the formation of soluble and insoluble protein–phenol complexes (Papadopoulou & Frazier, 2004). Phenolic compounds are known to form complexes with proteins leading to changes in the structural, functional and nutritional properties of both compounds (Papadopoulou & Frazier, 2004). Black beans are rich in phenolic compounds which possess antioxidant properties, but also can affect the functional properties of proteins like solubility, water binding capacity, gelation, emulsification, and foaming (Hosfield, 2001; Sęczyk et al., 2019). Questions remain concerning to what extent the protein–phenol interaction influences functionality.

The present study was undertaken with the following objectives:

1. To evaluate the effect of milling method on the physical, chemical and functional properties of whole black bean flour.
2. To compare the physical, chemical and functional properties of selected black bean flour fractions with the same particle size but milled using different mill settings.
3. To evaluate the effect of soluble phenolic compounds removal on functional and rheological properties of black bean protein isolates.

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CHAPTER 1: LITERATURE REVIEW

Dry Bean Production and Consumption

The *Fabaceae* family (which includes legumes) is the second largest source of human food and feed for livestock (Graham & Vance, 2003; Berrios, 2006). *Fabaceae* represents one of the most diverse families of plants that are grown in different parts of the world (Deshpande, 1992). More than 7,000 years ago, dry beans were domesticated in tropical and subtropical areas of Central and South America (Latin America) (Kaplan, 1965). Today, dry beans (*Phaseolus vulgaris* L.) are the world's second most important legume class, after soybeans [*Glycine max* (L.) Merr.], and are a staple food in Africa, India and Latin America (Xu & Chang, 2008). Dry bean consumption is highest in Latin America, where legume consumption ranges from 1-25 kg per capita per year, and where dry beans dominate, accounting for 87% of the total legume products consumed (Leterme & Muñoz, 2002). Dry beans are not a staple in the US where the per capita consumption was about 4.3 kg in 2018 (USDA-ERS, 2019).

Health consciousness and concern for the environment and animal welfare has encouraged consumer interest in alternative plant-based proteins. In US, sales of plant-based food, which is the largest source of alternative protein, rose 11%, from 2018 to 2019 and the use of alternative protein as a food ingredient in consumer products is predicted to continue grow (The Good Food Institute, 2019). The search for alternative protein sources to replace animal proteins has been a challenge due to the adverse functional and sensory properties of some of those alternative candidates in food systems (Henchion et al., 2017).

Pulses have gained increased attention as an alternative protein source due to their high level of protein (17-30%), low cost, and potential health benefits (Boye et al., 2010a; Papalamprou et al., 2010). Favorable functional properties in food systems by pulse proteins have gained interest from the food industry (Tiwari et al., 2011; Henchion et al., 2017). Meanwhile increased production, changes in ethnic groups and food patterns and interest in the health benefits of dry beans has enhanced the interest in utilizing dry beans as a food ingredient (Hall et al., 2017; Los et al., 2018).

Chemical Composition

Carbohydrate

Carbohydrates are an important chemical component that accounts for 70% of dry bean weight (Bennink & Rondini, 2008). Carbohydrates consist of starch and nonstarch polysaccharides, along with small amounts of oligosaccharides (Bravo et al., 1998).

Starch

Starch is the main nutrient in dry bean (42%) and accounts for approximately 60% of total carbohydrates present in the seed (Reddy et al., 1984). Raw dry bean starches appear to be smooth with an oval or elliptical shape (Gujska et al., 1994; Hoover & Ratnayake, 2002). Starch is mainly composed of amylose and amylopectin. The total amylose in black beans can range from 27-39% (Hoover et al., 2010) and is higher than the amylose content (≈ 20-25%) in cereal grains (Hu et al., 2010). Dry bean starches are known to develop greater viscosity than cereal starches (Lineback & Ke, 1975). The shape, size, and other morphological characteristics of dry bean starch granules vary depending on factors such as the botanical source and environmental conditions under which the crop was grown (Wang, 2013).

Non-starch polysaccharides

Dietary fiber represents non-starch polysaccharides. Dry bean non-starch polysaccharides mainly consist of cellulose, hemicellulose and pectin (Vasić et al., 2009). In dry beans, dietary fiber can range from 23-32% with about 80% being insoluble (Kutoš et al., 2003; Wang et al., 2010; Tiwari & Singh, 2012). Dry bean seed coat is comprised mainly of non-starch polysaccharides such as cellulose (70%) (Srisuma et al., 1991). Dry bean cotyledon mainly contains pectin (26%) and hemicellulose (22%) (Tiwari et al., 2011). Cellulose is the major cell wall component and has an unbranched linear chain of many glucose units with β-1, 4 glucosidic linkages. Hemicellulose also contains a backbone of β-1, 4 glucosidic linkages but has branches of β-1,3 glucosidic linkages. Hemicellulose is smaller than cellulose (Dhingra et al., 2012). Pectin contains D-galacturonic acid as the principal constituent and acts as intercellular bonding substance in the plant primary cell wall and the middle lamellae (Dhingra et al., 2012). Non-starch polysaccharides in foods are known for their swelling capacity, water-holding capacity, oil-binding capacity, and cation exchange capacity (Mudgil & Barak 2013).

Oligosaccharides

Dry beans contain 30-37 mg of oligosaccharides per gram of seed (Kuo et al., 1998). Oligosaccharides stabilize cell membranes during seed desiccation and when the seed rehydrates during germination (McPhee et al., 2002). Dry beans contain various oligosaccharides, mainly raffinose and stachyose, which cannot be hydrolyzed by the human digestive system (Shimelis & Rakshit, 2007). Raffinose is a trisaccharide composed of galactose, glucose, and fructose. Stachyose is a tetrasaccharide consisting of two α-D-galactose units, one α-D-glucose unit, and one β-D-fructose unit.

Microflora in the colon metabolize the oligosaccharides into carbon dioxide and water (Roberfroid & Slavin, 2000), which can result in intestinal discomfort and flatulence. Different processing methods can reduce oligosaccharide content (Barampama & Simard, 1994; Matella et al., 2005; Kelkar et al., 2012). The loss of oligosaccharides (raffinose and stachyose) from the beans increases the digestibility of the beans and leads to less gas formation. However, loss of oligosaccharides from dry beans will result in less oligosaccharides reaching the large intestine and serving as an energy-source for beneficial bacteria like bifidobacteria or lactobacilli that live there (Bornet & Brouns, 2002). Since robust concentrations of these bacteria are usually helpful to human health, this loss of oligosaccharides may not be desirable from an intestinal health standpoint.

Protein

Dry beans contain 20-30% protein on a dry weight basis. In dry beans, most of the protein is found in protein bodies located in cells of the cotyledon and embryonic axis (Van Der Poel, 1990). Dry bean protein bodies are small (1-10 μm) and spherical to oval and are found in the cell matrix between the starch granules (Berrios et al., 1998; Wood et al., 1998).

Dry beans contain storage proteins of albumins, globulins, glutelins and prolamins which are classified according to their solubility in various solvents based on the Osborne fractionation (Singhal et al., 2016). Globulins (soluble in dilute salt solutions) represent ∼70% and albumins (water soluble) account for 10–20% of the total dry bean proteins (Ma & Bliss, 1978; Boye et al., 2010b). Globulins include legumin and vicilin proteins and are characterized by multi-sub-unit molecules of high molecular weight (Karaca et al., 2011). Globulins have a relatively hydrophobic surface that limits their solubility in aqueous media (Boye et al., 2010b). In contrast, albumin fraction consists of relatively low to medium molecular weight enzymatic proteins, lectins and protease inhibitors (Boye et al., 2010b). Albumins have a hydrophilic surface that renders the proteins water soluble. Prolamins and glutelins are lesser in amount and soluble in ethanol solution and dilute alkali solutions, respectively (Oomah et al., 2011). Albumin/globulin and legumin/vicilin ratios are thought to be responsible for the differences in functionality of different dry bean proteins (Tiwari et al., 2011). Even though dry beans contain high levels of protein which is rich in lysine, the protein quality is considered low because it contains low amount of methionine, cysteine and tryptophan and has low true digestibility due to antinutrients such as lectins and protease inhibitors (Deshpande & Nielsen, 1987; Belitz et al., 2009).

Lipid

Dry beans have low lipid content, typically ranging from 1.8-2.6% and includes triacylglycerides, free fatty acids, sterols, glycolipids and phospholipids (Drumm et al., 1990). Lipid content can vary depending upon market class, cultivar, climate and other growing conditions (Worthington et al., 1972). Polyunsaturated fatty acids account for 74% of lipids in black beans and linolenic ($C_{18:3}$) (41.7%) and linoleic ($C_{18:2}$) (31.1%) acids are the major polyunsaturated fatty acids found in black beans (Sutivisedsak et al., 2010).

Micro-Nutrients

<u>Vitamins</u>

Black beans have water soluble vitamins especially thiamine, niacin, riboflavin and folate that can mostly be found in the cotyledons (Siddiq & Uebersax, 2013). Black beans also contain lipid soluble vitamins E and K (Campos-Vega et al., 2010). Gregory & Kirk (1981) reported the presence of non-digestible polysaccharides and lignin can reduce the availability of Vitamin B_6 for absorption.

<u>Minerals</u>

Minerals can be classified as macro-mineral nutrients and micro-mineral nutrients. Macro-mineral nutrients include: calcium, magnesium, phosphorus, potassium, and sodium. Micro-mineral nutrients include: copper, iron, manganese, selenium and zinc. Black beans are rich in minerals such as calcium, iron, copper, zinc, potassium, phosphorus and magnesium

(USDA-ARS, 2019). Dry beans contain low sodium levels and contain high amounts of iron, calcium, and zinc. Phosphorus in dry beans is present in the form of phytic acid. Storage iron in legumes is sequestered in ferritin, which is the major iron storage protein. However, 70–85% of the iron present in dry beans is in the form of non-ferritin-bound iron possibly bound to phytic acid (Petry et al., 2015). Calcium, copper, zinc, potassium, phosphorus and magnesium are found mainly in the seed coat (Deshpande & Damodaran, 1990).

Phytochemicals

Phytochemicals are secondary metabolites synthesized by plants to reduce the effects of oxidative stress-induced diseases (Isah, 2019). Phenolic compounds represent a group of phytochemicals that consists of bioactive structural phenolic units. Phenolic compounds contain several hydroxyl groups on one or more six-carbon aromatic rings and based on the number of aromatic rings and other structural elements, these phytochemicals can be divided into five main classes; flavonoids, phenolic acids, phenolic alcohols, stilbenes, and lignans (D'Archivio et al., 2007).

Phenolic compounds (polyphenols and simple phenols) are located predominantly in the seed coat of the dry bean (López et al., 2013). Phenolic acids and polyphenols such as tannins and flavonoids are responsible for the seed coat pigmentation. Diaz et al. (2010) examined the relationship between tannins and seed coat color and reported that black beans with a dark purple color have more tannins than did seed coats of white or light-colored beans. According to Chávez-Mendoza & Sánchez (2017), the seed coat contains most of the tannins in beans, whereas their concentration is low in cotyledons.

Dry beans contain various bioactive phytochemical compounds that are referred to as "antinutritional factors" due to their adverse impact on nutrient bioavailability (Ganesan & Xu, 2017). For example, black bean has phytochemicals such as tannin and enzyme inhibitors (trypsin and chymotrypsin) which could affect protein digestibility. Tannin is mainly present in the seed coat while phytic acids, trypsin, chymotrypsin and α–amylase inhibitors are present in the cotyledon (Deshpande et al., 1982). The inactivation of antinutritional factors is important to ensure nutrient absorption and proper contribution of health benefits by consuming beans (Uebersax, 2006).

Morphology and Anatomy of Dry Bean Seed

All dry beans possess a similar seed structure, which includes a seed coat, cotyledon and embryonic axis (Helm et al., 1990). The seed coat consists of approximately 8% of the total dry weight of the dry bean (Rahman, 2007; Carter, 2014). The main functions of the seed coat are to regulate movement of moisture into and out of the seed and to protect the cotyledon and embryo from microbial degradation especially during harvest and storage. The major components of the seed coat microstructure are; the waxy cuticle layer, palisade cell layer, the hour-glass cells, and the parenchyma layer (Ruengsakulrach, 1990). The 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). Phenolic compounds are concentrated in the dry bean seed coat. These compounds exhibit antioxidant activities and protect the seed from oxidative damage (Adebooye & Singh, 2007).

Cotyledon represents the largest portion of the black bean seed, accounting for 90% of the seed weight (Rahman, 2007) and contributes to the texture and nutritive value of the bean. Cotyledon is composed of an outer epidermal layer, hypodermis layer, and inner cotyledon layer. The epidermal layer is the outer most layer, which consists of outer cells that appear to be cubical and inner cells that are elongated. The hypodermis, which consists of large elliptical cells, is the next apparent layer. The inner cotyledon layer is composed of parenchyma cells that are bound by distinct cell walls and middle lamellae. The thick cell walls give rigidity to the cotyledon. The parenchyma cells in the cotyledon are packed with starch granules that are embedded in a matrix of storage proteins (Tiwari & Singh, 2012). Mature parenchyma cells are very thick with the secondary wall and pits in the wall that facilitate water diffusion during soaking. The middle lamella contains pectin, which binds cells together (Siddiq & Uebersax, 2013).

Dry bean cotyledons contain about 39% starch, 28% protein, 2% lipids, 4% ash and 20-30% non-starch polysaccharides (Powrie et al., 1960; Harvard, 2015). During seed maturation, cotyledon functions as a storage unit for nutrients and upon germination, the seedling uses the carbohydrate as a source of glucose and energy and protein as a source of amino acids. Cotyledon is also a photosynthetic structure that is responsible for the embryonic leaf tissue. Zimmermann et al. (1967) demonstrated the partitioning of nutrients within the cotyledon, with higher levels of protein and trypsin inhibitors in the outer layers compared to inner layers.

Embryonic axis consists of plumule, hypocotyl and radicle and serves as a nutrient-absorbing organ for the embryo during germination. Radicle is the rudimentary shoot and root of a plant that supports the cotyledons in the seed and from which the root is developed downward. Hypocotyl is the portion of the embryo between the root and cotyledons. Plumule consist of the apical meristem and the first true leaves of the young plant (Bio.libretext, 2019). The embryo is living tissue, relatively small and represents only 2% or less of the seed weight (Rahman, 2007). The embryo is rich in lipids, vitamins and enzymes required in growth and development (Siddiq & Uebersax, 2013).

The external seed structure also includes the hilum, micropyle, and raphe (Helm et al., 1990). Raphe, micropyle and hilum are entry points for water diffusion into the seeds (Tiwari & Singh, 2012). The hilum is the scar left when the ovule separated from the funiculus (stalk) which had supported and attached the seed to the pod during development. The micropyle is the site of pollen tube entry during fertilization. Raphe is the ridge on seed coat formed by the attachment of the funiculus to the seed coat (Helm et al., 1990).

Milling

Dry bean milling consists of all or a combination of the following steps: dehulling, splitting, grinding and separation (Vishwakarma et al., 2018). Dehulling separates seed coat from cotyledons. Each seed has two cotyledons that can be separated to produce ‘splits’. Pulse seed can be ground to produce whole seed or cotyledon flours (Wood & Malcolmson, 2011; Thakur et al., 2019). Dehulling efficiency, dehulling index, splits yield, percentage broken, and percentage loss have been used to assess the effectiveness of pulse milling test (Wood & Malcolmson, 2011).

Dehulling Pre-Milling Treatments

Dehulling is the removal of seed coat from the cotyledon. Removing the seed coat reduces cooking time and improves palatability and digestibility of pulses (Singh, 1995). Pre-milling treatments are employed to loosen the connection between seed coat and cotyledon and to reduce the breakage of the seed coat as to increase flour quality (Singh, 1995). Pitting, soaking and tempering with water or oil, enzyme treatments, and chemical treatments have been used as pretreatments to aid in dehulling during pulse milling (Verma et al., 1993; Tiwari et al., 2010; Scanlon et al., 2018; Vishwakarma et al., 2018).

Pitting

Scratching the seed surface by abrasion before any other pretreatment has been utilized by some research groups to improve the absorption of pretreatment agents (Narasimha et al., 2003). Efficacy of the pitting step can be dependent on the seed characteristics (seed moisture and size) and machine characteristics (roller speed, roller gap and pitting duration). The pitting step reduces the energy required for subsequent pretreatments and for dehulling process (Vishwakarma et al., 2018).

Soaking and tempering

Soaking and tempering can be used as a pretreatment. Soaking involves exposing seed to excess water while tempering is the addition of prescribe amount of water needed to increase moisture content to a desired level (Carter, 2014). Soaking and tempering cause the seed to expand and when seeds are dried, the cotyledons shrink more than the seed coat. This results in a gap between the cotyledon and the seed coat which favors the removal of the seed coat during dehulling (Sokhansanj & Patil, 2003). Optimal soaking or tempering time and final moisture levels can vary with the pulse type (Fernando, 2017). These wet pretreatments are labor intensive (Singh, 1995). Fernando (2017) reported that tempering combined with drying, can be used to remove the seed coat from the black beans with minimum quality changes in the flour.

Edible oil pretreatment

Many studies have evaluated different edible oils and their percentages on the dehulling efficiency (Perera, 2001; Tiwari et al., 2007). The edible oil penetrates through the pitted seed coat and loosens it from the cotyledon (Deshpande et al., 2007). Tiwari et al. (2007) suggested that edible oil can dissolve the gums or can help weakening the bonds between seed coat and cotyledon. Some commercial milling processes use edible oils to aid the dehulling of pulses

(Vishwakarma et al., 2018). The commercial process uses pitted seeds with 10-12% moisture. Edible oil (up to 1%, w/w) is applied. The seeds are then sun-dried, tempered, followed by a second sun drying to achieve 10-11% moisture and then milled using abrasive forces (Narasimha et al., 2003; Tiwari et al., 2007). This method only results in 40-50% dehulled seeds after the first pass. The seeds that were not dehulled are processed using the same procedure in order to achieve good yield.

Chemical pretreatments

Various chemicals like NaOH, $NaHCO_3$, Na_2CO_3, NaCl and acetic acid have been evaluated for use in improving the dehulling efficiency of pulses. This method involves pitting, mixing with chemical solution, conditioning, drying and abrasive milling (Vishwakarma et al., 2018). Srivastava et al. (1988) reported that dehulling is enhanced due to hydrolysis of gums by chemicals. This method is not commercially adopted due to nutrient loss, discoloration of seeds and residual chemicals in the seed coat which cannot be used for animal feed (Lal & Verma, 2007).

Enzyme pretreatments

Verma et al. (1993) used enzyme treatment to partially hydrolyze the cell wall of pigeon pea to increase the dehulling efficiency. Using this method, dehulling efficiency was reported to be 87 % (Verma et al., 1993). Xylanase and protease were used by Sreerama et al. (2009) on different pulses. They reported that protease was more efficient than xylanase in promoting dehulling. This method has not been adopted by the commercial pulse milling. No literature was found concerning the quality of the final pulse flour after enzyme treatment (Vishwakarma et al., 2018).

Mills Used for Size Reduction

Centrifugal mill, disc mill, cyclone mill, hammer mill, stone mill, and roller mill have been used to grind dry beans into flour. Milling is based on the principle of impact, cutting, compression, abrasion, and shear forces, or a combination of them. Each mill applies a different combination of forces to reduce dry bean seed into flour. During milling, the seed absorbs force as strain energy. When the local strain energy in the seed exceeds a critical level, fractures occur along lines of weakness and the stored energy is released as heat (Earle & Earle 2004; Schorno 2006). The efficiency of milling is related to uniformity of the particle size distribution and the differences in particle sizes.

<u>Centrifugal mill</u>

Whole bean flour can be produced by grinding the entire seed using a centrifugal mill. The milling chamber consists of the rotor and screen. Wedged shaped blades are located at the end of the rotor. The seeds are milled due to the impact by the blades 'throwing' the seed against a screen (Retsch, 2018). Shear and abrasion forces occur as the seed fragments are moved against the ring sieve. Thus, centrifugal mill uses the particle size reduction principle of impact, shear and abrasion forces. The ring sieve has openings or apertures. The size of the openings helps determines particle size of ground material. Centrifugal mill can grind any soft, medium-hard, brittle, and fibrous material (Carter, 2014). A vacuum system can be used to air cool the mill during milling.

<u>Disc mill</u>

Disc mills are used to dehull pulse grain but can also be used for the size reduction. Disc mills grind the sample between one vertical stationary and one rotating metal disc with sharp blades sometimes referred to as 'teeth'. Disc mills do not utilize screens. The particle size from the mill can be varied by adjusting the distance between the stationary and the rotating discs. Size reduction is due primarily to shear action between seed fragments and the teeth of the rotating disc.

Cyclone mill

Cyclone mill is designed for the rapid grinding of soft to medium-hard materials. High speed rotation of the impeller and air currents throw particles into and around the rough surface of the grinding ring. Particles remain in the grinding chamber until impact-shattering and abrasion make them small enough to flow through apertures of the screen covering the exit. The air flow removes the ground material out of the mill. The air flow also minimizes heating and therefore reduces the occurrence of thermal degradation.

Hammer mill

Milling chamber of the hammer mill contains a rotor with protruding metal strips, called hammers. Hammer mills grind by means of a high-speed rotating steel "hammer" and has a sieve in the periphery through which the sample leaves the grinding chamber. Sieves with different aperture sizes can be selected to generate the desired particle size distribution. Flat (blunt) or sharp (knife) edges of the hammers impart the impact force the grain which causes the grain to shatter. Impact force at the end of a hammer is four times the force at point halfway between the end of a hammer and the center of the shaft (Earle, 1987). This leads to more fine grinding at the end of a hammer than further down the shaft (Earle, 1987). Due to the force gradient, hammer mill can result in a wide range of particle size. Screen aperture size is important in determining the final ground particle size. According to Posner & Hibbs (2009) milling efficiency can be improved from 15 to 40% by the air flow induced from the inlet of the grinding chamber and vented at the discharged of the hammer mill. It also helps to remove the generated heat inside the mill.

Stone mill

In a stone mill, the grains are crushed between a horizontal stationary and a rotating stone and the broken material is pushed toward outside by spoke-like pattern grooves (Gray's Grist Mill, 2015; Inamdar & Prabhasankar, 2016). The gap between stationary and moving stones and grinding time affect the flour particle size. Fine particle size is achieved with the reduction in the gap and increased grinding time (Inamdar & Prabhasankar, 2016). Stone mill can have high-energy consumption (Özdemir, 2015). Due to the high temperature generation during grinding, Prabhasankar & Rao (2001) observed decreased total amino acid, free lipid content, unsaturated fatty acid content and high starch damage in wheat flour milled in stone mill compared to other mills.

Roller mill

The roller mill has the potential for mechanically removing the seed coat from the cotyledon of pulses as well as grinding them into whole pulse flour. Size reduction by a roller mill is done using a multiple stage approach. Roller mills contain cylindrical rolls that have different corrugation with a gap that can be adjusted. These cylindrical rolls are paired and spin in opposing direction. Based on the corrugation, rollers can crush the seed and remove the seed coat or break the seed into smaller particles.

The corrugated rolls are cut with a slight spiral to the roll axis. Increasing the spiral corrugation also increases the slicing action (Creason, 1975). Corrugations have sharp or dull angles that can have different roll configurations such as: sharp:sharp, sharp:dull, dull:sharp, or dull:dull. The roll configuration impacts shear and compression forces. For example, at the configurations progress from dull:dull to sharp:sharp, shear forces increase and compression forces decrease (Schorno, 2006). Seed coat can be removed using shear force while compression force is used to crush the seed into smaller particles. Each roll in a pair can rotate at the same speed or can rotate at different speeds (Posner & Hibbs, 2005). Differences in roll speed results in a shearing action that can aid in removing seed coat from the cotyledon. When material is drawn between the rolls having similar rotational speed, compression force is applied whereas, shearing forces result when roll-speed differential and roll corrugation are used (Posner & Hibbs, 2005; Schorno, 2006).

Effect of Grain Quality on Milling

There are no standard specifications or nomenclature describing the millability and milling performance of pulses. Grain quality parameters like foreign material content, dehulling efficiency, resistance-to-split, 100-seed weight, seed size, shape and hardness have a direct effect on milling yield and have been used to evaluate milling performance (Reichert et al., 1984; Ravi & Harte 2008; Thakur et al., 2019).

Seed size and shape

Seed size distribution through a stack of sieves, individual seed dimensions measured with Vernier calipers and various image analysis methods are currently use in characterizing seed size and shape (Harden & Wood, 2017). Uniform seed size is important in preventing over or under milling of grain, which affects flour particle size distribution. Roll gaps or disc gaps in the mills are adjusted based on the average seed size. Wide distribution in seed size would result in seeds smaller than average being under milled and seeds larger than average being over milled. A pre-sizing operation that segregates seed based on size can be attached to the milling process which would reduce the seed size variation when having a fixed mill setting (Watson et al., 1975).

Seed size can affect dehulling efficiency. Narasimha et al. (2003) has reported on the difficulty of dehulling small seeds. Different research groups have observed both positive and negative correlation between seed size and dehulling efficiency with different pulses (Singh et al., 1992; Black et al., 1998) indicating that dehulling characteristics are specific to the pulse type. Pulses can be spherical, cylindrical, pyramidal, flat oval or kidney shaped (Narasimha et al., 2003). Better dehulling efficiency has been observed in spherical seeds (Singh & Jambunathan, 1990), while flat seeds are difficult to dehull (Wood et al., 2012). High milling yields would be expected from round seeds, which would allow more seed surface to come in contact with the milling force.

Seed hardness

Reichert et al. (1984) reported a negative correlation between seed hardness and pulse milling efficiency. Milling yield can be decreased due to increased breakage and/or percentage powder when seeds are either too hard or too soft (Reichert et al., 1984). Lower dehulling yield has been reported with increased seed hardness (Reichert et al., 1984; Singh et al., 1992).

Seed composition

Chemical components like moisture, protein, and fiber can affect the milling quality of pulses. Some of these compositional variations can also lead to variations in the seed hardness. Moisture content affects the seed coat breakage, cotyledon strength and gum adhesiveness (Ehiwe et al., 1987; Vishwakarma et al., 2018). Erkinbaev et al. (2017) measured hardness of mung beans and showed that hardness index increased with increased moisture content up to 16%. Goyal et al. (2009) using pigeon pea and Wang (2005) using lentils both reported that seeds that are too dry or too moist can decrease dehulling yields due to increased breakage. Optimum moisture content for dehulling of pulses ranges between 8 and 11 % (Vishwakarma et al., 2018) and Goyal et al. (2008; 2009) showed that 10% moisture content was the best for dehulling with abrasion.

Dehulling can be affected by the quality and quantity of gums present between the seed coat and the cotyledon of pulses (Tiwari & Singh, 2012; Bravo et al., 1999). Gums are a network of cellulosic microfibrils that are embedded in protein and non-starch polysaccharide (NSP) network (Cosgrove, 1997). "Difficult to mill pulses" often have high contents of pectin, uronic acid and hexoses in between the seed coat and outer cotyledon region of (Ramakrishnaiah & Kurien, 1985; Wood et al., 2014). High lignin containing seed coats crack and separate easily from the cotyledon as lignin stiffens the cell walls and make seed coat stronger and brittle (Wood et al., 2014). High levels of minerals (calcium, magnesium, boron, and potassium) in the seed coat of "difficult to mill pulses" suggest the binding role of these minerals with pectin and other non-starch polysaccharides strengthen the cell wall.

Flour Quality

Particle size and shape

Methodical process of flour characterization is generally used when a new material is identified, when the existing substances are changed, or when two virtually identical materials behave differently (Olson, 2011). Flours can be characterized based on their particle size and particle shape (Mikli et al., 2001). Fine particles often are characterized by their particle size distribution as determined by various analytical instruments where particle shape is often ignored or disregarded (Olson, 2011). Both particle size and particle shape govern the technological properties of flours and their application in food systems (Tanguy et al., 1999).

Particle size of flours can be determined by different methods (sieve analysis, image analysis, and laser analysis). Particle size distribution depends on the nature of the grinding forces applied as well as the internal structure of the materials (Scanlon & Lamb, 1993, 1995; Vishwanathan & Subramanian, 2014). There is no official definition of pulse flour based on its particle size as Title 21 of the U.S. Code of Federal Regulations defines flour as a powder made from wheat grains where "not less than 98 percent of the flour passes through a cloth having openings not larger than those of woven wire cloth designated 212 µm (No. 70)". Across different studies, particle size of pulse flours in bakery applications has been reported as ranging from 17 µm up to 1,000 µm (Scanlon et al., 2018). This significant particle size variation hinders the comparisons of pulse flour functionality across different studies. Researchers have evaluated the effect of mill type on particle size distribution of pulse flours (Pelgrom et al., 2015; Maskus et al., 2016; Kaiser, 2019). Different mills and their specific mill settings produce a vast range of particle size distributions (Maskus et al., 2016; USA Pulses, 2020). It is important to establish specific standards for particle size, based on the desired end product. Particle size is also important in the efficacy of separating components like starch and protein (Pelgrom et al., 2015) and in mixing and hydration of ingredients. It is extremely difficult to compare milling effects on pulse flour functionality in studies where mill type and configuration differ.

Particle shape has an indirect effect on particle-size measurement. The size of a single particle can be defined by a single size parameter such as diameter or side length, only when that particle is a sphere or a cube (Saad et al., 2011). Several size parameters (length, width, thickness) are needed to define a particle size once particles depart from being a sphere. But for the simplicity, a shape factor, i.e., a combination of size parameters, can be used to define a particle. Although scientists have recognized the importance of morphology, application of image analysis to quantitatively characterize the shape of particles is not routinely done (Pons et al., 1999; Mikli et al., 2001). Improvement in available technology now allows for high speed and accurate image analysis (Mikli et al., 2001).

Starch damage

Starch damage is important to the functionality of pulse flours (Scanlon et al., 2018). Some studies have shown increased starch damage from high speed milling which creates small particle sizes (Kerr et al., 2000). Maskus et al. (2016) reported that significantly higher starch damage (2.8%) occurred with roller milling than with stone, hammer, or pin milling (1.2-1.5%). Sakhare et al. (2014) found high amount of damaged starch in the fine fractions of green gram (*Vigna radiata*). Gujska, et al. (1994) found higher starch damage for field pea compared to pinto and navy bean when milling with a hammer mill. A systematic comparison needs to be determined between different mills and mill settings on level of starch damage occur in pulse flour.

Flow properties

Flow properties of flour are important in moving of ingredients from the mill to storage and from storage to processing equipment. Proper flow is necessary to ensure mixing of desired proportion of ingredients during processing. Flow properties are governed by the flour physical properties (particle size and shape) and other external factors (moisture, temperature, flow material) (Abu-hardan & Hill, 2010; Jan et al., 2017).

Several research groups studied wheat flour flowability where they observed a decrease in flow and increase in cohesiveness of wheat flour with the reduction in particle size (Geldart et al., 2006; Landillon et al., 2008; Ambrose et al., 2015). Jan et al. (2017) observed that particle size greatly affected the rice flour flowability. In general, flour flowability decreases as the particle size becomes smaller. But sometimes similar sized particles have been found to have contrasting flowability. The reason for this might be due to differences in other flour properties like particle shape and chemistry.

In general, aspherical shape results in poor flour flowability due to intermittencies in flow rate (Fraige et al., 2008). Elongated or irregular particles tend to mechanically interlock or entangle with each other, thus obstructing flour flow and reducing flowability. Moisture content and lipid content have been shown to affect the flour flow properties (Dautant et al., 2007; de la Peña, 2014; Kaiser, 2019).

Protein Isolates

Pulse flour can be separated into pulse fiber, pulse starch, and pulse protein flours and concentrates (Farooq & Boye, 2011). The separation of these different components can be done by dry milling and air classification or by wet milling. The fractionation technique used affects the characteristics of the fractions (Boye et al., 2010b). Physical separation is energy efficient and results in high yields (Aguilera et al., 1982), whereas wet extractions have high product purity but have high energy requirements (Marki & Doxastakis, 2006).

Dry Separation

Dry fractionation consists of milling and air classification to separate the protein and starch fractions based on particle size and density (Pelgrom et al., 2013; Schutyser et al., 2015). Flours are first fractionated into starch and protein rich concentrates. Then starch fraction is re-milled and fractionated to give more starch and protein separation (Tyler, 1984).

The particle size distribution of flour has a significant role in fractionation. Pelgrom et al. (2015b) optimized the starch fractionation and protein fractionation in chickpea, pea and lentil by utilizing the differences in the particle size of starch (15 to 40 μm) and protein particles (~5 μm).

Air classification of pin-milled pinto bean (88% flour particles ≤ 44 μm) resulted in 80% being a starch-rich fraction (15 to 45 μm) and 20% being a protein-rich fraction (≤15 μm) (Simons et al., 2017).

Dry fractionation of pulses is affected by seed hardness. Pelgrom et al. (2015a) obtained higher protein content in the fine fraction from the lentil seeds which had a lower hardness compared to chickpea, pea and dry bean seeds.

Wet Extraction

Wet extraction is more labor intensive compared to the dry milling method and consists of several steps including soaking, homogenization, filtration, drying, grinding, and sieving (Hoover et al., 2010). The wet extraction processes include acid/alkaline extraction (isoelectric precipitation), ultrafiltration and salt extraction. In general, wet extraction results in protein concentrates and isolates at levels of 70% and 90% protein (or higher), respectively (Singhal et al., 2016).

<u>Acid/alkaline extraction-isoelectric precipitation</u>

Proteins are first dissolved under alkaline (alkaline extraction) or acidic (acid extraction) conditions, followed by a clarification step (centrifugation) and then precipitated by adjusting the pH to the isoelectric point of the protein (Han & Hamaker, 2002). Most dry bean proteins have acidic isoelectric points because of large amounts of glutamic and aspartic acid residues in their amino acid composition and they are efficiently solubilized by aqueous media at alkali pH (Sathe, 2002). Dry bean proteins typically have a bell-shaped curve protein solubility as a function of pH.

Ultrafiltration

Ultrafiltration method uses the particle size basis to separate proteins. Microfiltration can be used to separate particles larger than 0.1 μm, whereas ultrafiltration removes similar particles in the range of 0.001–0.02 μm (Koros et al., 1996). The protein content in concentrates obtained by the ultrafiltration method was found to be higher than in those obtained by isoelectric precipitation (Singhal et al., 2016). Boye et al. (2010b) obtained 83.9%, 88.6%, 82.7%, 76.5% and 68.5%, proteins from yellow pea, green lentil, red lentil, desi and kabuli chickpea, respectively, using ultrafiltration where isoelectric precipitation resulted in protein levels of 81.7%, 79.1%, 78.2%, 73.6% and 63.9%, respectively for the same legume crops. Ultrafiltration results in both globulins and albumins, whereas the isolates prepared by isoelectric precipitation method contain only globulins (Singhal et al., 2016).

Salt extraction

Salt extraction is a process where globulin proteins are separated from albumins on the basis of Osborne fractionation, where albumins are water soluble and globulins are soluble in dilute salt solutions (Boye et al., 2010a). At higher level of salt concentrations, the ions attract water molecules away from the surface of the proteins, protein-protein aggregation is favored due to hydrophobic interactions. Albumin aggregates continue to grow in size and number until they fall out of solution as a precipitate (Singhal et al., 2016). Globulins remain in solution.

Functional Properties of Dry Bean Proteins

In a food system, proteins have several functional properties such as solubility, water binding capacity, gelation, emulsification, and foaming (Malik et al., 2017). Hydration properties, surface properties and rheological properties influence the overall protein functionality of dry beans (Sathe, 2002).

Hydration properties include solubility, wettability, swelling, water absorption/adsorption, thickening and syneresis. The extent to which water and oil can be bound per gram of the protein material is referred to as water holding capacity and oil holding capacity, respectively (Boye et al., 2010a). The type and quantity of the protein as well as the presence of non-protein components in the preparation governs the water-holding capacity of dry bean proteins. Non-protein components like carbohydrates, increase the water holding capacity of bean protein extracts. Dry bean protein extracts typically can hold water 5 to 6 times of their own weight (Sathe, 2002). Water-holding capacity is partly dependent on the pH of the system where neutral or slightly alkali pH causes the proteins to have a net negative charge, while an acidic environment permits a net positive charge and this net charge determines the way that protein interacts with water (Sathe, 2002). Most dry bean proteins exhibit a wide range of the oil holding capacity where most can hold < 5 g oil/g. Based on oil holding capacity, dry bean materials can be selected to prepare fried products such as “dosa”, “vada” (Indian ethnic foods) as to provide a crisp, crunchy texture. Both protein and carbohydrates contribute to the overall texture of these products (Sathe, 2002).

Protein surface properties include emulsion, foam and film formation. Both foam and emulsion properties of proteins partly depend on protein molecular size, protein surface charge, pH of the medium, protein molecular flexibility, protein migration rate from bulk phase to the interface, degree of protein denaturation, relative proportions of immiscible phases, ability of the protein to form a “film” at the interface, and the protein solubility in the bulk phase (Mune & Sogi, 2016). Therefore, a large variability exists in foam and emulsion capacity of dry bean proteins (Sathe, 2002; Singhal et al., 2016).

Gelation, elasticity, grittiness, cohesiveness, chewiness, aggregation, stickiness, viscosity, texturization and adhesion can be classified under rheological properties. Heat-induced gelation is the most common type of gelation for most plant proteins (Yang, 2017). Protein gelation happens via protein denaturation, protein strand network formation, protein-protein and protein-non-protein component interactions, and gel setting. Type and source of protein, protein concentration, pH, ionic strength, temperature, presence of different proteins, presence of non-protein components and mechanical parameters affect the gel forming mechanism. Specific characteristics of dry bean protein gels allow them to be used in steamed pudding type products that have porous and spongy texture (Sathe, 2002).

Protein and Phenolic Interactions

Proteins can cause meaningful interactions individually and in combination with other molecules according to the changes in the processing environments (e.g. changes in pH and ionic strength) and composition, which can lead to diversified functional properties (Boye et al., 2010a). Phenol is a class of compounds that characteristically possess a significant binding affinity for proteins, which can lead to the formation of soluble and insoluble protein–phenol complexes (Papadopoulou & Frazier, 2004). Phenolic compounds are known to form complexes with proteins leading to changes in the structural, functional and nutritional properties of both compounds (Papadopoulou & Frazier, 2004).

Proteins have several functional properties in a food system such as solubility, water binding capacity, gelation, emulsification, and foaming, where solubility is the pre-requisite for other functional properties (Malik et al., 2017). Crosslinking between phenolic compounds and proteins, changes the net charge on the surface of protein molecule and lead to change the solubility of proteins (Prigent et al., 2003). Prigent et al. (2003) and Relkin & Shukat (2012) observed low solubility of proteins at the presence of phenols.

Binding of phenolic compounds to proteins can block some amino acids from reactions (Rawel et al., 2002; Rohn et al., 2006). Protein structure can change when phenolic compounds bind to hydrophobic sites of proteins as only weak hydrophobic sites could remain on the surface of the protein (Yuksel et al., 2010). Conversely, bio-accessibility and activity of phenolics can be influenced by phenolic complexation with proteins (Rawel et al., 2002; Sęczyk et al., 2019). These interactions can change the secondary and tertiary structure of proteins, and thereby influence the surface properties of molecules, and as a result, the functional properties like emulsification, foaming and gelation are affected (Malik et al., 2017).

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CHAPTER 2: EFFECTS OF MILLING METHOD ON THE PHYSICAL, CHEMICAL, AND FLOW PROPERTIES OF WHOLE BLACK BEAN FLOUR

Abstract

Dry bean milling has attracted interest due to the increased need for non-wheat ingredients available for food applications. The objective of this study was to examine the physical, chemical, and flow properties of whole black bean flour produced using different laboratory mills and mill settings. Flour from mills which utilized screens (cyclone mill, centrifugal mill, and hammer mill) showed significantly different characteristics than flour from mills without screen (disc mill and stone mill). Hammer mill, cyclone mill, and centrifugal mill 10,000/12,000 rpm-250 µm screen aperture settings had uni-modal distribution skewed toward fine particle size. Disc mill-coarse and stone mill-coarse settings had uni-modal distribution skewed toward coarse particle size. Disc mill-fine and stone mill-medium setting had bimodal particle size distribution curves, with an increase in coarse particles (600 µm) and an increase in medium size (250 µm) particles. Centrifugal mill (10,000 and 12,000 rpm with 500/1000 µm screen aperture) and stone mill-fine setting exhibited similar particle size distributions having bimodal distribution with peaks at fine (50 µm) and medium (250 µm) particle sizes. Geometric mean size of the flour was negatively correlated with starch damage (r = -0.92), L* (r = -0.94), angle of repose (r = -0.94), and angle of slide (r = -0.80 to -0.90) and positively correlated with moisture (r = 0.72), and loose bulk density (r = 0.72). Fine flour resulted in less circular and more elongated particles. Fine flour had difficulty in flowing. Milling method significantly affected ($P < 0.05$) several physical, chemical and flow properties of whole black bean flour. These findings will help food processors to process black bean ingredients using different mill settings to achieve different functionalities depending on the consumer requirements.

Introduction

Dry bean utilization by the food industry can be increased by developing value-added processing applications. Several studies have explored the utilization of dry bean flour in traditional products, such as bread, pasta, and snacks (Aguilera et al., 1982; Han et al., 2010; Hooper et al., 2019). The trend of incorporating non-wheat flours into food products has been driven by the increased demand for nutrient dense foods.

Centrifugal mill, disc mill, and hammer mill have been used to produce dry bean flour commercially and for research (Carter, 2014; Ardent mills, 2019). Different types of mills employ different size reduction forces to produce a flour. Primary size reduction forces applied during milling include impact, cutting, compression, abrasion, and shear. The effectiveness of a mill is determined by its ability to apply sufficient force to fracture or cut the seed into small particles. Within each mill type there are variations in how forces are applied to the grain.

Centrifugal mill can grind any soft, medium-hard, brittle, and fibrous material (Carter, 2014). The milling chamber of a centrifugal mill contains a horizontal rotor and a ring sieve. Wedged shaped blades are located at the end of the rotor. The seeds are milled due to impact by the blades 'throwing' the seed against a screen (Restch, 2018). Particle size can be altered by adjusting the rotational speed of the blade, number of blades on the rotor, and aperture size of the screen. Shear and abrasion forces occur as the seed fragments are moved against the ring sieve.

Cyclone mill, a variant of the centrifugal mill, is designed for the rapid grinding of soft to medium-hard materials. Cyclone mill has a horizontal impeller and a rough outer ring. High speed rotation of the impeller and air currents throw particles into and along the rough surface of the grinding ring. Particles remain in the grinding chamber until impact-shattering and abrasion make them small enough to flow through holes in the screen and out the exit with the air current.

Hammer mill utilizes a vertical rotor. Blades (hammers) are connected to the rotor in a staggered orientation. The impact surface of the blade can be blunt or beveled often referred to a knife surface. Grinding occurs by means of a high-speed rotating steel "hammer" and has a sieve in the periphery through which the sample leaves the grinding chamber. Rotational speed of the hammers and aperture size of the sieves can be selected to generate different particle size distributions.

Stone mill has a stationary and moving horizontal stone where the grains are crushed between them. Then the ground material is pushed outward along spoke-like grooves (Gray's Grist Mill, 2015). Particle size can be altered by adjusting the distance between the two stones and by regulating the speed of the rotating stone. Disc mill is a variant of the stone mill where the sample is ground between one stationary and one rotating metal disc with blades. In a disc mill, the discs are in a vertical position. The particle size from the mill can be varied by adjusting the speed of the rotating disc and the distance between the stationary and the rotating disc. Size reduction is due primarily to shear action between seed fragments and the blades of the rotating disc.

Most energy used during milling is converted to heat. Heat development during grinding is a disadvantage. For all mills, a vacuum system can be used to draw air through the milling chamber which will reduce the temperature increase during milling.

Flow properties of flour is important in moving ingredients from the mill to storage and from storage to processing equipment. Proper flow is necessary to ensure mixing of desired proportion of ingredients during processing. Flow properties are governed by the flour's physical properties (particle size and shape) and other external factors (moisture, temperature, flow material) (Abu-hardan & Hill, 2010; Jan et al., 2017). Flow properties are evaluated by determining the angle of repose and angle of slide. Angle of repose is the slope of a heap of flour on a given horizontal surface when dropped onto the surface under controlled conditions. Angle of slide is the minimum slope (relative to horizontal) required for a powder to flow under its own weight. This measurement can be used in the design of bins for grain and flour storage and hoppers for conveyance (Ambrose et al., 2015). Limited literature is available on flow properties of dry bean flour including angle of repose and angle of slide.

Particle size and particle shape are internal flour properties that affect the flour flowability. Several research groups studied wheat flour flowability where they observed a decrease in flow and increase in cohesiveness of wheat flour with the reduction in particle size (Geldart et al., 2006; Landillon et al., 2008; Ambrose et al., 2015). Jan et al. (2017) observed that particle size greatly affected the rice flour flowability. In general, flour flowability decreases as the particle size becomes smaller. But sometimes similar sized particles have been found to have contrasting flowability. The reason for this might be due to differences in other flour properties like particle shape and chemistry. Moisture content and lipid content have been shown to affect the flour flow properties (Dautant et al., 2007; de la Peña, 2014; Kaiser, 2019).

It has been shown both experimentally and computationally that particle shape has a significant effect on flour behavior (Roberts & Beddow 1968; Liu & Litster 1991; Wu & Cocks 2006). In general, aspherical shape results in poor flour flowability due to intermittencies in flow rate (Fraige et al., 2008). Elongated or irregular particles tend to mechanically interlock or entangle with each other, thus reducing flowability. Although scientists have recognized the importance of morphology, application of image analysis to quantitatively characterize the shape of particles is not routinely done (Pons et al., 1999; Mikli et al., 2001).

Dry bean milling has attracted interest due to the increased need for non-wheat ingredients available for food applications. However, dry bean milling has not been studied extensively. No studies have been done that compare physico-chemical and flow properties of black bean flours produced by different laboratory mills. Therefore, the objective of this study was to determine the effect of different laboratory type mill settings on the physico-chemical and flow properties of whole black bean flour.

Materials and Methods

Black Beans

Black beans were obtained from three dry bean companies. Seeds from each company were kept separate and treated as replications in the ensuing experiments. The black bean samples used in this experiment were typical of black beans grown in ND, USA having an average protein content of 21.8 %, test weight of 78.86 kg/hL (63.1 lb/bu), 100-seed weight of 19.7g, and average fracture hardness of 162 N.

Milling Methods

A laboratory-type cyclone mill (Direct drive model 3010-014, Colorado, USA), hammer mill (Perten LM 3100, Hägersten, Sweden), stone mill (KoMo classic grain mill, Penningberg, Austria), disc mill (Perten LM 3310, Hägersten, Sweden), and centrifugal mill (Centrifugal ZM 200, Haan, Germany) were used to mill 1 kg samples of black bean seeds. Feed rate was set for 40 g/min for all the mills. Mill room temperature and relative humidity were 25±2 °C and 20±5 %, respectively.

Physical and Chemical Flour Properties

Particle size distribution

Particle size distribution of black bean flours was determined using a vibratory shaker (Centrifugal AS 200 sieve shaker; Centrifugal International, Haan, Germany) configured with a stack of seven sieves and a vibratory amplitude displacement of 3 mm for 15 sec intervals for 5 min. Sieves used were 50, 100, 150, 250, 425, 500, and 600 µm.

Geometric mean size was calculated by using following equation.

$$d_{gw} = \log^{-1}\left[\frac{\sum_{i=1}^{n}\left(W_i \log \overline{d_i}\right)}{\sum_{i=1}^{n} W_i}\right]$$

Where *dgw* is the geometric mean diameter of the particles by mass (mm), *Wi* is the mass on the *i*th sieve (g), *n* is the number of sieves, and *di* is the nominal sieve aperture size of the *i*th sieve (mm).

D values were calculated by using the cumulative percent retained graphs for each mill setting. For example, the D_{50} would be the maximum particle diameter below which 50% of the sample exists (Figure 1). If the finest size does not correspond to 0 percent finer, or the coarsest grain size does not correspond to 100 percent finer, the missing sizes (D_0 and D_{100}) are computed by interpolation.

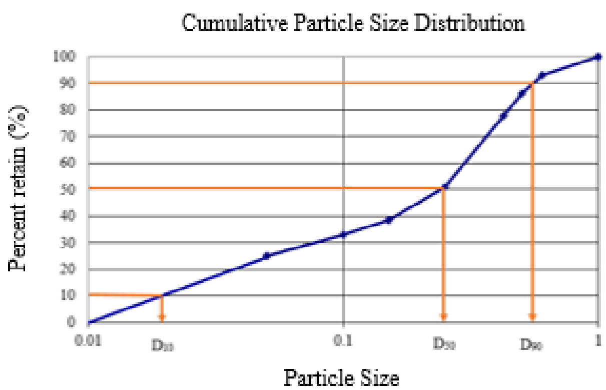

Figure 1. Calculation of D values

Particle shape

Particle imaging was done under 30 X magnification using OMNI Core Digital Microscope and Measurement System (Unitron, NY, USA) for each sieved fraction. Flour samples were dispersed onto microscope glass slides and applied dispersion conditions were optimized in order to obtain well separated particles before image acquisition. After particle dispersion, glass slides were positioned back under a digital microscope for observation.

Image analysis was carried out by Image Pro Premier 3D software (Media Cybernetics, MD, USA). Initially, images were pretreated with particle's border killing (removing particles that touch the border of the image), particle's silhouette hole filling (filling the holes within particle silhouette), separation (breaks narrow isthmuses and separates touching particles), and morphological cleaning. Calibration was done to translate pixel unit of the particle's silhouette into metric units and the silhouette dimensions were measured. Finally, shape factors were calculated by the Image Pro Premier 3D software.

Meso shape descriptors were defined by comparison with some referential shapes for elongation, circularity, compactness, and convexity. Values were normalized either between 0 and 1 (*e.g.* circularity, compactness and convexity) or between 1 and positive infinity (*e.g.* elongation). Initially, all the shape factors were measured for the flour fractions and later a weighted average value was calculated for the bulk flour samples.

Color

Flour fractions were evaluated for color (CIE L*, *a**, *b** values) using a Minolta 410 colorimeter (Konica Minolta Sensing Americas, Inc, NJ, USA). Flour was placed in a round black measurement cell (6 cm diameter x 2 cm deep) with a quartz glass window.

Moisture

Moisture content was determined according to AACC International Approved Method 44-15.02.

Starch damage

Starch damage was determined according to AACC International Approved Method 76-31.01.

Flow Properties

Bulk density

Bulk density was measured using Okaka & Potter (1997) method with modifications. Black bean flour (100 g) was measured into a calibrated measuring cylinder. For loose bulk density, the loose volume (mL) was recorded. The loose bulk density was calculated as the ratio of the weight of the sample to its loose volume. Then, the bottom of the measuring cylinder was tapped at a constant speed for 250 times at about 120 taps/min. The compact bulk density was calculated as the ratio of the weight of the sample to its compact volume.

Hausner Ratio was calculated by dividing the “loose volume” by the “compact volume”. Compressibility Index was calculated by 100 x (loose volume-compact volume)/loose volume.

Angle of repose

Angle of repose of flour (200 g) was determined as described by de la Peña (2014) using a stainless-steel cylinder (13.5 x 7.8 cm) placed on top of a stainless steel surface. The cylinder was then quickly lifted vertically to spread flour into a conical heap. The height and diameter of the conical heap were measured using a ruler. The poured angle of repose was calculated using the following equation:

$$\alpha = \tan^{-1}(h/\mathrm{r})$$

Where *h* is the height of the heap of material and r is the averaged radius of the heap obtained by recording the two diameters perpendicular from each other.

Angle of slide

Angle of slide was determined by as described by de la Peña (2014). The cylinder used in angle of repose was placed on the center of a sheet whose slope can be gradually increased using a manual screw connected platform. Flour (200 g) was poured into the cylinder. The cylinder was vertically lifted to make a conical heap. The slope of the platform was slowly increased until ≈ 75% of the material slid down. The angle formed between the slope of the platform and the horizontal base was measured using a protractor. Effect of the platform surface on the angle of slide was determined using surfaces made from aluminum, stainless steel, polyvinyl chloride (PVC) and polypropylene (PP).

Statistical Analysis

The experimental design was a randomized complete block except for the angle of slide. For the angle of slide, the experimental design was a randomized complete block with split plot arrangement where whole plot was milling method and sub plot was sliding surface material. Each treatment was replicated three times. Data were analyzed using SAS 9.4 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 $P=0.05$. Pearson correlations were computed between geometric mean of the flour and physico-chemical and flow properties.

Results and Discussion

Physical and Chemical Properties

Geometric means, D values (D_{10}, D_{50} & D_{90}) which are the intercepts for 10, 50 and 90% of the cumulative mass and range in particle size are summarized in the Table 1. Variation in milling method produced flour particles with significantly different sizes and size distributions. Centrifugal mill (10,000/12,000 rpm-250/500 µm screen aperture settings), cyclone mill and hammer mill produced the smallest geometric mean particles with no significant difference among the mills. These mills utilized high speed rotors (10,000-16,000 rpm) and a screen that kept the materials inside the milling chamber until they were small enough to pass through the screen apertures. Range, an indicator of particle size distribution, was calculated by using the equation: Range = (D_{90} – D_{10}). A large range value indicates a wide size distribution. Centrifugal-10,000 rpm/250 µm aperture and 12,000 rpm/250 µm aperture settings produced the narrowest range in particle size, 89 and 82 µm, respectively.

Geometric mean for mills utilizing a screen was substantially less than their aperture size. For example, the geometric mean size for flour produced by the hammer mill was 71 µm even though the screen aperture size was 800 µm. The functional aperture size would be smaller than the actual sieve aperture size, since particles in the milling chamber would interact with the screen at an angle which would reduce the apparent size of the opening. The smaller available space to pass through the screen would restrict movement of particles and increase the time the particles stayed in the milling chamber. Particles 'trapped' in the milling chamber were subjected to continued collisions with the rotors and with other particles which resulted in reduced particle size. When the particles hit the screen opening at an angle other than being perpendicular, that particle can break at the screen opening into several pieces, where small particles can pass through the opening and large particles remain in the chamber for further milling.

Table 1. Effect of milling method on particle size distribution[a]

Milling method	**Mill surface temperature (°C)**	**Geometric mean size**	**D_{10}**	**D_{50}**	**D_{90}**	**Range**
		(µm)				
Disc mill-Coarse-Gap 6	29 h	508 a	105 a	704 a	932 a	827 a
Disc mill-Fine-Gap 3	31 g	355 b	61 b	507 b	882 b	820 a
Stone mill-Fine	43 a	204 d	55 bc	264 c	506 d	451 d
Stone mill-Medium	40 b	301 c	45 b-d	475 b	818 c	773 b
Stone mill-Coarse	37 c	484 a	89 a	693 a	929 ab	840 a
Hammer mill 16,800 rpm – 800 µm	33 f	71 f	29 d-f	64 e	214 g	185 g
Cyclone mill 16,000 rpm – 500 µm	35 d	71 f	35 d-f	64 e	188 g	153 g
Centrifugal-10,000 rpm-250 µm	33 f	64 f	35 d-f	60 e	124 h	89 h
Centrifugal-10,000 rpm-500 µm	28 i	76 f	18 f	73 e	329 f	311 e
Centrifugal-10,000 rpm-1000 µm	26 k	153 e	23 ef	237 c	541 d	517 c
Centrifugal-12,000 rpm-250 µm	34 e	61 f	33 d-f	59 e	115 h	82 h
Centrifugal-12,000 rpm-500 µm	29 h	74 f	22 ef	70 e	289 f	267 f
Centrifugal-12,000 rpm-1000 µm	27 j	142 e	38 c-e	159 d	457 e	419 d

[a]Different lowercase letters across column indicates significant differences between milling method (P=0.05)

Disc mill and stone mill did not utilize a screen and produced larger particles than did the mills that utilized a screen (Table 1). It was observed that the time in the milling chamber of the disc mill or stone mill was less than that with mills that had screens. The lack of screen contributed in part to the large particle size of flour produced by these mills. The gap between the stationary and rotating disc or stone also affected particle size, with the smaller gap producing a finer particle size. In the disc mill, the materials were gravity fed and flour was gravity released. The time materials remained inside the milling chamber of the disc mill is comparatively short. The stone mill is also gravity fed but the ground material released through grooves in the horizontal stone via the centrifugal force.

Particle size distribution curves for the black bean flour (Figure 2) revealed that both disc mill-coarse setting (gap 6) and stone mill-coarse setting had uni-modal distribution skewed toward large particles (600 µm) over a narrow range of particle sizes. Similarly, disc mill-fine setting (gap 3) and stone mill-medium gap had similar bimodal particle size distribution curves, with an increase in coarse particles (600 µm) and an increase in medium size (250 µm) particles compared to the wide gap settings. Hammer mill, cyclone mill and centrifugal mill (10,000 and 12,000 rpm with 250 µm screen aperture) had similar uni-modal particle size distribution curves that were skewed toward fine particles (50 µm) over a narrow range of particle sizes. Centrifugal mill (10,000 and 12,000 rpm with 500/1000 µm screen aperture) and stone mill-fine setting exhibited similar particle size distributions having bimodal distribution with peaks at fine (50 µm) and medium (250 µm) particle sizes. These results indicate that mill type and settings determined particle size distribution and that depending on the settings, different mills can produce similar particle size distributions.

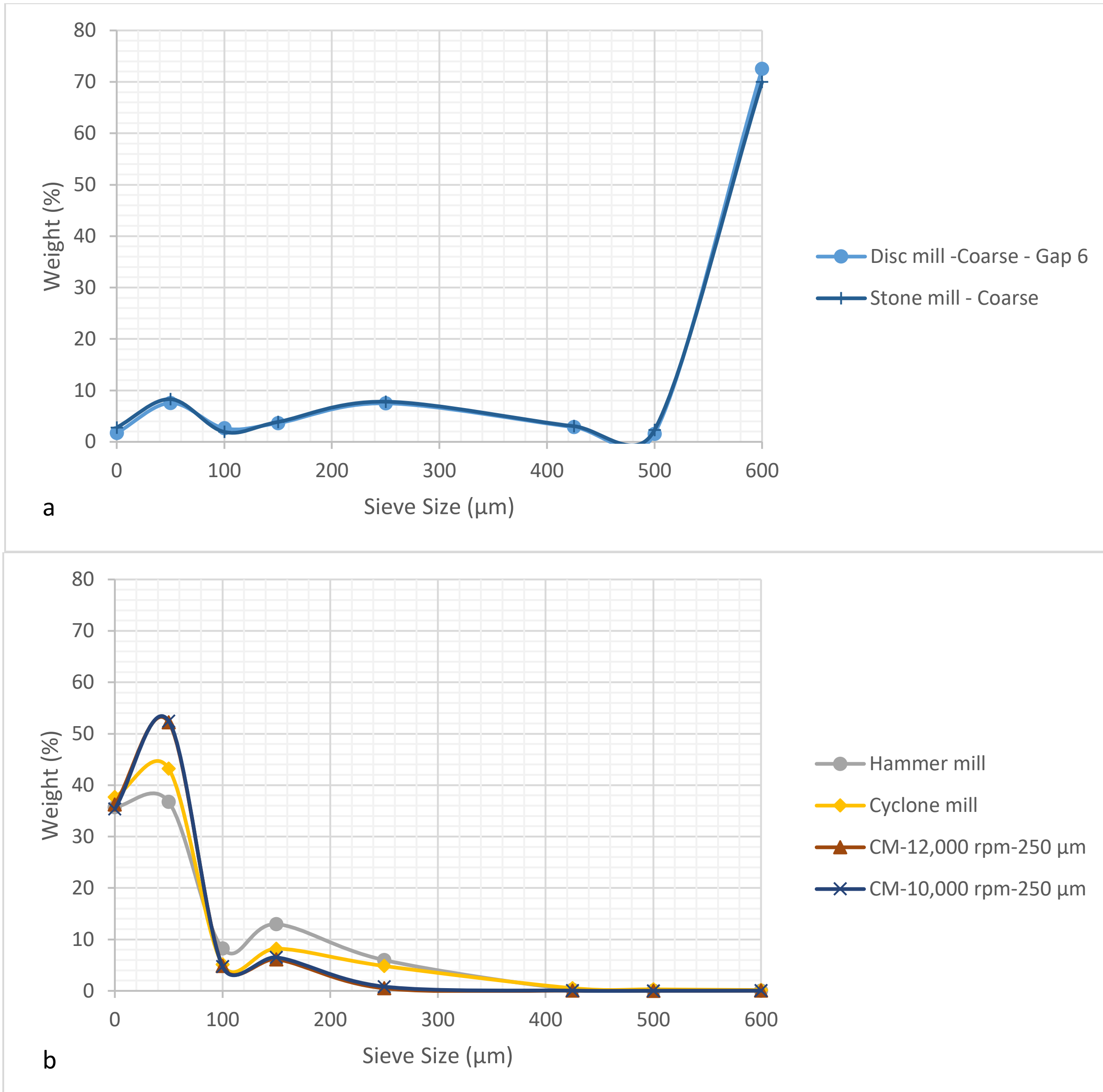

Figure 2. Particle size distribution curves (a: disc mill-coarse, gap 6 and stone mill-coarse; b: hammer mill, cyclone mill, centrifugal mill-12,000rpm-250µm and centrifugal mill-10,000rpm-250µm; c: disc mill-fine, gap 3 and stone mill-medium; d: stone mill-fine, centrifugal mill-12,000rpm-500µm, centrifugal mil-12,000rpm-1000µm, centrifugal mil-10,000rpm-500µm, centrifugal mil-10,000rpm-1000µm)

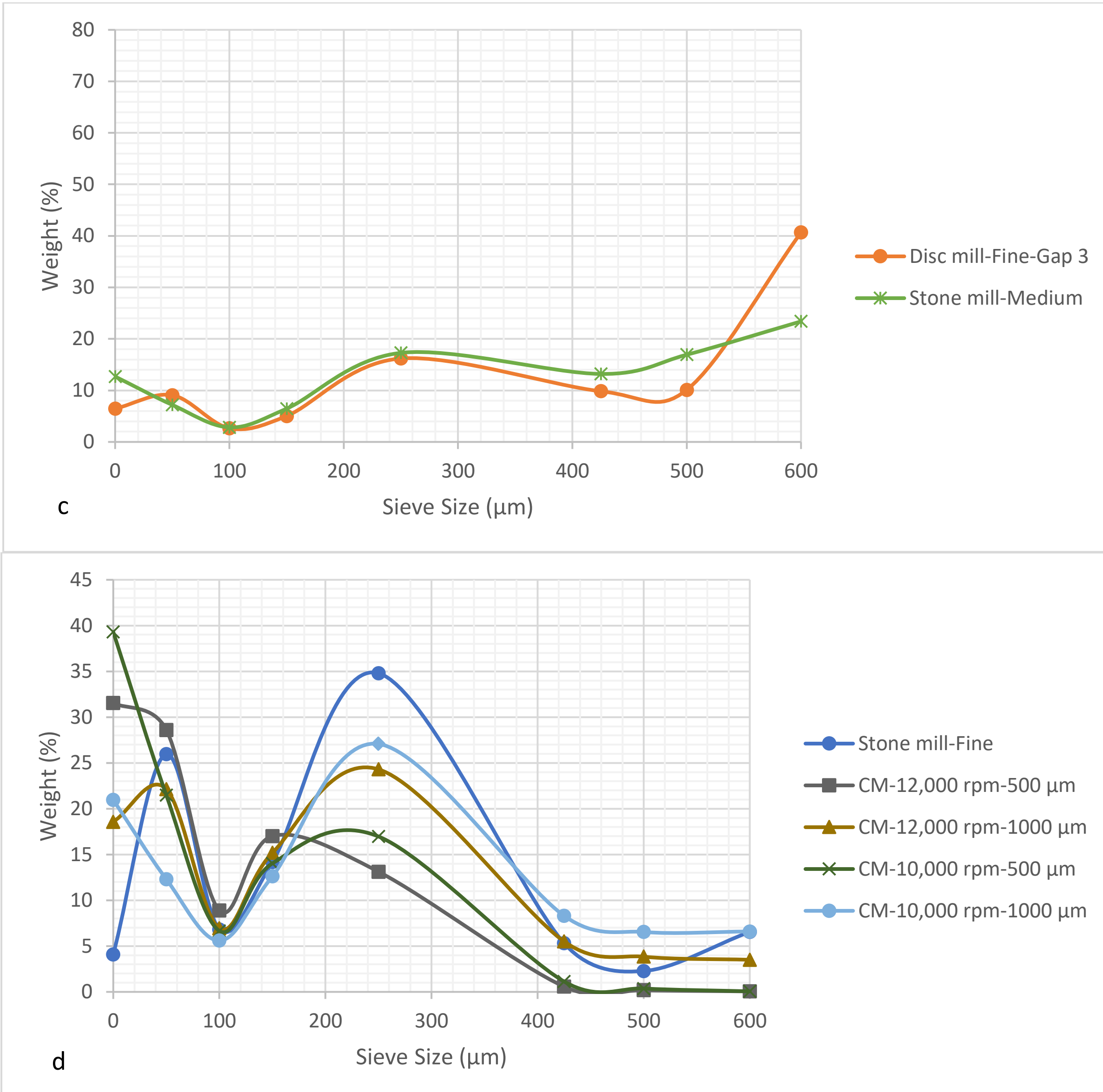

Figure 2. Particle size distribution curves (continued) (a: disc mill-coarse, gap 6 and stone mill-coarse; b: hammer mill, cyclone mill, centrifugal mill-12,000rpm-250µm and centrifugal mill-10,000rpm-250µm; c: disc mill-fine, gap 3 and stone mill-medium; d: stone mill-fine, centrifugal mill-12,000rpm-500µm, centrifugal mil-12,000rpm-1000µm, centrifugal mil-10,000rpm-500µm, centrifugal mil-10,000rpm-1000µm)

Particle size and particle size distribution are important in determining flour properties (Abu-hardan & Hill, 2010). Particle size is also important in the efficacy of separating components like starch and protein (Pelgrom et al., 2015). Different sieve aperture sizes, geometric mean, particle diameter or volumetric diameter have been used by researchers to define the particle size of various flours (Thakur et al., 2019).

Few studies have been done to compare the particle size with respect to different milling methods. Mills that lack a screen can still produce fine particles if the rotor speeds are high enough. High rotor speeds will result in more particle-rotor collisions than moderate or slow rotor speeds. Maskus et al. (2016) reported that pin milled (Hosokawa Alpine mill, 20,000 rpm, no screen) yellow pea flour had smaller particle sizes as compared to flours that were milled with a stone mill, hammer mill, or roller-mill. In another study on cowpea milling, Kerr et al. (2000) examined the effect of screen sizes on the distribution of particle sizes when using a knife mill. When a 500 μm aperture screen was used, the majority of particles (70%) were retained on the smaller sieves (less than 105 μm), while a 2,000 μm aperture screen produced flour with only 10% of particles less than 105 μm. Similar results were noted in this experiment, where 70-88% particles were retained on sieves with small aperture (less than 100 μm) when the cyclone mill (16,000 rpm) and the centrifugal mill (10,000 or 12,000 rpm) used 500 μm aperture screen in the milling chamber. Centrifugal mill (10,000 or 12,000 rpm) with 1,000 μm aperture screen produced a flour where 60% of particles were less than 100 μm.

Particle shape of the bulk sample was calculated based on the shape factor values recorded for the flour fractions (data presented in Chapter 3). Shape factors were evaluated for circularity, compactness, convexity, and elongation after visualization and segmentation of two-dimensional projection on a plane surface of the three-dimensional particle shape (Saad et al., 2011). A direct shape factor value could not be measured for the bulk sample due to the wide range of particle sizes, where single magnification cannot be assigned for the image analyzing microscope.

Compared to other mills, stone mill-fine setting generally produced flour with the most elongation and least circularity, convexity and compactness (Table 2). These values indicate that this flour has particles which are more elongated with rough edges and irregular boundaries. When comparing the particle shape of flour produced on the stone mill, circularity and compactness decreased as gap decreased, convexity was not affected but elongation increased with decline in stone gap or increase in fine particle size. When comparing the mills and settings that gave similar particle size distribution curves, the disc mill-coarse and stone mill-coarse produced similar particle shapes for circularity, compactness, convexity and elongation. Disc mill-fine and stone mill-medium produced similar circularity, convexity and elongation. Convexity was greater for flour produced with mills that had a screen compared to those without a screen. Particle shape values (compactness, convexity, elongation) for flour from centrifugal mill (10,000 or 12,000 rpm with 1,000 µm screen aperture settings) were similar whereas flour from the stone mill-fine had significantly different shape values than flour from centrifugal mill settings. Compared to centrifugal mill particle shape, particles from stone mill had lower circularity, compactness, and convexity and greater elongation. Hammer mill and cyclone mill flour particle shapes were similar. Centrifugal mill (10,000 or 12,000 rpm with 250 µm aperture screen) had similar shape and were less circular and compact than flour particles from hammer mill and cyclone mill. Overall, there were no correlations noted between the average shape factor values and bulk flour properties.

Table 2. Particle shape values[a] for bulk whole bean flour

Milling method	**Circularity**	**Compactness**	**Convexity**	**Elongation**
Disc mill-Coarse-Gap 6	0.605 bc	0.660 ab	0.485 cd	1.382 bc
Disc mill-Fine-Gap 3	0.579 de	0.637 b-d	0.486 cd	1.454 ab
Stone mill-Fine	0.550 f	0.602 e	0.484 d	1.513 a
Stone mill-Medium	0.578 de	0.652 a-c	0.487 cd	1.456 ab
Stone mill-Coarse	0.605 bc	0.664 a	0.487 cd	1.376 c
Hammer mill 16,800 rpm – 800 µm	0.596 cd	0.639 a-c	0.493 a	1.376 c
Cyclone mill 16,000 rpm – 500 µm	0.603 bc	0.657 a-c	0.491 ab	1.359 c
Centrifugal-10,000 rpm-250 µm	0.561 ef	0.612 ed	0.491 ab	1.385 bc
Centrifugal-10,000 rpm-500 µm	0.619 ab	0.643 a-c	0.490 ab	1.395 bc
Centrifugal-10,000 rpm-1000 µm	0.636 a	0.639 a-c	0.491 ab	1.350 c
Centrifugal-12,000 rpm-250 µm	0.582 de	0.633 cd	0.493 a	1.372 c
Centrifugal-12,000 rpm-500 µm	0.612 bc	0.653 a-c	0.491 ab	1.389 bc
Centrifugal-12,000 rpm-1000 µm	0.604 bc	0.649 a-c	0.488 bc	1.395 bc

[a]Different lowercase letters across column indicates significant differences between milling method (P=0.05)

Final flour temperature was greatest with stone mill and increased as gap between stones decreased (Table 3). Flour temperature was similar when milled with centrifugal mill at 10,000 or 12,000 rpm but for both rotor speeds the temperature increased as screen aperture size decreased from 1000 to 250 µm. High rotor speeds associated with the hammer mill and cyclone mill resulted in higher temperature than their corresponding centrifugal mill configuration indicating that high rotor speeds can contribute to heat buildup during milling. High temperatures associated with milling can be a concern for maintaining protein functionality and preventing lipid oxidation. Typically, black beans contain 0.2-0.5 % lipid and 20-25% protein (Fernando, 2017). Overall all mills and configurations, the flour temperature did not exceed 36 °C which is well below the temperature needed to cause protein denaturation. It is uncertain as to the consequence of short duration of exposure to 36 °C on lipid stability of black beans considering that black beans generally contain only 0.2-0.5% lipid.

Table 3. Effect of milling method on color, moisture and starch damage[a]

Milling method	**Final flour temperature (°C)**	**Color**			**Moisture %**	**Starch damage %**
		L*	**a***	**b***		
Disc mill-Coarse-Gap 6	26 h	73.09 f	0.61 ab	6.45 a	13.8 a	0.07 l
Disc mill-Fine-Gap 3	27 g	73.54 f	0.65 a	6.49 a	13.4 ab	0.13 i
Stone mill-Fine	36 a	78.04 b-d	0.29 f	6.49 a	13.4 a-c	0.15 h
Stone mill-Medium	34 b	77.29 d	0.40 e	6.61 a	12.9 cd	0.11 j
Stone mill-Coarse	32 c	74.90 e	0.54 bc	6.55 a	13.3 bc	0.09 k
Hammer mill 16,800 rpm – 800 µm	28 f	78.69 a-c	0.57 bc	4.46 d	12.7 de	0.43 a
Cyclone mill 16,000 rpm – 500 µm	29 e	79.17 a-c	0.57 bc	3.41 e	11.1 g	0.43 a
Centrifugal-10,000 rpm-250 µm	32 c	79.78 a	0.51 cd	3.34 ef	9.6 h	0.39 c
Centrifugal-10,000 rpm-500 µm	27 g	79.21 ab	0.46 de	4.85 c	11.8 f	0.37 e
Centrifugal-10,000 rpm-1000 µm	23 i	78.01 b-d	0.42 e	5.93 b	12.5 e	0.31 g
Centrifugal-12,000 rpm-250 µm	30 d	79.52 a	0.53 c	3.22 f	9.8 h	0.40 b
Centrifugal-12,000 rpm-500 µm	26 h	79.42 a	0.45 de	4.88 c	11.9 f	0.37 d
Centrifugal-12,000 rpm-1000 µm	23 i	77.88 cd	0.42 e	5.82 b	12.7 de	0.33 f

[a]Different lowercase letters across column indicates significant differences between milling method (P=0.05)

Flour moisture content varied with milling method (Table 3). Flour moisture was greater when milled on mills that did not use a screen compared to mills with a screen. Furthermore, moisture content declined as screen aperture size declined, regardless of type of mill. For example, flour moisture was 12.5-12.7% with 800-1000 µm, 11.8-11.1% with 500 µm and 9.6-9.8% with 250 µm screen aperture. Loss of moisture would be a consequence of heat generated and total exposed surface area of flour. Flour temperature increased and particle size decreased with decrease in aperture size (Table 1 and 3). When the geometric mean size is small the total surface area of the flour increase and more moisture can lose. Geometric mean size of the particle of black bean flour and the flour moisture had an $r = 0.72$ positive relationship (Table 4).

Table 4. Correlations between geometric mean size and black bean flour attributes

Attribute	**Correlation coefficient (r)**
Starch damage	- 0.92
Moisture	0.72
L*	- 0.94
b*	0.77
Loose bulk density	0.72
Hausner Ratio	- 0.86
Compressibility Index	- 0.87
Angle of repose	- 0.94
Angle of slide	
Stainless Steel	- 0.80
Aluminum	- 0.81
Polyvinyl Chloride	- 0.83
Polypropylene	- 0.90

Color differences were observed among flours produced at different mill settings. CM-10,000 rpm-250 µm had the highest L* (lightness) value and the b* (yellowness) values respectively (Table 3). Changes in flour lightness could be explained in simpler terms by the differences in sample particle sizes due to the various mill settings. Lightness was higher and yellowness was lower with flours that had small geometric mean particle sizes (Table 3). Liu (2009) and Ahmed et al. (2016) observed a similar relationship with flour lightness and yellowness with particle size in corn and lentil flours respectively.

Black beans typically have 35-40% starch. In this study, the grinding method had a significant effect on the starch damage (Table 3). Hammer mill and cyclone mill had the highest starch damage where disc mill coarse (Gap 6) had the lowest starch damage. However, overall starch damage was less than 0.5% regardless of mill or mill setting. In this study, starch damage of centrifugal mill was significantly increased when the rotating speed increased, and screen size decreased. Geometric mean size of the particle of black bean flour and the starch damage percentage had an $r = -0.92$ negative relationship (Table 4). Kerr et al., (2000) obtained similar results with cowpea flour where finely milled flour had greater starch damage. Regardless of milling method, black bean flour starch damage values were lower than values reported with pea flour starch damage (1-1.4%) (Maskus et al., 2016; Kaiser, 2019). This may associate with the initial lower seed hardness of the beans compared to peas (Pelgrom et al., 2015).

Flow Properties

Black bean flour flowability characterized by the angle of repose differed depending on milling method (Table 5). In general, angle of repose was greater with flour having fine than coarse particles (Table 1 and 5). Stone mill-coarse had the lowest angle of repose. Black bean flour made using mills that did not utilize a screen (disc and stone mills) had lower angle of

Table 5. Effect of milling method on flour flow properties[a]

Milling method	Flour flow properties				
	Angle of repose (°)	Angle of slide[a] (°)			
		SS	Al	PVC	PP
Disc mill-Coarse-Gap 6	26 dc	21 ef,B	24 ef,A	23 f,AB	23 ed,AB
Disc mill-Fine-Gap 3	30 bc	21 ef,B	25 de,A	23 f,AB	23 ed,AB
Stone mill-Fine	30 bc	20 e-g,B	24 ef,A	23 f,A	25 d,A
Stone mill-Medium	28 c	19 fg,C	22 fg,B	22 fg,B	24 d,A
Stone mill-Coarse	23 d	18 g,B	20 g,AB	19 g,AB	21 e,A
Hammer mill 16,800 rpm – 800 µm	34 a	28 c,C	38 a,A	28 e,C	35 c,B
Cyclone mill 16,000 rpm – 500 µm	34 a	35 a,C	41 a,B	40 ab,B	45 a,A
Centrifugal-10,000 rpm-250 µm	35 a	35 a,D	38 a,C	42 a,B	44 a,A
Centrifugal-10,000 rpm-500 µm	33 ab	30 bc,B	31 c,B	37 b,A	39 b,A
Centrifugal-10,000 rpm-1000 µm	33 ab	22 e,C	27 d,B	34 c,A	36 c,A
Centrifugal-12,000 rpm-250 µm	33 ab	36 a,C	39 a,B	42 a,A	44 a,A
Centrifugal-12,000 rpm-500 µm	36 a	31 b,C	35 b,B	38 b,A	40 b,A
Centrifugal-12,000 rpm-1000 µm	33 ab	25 d,D	28 d,C	30 d,B	34 c,A

[a]Different uppercase letters across rows indicates significant differences between slide surface (P=0.05). Different lowercase letters across column indicates significant differences between milling method (P=0.05)

SS = Stainless steel, Al = Aluminum, PVC = Polyvinyl Chloride, PP = Polypropylene

repose than those that used a screen (cyclone mill, hammer mill and centrifugal mills). Within the disc mill and stone mill, angle of repose was greatest with fine setting and least with coarse setting. The angle of repose of the flour produced from the mills that utilized a screen, was much similar. Goh et al. (2018) observed that angle of repose has been more associated with the D_{10} than with any other particle characteristics and samples with larger D_{10} values had better powder flow. Results from this experiment indicated that geometric mean had a high negative correlation of *r* = -0.94 (Table 4). Predicted flow properties based on angle of repose were all excellent for flours produced using a disc mill and stone mill and were fair to good for flours produced using a hammer mill, cyclone mill and centrifugal mills (Table 6).

Table 6. Particle size and flow property data from other legume flour

Flour type	**Milling method**	**Particle size (µm)**	**Surface used**	**Angle of repose (°)**	**Angle of slide (°)**	**Reference**
Adzuki bean	650 W dry grinder	103	Stainless steel	nd	40-46	Park et al. (2015)
		1599	Stainless steel	nd	29-33	
Black soybean	500 W dry grinder	150-250	Stainless steel	nd	43-47	Lee & Yoon (2015)
		1180-1400	Stainless steel	nd	39-42	
Soybean	ns	20	Aluminum	52	nd	Ricks et al. (2002)
	ns	39	ns	31	nd	Pordesimo et al. (2009)
Yellow split pea	Hammer mill (0.84-9.53 mm sieves)	99	Mean of Aluminum and Stainless steel	32	40	Kaiser (2019)
		214		32	36	
		300		35	33	
		1127		25	24	

na = Not specified, nd = Not determined

Milling method and surface material had an interaction effect on angle of slide of black bean flour (Table 5). Angle of slide is an indicator of the ease in which a material will move or slide across a surface. In general, angle of slide was less with coarse than with fine particles. Stone mill-coarse had the lowest angle of slide on all surface materials, indicating that it had the best flow property. Compared to other surface materials, angle of flow was the lowest or one of the lowest on stainless steel surface for all flours. Polypropylene and polyvinyl chloride are synthetic plastic polymers. Within the plastic surfaces, angle of slide on polypropylene was greater or equal to that for polyvinyl chloride. The magnitude in differences among surface materials was greater for fine than coarse particles. Surfaces with high angle of slide indicate that the flour flows or slides less easily than surfaces with low angle of slide. Pelgrom et al. (2015) observed poor flow characteristics of chickpea flour than that of pea, bean, and lentil of similar particle size, all decreasing as a function of average particle size. Kaiser (2019) showed that hammer-milled pea flour produced using screens with small apertures required a high angle of slide (Table 6). Results from this experiment indicated that geometric mean was highly and negatively correlated with angle of slide, r = -0.80 to -0.90 (Table 4).

The loose and compact bulk densities of black bean flour ranged from 0.44-0.66 and 0.67-0.84 g/cm^3, respectively, and were significantly different between the mill settings (Table 7). During loose bulk density measurement, flour was freely poured, and unsettled small particles tended to entrap more air. There was a positive correlation between geometric mean and loose bulk density (r = 0.72). Black bean flours with larger particle sizes have greater loose bulk density because they have very compact, un-milled large pieces that excluded air and the ability to settle with greater force in the cylinder which results in tighter packing. Kaiser (2019) observed that loose bulk density of pea flour decreased with reduction in particle size. Raigar & Mishra (2014; 2017) observed varying relationships between bulk density and particle size, based on the commodity. They noted an inverse relationship between bulk density (loose and compact) and particle size for roasted chickpea flour and for maize flour, while a direct relationship between bulk density (loose and compact) and particle size for roasted soybean flour.

Table 7. Effect of milling method on bulk density[a]

Milling method	Bulk density (g/cm^3)		Hausner Ratio	Compressibility Index
	Loose	Compact		
Disc mill-Coarse-Gap 6	0.63 ab	0.77 b	1.22 g	18 h
Disc mill-Fine-Gap 3	0.66 a	0.81 a	1.24 g	19 gh
Stone mill-Fine	0.50 de	0.71 cd	1.43 cd	30 cd
Stone mill-Medium	0.59 c	0.77 b	1.32 ef	24 ef
Stone mill-Coarse	0.61 bc	0.77 b	1.26 gf	21 f-h
Hammer mill 16,800 rpm – 800 µm	0.48 ef	0.71 c	1.49 a-c	33 a-c
Cyclone mill 16,000 rpm – 500 µm	0.45 fg	0.69 c-e	1.53 a	35 ab
Centrifugal-10,000 rpm-250 µm	0.44 g	0.67 e	1.52 ab	34 ab
Centrifugal-10,000 rpm-500 µm	0.53 d	0.75 b	1.41 d	29 cd
Centrifugal-10,000 rpm-1000 µm	0.66 a	0.84 a	1.28 gf	22 fg
Centrifugal-12,000 rpm-250 µm	0.44 g	0.68 de	1.55 a	35 a
Centrifugal-12,000 rpm-500 µm	0.51 de	0.75 b	1.45 b-d	31 b-d
Centrifugal-12,000 rpm-1000 µm	0.59 c	0.82 a	1.38 de	28 de

[a]Different lowercase letters across column indicates significant differences between milling method (P=0.05)

During compact bulk density measurement, tapping action added additional force to overcome cohesive attractions that caused particles to fall into void spaces. Previously, Du et al., (2014) had reported 0.54 g/cm^3 as compact bulk density of black bean flour. Flour from centrifugal mill with 10,000 rpm and sieve aperture of 1,000 µm had the highest compact bulk density (0.84 g/ cm^3). Interestingly, loose bulk density of flour from centrifugal mill with 12,000 rpm and 1,000 µm aperture screen was lower than loose bulk density of flour from centrifugal mill with 10,000 rpm and 1,000 µm even though both mill settings had similar particle size distribution and particle shape. Flour from both mill settings (12,000/10,000 rpm -1,000 µm aperture screen) had similar and high compact bulk densities. Flour from stone mill-fine setting had similar particle size distribution as flour from centrifugal mill (10,000 or 12,000 rpm) with 1000 µm aperture screen but had larger geometric mean along with more elongated particle shape which resulted in lower loose and compact bulk density. Heterogeneity in particle size allowed the filling of voids where specific surface area increased with a decrease in particle size (Maaroufi et al., 2000).

The Hausner Ratio and Compressibility Index provide an alternative index of the flow character of a powder and measures the propensity of a powder to be compressed, reflecting the relative degree of interparticulate interactions (Ambrose et al., 2015). Based on angle of repose, Hausner Ratio and Compressibility Index values, the flowability of powders could be classified as being 'excellent' to 'cohesive' (Table 8). More cohesive powders have greater surface attractions that help them overcome gravity, so that particles can support themselves around void spaces. Low Compressibility Index or low Hausner Ratios of a material indicate better flow properties than high values.

Table 8. Empirical relation between the flow properties and results

Flow classification	**Angle of repose (°) (Lumay et al., 2012)**	**Hausner Ratio (Lumay et al., 2012)**	**Compressibility Index (Eben, 2008)**
Excellent	25-30	1.00-1.11	<10
Good	31-35	1.12-1.18	11-15
Fair	36-40	1.19-1.25	16-20
Passable	41-45	1.26-1.34	21-25
Poor	46-55	1.35-1.45	26-31
Very poor	56-65	1.46-1.59	32-37
Cohesive	>66	>1.60	>38

Flow classifications were quite different when based on Compressibility Index and Hausner Ratio compared to angle of repose and angle of slide. None of the flours were classified as having excellent or good flow properties based on Compressibility Index and Hausner Ratio (Table 8). Flours from the disc mill were classified as having fair flow properties. Flour from stone mill coarse and medium setting had passable flow properties but flour from fine setting was classified as having poor flow properties. Mills with high speed rotors and screen (hammer mill, cyclone mill, and centrifugal mill) resulted in flours classified as having poor to very poor flow properties. However, based on the angle of repose, the disc mill and stone mill flours were classified as excellent flow properties and flour from the hammer mill, cyclone mill and centrifugal mill had fair to good flow properties. Overall, these results indicate that flours that had predominately large particles had better flow than did flours that had small particles. Small particle size has greater surface area than large particles per volume or weight. Greater number of particles and greater surface area allows particles to interact and prevent or restrict free flow of material.

Conclusions

Different laboratory type mill settings had a significant effect on the physico-chemical and flow properties of whole black bean flour. Flour from mills which utilized screens (cyclone mill, centrifugal mill, and hammer mill) showed significantly different characteristics (geometric mean size, particle size distribution, moisture, starch damage, flow properties) than flour from mills without screen (disc mill and stone mill). Geometric mean size of the flour was negatively correlated with starch damage, L*, angle of repose, and angle of slide and positively correlated with moisture, and loose bulk density. Black bean flour had an angle of repose of 26-36, which can be classified as having good or excellent flow behavior. Angle of slide was lowest on stainless steel regardless of flour sample. It is necessary to identify the optimum milling conditions on an industrial level to achieve flour properties that are required for optimal end product quality.

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CHAPTER 3: COMPARING THE PHYSICO-CHEMICAL AND FLOW PROPERTIES OF SELECTED BLACK BEAN FLOUR FRACTIONS FROM DIFFERENT MILLS

Abstract

Particle size and shape can influence inherent flour properties, and eventually the quality of the finished food product. The objective of this research was to determine the effect of mill type on the physical, chemical and flow properties of selected particle size fractions of black bean flour. Black bean flour produced using five different mills was sieved to form four (50 to <100, 100 to <150, 150 to <250, 250 to <425 µm) particle size fractions. Flour moisture content was significantly lower when milled with the centrifugal mill. Flour ash content was significantly higher when milled with the cyclone mill. Centrifugal mill caused the greatest starch damage among the mills. Stone mill resulted in the highest L* value and lowest *a** for any flour fraction. Disc mill and stone mill flour fractions had lower circularity, higher angle of repose and angle of slide than hammer mill, cyclone mill and centrifugal mill for flour fractions. Fine flour fractions had more starch damage, L* and less moisture, ash, *a** values, flowability, circularity than coarse fractions. Particle circularity had a negative correlation of r = -0.93, r = -0.81, r ≈ -0.95, r = -0.94 with L*, angle of repose, angle of slide and compact bulk density respectively. Particle circularity had a positive correlation of r= 0.93, r = 0.89 with average minimum particle size and loose bulk density respectively. Mill type and particle size interaction significantly affected the physical, chemical and flow properties of selected particle size fractions of black bean flour.

Introduction

Milling is a process where grains are reduced to meal or flour by processes involving grinding, sieving, and purifying (Limsangouan & Isobe, 2009). Wheat milling consists of all three steps whereas pulse milling generally utilizes only the grinding step. Milling process can be of two kinds, (1) wherein the whole grain is converted into flour without abstracting any parts or, (2) it could undergo differential milling to separate the grain into different parts (Oghbaei & Prakash, 2016). Therefore, dry beans could be milled into whole flour, cotyledon flour, different size fractions, starch/protein/fiber rich flour, isolates and concentrates (Roy et al., 2010; Thakur et al., 2019).

Particle size influences inherent flour properties, and eventually the quality of the finished food product. Particle size distribution depends on the nature of the grinding forces applied as well as the internal structure of the materials (Scanlon & Lamb, 1993, 1995; Vishwanathan & Subramanian, 2014). Effect of flour particle size of different commodities on its inherent properties and/or on the quality of the finished food products has been tested by different research groups. Borsuk et al. (2012) observed pulse flour with a coarse particle size was more desirable than a fine particle size for both pita bread and pan bread. Zucco et al. (2011) reported that incorporation of fine pulse flours increased cookies' hardness and decreased spread while coarse flours reduced both parameters. Across different studies, particle size of pulse flours in bakery applications has been reported to range from 17 to 1,000 µm (Scanlon et al., 2018). This significant particle size variation hinders the comparisons of pulse flour functionality across different studies. Therefore, each flour requires special attention and sought detailed study on particle size and its influence on functional and structural properties to elucidate its further use in food product development.

Particle size and distribution are often used to characterize the flour using various analytical instruments such as sieve sizing, laser diffraction and image analysis (Olson, 2011a). Particle size is based on a single parameter (diameter). But when the particle is not a perfect sphere or cube, several size parameters (length, width, thickness) are needed to define particle size (Olson, 2011a).

Shape factor determination depends on the method used (fractal or Fourier-based methods) by image analysis software to estimate the basic dimensions of the particle (Pons et al., 1999; Almeida-Prieto et al., 2007). There are two types of shape descriptors: macro and meso descriptors. Macro shape descriptors are calculated from size measurements made on the particle silhouette. Meso shape descriptors are calculated in comparison to a reference shape, such as the convex bounding polygon, and can give a more detailed appreciation of the shape (Pons et al., 1997; Pons et al., 1999).

There is no standard method and several descriptors are necessary to characterize the shape of particles. Shape descriptors can describe the shape at different levels of complexity. Shape descriptors are independent from the point of view of object size and position in the image (Pons et al., 2011). Shape can be characterized by elongation, circularity, compactness, and convexity.

Studies have been conducted in other fields (pharmaceutical, construction) on particle shape and product behavior and have shown both experimentally and mathematically that particle shape has a significant effect on flow properties, mixing properties and hydration properties (Olson, 2011b; Sinnott & Cleary 2016; Liu et al., 2018). Podczeck & Miah (1996) observed that flow rate increased as shape changed from needle shape, cubic, angular to round particles in eight different powders. Swaminathan & Kildsig (2002) described that when the shapes of particles in a mixture are extremely different from each other it can lead to segregation and the particles can also have hindered flowability and packing ability. Kuakpetoon et al.,

(2001) reported that wheat flour sample which had more spherical particles required longer mixing time than the sample containing more irregular, flour particles.

The relationship between particle shape and flour characteristics has not been extensively studied in food industry. During milling, a particle may fracture along large solid faces, forming two particles. Another possibility may be for small pieces to break off the particle, eventually forming a bimodal or skewed population of fine particles and larger, rounded particles (Olson, 2011b). The estimation of shape factors for black bean flour particles would be a valuable contribution to understanding the dynamics of black bean flour during handling and processing.

Proper understanding of how black bean flour fractions behave is necessary to design food applications and handling equipment. The previous chapter reported on the effect of different mills on black bean flour physical, chemical and flowability. In that chapter, the milling method significantly affected several physical, chemical and flow properties of whole black bean flour. Geometric mean size of the flour was negatively correlated with starch damage (r = -0.92), L* (r = -0.94), angle of repose (r = -0.94), and angle of slide (r = -0.80 to -0.90) and positively correlated with moisture (r = 0.72), and loose bulk density (r = 0.72). Fine flour resulted in more elongated particles and had difficulty in flowing. The properties of same particle size fractions made using different mills are still unknown. Therefore, the objective of this experiment was to characterize the particle shape, physical, chemical and flow properties of selected black bean flour fractions from different mills.

Materials and Methods

Black Beans

Black beans were obtained from three dry bean companies. Seeds from each company were kept separate and treated as replications in the ensuing experiments. The black bean samples used in this experiment were typical of black beans grown in ND, USA having an average protein content of 21.8 %, test weight of 78.9 kg/hL (63.1 lb/bu), 100-seed weight of 19.7g, and average fracture hardness of 162 N.

Milling Methods

A laboratory-type cyclone mill (Direct drive model 3010-014, Colorado, USA), hammer mill (Perten LM 3100, Hägersten, Sweden), stone mill (KoMo classic grain mill, Penningberg, Austria), disc mill (Perten LM 3310, Hägersten, Sweden), and centrifugal mill (Centrifugal ZM 200, Haan, Germany) were used to mill 1 kg samples of black bean seeds. Feed rate was set for 40 g/min for all the mills. Mill room temperature and relative humidity were 25±2 °C and 20±5 %, respectively. Following is the summary of mill settings used and the mill temperature after milling 1 kg of black beans (Table 9).

Table 9. Mill type and setting and temperature of milling surface and flour after milling 1 kg of black beans

Mill	**Mill settings**	**Mill temperature (°C)**	**Flour temperature (°C)**
Cyclone mill	16,000 rpm, 500 µm screen	35	29
Hammer mill	16,800 rpm, 800 µm screen	33	28
Stone mill	Fine	43	36
Disc mill	Fine disc, Gap 3	31	27
Centrifugal mill	12,000 rpm, 250 µm screen	34	30

Black bean flours were fractionated by particle size using a vibratory shaker (Centrifugal AS 200 sieve shaker; Centrifugal International, Haan, Germany) configured with a stack of seven sieves and a vibratory amplitude displacement of 3 mm for 15 sec intervals for 5 min. Sieves used had 50, 100, 150, 250, 425, 500, and 600 µm aperture size. Only particle size fractions of 50 to <100, 100 to <150, 150 to <250, and 250 to <425 µm were used in physical, chemical, and flow property testing due to sample size limitation and only 150 to <250, 250 to <425, 425 to <500, 500 to <600, and >600 µm were used in particle shape analysis due to limitation of particle dispersion method.

Physical and Chemical Flour Properties

For each particle size fraction, ash content, moisture content, protein content and starch damage were determined according to AACC International Approved Methods 08-01.01, 44-15.02, 46-30.01, and 76-31.01, respectively. Flour fractions were evaluated for color (CIE L*, *a**, *b** values) using Minolta 410 colorimeter. Flour was placed in a round black measurement cell (6 cm diameter x 2 cm deep) that had a quartz glass window.

The shape of the black bean flour particles was analyzed by an optical microscope with a magnification that was adapted to the particle size and image analysis software. Four meso shape factors (elongation, circularity, compactness, and convexity) which evaluate the differences between the measured shape of the particle and a regular shape, were used to characterize the fractions.

Particle imaging was done under 30 X magnification using OMNI Core Digital Microscope and Measurement System (Unitron, NY, USA). Flour samples were dispersed onto microscope glass slides and applied dispersion conditions were optimized in order to obtain well separated particles before image acquisition. After particle dispersion, glass slides were positioned under a transmitted light microscope for observation.

Image analysis was done by Image Pro Premier 3D software (Media Cybernetics, MD, USA). Initially, images were pretreated with particle's border killing (removing particles that touch the border of the image), particle's silhouette hole filling (filling the holes within particle silhouette), separation (breaks narrow isthmuses and separates touching particles), and morphological cleaning. Calibration was done to translate pixel unit of the particle's silhouette into metric units and the silhouette dimensions were measured. Finally shape factors were calculated by the Image Pro Premier 3D software.

Defining the minimum number of particles required to be analyzed, is necessary. Pons et al. (2002) has stated that a set of 1,000–1,500 particles is the best compromise between good statistical reliability and analysis duration. Observations of Saad et al. (2011) also agree with analyzing 1,000 particles to have good statistical reliability. To capture 1,000 particles from >600, 500 to <600, 425 to <500 μm fractions, 50 images originated from 10 glass slides were used and to capture 1,000 particles from 250 to <425 and 150 to <250 μm fractions, 8-10 images originated from 2 glass slides were required.

Flour fraction of 250 to <425 μm from centrifugal mill, 10,000 rpm with 1,000 μm aperture screen was used to determine the necessary number of particles to have stabilized shape factor values and standard deviation. This mill setting was selected due to the particle size distribution of the resulting flour, which had an even distribution over a wide range of size (Chapter 2). This step was done only to verify the number of particles to be analyzed stated in literature. Nearly 1,200 particles were analyzed according to the protocol. The effect of the number of particles on the average calculated circularity values and the standard deviation values for black bean flour 250 to <425 μm fraction is shown in Figure 3. The results show that the average value of circularity stabilized after analyzing more than 700 particles. The standard deviation starts to decrease after analyzing 800 particles. To be consistent with the values reported in literature, 1,000 particles were selected for the analysis (Pons et al., 2002; Saad et al., 2011).

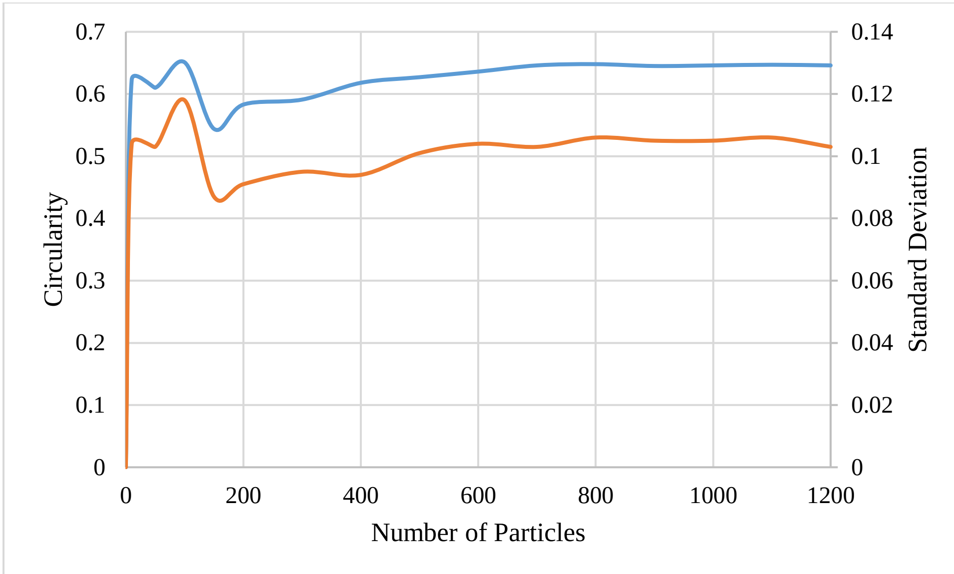

Figure 3. Effect of the number of particles on the calculated circularity values average (blue) and the standard deviation values (orange) for black bean flour 250 to <425 µm fraction from the centrifugal mill-10,000 rpm-1,000 µm setting

Particle morphology was expressed through several shape descriptors that were determined after visualization and segmentation of two-dimensional projection on a plane surface of the three-dimensional particle shape. The particle silhouette or its contour line was considered for the two-dimensional morphology characterization. The two-dimensional projection of a particle represents its largest silhouette (Saad et al., 2011). A single number can be assigned to express the size of spheres and cubes using diameter or side length. Shape descriptors are independent from object size and position in the image (Pons et al., 2011). There are two types of shape descriptors: Macro and meso descriptors. Macro shape descriptors are calculated from size measurements made on the particle silhouette. Meso shape descriptors are calculated in comparison to a reference shape, such as the convex bounding polygon, and can give a more detailed appreciation of the shape (Pons et al., 1997; Pons et al., 1999). In this experiment, meso shape descriptors were defined by comparison with some referential shapes for elongation, circularity, compactness, and convexity. Values were normalized either between 0 and 1 (*e.g.* circularity, compactness and convexity) or between 1 and positive infinity (*e.g.* elongation).

Flow Properties

Bulk density was measured using the method described by Okaka & Potter (1997) with modifications. Black bean flour (100 g) was measured into a calibrated measuring cylinder. The loose volume (mL) was recorded. Then the bottom of the measuring cylinder was tapped for 250 times in the same speed (≈120 taps/min). Loose bulk density was calculated as the ratio of the weight of the sample to its loose volume and the compact bulk density was calculated as the ratio of the weight of the sample to its compact volume.

Angle of repose and angle of slide were determined as described by de la Peña (2014). Angle of repose of flour (200 g) was determined using a stainless-steel cylinder (13.5 x 7.8 cm) placed on top of a horizontal stainless-steel surface. The cylinder was then quickly lifted vertically to spread flour into a conical heap. The height and diameter of the conical heap were measured using a ruler. The poured angle of repose was calculated using the following equation:

$$\alpha = \tan^{-1}(h/\mathrm{r})$$

Where h is the height of the heap of material and r is the average radius of the heap obtained by recording the two diameters perpendicular from each other.

The cylinder used in angle of repose was placed on the center of a sheet whose slope can be gradually increased using a manual screw connected platform. Cylinder was vertically lifted to make a conical heap after pouring 200 g of flour sample was poured into it. The slope of the platform was slowly increased until ≈ 75% of the material slid down. The angle formed between the slope of the platform and the horizontal base was measured using a protractor. Effect of the platform surface on the angle of slide was determined using surface made from aluminum and stainless steel.

Statistical Analysis

The experimental design was a randomized complete block with split plot arrangement where whole plot was mill type and sub plot was particle size of the fraction. Each treatment was replicated three times. Data were analyzed using SAS 9.4 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 $P=0.05$. Pearson correlations were computed between particle shape of the particles and physico-chemical and flow properties.

Results and Discussion

Physical and Chemical Properties

The moisture contents of different flour fractions from each mill are summarized in Table 10. Results indicate that flour moisture had an interaction effect of particle size and milling method. For each mill, the moisture content gradually decreased with decreasing flour particle size of fraction. Similar results were observed in the chapter 2 with the geometric mean size being positively correlated with moisture content, $r = 0.72$. Compared to large particles, small particles have more exposed surface area per unit weight which favors loss of moisture. Mills can be grouped based on moisture content of flour: high flour moisture, disc mill, stone mill and hammer mill (group 1) and low flour moisture, cyclone mill and centrifugal mill (group 2). The low flour moisture with cyclone mill and centrifugal mill can be attributed in part to their having greater air movement through the mill and longer retention time in the milling chamber. Within first group, moisture content was greater with stone mill than disc mill or hammer mill. Probably the least amount of air movement occurs within the stone mill. The grinding material is trapped between two horizontal stones which would not favor air flow. Within the second group, moisture content was greater with cyclone mill than centrifugal mill. Only the stone mill had different elevated temperatures, which supports the idea of poor air flow through the milling chamber (Table 9). The milling temperatures were similar with the other mills.

Table 10. Moisture content[a] of different flour fractions

Milling method	**Moisture content (%)**			
	50-100 µm	100-150 µm	150-250 µm	250-425 µm
Disc mill-Fine-Gap 3	12.7 b,D	12.9 b,C	13.1 b,B	13.3 b,A
Stone mill-Fine	13.4 a,C	13.5 a,B	13.7 a,A	13.7 a,A
Hammer mill	12.7 b,D	12.8 c,C	12.9 c,B	13.1 c,A
Cyclone mill	10.6 c,D	10.7 d,C	10.8 d,B	11.3 d,A
Centrifugal-12,000 rpm-250 µm	9.6 d,C	9.7 e,B	9.7 e,B	9.9 e,A

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

In a centrifugal mill, the seeds are initially broken due to impact by the blades ‘throwing’ the seed against a screen (Restch, 2018). Shear and abrasion forces also occur as the seed fragments are moved against the ring sieve. Cyclone mill uses similar force of action as in centrifugal mill, where high speed rotation of the impeller and air currents throw particles into and around the grinding ring. A screen covering the outlet ensures that particles remain in the grinding chamber until impact-shattering and abrasion make them small enough to flow through the screen apertures and out the exit with the air current. Disc mill and stone mill do not have a screen to retain the milled material for further grinding. The grinding forces are exerted only for a short time on the flour particles, since the retaining time of the flour inside the milling chamber is less.

The ash contents of different flour fractions from each mill are summarized in Table 11. Ash content had an interaction effect of particle size and milling method. For all milling methods, ash content was least with fine fraction, 50 to <100 µm and was significantly higher in the large flour fractions (150 to <250 and 250 to <425 µm). Higher ash content suggests that seed coat content was greater in the large particle fractions than with the small particle fraction. Maybe the seed coat itself is harder to mill into small particles since more fibrous material less prone to fracture.

Table 11. Ash content[a] of different flour fractions

Milling method	**Ash content (%)**			
	50-100 µm	100-150 µm	150-250 µm	250-425 µm
Disc mill-Fine-Gap 3	3.59 b,D	4.12 b,B	4.15 c,A	3.98 e,C
Stone mill-Fine	3.32 c,D	3.68 e,C	4.07 d,B	4.10 d,A
Hammer mill	3.58 b,D	3.87 c,C	4.26 b,A	4.20 c,B
Cyclone mill	3.86 a,D	4.18 a,C	4.40 a,A	4.29 b,B
Centrifugal-12,000 rpm-250 µm	3.58 b,D	3.78 d,C	4.09 d,B	4.37 a,A

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

Flour from the cyclone mill had the highest ash contents for any given flour fraction, except for 250 to <425 µm where flour from centrifugal mill had more ash. Ash content was greatest in 150 to <250 µm fraction for disc mill, hammer mill, and cyclone mill and 250 to <425 µm fraction for stone mill and centrifugal mill. Milling method may have an effect on how it loosens the seed coat from the flour particles.

The protein contents of different flour fractions from each mill are summarized in Table 12. Protein content had an interaction effect of particle size and milling method. Protein content was greater with intermediate particle size 100 to <150 and 150 to <250 µm than with smallest 50 to <100 µm or largest 250 to <425 µm fractions. Protein content was least with 50 to <100 μm for disc mill and stone mill and with 250 to <425 μm for hammer mill cyclone mill and centrifugal mill. Conversely, protein content was greatest with 150 to <250 μm for disc mill and stone mill and with 100 to <150 μm for hammer mill, cyclone mill, and centrifugal mill.

Table 12. Protein content[a] of different flour fractions

Milling method	**Protein content (%)**			
	50-100 μm	100-150 μm	150-250 μm	250-425 μm
Disc mill-Fine-Gap 3	18.9 d,D	22.5 a,B	22.8 b,A	21.2 b,C
Stone mill-Fine	17.5 e,D	19.7 e,C	23.4 a,A	22.7 a,B
Hammer mill	19.4 c,C	21.9 b,A	20.8 c,B	16.1 d,D
Cyclone mill	19.9 b,C	21.0 d,A	20.4 d,B	17.1 c,D
Centrifugal-12,000 rpm-250 μm	20.2 a,B	21.4 c,A	19.6 e,C	12.1 e,D

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

The starch damage of different flour fractions from each mill are summarized in Table 13. Results indicate that starch damage had an interaction effect of particle size and milling method. Starch damage was greatest with 50 to <100 μm fraction and decreased as particle size increased. In the previous chapter, it was noted that starch damage was negatively correlated with geometric mean size of the particle of black bean flour (r = 0.92). Kerr et al., (2000) obtained similar results with cowpea flour where finely milled flour had more starch damage than coarsely ground flour. In this study, the grinding method had a significant effect on the starch damage within the same flour fraction (Table 13). Centrifugal mill had the most starch damage among the mills for any flour fraction. Hammer mill and cyclone mill had intermediate level of starch damage among different flour fractions. Disc mill and stone mill had lowest levels of starch damage in each fraction. This might be due to the longer retaining time of particles inside the mills with screens, where it led to more particle collisions with the mill surface and each other. Overall starch damage was quite low and did not exceed 0.28% regardless of milling method or the flour fraction (Table 13). Black bean flour starch damage values were lower than values reported for starch damage in pea flour (1-1.4%) (Maskus et al., 2016; Kaiser, 2019). This might be associated with the initial lower seed hardness of the beans compared to peas (Pelgrom et al., 2015).

Table 13. Starch damage[a] of different flour fractions

Milling method	**Starch damage (%)**			
	50-100 µm	100-150 µm	150-250 µm	250-425 µm
Disc mill-Fine-Gap 3	0.05 e,A	0.04 d,B	0.02 d,C	0.02 b,C
Stone mill-Fine	0.06 d,A	0.04 d,B	0.02 d,C	0.01 c,D
Hammer mill	0.15 c,A	0.12 c,B	0.04 c,C	0.02 b,D
Cyclone mill	0.16 b,A	0.13 b,B	0.07 b,C	0.03 a,D
Centrifugal-12,000 rpm-250 µm	0.28 a,A	0.21 a,B	0.10 a,C	0.03 a,D

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

Color differences were observed among the flour fractions (Table 14). Results indicate that flour color had an interaction effect of particle size and milling method. Brightness was greatest with 50 to <100 µm fraction and decreased as size in fraction increased. Conversely, *a** value (redness) was greatest with 250 to <425 µm fraction and decreased as particle size decreased. Yellowness was greatest with 50 to <100 µm for centrifugal mill, 100 to <150 µm for hammer mill and cyclone mill, 150 to <250 µm for disc mill and 250 to <425 µm for stone mill. The least yellowness occurred with 50 to <100 µm for disc mill and stone mill and 250 to <425 µm for hammer mill cyclone mill and centrifugal mill. The increase of L* values with reduced particle size is due to increased surface area that allows more reflection of light (Ahmed et al., 2015). In chapter 2, it was observed that lightness was higher with flours that had small geometric mean particle sizes. Liu (2009) and Ahmed et al. (2016) observed a similar relationship with flour lightness with particle size in corn and lentil flours respectively. Particle shape might also impact flour brightness. Lightness (L*) had a negative correlation of *r* = -0.93 and *r* = -0.92 with circularity and compactness of the particles, respectively. L* had a positive correlation of *r* = 0.73 with elongation of the particles.

Circularity values of different particle fractions are shown in Table 15. Particle size and the milling method had an interaction effect on the circularity of particles. Circularity increased with increased particle size. Particles >600 µm had the highest circularity values for all mills except for stone mill which had highest circularity values with particle sizes 250 to <425, 425 to <500 and 500 to <600 um. Circularity was greater with hammer mill, cyclone mill and centrifugal mill than with disc mill or stone mill, regardless of particle size. Circularity value was positively correlated with average minimum particle size for all mills, *r* = 0.93. So, large particles are more circular than small particles (Table 16).

Table 14. Color values[a] of different flour fractions

Milling method	**L***				**a***				**b***			
	50-100 µm	100-150 µm	150-250 µm	250-425 µm	50-100 µm	100-150 µm	150-250 µm	250-425 µm	50-100 µm	100-150 µm	150-250 µm	250-425 µm
Disc mill-Fine-Gap 3	82.92 b,A	79.85 b,B	73.15 b,C	67.55 b,D	0.36 d,D	0.56 c,C	0.73 c,B	0.86 d,A	6.51 a,D	7.59 a,C	8.18 a,A	7.89 b,B
Stone mill-Fine	83.76 a,A	82.82 a,B	79.91 a,C	75.28 a,D	0.31 e,C	0.31 d,C	0.36 e,B	0.55 e,A	5.99 b,D	6.48 b,C	7.95 b,B	8.75 a,A
Hammer mill	81.41 c,A	78.08 c,B	68.04 d,C	56.31 d,D	0.73 a,B	0.73 a,B	0.76 b,B	1.35 b,A	4.78 c,B	5.20 c,A	4.52 c,C	3.34 c,D
Cyclone mill	81.10 d,A	76.90 e,B	66.31 e,C	55.97 e,D	0.45 c,D	0.58 b,C	0.88 a,B	1.52 a,A	3.81 d,B	3.87 d,A	3.58 d,C	2.90 d,D
Centrifugal-12,000 rpm-250 µm	80.89 e,A	77.25 d,B	70.40 c,C	57.64 c,D	0.50 b,D	0.56 c,C	0.64 d,B	1.27 c,A	3.75 e,A	3.41 e,B	2.53 e,C	1.11 e,D

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

Table 15. Circularity values[a] of different particle fractions

Milling method	Circularity				
	150-250 µm	250-425 µm	425-500 µm	500-600 µm	>600 µm
Disc mill-Fine-Gap 3	0.514 b,C	0.551 b,B	0.588 b,A	0.581 d,A	0.594 c,A
Stone mill-Fine	0.511 b,B	0.560 b,A	0.582 b,A	0.584 d,A	0.533 d,B
Hammer mill	0.579 a,D	0.630 a,C	0.670 a,B	0.732 a,A	0.717 a,A
Cyclone mill	0.578 a,B	0.640 a,A	0.648 a,A	0.633 c,A	0.644 b,A
Centrifugal-12,000 rpm-250 µm	0.577 a,D	0.622 a,C	0.674 a,B	0.702 b,A	0.719 a,A

[a]Different uppercase letters across rows indicates significant differences between particle sizes (P=0.05). Different lowercase letters across column indicates significant differences between milling method (P=0.05)

Table 16. Correlations between particle shape and black bean flour attributes

Attribute	Correlation coefficient (r)
Circularity	
Average particle size	0.93
L*	- 0.93
Loose bulk density	0.89
Compact bulk density	- 0.94
Angle of repose	- 0.81
Angle of slide – Stainless steel	- 0.96
Angle of slide - Aluminum	- 0.95
Compactness	
Average particle size	0.80
L*	- 0.92
Loose bulk density	0.91
Compact bulk density	- 0.86
Elongation	
Average particle size	- 0.90
L*	0.73
Loose bulk density	- 0.84
Compact bulk density	0.81

Compactness values of different particle fractions are shown in Table 17. Compactness of particles had an interaction effect of particle size and by milling method. Particle size > 600 µm had the highest compactness for all mills. Smaller particle fractions from each mill had a lower compactness value. So, large particles are more compact than small particles. Within each fraction, compactness was greatest with hammer mill and centrifugal mill, intermediate with cyclone mill and least with disc and stone mill. Compactness did not correlate with average minimum particle size.

Table 17. Compactness values[a] of different particle fractions

Milling method	Compactness				
	150-250 µm	250-425 µm	425-500 µm	500-600 µm	>600 µm
Disc mill-Fine-Gap 3	0.583 b,C	0.632 c,AB	0.635 c,AB	0.624 c,B	0.649 c,A
Stone mill-Fine	0.558 c,C	0.626 c,B	0.625 c,B	0.624 c,B	0.662 b,A
Hammer mill	0.626 a,C	0.667 b,B	0.659 ab,B	0.692 a,A	0.710 a,A
Cyclone mill	0.638 a,C	0.693 a,A	0.636 bc,C	0.658 b,BC	0.662 b,B
Centrifugal-12,000 rpm-250 µm	0.629 a,D	0.674 ab,BC	0.668 a,C	0.693 a,AB	0.700 a,A

[a]Different uppercase letters across rows indicates significant differences between particle sizes (P=0.05). Different lowercase letters across column indicates significant differences between milling method (P=0.05)

Convexity values of different particle fractions are shown in Table 18. Convexity of particles had an interaction effect of particle size and by milling method. Convexity was high in smaller particle fractions and among the mills that utilized screens (centrifugal mill, cyclone mill, and hammer mill). So, small particles were more convex than large particles. Convexity did not correlate with average minimum particle size.

Table 18. Convexity values[a] of different particle fractions

Milling method	Convexity				
	150-250 µm	250-425 µm	425-500 µm	500-600 µm	>600 µm
Disc mill-Fine-Gap 3	0.488 c,NS	0.486 bc,NS	0.486 b,NS	0.486 b,NS	0.486 b,NS
Stone mill-Fine	0.482 b,C	0.484 c,BC	0.489 ab,A	0.487 b,AB	0.478 c,D
Hammer mill	0.495 a,A	0.490 a,B	0.487 ab,B	0.490 ab,B	0.490 a,B
Cyclone mill	0.492 a,A	0.491 a,A	0.487 ab,B	0.487 b,B	0.478 c,C
Centrifugal-12,000 rpm-250 µm	0.493 a,A	0.489 ab,B	0.490 a,AB	0.492 a,AB	0.491 a,AB

[a]Different uppercase letters across rows indicates significant differences between particle sizes (P=0.05). Different lowercase letters across column indicates significant differences between milling method (P=0.05)
NS – Not Significant

Elongation values of different particle fractions are shown in Table 19. Particle size and the milling method interaction was not significant on the elongation of particles. Elongation values were highest in the stone mill flour fractions. Smaller flour particle fractions had a higher elongation. Elongation value was negatively correlated with average minimum particle size, (r = -0.90). So, small particles were more elongated than large particles (Table 16).

Table 19. Elongation values[a] of different particle fractions

	Elongation
Milling method [b]	
Disc mill-Fine-Gap 3	1.466a
Stone mill-Fine	1.495a
Hammer mill	1.284b
Cyclone mill	1.341b
Centrifugal-12,000 rpm-250 µm	1.292b
Fraction [c]	
600<	1.332b
500-600	1.350b
425-500	1.340b
250-425	1.386b
150-250	1.468a

[a] Values followed by same letter are not significantly different at P=0.05.
[b] Values averaged over milling method
[c] Values averaged over particle size

The results indicate that the large black bean particles are more circular and compact and less convex and elongated compared to small particles. The relationship between the size and the shape of black bean flour particles can be used to suggest the breaking behavior of black beans during milling. Black bean is initially broken into large circular particles and later into more elongated particles. The first step in black bean milling forms large particles with smooth surface and circular shapes can be due to erosion (Figure 4). Erosion cause the elimination of the irregularity on the surface of large particles and generate less circular small particles. Along with the milling process, the reduction of the large particles size is may be caused by shattering mechanisms. Shattering results in small particles with less circularity.

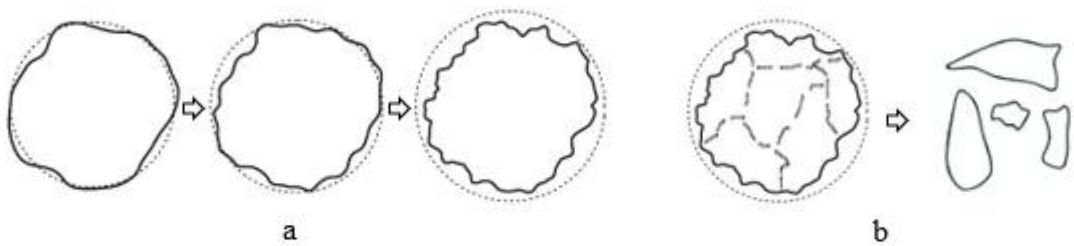

Figure 4. Suggested breakage pattern of black beans during single pass milling (a) Erosion and (b) Shattering.

Flow Properties

Loose bulk density values for flour from hammer mill, cyclone mill, and centrifugal mill were greater than for flour from disc mill or stone mill. Bulk density had an interaction effect of particle size and milling method. For each mill, loose bulk density significantly increased as the particle size of the fractions increased (Table 20). During loose bulk density measurement, flour was freely poured, and unsettled small particles tended to entrap more air. Black bean flour fractions with larger particle sizes had higher loose bulk density due to them having compact, un-milled large pieces that excluded air and have the ability to settle with greater force in the cylinder which results in tighter packing.

Particle shape seemed to affect loose bulk density. Loose bulk density had a positive correlation of $r = 0.89$, $r = 0.91$, $r = 0.71$ with circularity, compactness and convexity of the particles, respectively, and a negative correlation of $r = -0.84$ with elongation of the particles (Table 16). Disc mill and stone mill had lower circularity values where less spherical particles can settle freely during free pouring with anomalous packing behavior by entrapping more air.

Compact bulk density significantly decreased as the particle size of the fractions increased in each mill (Table 20). Interestingly, compact density was greater with disc mill than other mills at 50 to <100 µm. During compact bulk density measurement, tapping action adds additional force to overcome the cohesive attractions and causes particles to fall into void spaces.

In contrast to loose bulk density, compact bulk density had a negative correlation of $r = -0.94$ and $r = -0.86$ with circularity and compactness of the particles, respectively, and had a positive correlation of $r = 0.81$ with elongation (Table 16). No correlation with convexity was observed. Disc mill and stone mill had lower circularity and higher elongation values where less spherical particles can settle tightly during tapping while excluding air. Larger particle size fractions had higher circularity, where particles cannot fill voids to the optimum level as they can do with less spherical particles, as exhibited by higher compact density in smaller particle size fractions.

Angle of repose values for flour fractions from different mills are shown in Table 21. Angle of repose values had an interaction effect of particle size and milling method. The angle of repose is the steepest slope of the unconfined material, measured from the horizontal plane on which the material can be heaped without collapsing. Flour from the disc mill and stone mill had similar angle of repose values which were greater than angle of repose values for flour from hammer mill, cyclone mill and centrifugal mill (Table 21).

Table 20. Bulk density[a] of different flour fractions

Milling method	Bulk density(g/cm^3)							
	Loose				Compact			
	50-100 µm	100-150 µm	150-250 µm	250-425 µm	50-100 µm	100-150 µm	150-250 µm	250-425 µm
Disc mill-Fine-Gap 3	0.43 d,D	0.45 c,C	0.48 b,B	0.56 b,A	0.81 a,A	0.78 a,B	0.74 a,C	0.72 a,D
Stone mill-Fine	0.45 c,B	0.45 c,B	0.46 b,B	0.51 c,A	0.78 b,A	0.78 a,A	0.73 a,B	0.73 a,B
Hammer mill	0.47 b,C	0.48 b,C	0.57 a,B	0.59 a,A	0.77 b,A	0.70 b,B	0.69 b,C	0.62 b,D
Cyclone mill	0.45 c,D	0.50 a,C	0.57 a,B	0.59 a,A	0.78 b,A	0.70 b,B	0.68 b,C	0.62 b,D
Centrifugal-12,000 rpm-250 µm	0.48 a,C	0.48 b,C	0.56 a,B	0.58 a,A	0.78 b,A	0.76 a,B	0.71 a,C	0.62 b,D

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

The size and shape of the particles affect flour's angle of repose considerably (Al-Hashemi & Al-Amoudi, 2018). The angle of repose increased as the particle size fraction decreased. The angle of repose can indicate the cohesiveness of the granular material (Cain, 2002). Smaller particles have higher cohesiveness between particles leading to higher angle of repose (Al-Hashemi & Al-Amoudi, 2018). Angle of repose and the particle circularity had a negative correlation of r = -0.81. No correlations between angle of repose and convexity, compactness or elongation were observed (Table 16). When particles have more circular shape, they cannot fit on top of each other to form a heap without collapsing. But when particles have less circular shape, they can fill the voids to form a heap without collapsing.

Table 21. Angle of repose[a] of different flour fractions

Milling method	**Angle of repose (°)**			
	50-100 µm	100-150 µm	150-250 µm	250-425 µm
Disc mill-Fine-Gap 3	35.8 a,A	33.5 a,B	31.3 a, C	26.3 a,D
Stone mill-Fine	35.8 a, A	33.5 a,B	31.3 a,C	26.2 b,D
Hammer mill	34.6 b,A	32.8 b,B	29.9 b,C	23.8 d,D
Cyclone mill	34.5 c,A	32.7 c,B	29.8 c,C	23.4 e,D
Centrifugal-12,000 rpm-250 µm	34.6 b,A	32.7 c,B	29.7 d,C	24.3 c,D

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

Angle of slide values for flour fractions from different mills are shown in Table 22. Flour from the disc mill and stone mill had higher angle of slide values than flour from the hammer mill, cyclone mill and centrifugal mill (Table 22). Angle of slide (θ) is the minimum slope required for a powder to flow under its own weight. Angle of slide of black bean flour fractions had a negative relationship of $r \approx 0.95$-0.96 with the circularity of the particles (Table 16). No correlations between angle of slide and convexity, compactness or elongation were observed.

Table 22. Angle of slide[a] of different flour fractions

Milling method	Angle of slide (°)							
	Stainless steel				Aluminum			
	50-100 µm	100-150 µm	150-250 µm	250-425 µm	50-100 µm	100-150 µm	150-250 µm	250-425 µm
Disc mill-Fine-Gap 3	38.2 a,A	28.0 a,B	22.5 a,C	20.5 a,D	42.0 a,A	30.8 a,B	25.3 a,C	23.7 a,D
Stone mill-Fine	37.8 b,A	27.7 b,B	22.2 a,C	20.3 a,D	41.2 b,A	30.5 a,B	25.0 a,C	23.7 a,D
Hammer mill	36.5 c,A	25.5 c,B	20.5 b,C	19.2 b,D	40.0 c,A	28.2 b,B	24.0 b,C	22.3 b,D
Cyclone mill	36.7 c,A	25.0 d,B	20.5 b,C	18.8 c,D	40.0 c,A	28.2 b,B	23.7 b,C	21.8 b,D
Centrifugal-12,000 rpm-250 µm	36.5 c,A	25.5 c,B	20.5 b,C	19.5 b,C	40.0 c,A	28.3 b,B	24.0 b,C	22.8 b,C

[a]Different uppercase letters across rows indicates significant differences between particle size fractions (P=0.05). Different lowercase letters across column indicates significant differences between mills (P=0.05)

In general, aspherical shapes result in poor flour flowability due to intermittencies in flow rate (Fraige et al., 2008). Elongated or irregular particles tend to mechanically interlock or entangle with each other, thus obstructing flour flow and reducing flowability. A study by Pelgrom et al. (2015) showed poorer flow characteristics of chickpea flour than that of pea, bean, and lentil of similar size. Also, they observed poor flowability with decreasing average particle size. In this research, the angle of slide was significantly higher on the aluminum material compared to stainless steel. But Kaiser (2019) did not observe a significant difference in angle of slide for hammer milled pea flour on aluminum and stainless steel material. This may be due to differences in the commodity.

Conclusions

Thus, results indicate that milling method and particle size interaction affected the particle shape, physical, chemical and flow properties of selected black bean flour fractions. Flour moisture content was significantly lower when milled with the centrifugal mill. Flour ash content was significantly higher when milled with the cyclone mill. Centrifugal mill caused the greatest starch damage among the mills. Stone mill resulted in the highest L* value and lowest *a** for any flour fraction. Higher circularity, compactness, and convexity with hammer mill, cyclone mill and centrifugal mill than with disc mill or stone mill, suggest that mills having screens with round apertures favored passage of round or compact particles through the apertures and exiting the mill. Fine flour fractions had more starch damage, L* and less moisture, ash, *a** values, flowability, circularity than coarse fractions. Large particles tended to be more circular and compact and less convex and elongated compared to small particles suggesting that black beans may have initially broken into large circular particles and later into more elongated particles due to initial erosion and later shattering mechanisms during milling.

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CHAPTER 4: THE EFFECT OF REMOVING SOLUBLE PHENOLIC COMPOUNDS FROM FLOUR ON FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF BLACK BEAN PROTEIN ISOLATES

Abstract

Phenolic compounds are known to form complexes with proteins which cause changes in the structural, functional and nutritional properties of both compounds. The objective of this study was to evaluate the effect of removing soluble phenolic compounds from whole bean and cotyledon flours on the functional and rheological properties of black bean protein isolates. Whole bean flour and cotyledon flour were subjected to phenolic extraction resulting in whole bean flour free of soluble phenolics (WBFP) and cotyledon flour free of soluble phenolics (CBFP). Whole bean flour and cotyledon flour not subjected to phenolic extraction were categorized as whole bean flour with soluble phenolics (WBWP) and cotyledon flour with soluble phenolics (CBWP). Protein isolates were prepared from the four black bean flours described above. Protein isolates from CBFP had significantly lower ($P< 0.05$) ash content and phenolic content and significantly higher ($P< 0.05$) protein content compared to other protein isolates. The brightness of protein isolates from WBWP and CBWP improved considerably after removal of phenolic compounds. Protein isolates from CBFP had significantly higher ($P< 0.05$) wettability, dispersibility, foaming capacity, foaming stability, emulsion capacity, and emulsion stability than other protein isolates. Water binding capacity was significantly higher ($P< 0.05$) for protein isolates with high phenolic content. Storage modulus and loss modulus were lower for protein gels made from protein isolates containing phenolic compounds compared to those made from protein isolates without soluble phenolic compounds. Removal of soluble phenolic compounds improved the functional and rheological properties of black bean protein isolates.

Introduction

The search for alternative protein sources to replace animal proteins has been a challenge due to the adverse functional and sensory properties of the proteins in food systems (Henchion et al., 2017). Pulses represent a nutritious and inexpensive source of protein (Papalamprou et al., 2010; Henchion et al., 2017). Additionally, pulse proteins have gained importance as they have desired functional properties, including the ability to form gels and emulsions (Tiwari et al., 2011).

Protein isolates provide a sustainable way to utilize nutrient dense dry beans as a food ingredient (Pastor-Cavada et al., 2010). Physical separation and wet extractions are common methods to isolate proteins from dry beans (Siddiq & Uebersax, 2013). Dry physical separation is energy efficient and results in high yields (Aguilera et al., 1982). Wet extractions have high product purity but have high energy requirements (Marki & Doxastakis, 2006).

Proteins can interact individually and in combination with other molecules according to changes in the processing environment (e.g. changes in pH and ionic strength) and protein composition, which can lead to diversified functional properties (Sęczyk et al., 2019). Molecular properties like hydrophobicity, hydrogen bonds, ionic forces, and covalent bonds are involved in these interactions, which ultimately affect the behavior of proteins during food processing (Sathe, 2002).

Phenolic compounds are secondary metabolites that are synthesized by plants and consist of bioactive structural phenolic units. Phenolic compounds contain several hydroxyl groups on one or more six-carbon aromatic rings (D’Archivio et al., 2007). Phenolic compounds are predominantly located in the seed coat of the dry bean (López et al., 2013). Based on the extraction method, phenolic compounds can be categorized as non-extractable and extractable (Xu et al., 2020).

Phenolic compounds possess a significant binding affinity for proteins, which can lead to the formation of soluble and insoluble protein–phenolic complexes (Papadopoulou & Frazier, 2004). In a food system, proteins have several functional properties such as solubility, water binding capacity, gelation, emulsification, and foaming (Wouters et al., 2016). Protein solubility is reduced by its interaction with phenolic compounds. Phenolic compounds induce crosslinking between proteins, which changes the net charge on the surface of protein molecule and hence affects the solubility of proteins (Prigent et al., 2003).

Yuksel et al. (2010) reported that when phenolic compounds bind to proteins, the protein structure can change because only weak hydrophobic sites would remain on the surface of the protein leading to a possible change in the protein folding and in the protein functionality. Most of the phenolic compounds bind proteins non-covalently since phenolic hydroxyl groups are capable to form H-bonding with peptide carbonyl groups of proteins, where some oxidized phenolic compounds can also bind proteins covalently (Aydemir & Yemenicioglu, 2013). There is limited literature available related to black bean protein-phenolic interaction and effect of removing soluble phenolic compounds on protein functionality. The objective of this study was to determine the effect of removing soluble phenolic compounds on functional and rheological properties of black bean protein isolated from the whole bean and cotyledon.

Materials and Methods

Black Beans

Black beans were obtained from three dry bean companies. Seeds from each company were kept separate and treated as replications in the ensuing experiments. The black bean seeds were manually cleaned to remove all foreign matter. Seeds were dehulled to obtain cotyledons using the method described by Fernando (2017). Both the whole seeds and cotyledons were milled using a laboratory hammer mill (Perten LM 3100, Springfield, IL) to produce whole bean flour and cotyledon flour.

Extraction of Soluble Phenolic Compounds

Whole bean and cotyledon flours were initially defatted using hexane extraction (nonpolar solvent extraction) for 8 h. The defatted flours were dried at 45 °C temperature overnight in an oven to remove excess hexane. Whole bean and cotyledon flours were then subjected to phenolic extraction (polar solvent extraction) by dispersing the flours in 60% (v/v) methanol using a flour-to-solvent ratio of 1:20 (w/v) and stirring for 1 h. The suspension was filtered under vacuum using Whatman filter paper (No 1) and the supernatant was discarded. The extraction procedure was repeated four times. The extracted flours were dried at 30 °C overnight. The unextracted samples were designated as: whole bean flour with phenolics (WBWP) and cotyledon flour with phenolics (CBWP), while the extracted samples were designated as; whole bean flour free of soluble phenolics (WBFP); and cotyledon flour free of soluble phenolics (CBFP).

Preparation of Protein Isolates

Protein isolates were prepared from the four black bean flour samples described above using the method of Boye et al., (2010) with slight modification. Each flour sample was mixed with distilled water at 1:10 (w/v) flour-to-solvent ratio. To facilitate protein dissolution, the pH of mixture was adjusted to 9.0 with 1.0 M NaOH. The resulting mixture was stirred using a magnetic stirrer at 1,000 rpm for 1 h, and then centrifuged at 5,000 × *g* for 20 min at 4 °C. The process was repeated with a 1 to 5 (w:v) pellet-to-water ratio. Supernatants from both extractions were pooled and adjusted to pH 4.6 with 1.0 M HCl so as to facilitate protein precipitation. The precipitate was collected following centrifugation (5,000 × *g*, 20 min, 4 °C); washed with 25 mL distilled water, neutralized, frozen (−30 °C), and then freeze-dried to yield a free-flowing powder. Protein isolates were stored at 4 °C in sealed tubes for later use. This process resulted in four protein isolate samples: whole bean flour protein isolate with phenolics (WBPIWP); whole bean flour protein isolate free of soluble phenolics (WBPIFP); cotyledon flour protein isolate with phenolics (CBPIWP); and cotyledon flour protein isolate free of soluble phenolics (CBPIFP).

Chemical Analysis of Protein Isolates

Ash content, moisture content, protein content and total starch content were determined according to AACC International Approved Methods 08-01.01, 44-15.02, 46-30.01, and 76-13.01 respectively. Total phenolic content was determined using the Folin–Ciocalteu method as described by Singleton et al. (1999), using gallic acid standard (Sigma Chemical CO, St. Louis, MO, USA) as standard. The total phenolic content was expressed as gallic acid equivalent (GAE) in mg/g.

Available lysine content was determined using trinitrobenzenesulfonic acid (TNBS, Sigma Chemical CO, St. Louis, MO, USA) following the method of Kakade & Liener (1969) with some modifications. Protein isolate (10 mg) was mixed with 1 mL of 4% $NaHCO_3$ in a 50 mL test tube which was placed in a shaking water bath at 40 °C for 10 min. Then, 1 mL of 1.0% TNBS was added and the reaction was allowed to proceed for 2 h at 40 °C. The protein was hydrolyzed by adding concentrated HCl (3 mL) and incubating the reaction mixture in a boiling water bath for 2 h. The hydrolysate was cooled to room temperature and 5 mL of distilled water was added followed by filtration using Whatman filter paper (No 1) to remove any insoluble particulate matter. The supernatant was extracted twice with ethyl ether (10 mL) to remove the TNP-N-terminal amino acids or peptides, along with picric acid which is produced during the reaction. The residual ethyl ether was removed by placing the solution in hot water (40 °C) for 5 min. The solution cooled to room temperature and read at 346 nm, against a blank prepared by same procedure except that the concentrated HCl was added to the protein solution before the addition of the TNBS reagent. A molar absorptivity value of 1.46 x 10^{-4} M^{-1} cm^{-1} of 3-TNP-lysine was used for calculating the available lysine content.

Color

Color of the protein isolates was determined using a Minolta colorimeter CR410 (Konica Minolta Sensing Americas, Inc, NJ, USA). Protein isolates were placed in a sample cell that had a quartz glass window. The cell containing protein isolates was placed below the light source and L*, *a** and *b** color values were recorded.

Solubility

Protein solubility was determined according to the method of Wu et al., (1998). Four 20 mg samples of each isolate were dispersed in 20 mL of distilled water and stirred for 30 min to obtain uniform suspensions. Each suspension was adjusted with 1 M NaOH or 1 M HCl to pH 3, 5, 7, 9, and 11 stirred for 1 h and centrifuged for 15 min at 17,000×*g*. Protein content in supernatant was measured by using method of Bradford (1976). Solubility was expressed as a percentage of total protein content.

Wettability

Wettability of protein isolates was determined by static wetting test as described by Freudig et al., (1999) with some modifications. Distilled water (100 mL) was poured in a 250 mL beaker. A glass funnel with opening blocked by a test tube was placed above the beaker in ring stand. About 1 g of protein isolate was placed in the funnel and test tube was removed to allow the protein powder to move inside the beaker and a stopwatch was used to record time needed for all powder particles to penetrate the water surface.

Water Binding Capacity (WBC)

Water binding capacity of black bean protein isolates was determined using the method of Shchekoldina & Aider (2014) with modification. Protein isolate (1 g) was mixed with 10 mL of distilled water and stirred for 5 min. The mixture was left to stand for 30 min and then centrifuged at 1,100 x *g* for 30 min. The supernatant water was discarded, and tube was inverted at 45° angle for 25 min to drain the remaining water from the protein sediment. The water binding capacity was calculated as:

WBC = (Weight of the tube with hydrated protein isolate – Weight of the tube and dry protein isolate) / Weight of dry protein isolate

Dispersibility

Dispersibility of black bean protein isolates was determined using the method of Kullarni & Ingle (1991) with modification. Protein isolate (3 g) was mixed with 30 mL of distilled water in a measuring cylinder. The mixture was vigorously stirred and allowed to settle for 2 h. The volume of settled particles was measured and dispersibility was calculated as:

Dispersibility (%) = (Total volume – Settled volume) / Total volume X 100

Emulsification Properties

Emulsifying capacity and stability were determined using the method described by Lawal et al., (2007) with modifications. Black bean protein isolates (100 mg) were mixed with 5 mL of distilled water to make 2% aliquots that were placed in centrifuge tubes (50 mL) and thoroughly mixed with 5 mL of canola oil for 1 min using a vortex mixer (VWR Analog Mixer - speed 6).

The emulsions were centrifuged at 1,100 x *g* for 5 min. The height of emulsified layer (mm) separated from the sample and the height of the total content were measured. The emulsion capacity was calculated according to the following equation.

Emulsifying capacity = (Height of the emulsified layer / Height of the total content) X 100

The emulsion was heated in a water bath at 80 °C for 30 mins to determine the emulsion stability. Then the emulsion was centrifuged at 1,100 x *g* for 5 min. Emulsion stability was calculated using the following equation.

Emulsion stability = (Height of the emulsified layer after heating/ Height of the total content) x 100

Foaming Properties

Foaming capacity (FC) and foam stability (FS) was determined using the method of Wani et al., (2015) with modifications. Protein isolate (2 g) was dispersed in 100 mL distilled water. The solution was whipped for 1 min at 10,000 rpm using a homogenizer. The whole content was transferred to a measuring cylinder and the volume of foam was measured immediately to determine the foam capacity. The change in volume of foam with time was measured at 15, 30, 45, 60 and 120 min. The volume of foam at each time interval gives a measure of foam stability for that time. The foaming capacity and foaming stability were calculated according to the following equation.

FC (%) = (Volume after whipping - Volume before whipping) / Volume before whipping X 100

FS (%) = Foam volume after time (t) / Initial volume X 100

Gelation

Gelation was quantified by the procedure reported by Sathe & Salunkhe (1981). Protein solutions (5 mL) at concentration of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20% (w/v) were heated in a boiling water bath for 1 h followed by rapid cooling under running water (23 °C). The solutions were further cooled at 4 °C for 2 h. The concentration above which the sample did not collapse or slip when the tube was inverted was noted as the least gelation concentration.

Gelation Dynamics

The gelation dynamics of the protein isolates were determined using the Discovery HR 2 hybrid rheometer (Eden Prairie, MN) equipped with parallel plates geometry. The effect of thermal treatment on the rheological properties of protein isolate dispersion was studied by monitoring the storage (G') modulus, loss modulus (G") and loss tangent (tan δ) of the gels. Linear viscoelastic region (LVR) was determined by performing the amplitude and frequency sweep tests. The linear region of 0.1% of strain and 1 Hz of frequency was used for the measurements. Protein solution (20% w/v) was heated from 25 to 95 °C at 10 °C/min. Storage modulus (G'), loss modulus (G") and loss tangent (tan δ) were continuously recorded as function of temperature. After cooling the sample, rheological behavior was studied by comparing the dependence of storage modulus (G') and loss modulus (G") with frequency. Frequency sweep was performed in the range of 0.05-10 Hz at constant strain of 0.1% and storage and loss modulus were recorded as a function of frequency. The frequency sweep was performed at 25 °C.

Statistical Analysis

The experimental design was a randomized complete block. Each treatment was replicated three times and replicates were based on commercial source of seed. Data were analyzed using SAS 9.4 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

Proximate Composition

Proximate compositions of the four black bean flours WBWP, WBFP, CBWP and CBFP are shown in Table 23. Ash and protein contents were less, and total starch content was more in cotyledon flour compared to whole bean flour. Whole black bean flour and cotyledon flour contained 74.99 mg (GAE)/g and 1.62 mg (GAE)/g of total phenolic values, respectively. These values are similar to the values reported in Yang et al. (2018). Removal of the seed coat reduced the total phenolic content 98% from WBWP to CBWP. Thus, phenolic compounds are predominantly located in the seed coat of the dry bean (López et al., 2013).

Phenolic extraction reduced the ash content and total starch content in both whole bean and cotyledon flours (Table 23). Moisture values were higher after the extraction compared to the original flours. Protein content of both whole bean and cotyledon flours were increased after the phenolic extraction. After the soluble phenolic removal, WBFP and CBFP contained 4.76 mg (GAE)/g and 0.11 mg (GAE)/g of total phenolic values, respectively. Interestingly, extraction step removed 93.7% of phenolic compounds from whole black bean flour and 93.2% from cotyledon flour. The total phenolic content observed after extraction process is attributed to the unextractable bound phenolics present in dry beans (Gao et al., 2017).

Table 23. Chemical composition[a] of black bean flour

Flour type	**Moisture (%)**	**Ash[b] (%)**	**Protein[b] (%)**	**Total starch[b] (%)**	**Total phenolic content[b] (mg (GAE)/g)**
WBWP	6.5 b	3.62 a	22.67 b	40.74 b	74.99 a
WBFP	7.0 a	2.34 c	23.97 a	37.96 c	4.76 b
CBWP	6.8 b	3.48 b	22.39 c	41.52 a	1.62 c
CBFP	7.1 a	1.87 d	23.52 ab	37.24 d	0.11 d

[a]Different lowercase letters across column indicates significant differences between flour type (P=0.05)
[b]Dry weight basis
WBWP = whole bean flour with phenolics; WBFP = whole bean flour free of soluble phenolics; CBWP = cotyledon flour with phenolics; CBFP = cotyledon flour free of soluble phenolics.

Proximate compositions of the four protein isolates WBPIWP, WBPIFP, CBPIWP and CBPIFP are shown in Table 24. Moisture content was lower in protein isolates than the flour (Tables 23 and 24). This may be due to the freeze-drying step during protein isolate preparation. Ash content and protein content were higher and total starch content and total phenolic content were lower in protein isolates than in the flours. In this experiment the process of converting whole bean and cotyledon flour to their respective protein isolates reduced total phenolic content up to 72.9 and 79.7%, respectively. Deshpande & Cheryan (1982) observed a reduction up to 60% in total tannin and phytic acid (phenolic compounds) content in protein concentrates compared to whole dry beans.

Cotyledon protein isolates had higher percentage of protein compared to whole bean protein isolates 90.31 vs 84.42 %, respectively. Seed coat represents nearly 8% of weight of the seed. Removal of seed coat reduced the phenolic content 96% from WBPIWP to CBPIWP. The differences in protein percentages of cotyledon protein isolates and whole bean protein isolates are mainly due to seed coat containing non-protein components. Ash content was higher in whole bean protein isolates than cotyledon protein isolates. This may be due to the presence of seed coat in the whole bean flour which has been reported to contain high amount of minerals (Tiwari & Singh, 2012; Ribeiro et al., 2012).

Table 24. Chemical composition[a] of protein isolates

Protein isolate	Moisture (%)	Ash[b] (%)	Protein[b] (%)	Total starch[b] (%)	Available lysine[b] (g/100g)	Total phenolic content[b] (mg (GAE)/g)
WBPIWP	2.5 b	5.93 a	83.18 b	0.77 a	9.44 d	13.06 a
WBPIFP	2.4 bc	5.64 b	85.66 b	0.33 c	9.94 c	1.75 b
CBPIWP	2.3 c	5.47 c	88.84 a	0.60 b	10.33 b	0.51 c
CBPIFP	2.7 a	5.44 d	91.78 a	0.23 d	11.00 a	0.01 d

[a]Different lowercase letters across column indicates significant differences between protein isolate (P=0.05)
[b]Dry weight basis
WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

Removal of soluble phenolics showed a significant ($p < 0.05$) effect on compositional profile of protein isolates. Phenolic content was 86.6% less in WBPIFP than in WBPIWP. The extraction procedure resulted in a 4.9 and 0.5% decrease in ash content and a 3.1 and 3.3% increase in protein content and 5.3 and 6.5% increase in available lysine in both WBPIFP and CBPIFP, respectively. Interaction of phenolics with proteins at alkaline pH during extraction process may have led to reduced protein percentage in protein isolates with phenolics (Malik & Saini, 2017). Increase in available lysine suggests that the extraction process altered the configuration of proteins which caused lysine to become more exposed and so available for detection.

The available lysine content of protein isolates is shown in Table 24. Protein isolates (WBPIWP and CBPIWP) with phenolic compounds were found to have less available lysine content than the protein isolates after soluble phenolic extraction. Cotyledon protein isolates had higher available lysine content as compared to the whole bean protein isolates. This reflects low content of phenols in cotyledon than in whole bean flours. The lower level of lysine in isolates containing phenols might be due to the presence of quinones, which are oxidized forms of phenolic compounds. Quinones (electrophilic) interact with the nucleophilic Ɛ -amino group on lysine. TNBS binds to the nucleophilic nitrogen of the Ɛ-amino side chain of lysine while estimating available lysine content. Interaction of oxidized phenolic compounds with proteins block the reaction sites for TNBS and decrease the available lysine content (Kroll et al., 2003). Oxidized phenolic compounds can also react with other nucleophilic groups such as cysteine, tryptophan, methionine, tyrosine, histidine, and N terminal proline of protein molecules (Kroll et al., 2003). These results indicate the possible nutritional consequence in the food systems resulting in the limited availability of the essential amino acids (Rawel et al., 2002). The Ɛ-amino group of protein becomes modified upon the interaction of phenolics, which decreases the free amino groups in the modified proteins (Ali et al., 2013). Rawel et al. (2002) working with soy protein isolates observed similar results. Aggregation of proteins by phenol binding can also explain the reduction in available lysine content where the protein aggregation causes low accessibility of test reagent.

Physical and Functional Properties

Color values (L*, *a** and *b**) of whole black bean flour and cotyledon flour are presented in Table 25. Cotyledon flour had significantly higher brightness (L*) and yellowness (*b**), and lower redness (*a**) than whole bean flour. This reflects the differences in phenolic content found in the seed coat and cotyledon. The color of the dry bean seed coat is due to the presence of phenolics including anthocyanins, flavonols glucosides, and condensed tannins (Ganesan & Xu, 2017). High anthocyanin content is normally present in dark-colored beans whereas condensed tannins are responsible for light yellow or pink appearance of the seed coat of certain market classes of dry beans (Ganesan & Xu, 2017). After the soluble phenolic extraction, brightness of whole bean flour was reduced and had a gray to brownish gray appearance. The supernatant from the whole black bean was purple in color (Figure 5a). After removing the soluble phenolics, the cotyledon flour changed from creamy white to white. The supernatant from the cotyledon flour was creamy in color (Figure 5b). Carter (2014) reported that anthocyanins leached from the seed coat into the cotyledon during soaking or cooking causing the cotyledon flour to lose its brightness and take on a light purple appearance. Bushey et al. (2000) also reported excessive leaching of soluble phenolics resulting in a flat brown appearance.

a b

Figure 5. Filtrate and the flour color after soluble phenolic extraction (a) whole bean flour and (b) cotyledon flour

Color values (L*, *a** and *b**) of whole black bean and cotyledon protein isolates are presented in Table 25 and Figure 6. Brightness of protein isolates made from whole bean was lower than when made from cotyledon flour. This was also reflected in the black bean flours (Table 25) due to the differences in phenolic content found in the seed coat and cotyledon. Both whole bean and cotyledon protein isolates showed significant ($p < 0.05$) variation in the color with and without phenolics. Removal of phenolics increased the brightness of cotyledon protein isolates to a large extent, as is evident from the L* value of CBPIWP (72.98) and CBPIFP (84.00).

Table 25. Color[a] of black bean flour and protein isolates

Sample	L*	a*	b*
WBWP	79.00 c	0.76 b	4.51 c
WBFP	70.51 d	1.51 a	2.03 d
CBWP	88.49 b	-0.82 d	7.74 a
CBFP	89.09 a	-0.42 c	6.46 b
WBPIWP	45.77 d	1.69 a	-0.36 c
WBPIFP	51.25 c	1.33 b	-0.29 c
CBPIWP	72.98 b	0.87 c	13.61 a
CBPIFP	84.00 a	-0.04 d	8.23 b

[a]Different lowercase letters across column indicates significant differences between protein isolates (P=0.05)
WBWP = whole bean flour with phenolics; WBFP = whole bean flour free of soluble phenolics; CBWP = cotyledon flour with phenolics; CBFP = cotyledon flour free of soluble phenolics; WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

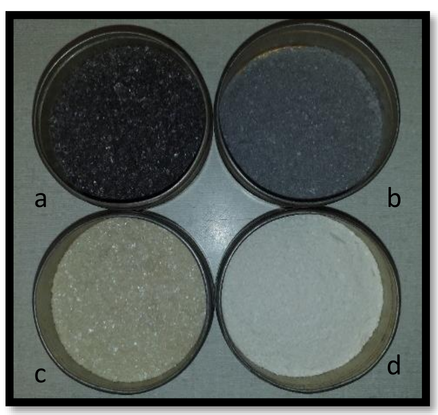

Figure 6. Color of protein isolates (a) WBPIWP, (b) WBPIFP, (c) CBPIWP, and (d) CBPIFP

WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

a b c d

Figure 7. Color changes during protein extraction from whole black bean flour (a) pH=6.5, (b) pH=9.0, (c) pH=4.6, and (d) pH=7.0

When whole bean flour with phenolics was mixed with distilled water, the initial pH was 6.5 and had a blue color (Figure 7a). The color of the solution changed to green when pH was adjusted to 9.0 with NaOH (Figure 7b). Later, when the pH of the supernatant was adjusted to 4.6 with HCl, the color turned to pink (Figure 7c) and when the pH was adjusted to 7.0 the supernatant turned dark purple (Figure 7d). There were no distinct color changes in the cotyledon flour supernatant except for variations in between white and a creamy color. This suggests that less anthocyanin was present in the cotyledon. Saeed & Cheryan (1988) also observed similar color changes during sunflower protein extraction due to the oxidation of phenolic compounds to o-quinones at alkaline pH. Under different pH conditions, the hydroxyl (OH) and/or methyl ether ($O-CH_3$) groups attached to the carbon rings undergo reversible structural transformations and ionizations. Restructuring a molecule changes the way it absorbs light, giving rise to color changes (Cavalcanti et al., 2011).

Wettability of protein isolates both from whole bean and cotyledon showed significant ($p < 0.05$) variation with and without phenolics (Table 26). Protein isolates with phenolics took less time to wet than protein isolates that were free of soluble phenolics. Cotyledon protein isolates took longer for complete wetting compared to their corresponding whole bean protein isolates.

This may be due to the low phenolic content and to higher percentage of proteins in cotyledon protein isolates (Average = 90%) than whole bean protein isolates (Average = 84%). Exposure of more protein results in increased time for complete wetting (Kim et al., 2002).

Table 26. Wettability, dispersibility, water binding capacity and least gelation concentration values[a] of protein isolates

Protein isolate	Wettability (Sec)	Dispersibility (%)	Water binding capacity (%)	Least gelation concentration (%)
WBPIWP	1,302 d	79 d	343 b	10 a
WBPIFP	2,138 b	90 b	292 c	8 b
CBPIWP	1,719 c	88 c	367 a	6 c
CBPIFP	2,899 a	91 a	334 b	4 d

[a]Different lowercase letters across column indicates significant differences between protein isolates (P=0.05)
WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

Protein isolates from cotyledon had better dispersibility than the protein isolates from whole bean (Table 26). Higher dispersibility of cotyledon protein isolates can be attributed to their higher solubility and higher percentage of proteins (Table 24). Dispersibility of protein isolates showed significant ($p < 0.05$) variation with phenolics. Protein isolates containing phenolics had decreased solubility leading to decreased dispersibility, as discussed below.

Solubility of protein isolates from whole seed and from cotyledon flour varied significantly ($p < 0.05$) with the pH (Figure 8). Minimum solubility was reported at pH 5.0 for all the types of protein isolates. Kudre et al. (2013) also obtained similar results for black bean protein isolates. Protein isolates were more soluble in alkaline pH than acidic pH. These results support the use of alkaline extraction of protein isolates over acidic extraction during sample preparation. The minimum solubility of WBPIWP, WBPIFP, CBPIWP and CBPIFP at pH 5.0, was 11.0, 14.8, 17.8 and 20.8 %, respectively (Figure 8). Protein isolates were more soluble in alkaline pH than acidic and maximum solubility was 69.3, 73.1, 75.5 and 79.0% for WBPIWP, WBPIFP, CBPIWP and CBPIFP, respectively. Cotyledon protein isolates had greater solubility than their corresponding whole bean protein isolates. The removal of soluble phenolics had a significant ($p < 0.05$) effect on the solubility of black bean protein isolates. Whole bean and cotyledon protein isolates without phenolic removal had a lower solubility than the protein isolates after phenolic removal.

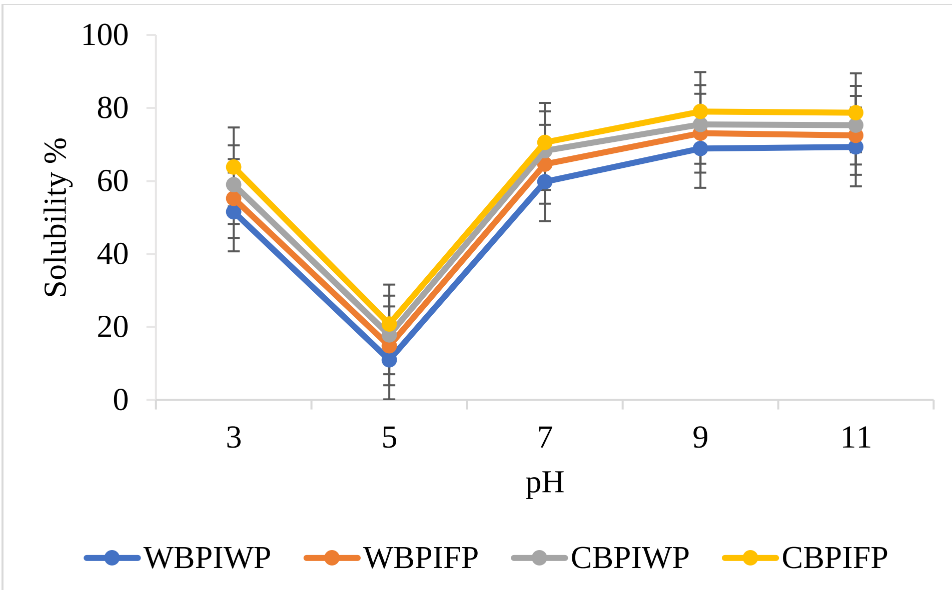

Figure 8. Solubility of protein isolates

WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

Solubility is governed by the balance of protein-protein and protein-solvent interactions. Solubility of protein depends on polar residues interacting with water via hydrogen bond while hydrophobic part of protein folds to avoid contact with water. Protein-solvent interaction promotes solubility, and further can be affected by environmental factors like pH and processing (Kinsella 1979; Sęczyk et al., 2019). Variations in protein isolate solubility leads to variations in protein functionality in food systems (Kinsella & Melachouris, 1976). Electrostatic repulsion between proteins due to high surface charges helped to overcome electrostatic and van der Waals attractive forces to remain soluble in solution. Crosslinking can form a barrier to water movement on protein. Ali et al. (2013) and Sęczyk et al. (2019) described protein denaturation by polyphenol interaction that led to reduced solubility of phenol modified proteins. Also, Rawel et al. (2002) explained that phenolic interaction reduced the net charge on the protein surface that led to decrease in the protein solubility. At the isoelectric point, there is no net charge on the protein thus reducing or eliminating repulsive force between proteins and minimizing solubility (Singh et al., 2005). Phenolic compounds can block the hydrophilic groups of protein and introduce an aromatic structure and decrease the solubility of proteins (Kroll et al., 2001; Rohn et al., 2005).

Water binding capacity is important for texture, mouthfeel, starch retrogradation, and staling of product (Sathe, 2002). The values for water binding capacity of protein isolates of WBPIWP, WBPIFP, CBPIWP and CBPIFP are shown in Table 26. Presence of phenolics in protein isolates showed significant ($p < 0.05$) variation in water binding capacity. Protein isolates with phenolics had greater water binding capacity than the protein isolates free of soluble phenolics. This is due to the reduced hydrophobicity of proteins upon interaction with phenolics (Salgado et al., 2011; Yüksel et al., 2010). The phenolic compounds possess many water binding sites, which bind water mainly through hydrogen bonding. This enhances the water binding capacity of protein isolates (Malik & Saini, 2017). Protein isolates from cotyledon showed greater water binding capacity than protein isolates from the whole seed. Higher protein content in cotyledon than in whole bean protein isolates (Table 24) might have led to these results. Chavan et al. (2001) also explained that differences in water binding capacities of protein isolates can be related to protein concentration and their conformational characteristics.

Removal of soluble phenolic compounds significantly ($p < 0.05$) increased the foaming capacity and stability of whole bean and cotyledon protein isolates (Table 27). Phenolic compounds can block the hydrophilic groups and decrease the solubility of proteins which leads to a reduction of foaming properties of protein isolates (Rohn et al., 2005).

Table 27. Foaming capacity and stability values[a] of protein isolates

Protein isolate	**Foaming capacity**	**Foaming stability**				
		15 min	**30 min**	**45 min**	**60 min**	**120 min**
WBPIWP	63 d	53 d	51 d	49 d	45 d	45 c
WBPIFP	71 c	58 c	58 c	54 c	50 c	46 b
CBPIWP	98 b	78 b	70 b	66 b	62 b	58 a
CBPIFP	118 a	94 a	85 a	77 a	67 a	43 d

[a]Different lowercase letters across column indicates significant differences between protein isolates (P=0.05)
WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

Foaming capacity and stability of protein depends upon their ability to move quickly to the air/liquid interface and solubility in liquid phase so that it is able to rearrange itself at the interfacial film (Toews & Wang, 2013). When the net charge on protein is increased, there is a weakening of hydrophobic interactions and an increase in the flexibility of proteins (Aluko and Yada, 1995). Phenolic compounds can block the hydrophilic groups on the protein, reduce the net charge and decrease the solubility of proteins (Rohn et al., 2005; Kroll et al., 2001). Therefore, presence of phenolics might have reduced the net charge of proteins leading to lower flexibility. Flexible structure of proteins leads to good foaming properties (Hailing & Walstra, 1981). According to Damodaran (1997) only soluble proteins are involved in foam formation and since at isoelectric point, the concentration of these soluble proteins is very low, foam formation will be less. Increase in protein flexibility helps protein to diffuse more quickly to the air-water interface to encapsulate air and enhances the foam formation (Yuliana et al., 2014).

Data indicating the capacity of protein isolates from whole bean and cotyledon to form and stabilize emulsions are shown in Table 28. Cotyledon protein isolates had better emulsion activity and stability than their corresponding whole bean protein isolates. Higher solubility and higher percentage of proteins account for the better emulsion properties of cotyledon protein isolate compared to whole bean protein isolates. The presence of high phenolic compound content showed significant ($p < 0.05$) reduction in the emulsion activity and emulsion stability of protein isolates. Phenolic compounds decreased the solubility and hydrophobicity of protein isolates. The reduced solubility hinders the rapid movement of peptides to the oil-water interface. The decrease in the surface hydrophobicity of protein isolate might reduce the ability of protein to localize at the oil-water interface. Salgado et al. (2011) reported that phenolic compounds enhanced protein aggregation, which resulted in diminished hydrophobicity and hence reduced unfolding at the oil-water interface.

Table 28. Emulsion capacity and stability values[a] of protein isolates

Protein isolate	**Emulsifying capacity**	**Emulsifying stability**
	(%)	
WBPIWP	41.3 c	40.1 c
WBPIFP	43.1 b	40.3 c
CBPIWP	44.0 ab	40.7 b
CBPIFP	45.7 a	42.6 a

[a]Different lowercase letters across column indicates significant differences between protein isolates (P=0.05)
WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

Gelling capacity of protein can be expressed by using the least gelation concentration (LGC) which is defined as the lowest protein concentration required to form a self-supporting gel. Thus, a lower the LGC indicates better gelating ability of the protein ingredient (Akintayo et al., 1999; Kaushal et al., 2012).

Covalent and non-covalent interactions are involved in gel formation. Gelation of proteins depends on the critical concentration of protein, protein-protein interaction and protein interaction with other non-protein components (Sathe & Salunkhe, 1981; Foegeding & Davis, 2011). Hence, more protein isolate was needed to form gels with protein isolates having low protein percentage. Presence of phenolics and the protein concentration in the protein isolates had an effect on the least gelation concentration (Table 26). Protein isolates with high concentration of proteins (cotyledon derived) and low concentration of phenolics had a low least gelation concentration (LGC). Rubino et al. (1996) observed a lower gelation ability in canola protein with addition of polyphenols. Protein-protein interactions for gelling are hindered by the phenolic compounds that are attached to proteins. CBPIFP having high protein content and low phenolic content had a 4% LGC and WBPIWP having low protein and high phenolic content required 10% to form a self-supporting gel. CBPIWP and WBPIFP formed gels at 6% and 8%, respectively. Sathe & Salunkhe (1981) reported 8% LGC for great northern bean protein concentrates (85.4% protein) and Coffman & Garcia (1977) has reported 10% LGC for mung bean protein isolates (92.8% protein).

The storage modulus (G') and loss modulus (G") showed a similar pattern for gels made with the four protein isolates studied (Figures 9 and 10). G' represents the strength of the gel structure and measures the elastic component of the network. G" measures the viscous component and represents interactions which do not contribute to the three-dimensional nature. For all gels, G' was greater than G", throughout the test, suggesting a predominant elastic behavior. G' of all the four protein isolates (WBPIWP, WBPIFP, CBPIWP and CBPIFP) increased during the heating, holding and cooling periods, which indicates network strengthening. During heating, proteins become denatured and consequently their hydrophobic areas are exposed. These hydrophobic areas facilitate the subsequent peptide association and gel network structure formation (Lamsal et al., 2007). The new regions previously involved in stabilizing native structure of protein become available for inter-molecular interactions (Malik & Saini, 2017).

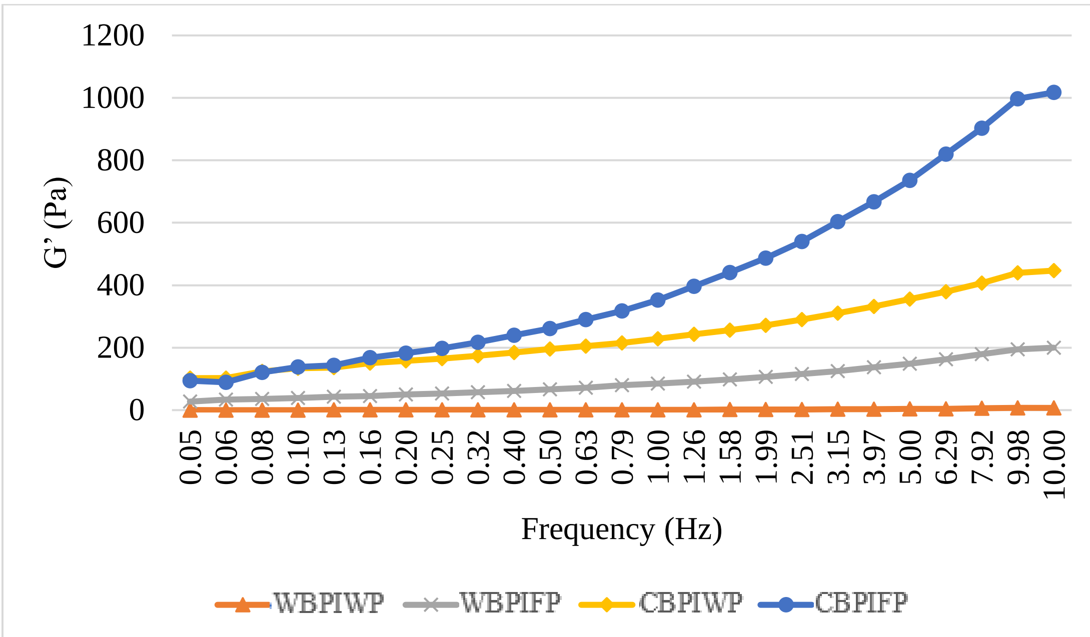

Figure 9. Variation of storage modulus (G') with frequency at controlled strain of 0.1 %

WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

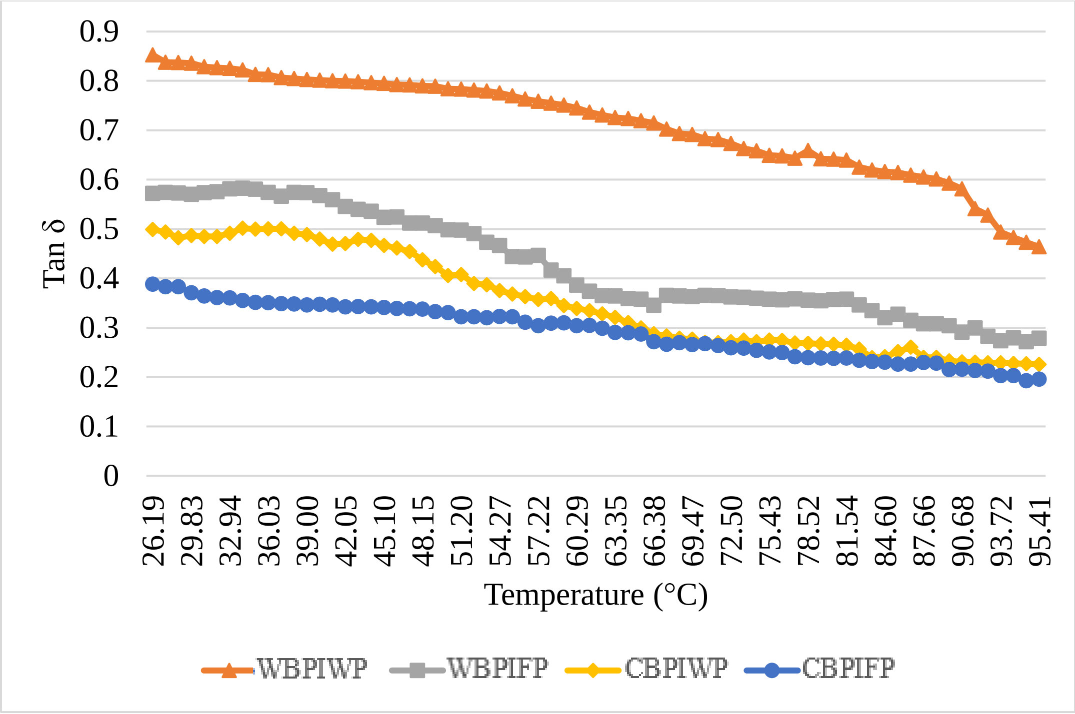

Figure 10. Change in Tan δ of protein isolates with temperature

WBPIWP = whole bean protein isolate with phenolics; WBPIFP = whole bean protein isolate free of soluble phenolics; CBPIWP = cotyledon protein isolate with phenolics; CBPIFP = cotyledon protein isolate free of soluble phenolics.

Gelation behavior of protein was affected by the presence of phenolic compounds. WBPIFP and CBPIFP showed higher G’ values compared to WBPIWP and CBPIWP samples, which indicates that interaction of proteins with phenolics impaired their elastic behavior (Figure 9). Phenolic compounds might be occupying the reaction sites required for the gel formation during heating resulting in the gels having a weak network structure. The better gelation properties of WBPIFP and CBPIFP samples might also be due to their higher solubility (Figure 8). A high concentration of protein in solution would favor gels having a good three-dimensional network structure which would resist breakdown by the application of shear stress and the oscillatory frequency. Tan et al. (2014) reported similar results on canola proteins protein isolates.

A greater proportion of β-sheets associated with WBPIFP and CBPIFP compared with WBPIWP and CBPIWP might also be responsible for the higher values of G' for WBPIFP and CBPIFP (Malik & Saini, 2017). Large surface area of β-sheets could provide more opportunities for intermolecular hydrogen bonding. Wang & Damodaran (1991) reported that gel networks were stabilized by the junction zones made out of intermolecular hydrogen bonds between β-sheets. Since exposure of hydrophobic sites is a prerequisite for gel formation, phenolic interactions with proteins that results in conformational changes and reduction in hydrophobicity, might also contribute to the weak gelation properties of WBPIWP and CBPIWP. The presence of a large number of exposed hydrophobic sites promotes gel formation by favoring protein-protein interactions and aggregate formation. According to Arzeni et al. (2012) an increase in hydrophobicity would result in the formation of more structured gels.

The loss tangent value (Tan δ= G''/G') provides information on the type of network structure formed. The energy lost due to viscous flow compared to energy stored due to elastic deformation in a single deformation cycle is measured by Tan δ. A high Tan δ value indicates a weak gel structure (more viscous) and conversely, a low Tan δ value indicates a strong gel structure (more elastic) (Douglas, 2018). Tan δ of all the protein isolates decreased with increased temperature, indicating that a stronger gel is formed as temperature increased (Figure 10). Protein isolates with low phenolic compound content had lower Tan δ values compared to their corresponding high phenolic content protein isolates, which indicated that gel strength increased as phenolic content decreased.

Conclusions

Removal of soluble phenolic compounds had a considerable effect on the functional and rheological properties of protein isolates from both whole bean flour and cotyledon flour. Protein isolates obtained from cotyledon had higher protein content and lower ash content than protein isolates from the whole seed. High available lysine content was observed in protein isolates from cotyledon due to lack of phenolics. Removal of phenolic compounds increased the available lysine content of protein isolates. Protein isolates from cotyledon had lighter color than the protein isolates from the whole seed and color of protein isolates improved significantly after removal of phenolics. All protein isolates had the lowest solubility at pH 5.0. Protein isolates with higher percentage of protein and lower percentage of phenolic compounds had the highest wettability, dispersibility, foaming capacity, and emulsifying capacity and had the best gel forming properties as indicated by low least gelation concentration. Gel strength of protein isolates was reduced by the presence of phenolic compounds. Gel formed from protein isolates with phenolics was more viscous and less elastic. Removal of soluble phenolic compounds improved the functional and rheological properties of black bean protein isolates.

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OVERALL CONCLUSIONS

Variation in milling method produced flours with significantly different particle size and size distribution. Centrifugal mill (10,000 or 12,000 rpm and 250 or 500 μm screen aperture), cyclone mill and hammer mill produced the smallest geometric mean particles. Disc mill and stone mill had similar particle size distributions as they do not utilize a screen. Geometric mean size had strong positive correlation with moisture, and loose bulk density and negative correlation with starch damage, L* and flow properties. Functional aperture size was smaller than the actual screen size of the mill. Black bean flour had an angle of repose of 26-36, which indicates that it has good or excellent flow behavior.

Interaction of particle size of the fraction and the milling method had a significant effect on characteristics of black bean flour fractions. Smaller particle fractions from each mill had a lower circularity value and higher elongation. Black beans may have initially broken into large circular particles and later into more elongated particles due to initial erosion and later shattering mechanisms during milling. Flour particle circularity had a strong positive correlation with particle size, loose bulk density and negative correlation with angle of slide, angle of repose, compact density and L* value.

Removal of soluble phenolics showed considerable effect on the functional and rheological properties of protein isolates from both whole bean flour and cotyledon flour. Protein isolates with higher percentage of protein and lower percentage of phenolic compounds had the highest wettability, dispersibility, foaming capacity, and emulsifying capacity and had the best gel forming properties as indicated by low least gelation concentration. Gel strength of protein isolates was reduced by the presence of phenolic compounds.

FUTURE RESEARCH AND INDUSTRIAL APPLICATION

There are several research gaps that can be identified as potential hindrances towards better utilization of dry beans. More research needs to be done on milling performance with regards to variability in seed characteristics and dry bean market class. Seed characteristics include seed size, shape, moisture content, seed coat content, cell wall composition and thickness, seed chemical composition, and seed hardness. Further investigation on genotype by environment effect on milling performance should be further investigated as to support the breeding programs to produce dry bean cultivars with good milling quality. More research is needed concerning post-harvest handling and storage conditions of pulses and pulse flour. The greatest gap in dry bean milling is available information on different milling methods/machines used to produce a defined dry bean flour to a standard particle size as to optimize the flour quality. As dry beans vary in shape, size, and hardness, it is difficult to employ a single machine or a given set of machine parameters for better milling efficiency. Identifying the suitable mill and the mill settings linked to the final product quality is much needed. The effect of removing phenolics from the dry bean proteins needs to be further studied in specific food systems.

The findings of this research can be used as a model to evaluate commercial dry bean milling. Also, the removal of phenolic compounds can be used to enhance the functional properties of dry bean proteins in different food products.