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IMPROVING THE UTILIZATION OF DRY EDIBLE BEANS IN A READY-TO-EAT SNACK PRODUCT BY EXTRUSION COOKING

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by

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The growing snacking habit and steady increasing demand for healthy snacks have drastically changed the ready-to-eat snack market in recent years. While the current healthy, ready-to-eat snack products are still dominated by whole cereal grains, legumes, especially dry edible beans, have a high potential to emerge in nutritional, novel food. Dry edible beans (*Phaseolus Vulgaris* L) are not only economically valuable but also nutritionally important, since they are important sources of proteins, B vitamins, mineral elements, and soluble dietary fibers even when compared to whole grain cereals. Recent studies have shown extrusion processing is not only effective at producing an acceptable ready-to-eat snack product but also in altering the *in vitro* digestibility (starch, protein, and mineral elements) of bean flour. The present thesis describes two research projects to improve the utilization of pinto bean by extrusion technology. The first study was conducted to investigate the effects of pinto bean flour and feed moisture on the physical properties and *in vitro* digestibility of rice-bean extrudates. Addition of bean flour adversely affected the physical properties of rice-bean extrudates, but was partially alleviated by decreasing feed moisture. Rapidly digestible starch (RDS) decreased and resistant starch increased with increasing bean flour and feed moisture. *In vitro* protein digestibility increased as feed moisture decreased. For the second study, the effects of extrusion parameters, namely barrel temperature (^{o}C), screw speed (rpm), and moisture content (%), on *in vitro* element bioaccessibility were investigated. Although extrusion

parameters were found to not significantly affect the bioaccessibility of mineral elements, extrusion processing significantly increased the bioaccessibility of elements compared to flour. The average increase was higher for essential elements (Fe, Mg, P, and K) than for toxic elements (Cd and Pb) in the extruded product. The correlation analysis suggested that the increase of bioaccessible elements by extrusion was unique depending on the binding mechanism to chemical substances (phytic acid, tannins and dietary fiber components).

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Table of Contents

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The growing demand for healthy snack foods has been increasing over the past five years. According to IBIS world's market research (2014), North America's healthy snack production grew 3.6% from 2009 to 2014 [1], in addition to the proliferation of snacking habits since 2010 [2]. This has pressured the ready-to-eat snack market to deliver healthier snack products.

Dry-edible beans (*Phaseolus Vulgaris* L) have recently gained attention as functional foods. The superior nutritional value of bean flour compared to whole grain cereals is mainly attributed to high protein content, mineral nutrient content, and soluble dietary fiber profile [3]. Although beans have a great potential for improving the nutritional quality of ready-to-eat products, they are still underutilized due to limited processing methods for bean [4, 5]. The nutritional quality of beans however, are not only dictated by the concentration of nutrients in raw flour, but also their digestibility and bioaccessibility. The presence of antinutritional substances, for instance phytic acid, polyphenols and trypsin inhibitors can impair the digestibility of protein and mineral element bioaccessibility [5].

The combination of high temperature and high shear in extrusion cooking can be used as an effective cooking method to transform raw ingredients into ready-to-eat snack products [6, 7]. Extrusion cooking offers several advantages over traditional cooking including reduction of processing duration [5], alteration of *in vitro* starch and protein digestibility profiles [8, 9], improvement of *in vitro* element bioaccessibility [10] , and alteration of dietary fiber profile [11].

Extrusion cooking is an integrated system that depends on many parameters to produce acceptable results. The acceptability and nutritional quality of the final product is not only determined by feed ingredient, but also manipulation of extrusion parameters including feed moisture, screw speed, screw configuration, and temperature [12].

Objectives and Hypotheses

The overall objective was to show that extrusion cooking can be used as a promising technology to improve the utilization of dry edible bean. One of the most important extrusion parameters, feed moisture, plays an essential role in governing the degree of cooking in an extruded product. We hypothesized that through the manipulation of feed moisture, the overall acceptability of a cereal-bean snack is improved and the *in vitro* starch and protein digestibility is altered. Since extrusion parameters also play substantial roles in modulating chemical properties for phytic acid, polyphenols, and dietary fibers that dictate the bioaccessibility of mineral elements, it is further hypothesized that extrusion parameters can be used to alter the chemical properties of bean flour to change the *in vitro* element bioaccessibility.

Organization

This Thesis is organized as follows: a literature review (Chapter 1) followed by manuscripts describing two research projects (Chapters 2 and 3). All chapters have been formatted using guidelines for *Food Chemistry*. References can be found at the end of each chapter.

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CHAPTER 1. ADDITION OF DRY EDIBLE BEANS AND USE OF EXTRUSION COOKING IN PRODUCTION OF READY-TO-EAT SNACK AS A STRATEGY TO IMPROVE ITS NUTRITIONAL QUALITY

1.1. Abstract

The dramatic increase in metabolic disease in conjunction with an increasing trend of snacking has led to a demand for healthy snacks. Dry edible bean flour offers an excellent opportunity for the development of healthy snacks considering its superior nutritional quality and economical value. Apart from the health benefits of bean flour, processing technology can also play a substantial role affecting its nutrition. Extrusion technology is a promising method for the development of ready-to-eat snacks made by pinto bean flour. The benefits of extrusion cooking include: 1) improving the texture of pinto bean snack by modification of extrusion parameters, 2) altering *in vitro* protein and starch digestibility profile, and 3) increasing *in vitro* bioaccessibility of elements by degradation of anti-nutritional substances for instance phytic acid and tannins, and also solubilization of dietary fiber component from insoluble to more soluble fractions.

1.2. Introduction

Snacking has become increasingly prevalent in recent years; the trend has steadily grown 2% annually since 2010 and accounts for nearly half of all eating occasions for consumers (Hartman, 2013). In the US, 63% of snacks consumed belong to ready-to-eat snack products and breakfast cereals (Nielson, 2014). This may lead to public health issues, as current snack products are still dominated by refined grain cereal that are inferior in nutritional quality. Proliferation of these dense energy snack products has been associated with increased prevalence of obesity (Lobstein & Wang, 2006).

Concurrently, the snack food industry has been facing pressure from regulatory agencies to deliver healthier snack products. The 2015 Dietary Guidelines released by USDA, indicated a need for increased consumption of whole grain to increase dietary fiber as a dietary improvement for individuals with risks for chronic diseases, as well as healthy individuals, in order to help reduce the risks of heart disease and certain cancers (USDA, 2015). The demand for healthy ready-to-eat snack products has therefore remarkably grown by 33% in 2015 as a consequence of metabolic disease related to a ubiquitous of unhealthy snacks (Mintel, 2015).

The snack industry has been linked closely to the development and use of extrusion technology for over sixty years. Ready-to-eat snack products, or generally known as directly expanded extruded snack, were developed in the early 1940's. The process involves feeding the raw ingredients into an extrusion barrel under limited moisture conditions, which creates intense cooking and pressure. The expanded snack product is generated by releasing pressure at the exit die causing water vapor to escape instantaneously forming a foamy structure. The expanded snack is then cut into portions by a rotating knife (Guy, 2000b).

Whole grains, for instance whole wheat and corn, are often used to formulate healthy snack products using extrusion, while brown rice is becoming more attractive due to its relatively high starch content, bland taste and hypoallergenicity (Chaiyakul, Jangchud, Jangchud, Wuttijumnong, & Winger, 2009). These whole grains cereals, however, are still considerably lower in dietary fiber and protein content compared to legumes (Tharanathan & Mahadevamma, 2003). Entire or partial incorporation of legume flour into ready-to-eat snack product can desirable. However, several studies have indicated that partial

substitution of cereal flour with high fiber and protein ingredients, for instance legume flour, can negatively affect the texture, expansion and overall acceptability of ready-to-eat snack products (Anton, Gary Fulcher, & Artfield, 2009; Pastor-Cavada et al., 2011). In extrusion cooking, not only feed ingredients can significantly affect the product characteristics, but also extrusion parameters for instance feed moisture, screw speed, barrel temperature, and feed ratio. These extrusion parameters are essential as extrusion system variables controlling heat transfer of materials, mechanical energy transferred from movements of screw, viscosity of fluid, and residence time which eventually governs the degree of cooking of starchy materials. The quality of extrudate is largely depend on the degree of cooking. Therefore by manipulating this extrusion conditions, it can be useful in alleviating the negative effects posed by addition of legume flour (Anton et al, 2009; Pastor-Cavada et al., 2011; Pérez-Navarrete, González, Chel-Guerrero, & Betancur-Ancona, 2006).

In this article, the benefits of pinto bean (Phaseolus vulgaris L.) flour on as well as the effect of extrusion conditions on the physical and nutritional quality of snack products will be reviewed, followed by a discussion on nutritional qualities that can be potentially improved by extrusion technology and inclusion of bean flour.

1.3. Benefits and challenges of incooperating pinto bean (*Phaseolus Vulgaris* L.) flour into ready-to-eat snack products.

Dry edible beans (*Phaseolus vulgaris* L.) are a main sector of legumes, accounting for almost 50% of total legume production (USDA-ERS, 2012). The nutrients values of legumes and several major cereal grains is shown on Table 1.1. Pinto beans deliver an important source of protein, dietary fibers, B vitamins and elements in human diets, with

relatively low amount of lipids (Tharanathan & Mahadevamma, 2003). Several reports have demonstrated the health benefits of pinto bean such as prevention of metabolic diseases (Basset, Boye, Tyler, & Oomah, 2010; Tharanathan & Mahadevamma, 2003) and regulation of normal gastrointestinal function (Cardador-Martínez, Loarca-Piña, & Oomah, 2002). Most health benefits from pinto bean are attributed to the higher content of both soluble and insoluble fiber, prebiotic oligosaccharides, and phenolic compounds as compared to cereal grains (Bassett et al.. 2010; Cardodor-Martínez et al 2002).

Apart from their nutritional quality, pinto beans are also economically valuable. Pinto beans are a leading variety in the production of dry edible bean in the US, according to 2006-2008 census of agriculture and account for 42 percent of total dry bean production (Cămara, Urrea, & Schlegel, 2013; USDA-ERS, 2012).

Considering the superior nutritional quality as well as the economic advantages of pinto bean, it has great potential as a novel ingredient to improve the nutritional quality of ready-to-eat snack products. Although the nutritional benefits of pinto beans have been extensively recognized by research and health organizations globally, some researchers have reported a downward trend in pinto bean consumption globally and underutilization in the U.S (Balandrán-Quintana, Barbosa-Cánovas, Zazueta-Morales, Anzaldúa-Morales, & Quintero-Ramos, 1998; Codex, 2009).

Nevertheless, several compounds that are considered to confer health benefits, including tannins, oligosaccharides, and polyphenols can also act as anti-nutritional factors (Díaz-Batalla, Wildhom, Fahey, Castăno-Tostado, & Paredes-López, 2006). The presence of these compounds may pose challenge in utilization of pinto bean flour in ready-to-eat snacks, by reducing its nutritional values (Farooq & Boye, 2011). Anti-nutritional

compounds can lead to reduced mineral bioavailability and protein digestibility (Díaz-Batalla et al, 2006). The abundance of oligosaccharides in bean can lead to flatulence, while traditional processing method for instance boiling and roasting may require extensive cooking duration to eliminate this substance (Khattab & Artnfield, 2009). Moreover, tannins found in pinto bean might also contribute to bitterness and astringency of the snack foods and thus reduce food acceptance (Cox, Melo, Zabaras, & Delahunty, 2012).

One of the most efficient technologies to overcome the adverse effects of pinto bean flour is extrusion cooking (Balandran-Quinatana et al., 1998). The combination of several unit operations including mixing, heating, conveying, puffing and drying into a single process causes an intense cooking process inside an extrusion barrel (Colonna, Tayeb, & Mercier, 1989). This intense cooking condition can significantly reduce the adverse effects of anti-nutritional factors found in pinto bean flour (Balandran-Quinatana et al., 1998).

1.4. Basic principle of direct expansion snack extrusion

The basic process used for ready-to-eat snack foods involves three stages: forming and heating the dough mass and drying of snack (Matz, 1993). In the first step, the dough is formed by hydration of starch polymers into a fluid mass that can be shaped into intended snack pieces. Next, the hydrated dough is subjected to intense temperatures and shear until the water turns superheated and releases rapidly as vapor. The sudden release of water vapor causes the mass dough to puff instantly. Lastly, the snack is stabilized into a hard brittle structure by being dried to a low moisture level.

Raw materials have the most influential effect on the overall acceptability of extruded product (Colonna et al., 1989, Valle, Vergness, Colonna, & Patria, 1997). The structure of an extruded snack product is created by forming a melt fluid from biopolymers

and blowing bubbles of water vapor into the fluid to generate a foamy structure (Guy, 2000). Starch polymers, for instance wheat, maize, rice or potato, are very good at this function. Since the structure-forming materials are mainly starch, incorporation of high protein and high fibers flour can negatively affect the texture, expansion, and hardness of the snack product (Li, Wei, White, & Beta., 2007; Obatolu Veronica, Omueti Olusa, & Adebowale, 2006). Protein and fiber can act as a dispersed phase within the continuous starch structure. The presence of dispersed-phase materials will negatively affected the expansion by disrupting cell wall formation by the starch film (Guy, 2000) .Additionally, the dispersed substances can reduces the elastic recoil and die swell effect of fluid as it leaves the die exit, which eventually reduces the expansion and pore structure of the product. Other substances that may substantially influence the physical quality of extruded product are categorized as plasticizers and lubricants, which include water, oils, sugars, and fats. The lubricating action decreases the mechanical shear exerted by screw system which may eventually reduce the degree of cooking in the extruder. The reduction in degree of cooking can results in low expanded and dense product. Low molecular weight materials, such as sugars and salts, can dilute the other ingredients and affect the viscosity of the fluid which will govern the degree of cooking inside the extruder (Z Jin, 1994)

Successful production of ready-to-eat snack products requires close control of many extrusion parameters including feed moisture, feed rate, barrel temperature, screw speed and barrel temperature (Meng, Threinen, Hansen, & Driedger, 2010). These process variables determine the transformation of raw materials during processing, which then influence the rheological properties of plasticizer melt in the extruder. It is important to understand the balance of thermal effects and rheological properties in extrusion cooking,

and therefore choosing the most suitable thermal treatment for conferring the require qualities on the final product (Mottaz, Bruyas, Cextral, & Firmniny, 2001). A large variance of viscosity occurs from the heat and pressure in the extruder caused by changes in raw materials and alteration of extrusion parameters (Battacharya & Hanna, 1985; Grenus, Hsieh, & Huff, 1993). Research has investigated the melt viscosity of ready-to-eat snack product pertaining to extrusion parameters (Chinnaswamy & Hanna, 1990; Grenus et al., 1993; Ilo et al., 1996) found that the apparent viscosity has been substantially related to barrel temperature, flow shear rate, and feed moisture level

Feed moisture has been found to be the main parameter in governing the texture of extrudates because it substantially influences the rheological properties of the molten starch (Ding, Ainsworth, Trucker, & Marson, 2005; Ilo, Tomschik, Berghofer, & Mundigler, 1996; Launay & Lisch, 1983). Increased feed moisture caused a lower melt viscosity (Ilo et al., 1996). Feed moisture was found to be the most influential effect on the apparent melt viscosity (Battacharya & Hanna, 1985). Moisture acts as a plasticizer, which reduces the viscosity and mechanical energy dissipation during extrusion.

The effect of screw speed on the quality of extrudate is complex and highly dependent on temperature (Ilo et al, 1996). A higher screw speed results in greater mechanical energy, or frictional heat, which leads to an increase in product temperature (Valle et al., 1997). The combination of shear and temperature can lead to a change in rheological properties of the melt and therefore affect the texture of extrudate. Increasing screw speed can lead to increased shear forces causing a fragmentation of starch granules, which can eventually lower the melt viscosity. Melt viscosity substantially relates to the degree of gelatinization of starch and, therefore, influences the physical quality of the

product, especially volumetric expansion (Battacharya & Hanna, 1985; Lai & Kokini, 1990).

Feed rate influences the degree of fill and residence time of raw material inside an extruder (Ding et al, 2005). As feed rate increases, the extrusion barrel is filled with more food material, and consequently, more thermal energy is received by the food material causing greater gelatinization (Mercier & Feillet, 1975).

Choosing a proper extruder configuration is critical for successful extrusion. All extruders consist of a screw(s), which convey the premixed ingredients through the barrel (Riaz, 2000). Apart from raw materials, basic components in the extruder, including screw configurations and optimization of extruder parameters, are the most important factors in the final texture of ready-to- eat snack product. The extruders screw configuration affects the degree of mixing, shear forces, amount of heat generated by friction and the residence distribution time (Guy, 2000b). The most commonly used types of extruders are single screw extruders, single screw interrupted flight extruder, and twin-screw extruders (Riaz, 2000a). Single screw extruders are relatively economical compared to twin-screw extruder. However, as consumer demand for innovative food product is constantly increasing, twin-screw extruders have become popular as they have a greater flexibility for controlling both product and process parameters (Riaz, 2000). Twin-screw extruders are also more preferable for more uniform size and shape products and is suitable for raw ingredients with higher internal fat content and ultra-small flour sizes (Riaz, Lucas, & Mohyudin, 1996).

1.5. Physical Characteristics of extruded product affected by extrusion parameters

Physical Characterization is an important quality parameters for extruded snack product. The physical quality is conducted for estimate consumer acceptability of the product. Some of the most common physical characteristics are volumetric expansion, density, hardness and porosity of the product.

Volumetric expansion represents overall expansion of the product and is determined by taking into account cross sectional (radial) and longitudinal (axial) expansion (Launay & Lisch, 1983; Valle et al., 1997). Overall expansion is reported to reach its highest at high melt viscosity. Several researchers have reported that axial expansion is inversely related to radial expansion (Lai & Kokini, 1990; Meng et al., Valle et al., 1997). According to Mercier & Fillet (1975), there are two forces, viscous and elastic, that govern the direction of expansion (axial and radial). The elastic force is more dominant at low barrel temperatures and low feed moistures, while high feed moistures and high temperatures will favor viscous force (Őzer, Îbanoğlu, Ainsworth, & Yağmur, 2004). The elastic force is mainly responsible for longitudinal expansion whereas, cross sectional expansion is mostly dictated by viscosity forces (Ding et al., 2005).

The density of the extrudate was found to be most dependent on feed moisture and barrel temperature (Ding et al., 2005; Grenus et al., 1993; Meng et al., 2010). Increasing feed moisture caused a drastic increase in extrudate density, whereas increasing barrel temperature resulted in a decrease in density (Ding et al., 2005). Another study found that only feed moisture and screw speed significantly affected the density of the product (Őzer et al., 2004). Nevertheless, feed moisture has been reported to be the most influential parameter whereas, feed rate was found to be not have a significant effect on extrudate density (Ding et al., 2005; Grenus et al., 1993; Meng et al., 2010).

Hardness of an extrudate is significantly affected by feed moisture, screw speed and barrel temperature (Chinnaswamy & Hanna, 1990). Hardness generally decreases as feed moisture decrease, and increasing screw speed and barrel temperature (Meng et al., 2010). Hardness is the measured peak force of the probe penetrating the extrudate, whereas the perception of crispiness is a correlation of hardness and porosity of the product (Őzer et al., 2004). Porosity is related to air cells created during extrusion process (Őzer et al., 2004) .An increase in porosity of the product is desirable since it is associated with highly expanded and low-density products. Several researchers have reported only feed moisture and screw speed having a significant effect on the porosity for the product (Chinnaswamy & Hanna, 1990; Meng et al., 2010; Őzer et al., 2004).

1.6. *In vitro* digestion and bioaccessibility

The nutritional superiority of pinto bean flour as compared to cereal flours is due to its protein content, dietary fibers, polyphenols, and mineral content. However, presence of anti-nutritional factors, especially from phytic acid and tannin, can negatively affect the absorption of the nutrients (Bassett, Boye, Tyler, & Oomah, 2010). Addition to the high protein content, several studies have reported that peptides degradation from bean protein can confers health benefits (Duranti, 2006; Vermeirssen, Van Camp, & Verstraete, 2005). The study of dietary fiber fractions in legumes is interesting due their important physiological properties. Pinto beans are considered to be good sources of fibers due to their higher amount of soluble dietary fiber compared to that of cereals (Esteban et al., 1998).

The combination of high temperature and shear cooking during extrusion make this technology not only affective in modifying the texture of the product, but also the intense

cooking can render a number of modifications to the raw ingredients (Carvalho & Mitchell, 2000). The application of extrusion is becoming popular due to the destruction of anti-nutritional value and improvement of digestibility of starch and protein with minimal destruction of essential nutrients. Other benefits of the nutritional aspects of food extrusion including improving dietary fiber profile by increasing soluble dietary fibers (Arcila, Weier, & Rose, 2015) and reduction of lipid oxidation (Imran et al., 2015). However, some of the heat labile vitamins may be reduced during intense extrusion process (Camire, 2001).

1.6.1. *In vitro* starch digestibility

Incorporation of legume flour into snack products may improve the starch digestibility by lowering the glycemic index (Rizkalla, Bellisle, & Slama 2002). The rate of starch digestibility can be advantageous depending on the consumers; for instance, infant snacks should be highly digestible, whereas adult snack foods should be slowly digestible for prevention of metabolic disease (Camire, 2001).

Based on rate of in vitro digestion, starch can be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst, Kingman, & Hummings, 1992). RDS is the starch fraction that may contribute to a sudden increase blood glucose level after ingestion, whereas SDS is completely digestible in a much slower rate than RDS. Lastly, RS is a fraction that may not be digested in the small intestine. Several studies reported that pinto bean starch is less digestible than cereal starches (Chung et al., 2008; Ring, Gee, Whittman, Orford, & Johnson, 1988). Bean starch also shows low digestibility with high SDS and RS content but low in RDS. The digestibility profile of pinto bean starches can be attributed to several factors including an absence of pores on

the granules surface, higher amylose content, B-type crystallites structure, and strong interaction among amylose chains (Chung et al., 2008; Hoover & Zhou, 2013).

Extrusion technology is considered unique because gelatinization of starch can occur at much lower moisture levels than is necessary in other food processing techniques (Qu & Wang, 1994). Since gelatinization of starch is one of the requirement in digestion of starch, extrusion can effectively increase the digestibility of starch. Extrusion can greatly increase starch susceptibility for enzymatic attack and therefore increase starch digestion. Barrel temperature, feed moisture and screw configuration can be carefully designed to alter the *in vitro* starch digestibility (Gautam & Choudhury, 1999). Study by Gautam & Choudhury (1999) indicated the digestibility of starch is directly related to the severity of extrusion cooking. This can be due to the rupture of granules is making the starch more accessible and facilitate enzymatic digestion. In addition study by Hagenimana, Ding, & Fang (2006) showed that RDS is decreased while SDS is increased at higher feed moisture and lower screw speed while temperature has less apparent effect.

In addition to rapid molecular degradation, the formation of amylose-lipid complexes can also occur during the extrusion process, although the formation of this complexes might not always occur. This complex can resist digestion and be considered as RS (Gautam & Choudhury, 1999). The extent of amylose-lipid complex formation is dependent upon both starch structure and type of lipid in the food matrix. High amylose starch (pinto bean starch) with monoglycerides or free fatty acid is most likely to increase RS. Low feed moisture (19%) and barrel temperature ranging from 110-140 °C exerts the greatest RS formation. Moreover, high viscosity and extended residence time may favor RS formation (Bhatnagar & Hanna, 1994).

1.6.2. *In vitro* protein digestibility

The quality of protein is not only determined by the amount but also its digestibility and bioavailability of amino acid. The digestibility of protein in bean flour is generally highly digestible. However due to the presence of anti-nutritional substances in bean flour, the digestibility of unprocessed bean flour can be adversely affected (Balandrán-Quintana et al., 1998). Several studies found no significant difference in the digestibility of bean protein compared to that of cereal (Balandrán-Quintana et al., 1998; Pastor-Cavada et al., 2013). Additionally, the results of *in vitro* protein digestibility of bean and cereals flour are widely varied. This can be attributed to differences in methodology used in measuring the digestibility profile (Pastor-Cavada et al., 2013).

Extrusion technology has been widely used to enhance the digestibility of protein. The improvement of protein digestibility by extrusion cooking involves several factors including inactivation of anti-nutritional substances, inactivation of enzymes inhibitors, and denaturation of protein, which might expose new sites for enzymatic attack (Colonna et al., 1989). It has been found that most of extrusion parameters including feed moisture, barrel temperature, and feed ratio have significant effects on protein digestibility. Decreasing feed moisture and increasing barrel temperature improves the digestibility of protein by enhancing inactivation of protease inhibitor (Camire, 2001). A study by Bhattacharya & Hanna (1985) reported that length to diameter ratio of extrusion screw has no significant effect on protein digestibility of wheat flour, whereas feed moisture has the maximum effect on protein digestibility followed by barrel temperature.

The main anti-nutritional substances that hinder the digestibility of protein in pinto bean flour are trypsin inhibitors and phytic acid (Cardador-Martínez et al., 2002). The

destruction of anti-nutritional factor mainly depends on barrel temperature and feed moisture. At constant barrel temperature however, the inactivation of anti-nutritional factor is mainly affected by residence time and moisture content (Cardador-Martínez et al., 2002). A similar study also reported feed rate having a significant effect on the inactivation of this substances, as feed rate are closely related to residence time of raw material inside the extruder (Asp & Björck Asp, 1983) Thus, anti-nutritional factor inactivation is increased with increased barrel temperature, longer residence time, and lower feed moisture.

1.6.3. *In vitro* element bioaccessibility

Dietary mineral elements play an important role in food chemistry and nutrition although they represent a minor portion of food compositions. A deficiency in any one of essential micronutrients can result in severe metabolic diseases (Alonso, Rubio, Muzqui & Marzo, 2001). Absorption of nutrients insiofde the human is often complex and not easy to be investigated. In order to study the intestinal absorption of elements, both *in vitro* and *in vivo* models have been used to investigate true absorption. *In vivo* studies are useful in investigating the mechanism of absorption of minerals, commonly refers as bioavailability, whereas *in vitro* studies, referred to as bioaccessibility, represent the amount of elements that pass through a dialysis tube and are potentially available for absorption (Glahn, 2009).

Bean flour has been known to contain higher amount of minerals compared to some cereal grain flour (Tharanathan & Mahadevamma, 2003). The content of mineral elements, however, does not reflect their absorption in the human intestine. Bean flour contains high amounts of phytic acid, phenolic acids and dietary fiber; these substances can form a complex with elements causing a reduction in element bioaccessibility (Díaz-Batalla, Widhlom, Fahey, Castańo-Tostado, Paredes-López, 2006). Modification of raw bean flour

is required to improve the solubility, dispersibility and fractional dialyzability of elements. The bioaccessible elements in raw pinto bean ranges from 3-35%, depending on the type of element (Lee & Garcia-Lopez, 1985; Hemalatha, Platel, & Srinivasan, 2007).

Extrusion technology is commonly used to improve the absorption of elements by solubilization of dietary fiber and degradation of inhibitory factors such as phytic acid and phenolic compounds. A study by Alonso et al (2001) reported that extrusion process can significantly increase the absorption of Ca, Mg, Zn, Cu and P in bean flour. Extrusion cooking has been reported to reduce phytic acid by 13-50% (Alonso et al., 2001; Anderson, Conway, & Peplinski, 1970; Anton et al, 2009; Pĕrez-Navarrete, González, Chel-Guerrero, & Betancur-Ancona, 2006). Temperature and moisture content are two extrusion parameters that have been reported to significantly reduce phytic acid. Extrusion also exerts an effect on the modification of dietary fiber components, causing it increase soluble fraction while decrease insoluble fraction of dietary fibers (Alonso et al., 2001; Martín-Cabrejas et al., 1999). The combination of high temperature and high shear during extrusion cooking causes a significant solubilization of insoluble dietary fiber. Total dietary fiber however is not significantly affected by extrusion technology (Anderson et al., 1970; Martín-Cabrejas et al., 1999).

Phytic acid is the major phosphorous storage compound in plant seeds, which accounts for up to 80% of total phosphorous. The content of Phytic acid for some legumes and cereal grains is presented on Table 1.2. It is highly negatively charged which can have high affinity to mineral cations and form insoluble complexes (Lopez, Leenhardt, Coudray, & Remesy, 2002). Phytic acid forms very stable complexes with elemental ions causing it not to be available for intestinal absorption. It is reported that phytic acid binds strongly to

zinc, iron and calcium (Davies & Nightingale, 1975). In general, degradation of phytic acid increases element solubility; nevertheless phosphates from phytic acid hydrolysis can also bind with calcium, magnesium, zinc and iron to form insoluble complexes (Lopez et al., 2002).

Dietary fiber, especially cell wall materials, can bind to metallic polyvalent cations. Understanding the individual fiber components is crucial to study the relationship between element bioaccessibility and dietary fiber (Table 1.2). Dietary fiber can be isolated into three main fractions including cellulose, lignin and pectin (Toma & Curtis, 1986). Cellulose is one of the constituents of insoluble fiber fraction made from linear polymers of glucose. The presence of hydroxyl functional group in cellulose causes chemical binding with elements (Krassig, 1985). Pectin is a heteropolysaccharide with a backbone made of D-galacturonic acid with fucose, xylose, and galactose branches. Pectin has the capacity to bind with divalent elements especially iron, calcium, cooper, zinc, and magnesium. The interaction of pectin to elements is reported to be due to electrostatic forces and complex formation between cation and carboxylic functional group (Khon, 1987). Lignin is a cementing intracellular substance. It is quite complex in nature and can associated with polysaccharides, protein, polyphenols, methoxyl and hydroxyl groups (Toma & Curtis, 1986). Lignin is reported to form strong complexes with transitional metal ions by forming multidentate complexes that may come from methoxyl and hydroxyl groups (Toma & Curtis, 1986). Extrusion cooking has been reported to effectively degrade pectin and cellulose and therefore can potentially increase the accessibility of elements (Krassig, 1985).

Pinto bean, like other legumes contain high proportion of phenolic compounds (Table 1.3). The presence of this phenolic compounds can be act as natural antioxidant, however it can also inhibit the absorption of mineral elements in the body (Díaz-Batalla et al., 2006). Tannins are one of the most common phenolic compound found in pinto bean (Reddy, Pierson, Sathe, & Salunkhe 1985). Tannins, like most of phenolic acids have high affinity to divalent ions and can form a stable insoluble complexes (Alonso et al., 2001). Because tannins are commonly present in high amounts in pinto bean, traditional processing methods, for instance boiling, is often not sufficient in reducing tannins (Mondor et al., 2009). A combination of high shear and high temperature in extrusion however, has been reported to significantly reduce the amount of tannins in legumes (Alonso et al., 2001).

1.6. Conclusion

The nutritional qualities of pinto bean flour have been supported by numerous studies. Incorporation of pinto bean flour can be potentially used as a strategy to create development of healthy snack product. The texture of a pinto bean snack, however, may not be as comparable to other snack made with cereal grains. Through modulation, extrusion cooking is a promising technology to improve the utilization of pinto bean flour not only generating an acceptable snack texture, but also to improve the nutritional qualities of the product.

1.7. References

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Table 1.1. Nutrients composition of selected legumes and cereal grains. [a]

Type	Macronutrients (g/100g)			Mineral elements (mg/100g)						Vitamins (mg/100g)			
	Protein	Starch	Lipids	Fe	Ca	Mg	Zn	K	P	C (Ascorbic acid)	B1 (Thiamine)	B2 (Riboflavin)	B3 (Niacin)
Pinto bean	21.5	62	0.90	5.07	113	176	2.28	1393	411	6.30	0.71	0.21	1.17
Great Northern Bean	21.9	62.3	1.14	5.47	175	189	2.31	1387	447	5.30	0.65	0.24	1.96
kidney bean	23.6	60.0	0.83	8.20	143	140	2.79	1406	407	4.50	0.53	0.22	2.06
Black bean	21.7	62.4	1.42	5.02	123	171	3.52	1483	352	-[b]	0.90	0.19	1.96
Chickpea flour	22.4	57.8	6.69	4.86	45.0	166	2.81	846	318	-	0.49	0.11	1.76
Soybean	36.9	30.2	19.9	15.7	277	280	2.01	1797	280	6.05	0.87	0.87	1.62
Brown rice flour	7.23	76.5	2.78	1.98	11.0	112	2.45	289	337	-	0.44	0.08	6.34
White rice flour	5.95	80.1	1.42	0.35	10.0	35.0	0.80	76	98	-	0.14	0.02	2.59
Whole wheat flour	13.2	71.9	2.50	3.60	34.0	137	2.60	363	357	-	0.50	0.17	4.96
Corn flour	9.42	74.3	4.74	2.71	7.00	127	2.21	315	273	-	0.39	0.20	3.63
Oat	16.9	66.3	6.90	4.72	54.0	177	3.97	429	523	-	0.76	0.14	0.96

[a] references; USDA-Nutrient database, 2015

[b] the concentration is too low to be detected.

Table 1.2 Dietary fiber composition and phytic acid content of selected beans and cereal grains

	Dietary fiber profiles (g/100g dry basis)						PA	References
Type	TDF	TNSP	SNSP	INCP	CELL	LIG	(g/100g)	
Pinto bean	21.18	19.6	8.15	7.72	3.73	1.58	1.95	Anderson & Bridges, 1988; Lolas & Markakis, 1975
Kidney bean	20.9	17.53	5.26	6.85	5.42	3.37	2.06	Anderson & Bridges, 1988; Lolas & Markakis, 1976
Navy bean	23.02	21.8	7.76	7.08	6.96	1.22	1.58	Anderson & Bridges, 1988; Lolas & Markakis, 1977
White bean	18.31	17.27	4.54	8.6	4.14	1.04	1.63	Anderson & Bridges, 1988; Lolas & Markakis, 1978
Oat Bran	15.72	14.49	7.84	5.86	0.52	0.15	0.79	Anderson & Bridges, 1988; Lolas et al, 1976
Brown rice flour	-[a]	2.87	0.89	1.37	0.61	-[a]	0.89	Anderson & Bridges, 1988; Reddy & Salunkhe, 1980
White rice flour	1.4	1.19	0.41	0.47	0.31	0.21	0.34	Anderson & Bridges, 1988; Reddy & Salunkhe, 1980
Wheat flour	7.2	6.68	3.4	2.04	1.24	0.52	0.98	Anderson & Bridges, 1988; Lolas et al., 1976

TDF, total dietary fiber, TNS, total non-starch polysaccharides; SNSP, soluble non-starch polysaccharides; INCP, insoluble non cellulose polysaccharides; CELL, cellulose; LIG, lignin; PA, Phytic acid.

[a] the component is not reported.

Table 1.3 Total phenolic and tannin content of selected legumes and cereal grains.

Type	Total phenolic (mg/g)	Tannin content (g/100g)	References
Pinto bean	93.6	2.65	Amarowicz & Pegg, 2008; Reddy et al., 1985
White bean	1.10	- [a]	Amarowicz & Pegg, 2008; Reddy et al., 1985
Kidney bean	6.30	1.02	Amarowicz & Pegg, 2008; Reddy et al., 1985
Black bean	44.0	33.7	Amarowicz & Pegg, 2008; Reddy et al., 1985
Wheat	1.30	-	Kulp & Ponte, 2000; Serrano et al, 2009
Brown rice	3.90	-	Kulp & Ponte, 2000; Serrano et al, 2009
Corn	0.61	-	Delcour & Honesey, 2010; Serrano et al, 2009
Sorghum	0.79	136	Delcour & Honesey, 2010; Radhakrishnan & Sivaprasad, 1980

[a] the component is not reported

CHAPTER 2. EFFECTS OF FEED MOISTURE ON THE PHYSICAL PROPERTIES AND *IN VITRO* DIGESTIBILITY OF EXTRUDED BROWN RICE AND PINTO BEAN COMPOSITE FLOURS

2.1. Abstract

The incorporation of pinto bean flour into extruded snack foods offers the opportunity to increase dietary fiber and protein contents of the snacks. The objective of this study was to investigate the effects of pinto bean flour and feed moisture on the physical properties and *in vitro* digestibility of rice-bean extrudates. Four blends of brown rice and pinto bean flour (0%, 15%, 30% and 45% bean flour) were extruded using a pilot scale twin screw extruder under 5 moisture conditions (17.2%, 18.1%, 18.3%, 19.5%, and 20.1%). Physical properties [bulk density, unit density, radial expansion, axial expansion, overall expansion, specific volume, hardness, color, water solubility index (WSI), and water absorption index (WAI)] and *in vitro* starch and protein digestibility were determined. The percentage of bean in the composite flour and feed moisture of the extruder had significant ($p<0.05$) effects on all physical characteristics and *in vitro* digestibility profiles, except for redness and slowly digestible starch which were only significantly affected by bean flour substitution. Increasing bean flour and feed moisture increased density and hardness while decreasing expansion. Rapidly digestible starch (RDS) decreased and resistant starch (RS) increased as bean substitution and feed moisture increased. *In vitro* protein digestibility increased with increasing bean flour or with decreasing feed moisture. Incorporating bean flour into brown rice-based extruded snacks can negatively affect the physical attributes (hardness, density and expansion) while positively affecting *in vitro*

starch (decrease) and protein (increase) digestibility. The negative impact of bean flour on physical properties can be partially overcome by decreasing feed moisture content.

2.2. Introduction

Most ready-to-eat snack products on the market are made from refined cereal grains and are generally low in dietary fiber and protein (Anton, Gary Fulcher, & Arntfield, 2009). However the demand for nutritionally improved snacks increased by 33% in 2015 (Mintel, 2015). Therefore, improving the nutritional value of ready-to-eat snacks is desirable.

Whole grains are often used to formulate healthy ready-to-eat snacks. Although whole grain extruded snacks are generally produced from whole wheat or corn flour, brown rice flour is becoming more attractive due to its relatively high starch content and bland taste (Chaiyakul, Jangchud, Jangchud, Wuttijumnong, & Winger, 2009). Unfortunately, this flour is also lower in dietary fiber and protein compared with wheat and corn. Legumes, on the other hand, contain high dietary fiber and complimentary to those in cereals (Tharanathan & Mahadevamma, 2003). Thus, production of a ready-to-eat snack made with a blend of brown rice flour and legume flour may produce a very desirable extruded product containing both high dietary fiber and complete protein.

The nutritional advantages of a rice-bean extrudate are dependent not only on the increase in dietary fiber and complete protein, but also on the digestibility of protein and starch. Some research has indicated that extrusion cooking increases the *in vitro* protein digestibility more efficiently than conventional cooking methods (Alonso, Aguirre, & Marzo, 2000), while Mertz et al (1984) indicated that *in vitro* protein digestibility varies across types of cereals grains. Mahasukhonthachat, Sopade, and Gidley (2010) indicated

that *in vitro* digestibility starch was enhanced under low moisture conditions when extruding sorghum flour, while another study on sweet potato flour showed that the rate of starch digestion was not significantly affected by moisture content (Warambi, Gidley, & Sopade, 2014). Thus, the digestibility of starch and protein may not be consistently affected when different raw materials are used.

When producing extruded snack products, there are many variables that affect the characteristics of the final product, such as moisture content, feed rate, screw speed, and barrel temperature. Among these variables, however, moisture content is one of the most important aspects in manipulating the physical characteristics of extruded products because it can substantially influence the melting temperature, viscosity, and shear stress experienced by the material inside the extrusion barrel (Thymi, Krokida, Pappa, & Maroulis, 2005). Therefore the aim of this study was to investigate the effects of pinto bean flour and feed moisture on the physical characteristics and *in vitro* starch and protein digestibilities of rice-bean extrudates

2.3. Materials and methods

2.3.1. Brown rice flour and pinto bean flour preparation and composition

Brown rice flour was obtained from Sage Foods (Los Angeles, California, USA). Pinto beans (cultivar ‘Poncho’) were obtained from Stateline Bean (Gering, Nebraska, USA). Whole pinto bean seeds were ground in a pilot plant scale hammer mill (C.S. Bell, Tiffin, Ohio, USA) with 1 mm screen size. Brown rice flour: bean flour composites were prepared by using a pilot plant ribbon blender (Wenger, Sabetha, KS, USA) at 4 ratios: 100:0, 85:15, 70:30, and 55:45, respectively. These flours were named based on the percentage of pinto bean flour in the treatment (i.e., 0%, 15%, 30%, and 45%, respectively).

Salt was added to the flours at 1% (by total weight of flour) after composites were made. The flours were then stored at 4°C until extruded.

Proximate composition, including moisture content, total starch, ash, and total dietary fiber, were analyzed on composite flours using approved methods (AACC International, 2015). Total starch was analyzed using a Megazyme total starch assay kit (K-TSTA, Megazyme, Bray, Ireland). Crude fat was determined using an extraction unit (HT 1043, Soxtec, CA, USA) with hexane as the extraction solvent. Protein content was analyzed using a nitrogen analyzer unit (Leco FP-528, Leco Corporation 3000, St Joseph, MI, USA) with 6.25 as nitrogen conversion factor. Analyses were performed in triplicate.

2.3.2. Extrusion process

Nighty kilograms of each composite flour were extruded using a pilot scale twin screw extruder (TX-57, Wenger, Sabetha, KS, USA). The screw length to diameter ratio was 13.5 to 1. The barrel temperatures at each zone were 80/90/110 °C. The barrel screw speed was 300 rpm and the diameter of die opening was 4.08 mm. The flours were fed into the extrusion barrel at 1.3 kg/min. Water was injected into the screw zone at different rates, resulting with five different processing moisture contents: 17.2%, 18.1%, 18.3%, 19.5% and 20.1%. Upon exit from the extruder die, materials were conveyed to a belt dryer at 103 °C for 10 min. The samples were stored in plastic bags at room temperature. Moisture content was analyzed within 24 h of production. Texture analysis was conducted within 3 days after production. For chemical analysis, the extrudate was ground with food processor (Waring, New Hartford, CT) and stored at 4 °C until analysis.

2.3.3. Physical analyses

Bulk density was determined by adding 50 g of extrudate into a 2 L graduated cylinder and inverting the filled cylinder once. The volume was noted and the bulk density was calculated by dividing the exact weight of extrudate (in mg) by the recorded volume (in cm^3).

Unit density and true solid density were measured according to Ali, Hanna, and Chinnaswamy (1996), with minor modifications. For unit density, 5 g of extrudate were placed into a cylindrical canister (9.1 cm in diameter X 9.3 cm in height) and then the canister was filled with rapeseed and leveled off. The rapeseed filling the container was then weighed. Unit density was then calculate by the equation:

$$\rho_u = (W_{ex} \text{ x } W_{rs}) / (W_{rf} \text{ x } V_c)$$

Where ρ_u = unit density (mg/cm^3), W_{ex} = mass of extrudate (mg), W_{rs} = mass of rapeseed replaced (mg), W_{rf} = mass of the rapeseed on full container (mg), V_c = Volume of canister (cm^3). True solid density was determined by milling the extrudates with a food processor (Waring, New Hartford, CT) and sieving to pass through a 0.21 mm opening (US standard mesh number 70). The flour was then added to a cylindrical canister (3.6 cm diameter X 6.4 cm height) until the canister was completely filled. The container was tapped 10 times against the benchtop and, if the height decreased, more flour was added to fill the container. This `tap-and-fill' process was continued until no change in fill height was observed upon tapping 10 times. The density was calculated by dividing the weight of flour (g) by the volume of the canister (cm^3). True solid density was not reported, but data were collected for overall expansion ratio calculation (see below). Unit, bulk, and true solid density were analyzed 5 times for each sample.

Three expansion characteristics were measured: radial expansion ratio, axial expansion ratio, and overall expansion ratio. Radial expansion ratio was the cross-sectional diameter (mm) of each extrudate divided by the diameter of the die opening (mm). Radial expansion was measured 15 times for each sample. The overall expansion was determined as the ratio of true solid density to unit density. Axial expansion was the ratio of overall expansion to radial expansion (Ali et al., 1996). Axial and overall expansions were calculated 5 times for each sample.

Water absorption index (WAI) and water solubility index (WSI) of extrudates were measured three times per sample as reported previously (Anderson, Conway, & Peplinski, 1970). Color was analyzed 5 times for each sample using a colorimeter (Konika Minolta Chroma Co, Osaka, Japan) and expressed as L* (lightness/darkness), a* (redness+/greenness-) and b* (yellowness+/ blueness-).

The texture of extrudates was characterized in terms of hardness (N) and jaggedness (N·s) and was measured 5 times for each sample. The analysis was performed using a texture analyzer (TA.XT plus, Stable Micro systems, Godalming, UK) with a 5 blade Kramer shear cell as the probe. Extrudates were added to a height of 4 cm in the canister, and were cut with the blades at speed of 2 mm/s and a distance of 48 mm. Hardness was the maximum force (N) achieved during the run.

2.3.4. *In vitro* digestion

In vitro starch digestion was determined following Mkandawire, Weier, Weller, Jackson, and Rose (2015). The fraction of starch digested after 20 min of incubation was expressed as rapidly digestible starch (RDS), after 120 minutes was total digestible starch (TDS), and the digested starch fraction between 20 and 120 minutes was defined as slowly

digestible starch (SDS). Resistant starch (RS) was the starch that remained undigested after 120 minutes of digestion. *In vitro* protein digestion was determined using pepsin according to Mkandawire et al. (2015). All *in vitro* digestibility analyses were performed 3 times for each sample.

2.3.5. Data analysis

Data for each treatment combination were averaged and treated as continuous variables for data analysis following response surface approach. Regression analysis (Table 2.3) was first conducted on each factor (percentage of bean flour and feed moisture) and their interaction, allowing main effect terms from linear to cubic and all possible interaction. Next, a new regression model was created using only significant terms ($P<0.05$) from this initial model and all non-significant terms were pooled into the error term. Lastly, the significant terms tested from the new regression model were then used in a final model from which prediction equations were generated (Ali et al., 1996). Statistical analyses were performed using statistical software (version 9.4, SAS Institute, Cary, NC, USA). Response surface figures were constructed from the prediction equations with statistical software (R, version 3.2.2, Vienna, Austria).

2.4. Results and Discussion

2.4.1. Composition of rice-bean composite flours

Among brown rice: pinto bean composite flours, starch content ranged from 68 to 80%, protein from 9 to 13%, and total dietary fiber from 6 to 15% (Table 2.1.). the composition of 100% pinto bean flour contained 62% of starch, 22% of protein and 2% of fat. As expected, increasing the percentage of pinto bean flour in the composite increased dietary fiber, protein, ash, and lipid concentrations while decreasing starch, since bean

flours have higher protein, fat, ash and dietary fiber and lower starch compared to brown rice (Balasubramanian, Borah, Singh, & Patil, 2012). The compositions reported were similar to those reported for brown rice and wild legume composites (Pastor-Cavada et al., 2011).

2.4.1. Physical characteristics of rice-bean extrudates

The overall expansion ranged from 3.5 to 4.2 (Table 2.2). The overall expansion from our study is comparable to other studies: Pastor-Cavada et al. (2013) reported an expansion ratio of 3.2 to 3.4 when extruding rice and wild legume, and Chaiyakul et al. (2009) found an expansion between 2.7 to 3.9 when extruding glutinous rice.

From the regression equations, it was observed that bean flour percentage had a cubic trend on overall expansion (Table 2.3). Overall expansion was negatively affected by the addition of bean flour, where the lowest overall expansion was reported for 15% and 45% bean flour substitution and the highest at 0% bean flour (Fig. 2.1A). The higher overall expansion of 30% flour as compared to 15% was likely due to high axial expansion at higher bean flour substitutions (Table 2.2). Although high axial expansion was also the case for 45% bean flour, the accompanying decrease in radial expansion resulted in a decrease in overall expansion. Elastic forces and viscous forces are the two main factors governing extrusion expansion. While radial expansion is mainly driven by elastic forces, axial expansion is dependent on viscous forces (Pitts, Favaro, Austin, & Day, 2014). The addition of bean flour may have increased the viscous forces inside the extruder to be more dominant than the elastic forces, which caused the product to be more axially expanded. Higher axial expansion may be due to a reduction in flow properties due to the presence of

increased hydrophilic groups from bean flour constituents (such as protein) that compete with starch for water availability (Pitts et al., 2014).

An increase in feed moisture resulted in an increase in radial expansion but decreased axial and overall expansion (Table 2.3). Expansion is usually the highest with lower feed moisture (Pastor-Cavada., et al, 2013; Chaiyakul., et al, 2009) as was depicted for overall expansion in our study. The increase in radial expansion at lower feed moisture in our study was unexpected. Expansion properties can occur both radially and axially. In our study, axial expansion seemed to have a more predominant effect on the overall expansion properties than radial expansion.

Two methods for measuring density of the extrudates were used: bulk density and unit density. Bulk density was the density of a filled container of extrudates, which took into account the porous nature of the extrudates as well as the shape of the extrudates and how they packed together. Unit density was the density of the extrudates excluding the effect of shape. Bulk density ranged from 33.4 to 60.1 mg/cm^3, while unit density ranged from 98.1 to 161 mg/cm^3 (Table 2.2). Density properties of the extrudates were increased as feed moisture and percentage of bean flour increased (Fig 2.1B), as has been reported in others studies (Chaiyakul et al., 2009; Pastor-Cavada et al., 2011). Increases in density due to higher fiber content in the flour have been reported (Anton, Gary Fulcher, & Artnfield, 2009). The addition of fiber can interfere with the formation of the porous structure by disrupting pore walls during the extrusion process and result in a denser product (Pérez-Navarrete, González, Chel-Guerrero, & Betancur-Ancona, 2006). Additionally, a displacement of starch content and higher protein content on higher bean substitution may

also negatively affect the density properties of extrudates (Obatolu Veronica, Omueti Olusola, & Adebowale, 2006).

Texture analysis indicated that the hardness of rice bean extrudate ranged from 139 to 188 N, whereas the jaggedness ranged from 1.39 to 2.44 $x10^3$ N·s (Table 2.2). The highest hardness was reported on the 45% bean flour extrudates at the highest feed moisture (20.1%). The incorporation of higher concentrations of fiber can rupture the cell walls of expanding extrudates and therefore interfere with the expansion of air bubbles (Pérez-Navarrete et al., 2006). A higher concentration of protein can also result in fluctuations in pressure and temperature as the material moves through the extrusion barrel, which can negatively affect the texture of the extrudates (Obatolu Veronica et al., 2006). Furthermore, the reduced shear of the plasticized mass inside the extrusion barrel due to higher feed moisture can result in less gelatinized starch and therefore may impede bubble growth of the extrudate (Stojceska, Ainsworth, Plunkett, & Ibanoğlu, 2009).

The water solubility index (WSI) of the extrudates ranged from 30.6 to 48.5 (Table 2.2), with a decreasing cubic trend at higher feed moisture and increasing linear trend with addition of more bean flour (Table 2.3). The increase in WSI at lower feed moisture may be related to higher starch degradation due to higher shear (Stojceska et al., 2009). WSI also increased as the proportion of bean flour increased. In pure starch systems, WSI is a measure of the mechanical degradation of amylopectin released from starch granules during extrusion (Seth, Badwaik, & Ganapathy, 2015). However, samples with higher bean flour content had both lower starch and higher WSI. Thus, the increase in WSI at higher bean substitution may be related to the increase in hydrophilic groups associated with dietary fiber, protein, or other components in the bean flour. The increase in hydrophilic

groups with higher bean flour could further explain the increase in viscous forces inside the extrusion barrel that led to the higher axial expansion mentioned previously. These results were similar to a previous study with brown rice and wild legume flours (Pastor-Cavada et al., 2011).

WAI ranged from 3.6 to 6.0 (Table 2.2). WAI followed a linear trend for both feed moisture and bean flour substitution, with feed moisture positively affecting WAI and bean percentage negatively affecting WAI. The decrease in WAI as bean percentage increased may be due to the reduction in starch content of composite flour (Table 2.1). Similar results were reported by Lazou and Krokida (2010), who showed that an increase in protein upon addition of legume flour to yellow corn flour increased the availability of hydrophilic groups that bind water and, therefore, increase WAI.

The brightness of the extrudate decreased and redness increased with the addition of more bean flour or less moisture (Table 2.3). Yellowness of the extrudates was only significantly affected by bean percentage. The color analysis from our study was in agreement with a previous report on extruded corn starch and common bean flour blend (Anton et al., 2009). Changes in color characteristics caused by lowering the feed moisture may be due to the maillard reaction which causes the product to decrease in brightness and increase in yellowness (Bates, Ames, & MacDougall, 1994).

2.4.2. *In vitro* digestibility of rice-bean extrudates

Rapidly digestible starch (RDS) ranged from 67 to 74%, while resistant starch (RS) ranged from 1.8 to 12.6 % (Table 2.2). *In vitro* protein digestibility ranged from 67% to 78%. These ranges were similar to other studies (Pastor-Cavada et al., 2011; Pérez-Navarrete et al., 2006).

RDS decreased as bean flour increased (Table 2.3). From the regression equations, it was observed that bean flour had a cubic trend and feed moisture had a linear trend with RDS (Table 2.3; Fig 2.2A). The increase in RDS by lowering the feed moisture in our study is in agreement with a previous study extruding hard-to-cook bean and maize flour (Ruiz-Ruiz et al., 2008). Therefore, the increase in RDS may be due to higher mechanical shear exhibited by lower feed moisture which provided a higher mechanical rupture on the plant cell wall. This would create a larger surface area for digestive enzymes and also an increase in gelatinization of the starch (Mahasukhonthachat, Sopade, & Gidley, 2010).

Slowly digestible starch (SDS) was only significantly affected by bean flour substitution (Table 2.3). However, the adjusted R^2 (0.49) was considerably low. This may be because the SDS was calculated by subtraction of RDS from total digestible starch. Thus, because the total error remained constant, the relative error was large and could have resulted in a lower coefficient of determination.

RS increased as percentage of bean flour increased and also increased as feed moisture increased (Table 2.3; Fig 2.2B). The increase in RS as bean flour increased may be attributed to increasing total dietary fiber in higher levels of bean flour. The reduction in RS as the feed moisture decreased was in agreement with a previous study that reported a decrease in RS on a higher mechanical degradation of waxy and regular barley flours (Faraj, Vasanthan, & Hoover, 2004). However, increase in RS may also occur at lower feed moisture due to fragmentation of starch into low molecular weight oligomers that retrograde easily (Mahasukhontachat et al., 2010). This does not appear to be the case for brown rice: bean flour extrusion.

In vitro protein digestibility of the extrudate increased linearly as feed moisture decreased and bean substitution increased (Table 2.3; Fig. 2.3). In agreement with these reports, Balandrán-Quntnana, Barbosa-Cánovas, Zazueta-Morales, Anzaldúa-Morales, and Quentero –Ramos (1998) reported that protein digestibility increased as barrel temperature increased from 140 °C to 160 °C from extruding pinto bean flour. Similar results were also reported by Alonso et al (2000). Results from our study also indicated that the protein digestibility increased with the addition of bean flour. Other studies have found no significant differences in percentage of protein digestibility upon the addition of legume flours (Alonso et al., 2000; Pérez-Navarrete et al., 2006). This may be due to different digestion methods. Multienzyme methods containing trypsin, chymotrypsin and peptidase were applied in the previously mentioned studies, whereas in our study the extrudates were digested using pepsin. Romero and Ryan (1978) compared the susceptibility of phaseolin, the major storage protein in beans, to pepsin and trypsin digestion. They found that pepsin was more efficient at hydrolyzing phaseolin than trypsin. In contrast, Chandi and Sogi (2007) reported that trypsin and pepsin digestibility of rice proteins were similar.

2.5. Conclusion

The use of pinto bean flour in extruded brown rice-bean composites can improve the nutritional profiles of the extrudates by improving the *in vitro* protein digestibility and increasing RS, while some physical attributes, namely, hardness, densities, and overall expansion, may be negatively affected. Reducing the feed moisture inside the extrusion barrel increased *in vitro* protein digestibility and partially alleviated the negative effects of bean flour on the physical characteristics, but decreased RS.

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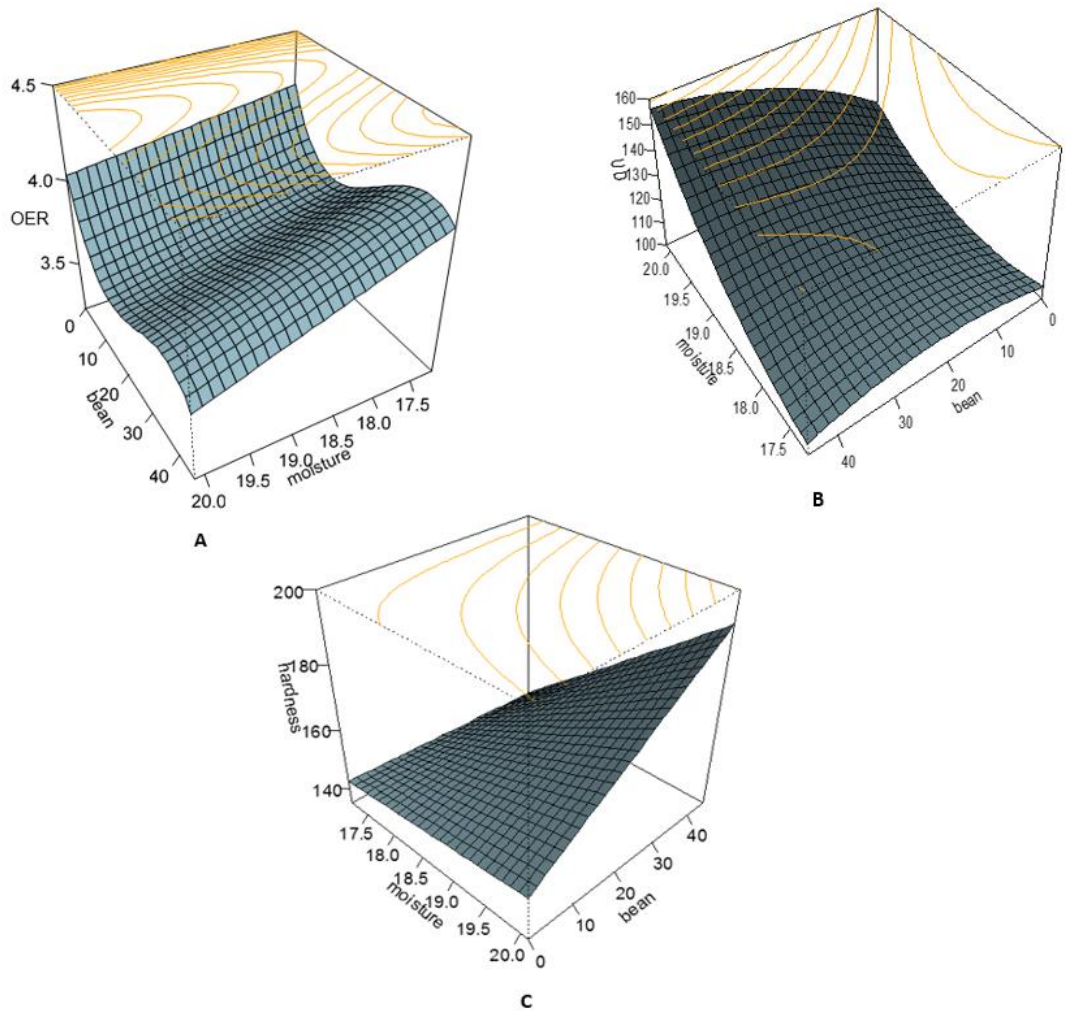

Figure 2.1. Effect of feed moisture (%) and percentage of bean flour in the brown rice: bean flour composite (bean, %) on (A) OER, (B) UD, and (C) hardness of brown rice-bean extrudates; OER= Overall expansion ratio, UD= unit density.

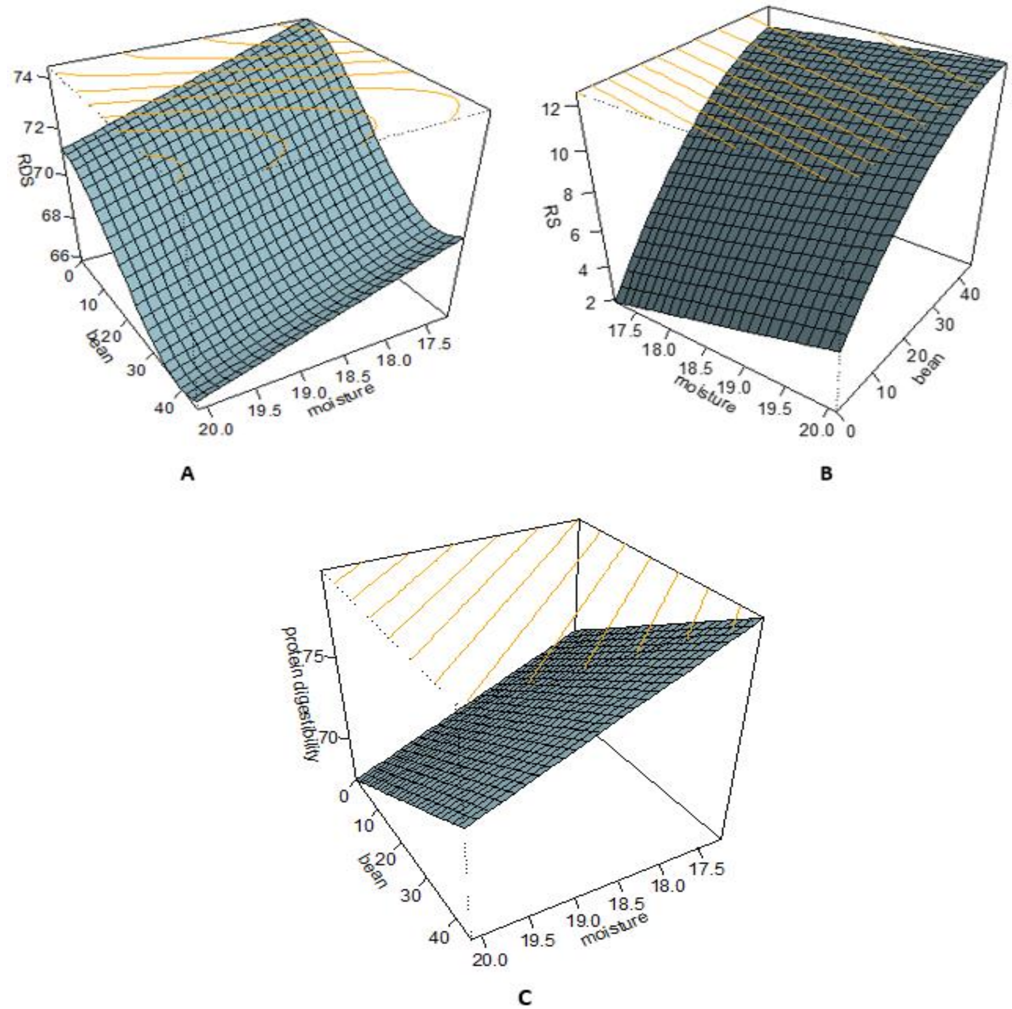

Figure 2.2. Effect of feed moisture (%) and percentage of bean flour in the brown rice: bean flour composite (bean, %) on (A) rapidly digestible starch (RDS, %) and (B) resistant starch (RS, %) (C) *in vitro* protein digestibility (%) of brown rice-bean extrudates.

Table 2.1. Composition (% dry basis) of brown rice: pinto bean composites flours. [A]

Bean flour in composite (%)	Starch	Protein	Fat	Ash	SDF	IDF	TDF
0	$80.1^{a} \pm 3.5$	$9.25^{a} \pm 0.16$	$1.46^{a} \pm 0.04$	$2.34^{a} \pm 0.06$	$0.90^{a} \pm 0.15$	$5.91^{a} \pm 0.36$	$6.82^{a} \pm 0.51$
15	$76.7^{ab} \pm 2.4$	$9.96^{b} \pm 0.25$	$2.14^{b} \pm 0.03$	$2.83^{b} \pm 0.03$	$1.08^{a} \pm 0.36$	$7.13^{b} \pm 0.69$	$8.21^{a} \pm 0.62$
30	$72.4^{b} \pm 6.9$	$12.4^{c} \pm 0.30$	$2.82^{c} \pm 0.15$	$3.37^{c} \pm 0.05$	$2.22^{b} \pm 0.51$	$8.77^{bc} \pm 0.77$	$10.9^{b} \pm 0.83$
45	$68.6^{b} \pm 4.4$	$13.7^{d} \pm 0.11$	$3.02^{c} \pm 0.02$	$3.59^{d} \pm 0.01$	$3.24^{c} \pm 0.41$	$9.70^{c} \pm 0.24$	$13.0^{b} \pm 0.29$

[A] Mean ± standard deviation; means with the same superscript letters within same column are not significantly different ($p>0.05$, $N=3$); SDF, soluble dietary fiber; IDF, insoluble dietary fiber; TDF, total dietary fiber.

Table 2.2. Physical characteristics of rice-bean extrudates.[A]

Moisture/ Flour blend (%)	RER	AER	OER	BD (mg/cm^3)	UD (mg/cm^3)	Hardness (N)	WSI	WAI	L*	a*	b*
17.2											
0	2.54 ± 0.03	1.65 ± 0.01	4.19 ± 0.01	33.4 ± 0.49	98.1 ± 1.75	39.8 ± 0.1	5.01 ± 0.19	77.6 ± 0.6	2.31 ± 0.13	19.4 ± 0.3	
15	2.62 ± 0.02	1.46 ± 0.10	3.84 ± 0.13	39.3 ± 0.71	112 ± 3.01	40.9 ± 1.5	4.82 ± 0.01	73.8 ± 0.3	3.06 ± 0.11	19.9 ± 0.3	
30	2.46 ± 0.03	1.64 ± 0.05	4.03 ± 0.15	34.3 ± 0.41	105 ± 2.67	45.3 ± 1.6	4.24 ± 0.15	73.6 ± 0.3	3.77 ± 0.08	21.0 ± 0.6	
45	2.41 ± 0.03	1.66 ± 0.04	4.00 ± 0.05	33.8 ± 0.48	106 ± 3.77	48.5 ± 3.0	3.60 ± 0.25	71.1 ± 0.5	4.15 ± 0.17	20.8 ± 0.3	
18.1											
0	2.66 ± 0.07	1.55 ± 0.08	4.13 ± 0.12	37.3 ± 0.56	103 ± 7.83	36.3 ± 0.2	5.38 ± 0.03	77.6 ± 0.2	1.50 ± 0.14	19.3 ± 0.1	
15	2.65 ± 0.01	1.42 ± 0.08	3.77 ± 0.09	41.9 ± 1.09	113 ± 6.22	37.5 ± 0.9	5.17 ± 0.01	74.6 ± 0.4	3.10 ± 0.16	20.5 ± 0.8	
30	2.48 ± 0.02	1.61 ± 0.07	3.99 ± 0.17	38.9 ± 0.41	114 ± 5.16	44.4 ± 0.3	4.25 ± 0.08	74.0 ± 0.3	3.84 ± 0.09	21.1 ± 0.3	
45	2.37 ± 0.02	1.67 ± 0.07	3.96 ± 0.10	40.1 ± 0.52	114 ± 5.48	48.3 ± 0.8	3.63 ± 0.29	71.8 ± 0.3	4.52 ± 0.15	21.5 ± 0.5	
18.3											
0	2.67 ± 0.05	1.54 ± 0.08	4.11 ± 0.16	38.2 ± 0.64	103 ± 7.38	36.0 ± 0.7	5.21 ± 0.03	78.3 ± 0.2	1.13 ± 0.09	19.4 ± 0.3	
15	2.69 ± 0.02	1.40 ± 0.03	3.77 ± 0.08	39.8 ± 0.71	113 ± 6.22	37.6 ± 0.9	5.17 ± 0.04	76.4 ± 0.4	2.75 ± 0.17	19.9 ± 0.3	
30	2.52 ± 0.03	1.53 ± 0.02	3.85 ± 0.01	38.4 ± 0.61	119 ± 2.61	44.0 ± 0.2	4.31 ± 0.03	75.2 ± 0.3	3.65 ± 0.09	20.7 ± 0.3	
45	2.39 ± 0.02	1.62 ± 0.07	3.88 ± 0.09	41.1 ± 0.52	115 ± 7.11	47.1 ± 1.7	3.84 ± 0.22	72.4 ± 0.3	4.34 ± 0.16	21.6 ± 0.5	
19.5											
0	2.82 ± 0.02	1.45 ± 0.06	4.08 ± 0.07	46.2 ± 0.88	109 ± 5.71	33.1 ± 0.2	5.69 ± 0.05	78.4 ± 0.2	0.93 ± 0.002	18.8 ± 0.3	
15	2.80 ± 0.02	1.28 ± 0.01	3.59 ± 0.05	48.5 ± 1.37	122 ± 0.63	35.5 ± 0.7	5.24 ± 0.14	74.2 ± 0.4	3.05 ± 0.21	20.3 ± 0.5	
30	2.76 ± 0.02	1.33 ± 0.10	3.67 ± 0.13	48.3 ± 1.91	126 ± 4.21	39.0 ± 0.6	4.53 ± 0.04	74.0 ± 0.9	3.63 ± 0.29	20.2 ± 1.0	
45	2.51 ± 0.03	1.49 ± 0.03	3.73 ± 0.08	55.1 ± 1.48	135 ± 3.79	41.2 ± 1.8	4.25 ± 0.03	72.5 ± 0.5	4.32 ± 0.16	20.8 ± 0.5	
20.1											
0	2.81 ± 0.03	1.45 ± 0.05	4.07 ± 0.14	53.1 ± 1.03	117 ± 3.01	30.6 ± 1.3	6.06 ± 0.07	77.6 ± 0.3	0.93 ± 0.00	19.4 ± 0.4	
15	2.81 ± 0.03	1.24 ± 0.05	3.48 ± 0.13	54.2 ± 0.21	141 ± 4.67	32.5 ± 1.2	5.49 ± 0.15	76.0 ± 0.5	2.88 ± 0.23	20.0 ± 0.9	
30	2.72 ± 0.02	1.37 ± 0.08	3.74 ± 0.08	57.1 ± 2.08	151 ± 7.14	36.6 ± 1.7	4.63 ± 0.17	74.6 ± 0.1	3.63 ± 0.18	20.1 ± 0.6	
45	2.49 ± 0.01	1.44 ± 0.04	3.57 ± 0.01	60.1 ± 0.79	161 ± 9.74	42.0 ± 1.9	4.30 ± 0.07	74.0 ± 0.3	4.38 ± 0.23	20.8 ± 0.5	

[A] Mean ± standard deviation; flour blend represents the percentage of bean flour in the brown rice: bean flour composite; BD, bulk density; UD, unit density; RER, radial expansion ratio; AER, axial expansion ratio; OER, overall expansion ratio; WSI, water solubility index; WAI, water absorption index.

Table 2.3. Regression equations of response variables.[A]

Responses	Regression Model	Adjusted R^2	$\sqrt{MSE}$
RER	$-4.97 + 0.32B + 0.72M - 0.0002B^2 -0.016M^2 -0.03MB + 0.0008M^2B$	0.89	0.049
AER	$3.18 + 0.031B - 0.089M + 0.002B^2 -0.00002B^3$	0.88	0.045
OER	$5.24 - 0.02B - 0.06M - 0.002MB + 0.003B^2 - 0.00003B^3$	0.93	0.05
BD	$551 - 0.91B – 60.4M + 1.77M^2 + 0.51MB$	0.94	1.82
UD	$1681 – 4.36B – 174.2M - 0.013B^2 + 4.79 M^2 + 0.288MB$	0.92	4.65
WAI	$1.07 + 0.036B + 0.24M$	0.94	0.16
WSI	$-1457 + 0.23B + 242M - 12.9M^2 + 0.22M^3$	0.94	1.23
Hardness	$107 - 5.88B + 2.09 M +3391MB$	0.89	4.83
Jaggedness	$304062 - 48324M + 2550M^2 - 44.4M^3$	0.96	77.2
L*	$85 - 0.114B - 0.42M$	0.84	0.87
a*	$3.68 + 0.112B - 1.13M - 0.001B^2$	0.93	0.29
b*	$19.3 + 0.004B$	0.79	0.35
RDS	$94 - 0.002B - 1.13M - 0.01B^2 + 0.0002B^3$	0.95	0.54
SDS	$23.5 - 0.17B + 0.002B^2$	0.49	1.09
RS	$-17.9 + 0.67B + 1.15M - 0.003B^2 - 0.017MB$	0.98	0.45
IVPD	$108 + 0.14B - 2.01M$	0.92	0.96

[A] MSE, mean square error; *B*, percent bean flour in composite flour; *M*, feed moisture content (%);BD, bulk density; UD, unit density; RER, radial expansion ratio; AER, axial expansion ratio; OER, overall expansion ratio; WSI, water solubility index; WAI, water absorption index.

Table 2.4. In vitro digestibility of starch (% of total starch) and protein (% of total protein) of rice-bean extrudates.[A]

Moisture/ Flour blend (%)	RDS	SDS	RS	IVPD
17.2				
0	74.8 ± 3.6	23.3 ± 2.0	1.80 ± 2.4	73.6 ± 1.7
15	72.7 ± 0.6	20.9 ± 1.1	6.30 ± 0.5	75.2 ± 1.6
30	69.5 ± 1.7	20.3 ± 1.2	10.1 ± 0.9	77.9 ± 1.5
45	69.1 ± 0.2	24.0 ± 0.3	11.1 ± 0.2	78.5 ± 1.6
18.1				
0	73.4 ± 1.3	23.6 ± 0.6	2.90 ± 0.7	72.5 ± 1.4
15	71.5 ± 1.3	21.3 ± 1.0	7.10 ± 0.3	73.3 ± 2.3
30	69.5 ± 1.1	19.9 ± 0.6	10.5 ± 1.7	75.4 ± 0.6
45	68.8 ± 1.0	19.4 ± 1.4	11.8 ± 0.4	77.9 ± 0.4
18.3				
0	73.7 ± 2.0	22.4 ± 1.2	3.90 ± 1.5	69.0 ± 0.2
15	71.3 ± 0.7	21.4 ± 1.1	7.10 ± 0.9	72.2 ± 1.9
30	67.8 ± 0.6	20.9 ± 0.4	11.2 ± 0.3	76.9 ± 1.0
45	68.1 ± 1.2	19.3 ± 1.8	12.1 ± 0.6	76.9 ± 0.6
19.5				
0	71.9 ± 0.4	24.4 ± 0.6	3.60 ± 0.4	68.1 ± 1.5
15	70.4 ± 1.5	21.0 ± 1.8	8.60 ± 0.3	70.0 ± 1.4
30	66.9 ± 1.8	21.6 ± 2.2	11.4 ± 0.5	74.3 ± 1.3
45	66.4 ± 0.3	21.4 ± 0.5	12.1 ± 0.7	74.5 ± 0.3
20.1				
0	70.1 ± 2.6	24.0 ± 2.8	5.80 ± 0.4	66.7 ± 2.5
15	69.1 ± 0.6	21.7 ± 1.0	9.10 ± 0.4	69.0 ± 0.4
30	67.1 ± 1.7	21.5 ± 1.8	11.3 ± 0.7	72.8 ± 1.6
45	67.0 ± 0.2	20.3 ± 0.5	12.6 ± 0.3	73.3 ± 0.2

[A] Mean ± standard deviation; flour blend represents the percentage of bean flour in the brown rice: bean flour composite; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; IVPD, *in vitro* protein digestibility

CHAPTER 3. EXTRUSION INCREASES *IN VITRO* BIOACCESSIBILITY OF MINERAL ELEMENTS IN PINTO BEAN FLOUR

3.1. Abstract

Pinto beans (*Phaseolus vulgaris*) are "good sources" (>10% of the US Daily Value per serving) of Fe, Mg, P, and K. However, previous reports have suggested low bioavailability, especially of the divalent cations, due to the chelating effects of phytic acid, tannins, and dietary fibers. Extrusion may be a strategy to improve the bioaccessibility of these elements. In this study, *in vitro* bioaccessibility of Fe, Mg, P, and K, as well as two heavy metals (Cd and Pb) were analyzed in extruded pinto bean and unprocessed flours. Chemical (phytic acid, dietary fiber, and tannins) and physical properties (radial expansion, apparent bulk density, water solubility index and water absorption index) were also determined. Extrusion parameters (temperature, moisture content, screw speed) significantly affected physical properties, but not element bioaccessibility or chemical properties, except in very few isolated cases. Extrusion did, however, increase the bioaccessibility of all essential elements (Fe, Mg, P, and K) by 1-5% without a comparable increased in toxic elements (Cd ; 0.5%). A significant reduction in phytic acid and tannins together with an increase in soluble dietary fiber was found after extrusion. Correlation analysis suggested that bioaccessibility of Fe is influenced by phytic acid concentration, while Mg is influenced by tannins and heavy metals are influenced by soluble dietary fiber. The increased of elements bioaccessible by extrusion cooking is highly related to the reduction of chemical factors, whereas the severity of extrusion process only affected physical properties of pinto bean extrudates.

3.2. Introduction

Pinto beans (*Phaseolus Vulgaris* L. Pinto) are among the most prominent dry edible bean varieties produced in the US, with an estimated economic value of $320 million (USDA-ERS, 2012). In terms of nutritional quality, pinto beans, like other dry beans, are high in protein, dietary fiber, and an excellent source of vitamins and mineral elements (Tharanathan & Mahadevamma, 2003). According to the US Department of Agriculture (USDA) Nutrient Database, pinto beans contain >10% of the daily value for Fe, Mg, P, and K per serving (2.09 mg, 50 mg, 147 mg, and 436 mg, respectively, per 85.5 g of cooked beans; (Nutrient data based.USDA, 2016). In comparison to whole grain flours (wheat, oats, and rice), which are also considered good sources of essential elemental nutrients, pinto bean flour contains about 35% more Fe, 25% more Mg, 10% more P, and 75% more K on a same weight basis (USDA, 2016).

However, the total concentration of these nutrients does not necessarily reflect the quantity available for human absorption. Many factors including physiological state of the element (e.g. solubility, dispersibility, and ligand binding), presence of enhancers (e.g. ascorbate for Fe) or inhibitors (polyphenols and phytic acid), and competitive inhibitors (e.g. transport binding protein) (Fairweather-Tait, 1987) come together to increase or decrease the bioavailability of these elements. Bioavailability is defined as the proportion of an element that can be absorbed from the gastrointestinal tract (Glahn, McClements, & Decker, 2009).

In vitro models can be used to estimate the proportion of elements in a material that may be available for absorption. These models involve enzymatic digestion under controlled pH that closely resembles the physiological conditions in stomach and

intestines (Luten et al., 1996). During this process, a fraction of the elements in a sample diffuse into a dialysis tube and represent the elements that are potentially bioaccessible to absorption from the gastrointestinal tract (Etcheverry, Grusak, & Fleige, 2012).

The presence of anti-nutritional factors in pinto beans can negatively affect their absorption in the body and hence affect element bioaccessibility (Sahuquillo, Baeberá, Farré, 2003). These anti-nutritional factors, especially phytic acid and tannins in pinto bean, can form insoluble complexes with elements and inhibit absorption (lopez, Leenhardt, Couray, & Remesy, 2002; Akande, Doma, Agu, & Adamu, 2010). Tannins can also form insoluble complexes with elements through hydrogen bonding and hydrophobic interactions (Butler, 1989), and dietary fibers can affect the bioaccessibility of elements by forming electrostatic interactions with polyvalent ions (Alonso, Orúe, Zabalza, Grant, & Marzo, 2000).

Pinto beans must be processed before consumption. While the traditional processing of pinto beans (i.e., soaking and boiling) has been reported to modify factors related to element absorption (Alonso, Aguirre, & Marzo, 2000), this process is often not effective in deactivating phytic acid because it is relatively heat resistant. (Wang, Hatcher, Tyler, Toews,& Gawalko, 2010). While other studies indicated a slightly decreased of tannins and solubilization of dietary fiber after boiling. (de Almeida Costa, da Silva Quiroz-Monici, Pissini Machado Reis, & de Oliveira, 2006; Wang et al., 2016)

Extrusion combines high temperature and shear to modify the physical and chemical properties of agricultural products. The intensive shearing forces combined with high temperature cooking are effective at deactivating anti-nutritional factors in bean flours, especially phytic acid (Alonso, Rubio, Muzquiz, & Marzo, 2001). Additionally,

extrusion can modified dietary fiber components by the formation of soluble complexes from insoluble fibers which may release elements that were bound to insoluble fiber (Alonso, Orúe, et al., 2000). Ummadi et al (1995) compared the dialyzable iron in boiled versus extruded navy bean and concluded that extrusion cooking resulted in 1.5% and 2% higher of dialyzable iron for low and high shear condition respectively.

Extrusion involves many processing parameters including screw configuration and feed rate, but the parameters that influence the properties of the final product the most are barrel temperature, screw speed, and flour moisture content (Harper, 1989). Therefore, the objective of this study was to investigate the effects of extrusion parameters on *in vitro* element bioaccessibility and to link these changes with changes in phytic acid, tannins, and dietary fiber concentrations upon extrusion. A secondary objective was to determine how extrusion parameters change physical properties of pinto bean extrudates.

3.3. Materials and Methods

3.3.1. Pinto bean flour

Whole pinto beans were milled on a pilot scale hammer mill (C.S Bell Co, Ohio, USA) equipped with a 1.0 mm screen. Moisture content of the flour was measured by the air oven method (44-15-02, AACC International,2015). Two kg batches of pinto bean flour were then blended with distilled water in a mixer (Kitchen Aid KV25G0X, Benton Harbor, MI, USA) for 10 min to achieve a prescribed moisture content (as described in the *Experimental Design* section). The blended flour was then transferred to a zipper-top plastic bag and equilibrated for at least 16 h at 4 °C.

3.3.2. Experimental design

Three variables were selected to study the effects of extrusion parameters on *in* vitro element bioaccessibility during extrusion of whole pinto bean flour: flour moisture content and extruder barrel temperature and screw speed. Because randomization of barrel temperature was impractical due to the time required to heat and cool the extruder barrel, the experiment was arranged into a split plot design, where barrel temperature was assigned as the whole plot factor and moisture content and screw speed were randomly arranged within the whole plot. A central composite rotatable design (CCRD) was thus generated (JMP version 10.0.0, SAS Institute, Cary, NC.USA) on three variables at three levels with rotatability values of α and β of 1.43 and 0.7, as recommended for an optimization design where one factor is difficult to change (Draper & John, 1998). The final design consisted of 8 factorial points, 4 axial points, and 5 center points for a total of 17 runs (Table 3.1).

3.3.3. Extrusion processing

Moisture-adjusted pinto bean flours were extruded using a laboratory scale twin screw extruder (model CTSE-V, CW Brabender, Hackensack, NJ, USA). The extruder was operated by a direct current drive unit (Intelli-Tourque, plastic Corder Lab-Station, C.W Brabender) consisting of a 7.5 hp motor. The flours were fed into the extruder by means of a mechanical volumetric feeder (FW 40 Plus, C.W Brabender, Hackensack, NJ, USA) at a constant speed of 165 g/min. The screw configuration was co-rotating with the screw length to diameter ratio (L/D) ranging from 13.2/1 to 8.60/1. The barrel was divided into four zones with the first two zones maintained at constant at 60 °C and 90 °C, while the last two zones were set to the temperature according to the experimental design. The actual temperatures of last two zones were slightly higher than the set temperature due to frictional heat generated during extrusion and they were recorded and used in all

subsequent statistical analyses. The diameter of the die opening was 3.0 mm. The torque was measured by measurement control software version 3.0.2 (C. W Brabender, Hackensack, NJ, USA). The extrudates were placed on a belt dryer at 103 °C for 10 min and stored for further analyses. The samples were stored in plastic bags in room temperature.

3.3.4. Physical Analysis

Radial expansion was calculated as the ratio of the cross-sectional diameter of the extrudate to the diameter of the die opening. Radial expansion was measured 15 times for each sample. Apparent bulk density was determined as mass per unit volume of extrudates by rapeseed displacement. Five grams of extrudate were weighed into a cylindrical canister and then the canister was filled to overflowing with rapeseed and then the top was leveled with a wooden rod. The density was calculated with the equation:

$$\rho_a = (W_{ex} \text{ x } W_{rs}) / (W_{rf} \text{ x } V_c)$$

Where ρ_a = unit density (g/cm^3), W_{ex} = mass of extrudate (g), W_{rs} = mass of rapeseed replaced (g), W_{rf} = mass of the rapeseed on full container (g), V_c = volume of canister (cm^3). Apparent bulk density were conducted in triplicates for each sample. Water absorption index (WAI) and water solubility index (WSI) of the extrudates were measured according to (Anderson, Conway, & Peplinski, 1970). WAI and WSI were performed in triplicate for each sample.

3.3.5. Compositional analysis

For all chemical analyses, whole extrudates were ground with cyclone mill (Udy Corporation, Fort Collins, CO,USA) equipped with 1 mm screen. All chemical analysis were conducted at triplicate and adjusted to dry basis after measurement of the moisture

content (AACC International, 2015). Dietary fiber was partitioned into soluble and insoluble dietary fiber according to method by (Arcila, Weier, & Rose, 2015). The cell wall components of soluble and insoluble dietary fiber were calculated according to composition analysis of commons beans (*Phaseolus vulgaris* L) by Shiga & Lajolo (2006) and Shiga, Lajolo, & Filisetti (2003). The study describes that two soluble main polymers were associated with fiber fraction of bean (Shiga & Lajolo, 2006). The first polymer suggested the presence of xyloglucans (XGs) and contained glucose (18%), xylose (12%), arabinose (18%), galactose (14%) and uronic acid (4%), whereas the second polymers were highly associated with pectic substances which composed of Galactose (11%), xylose (8%), rhamnose (1.5%) and uronic acid (26%). For the insoluble dietary fiber, the cell wall materials were classified based on extraction solvents (Shiga et al., 2003). To simplify the fractions, the fractions was divided into cellulose (accounted for 5.6% of total insoluble fiber fractions) and non cellulose insoluble fiber.

Phytic acid was determined as phytate phosphorus using a colorimetric method (Haug & Lanstzsch, 1983) with slight modifications. In short, 250 mg of extrudate was suspended in 10 mL of 0.2 N HCl in a 15 mL tube and incubated overnight on shaking water bath at 4 °C with addition of ice. The tube was centrifuged at 4500 x g for 15 min at 4 °C. The supernatant was quantitatively transferred into a 50 mL tube and diluted to 25 mL with distilled water. For pinto bean flour, 0.25 mL of the extract was mixed with 0.75 mL of 0.2 N HCl in a clean 15 mL tube; for extrudates, 0.5 mL of the extract was mixed 0.5 mL of 0.2 N HCl. One mL of 415 µM $Fe(NH_4)(SO_4)_2$ was then added and placed on a boiling water for 30 min. The tube was cooled on ice for 10 min and then centrifuged at 3000 x g for 30 min at 4 °C. One mL of the supernatant was transferred into a new tube

and mixed with 1.5 mL of 2, 2'-bipyridine. The color developed was measured at 530 nm with a spectrophotometer (Lambda XLS, Perkin Elmer, Waltham, MA, USA). The standard solution was sodium phytate dodecahydrate which contained 19% of phytate phosphorus as measured with inductive coupled plasma mass spectrometry (ICP-MS) as described below.

Tannins were determined by the vanillin-HCl assay according to Prince, Van Scoyoc, & Butler (1978) using a catechin as the reference standard. Flour or extrudate (150 mg) was extracted with 4 mL of 1% (v/v) HCl in methanol for 20 min. Then duplicate 0.2 mL extracts were transferred to microcentrifuge tubes and kept at 30 °C. One tube was reacted with 1% (1g of vanillin in 100 mL of 4% HCl-methanol) vanillin for exactly 20 min for color development, while 1 mL of 4% (v/v) HCl in methanol was added to another set of extract as blank and reacted for exactly 20 minutes. Tannins was measured by subtracting the blank after reading the extracts at 500nm with spectrophotometer.

3.3.6. *In vitro* element bioaccessibility

In vitro element bioaccessibility was measured after Luten et al (1996) with some modifications. In short, 2 g of sample was suspended in 16 mL of water and placed in an orbital shaking water-bath (195-200 rpm) at 37 °C for 5 min. Gastric digestion was then initiated by adjusting the pH of the mixture to 2.0 with 6 M HCl and the volume noted. Pepsin was then added (0.6 mL of 160 mg/mL in 0.1 N HCl; P-7000, Sigma, St Louis, MO, USA) and additional water was added to adjust the final solids concentration to 10% (w/v). The mixture was then incubated in the shaking water-bath for 2 h. The pH was then raised to 5 over a period of 55 minutes by inserting dialysis tubing (Fisher brand, molecular weight cutoff 12,000-14,000 Da) containing 25 mL of 0.0067 M sodium bicarbonate. The

concentration of sodium bicarbonate was previously determined by measuring the titratable acidity of the gastric mixture plus the pancreatin-bile acid solution added next. After 55 min, 5 mL of bile-pancreatin solution [0.4 g of pancreatin (Sigma, P7545, Sigma, St Louis, MO, USA) and 2.5 g of bile salt (Oxoid, LP0055, Hants, UK) dissolved in 100 mL of 0.1 M $NaHCO_3$, were then added and the digestion was continued for 2 h (the digestion duration was determined previously by averaging the time needed for the mixture to reach pH 5.0 and 7.0 respectively). During the digestion, bioaccessible elements diffused into the dialysis tube. At the end of digestion, the dialysis tube was removed and washed with water. The retentate was transferred to a new 50 mL tube and weighed as the bioaccessible fraction. The remaining digestion slurry was centrifuged at 4500 x g for 25 min at 4 °C. The pellet and supernatant were separated and weighed. These fractions represented soluble bio-inaccessible and insoluble bio-inaccessible fractions, respectively. The bioaccessible element represent the amount of element that was absorbed, soluble bio-inaccessible represent the fraction of element that was potentially absorbable but it formed complexes with component that made it not available for absorption, and insoluble bio-inaccessible represent the fractions of elements that was not absorbable (Icard- Verniére et al., 2013).

To determine the element composition in each of the three digestion fractions, the insoluble bio-inaccessible pellet was dried overnight at 80 °C and then wet ashed. The wet ashing procedure was initiated by overnight pre-digestion of the dried pellet with 3 mL of concentrated HNO_3 followed by heating at 100 °C for 2 h. The mixture was then cooled to room temperature and 2 mL of 30% H_2O_2 was added. The sample was then heated uncovered at 165 °C overnight or until dryness. The sample was then re-suspended in 5

mL of 1% HNO_3. For soluble bio-accessible and soluble bio-inaccessible fractions, the samples were diluted with 10% HNO_3 to make the final concentration of 1% HNO_3. Lastly, 50 ppb Ga was added as an internal standard. Elements for all the dialysis fractions were analyzed with ICP-MS (7500cx, Agilent Technologies, Santa Clara, CA, USA) operating in 5 mL/min of collision cell mode. The conditions of plasma were 15L/ min for plasma gas, 1 L/min for carrier rate, 100µL/min for uptake rate with 100 mSec as dwell time. Each sample was injected in triplicate

3.3.7. Statistical analysis

Response surface methodology was employed to fit the responses (physical properties and chemical and elemental data) as a function of the process variables (temperature, screw speed, and moisture content). The data were fitted into second order polynomial models. The adequacy of the models were evaluated by the adjusted R^2. Model fitted error (MRE) was calculated to assess the relative error between actual and fitted model. Error range of ±5% was considered acceptable. The response surface analysis for second order polynomial models showed that the process variables (temperature, screw speed, and moisture content) did not affect element bioaccessibility or chemical composition except in the case of only 3 responses: a significant moisture effect on tannins and moisture and screw speed effects on soluble bio-inaccessible lead and insoluble bio-inaccessible potassium (Table 3.5 & 3.6). Thus, the response surface approach was not used for expression of element bioaccessibility or chemical composition results. Instead, the data from each extrusion run and the flour were compared by ANOVA followed by Fisher's protected least significant difference (LSD) test to determine significant differences among samples. Error was calculated from replications at center the point

(N=5). Correlation between *in vitro* bioaccessible elements and chemical composition were calculated using Pearson's method. The response surface model, ANOVA, and Pearson's correlation were calculated and generated by statistical software (JMP version 10.0.0, SAS institute)

3.5. Results and discussion

3.5.1. Physical properties

Extrusion parameters significantly affected the physical responses (Table 3.2). All extrusion parameters significantly impacted the unit density with temperature and moisture also showing significant quadratic effects. The density decreased as the moisture content decreased or screw speed or temperature increased. RER was significantly affected by the liner and quadratic effects of barrel temperature and flour moisture content. The highest RER was reported at high temperature and low moisture content. The trends for unit density and RER were similar to previous reports (Balandrán-Quintana, Barbosa-Cánovas, Zazueta-Morales, Anzaldúa-Morales, & Quintero-Ramos, 1998; Sachetti, Pinnavaia, Guidolin, & Rosa, 2004; Ding, Ainswoth, Tucker, & Marson, 2005).

All extrusion parameters had both linear and quadratic effects on WAI, as well as an interaction of moisture and screw speed (Table 3.2). WAI increased as barrel temperature increased up to about 135 °C and then slightly decreased. This result suggested that starch gelatinization increased as barrel temperature increased until it slightly decreased due to excessive dextrinization. WSI was significantly affected by moisture content, screw speed and their interactions. Increasing moisture content significantly decreased the WSI whereas, screw speed had the opposite effect. The WAI and WSI trends

from our study were similar to other studies from extruding durum wheat (Yağci & Gőğűş, 2008) and rice flour (Ding et al., 2005).

3.5.2. *In vitro* element bioaccessibility

Pinto bean extrudates were high in Fe, Mg, P, and K, as expected, with low concentrations of the heavy metals, Cd and Pb (Fig. 3.1A). The concentration of these elements was not significantly different between extrudate and flour samples, which is in agreement with Alonso et al (2001), who reported similar element composition between extrudates and flour in pea and kidney bean. Furthermore, element concentration was comparable to the values reported in the US Department of Agriculture Nutrient Database (USDA, 2015).

Although extrudates with a wide range of physical properties were created by varying moisture, screw speed, and temperature according to our experimental design (as described above), response surface methodology indicated that these parameters did not have significant effects on element bioaccessibility or chemical properties, except in a few isolated cases (Table 3.5). However, there were significant differences among extrudates and the unextruded pinto bean flour as assessed by ANOVA (Table 3.2). Various studies have reported the effects of extrusion conditions to elements bioaccessibility. Drago, Velasco-González, Torres, Gonzáles, & Valencia (2007) indicated that bioaccessibility of iron and zinc were not affected by extrusion parameters on extrusion of common bean grits, while Ummandi et al. (1995) showed that iron bioaccessibility was dependent on severity of the extrusion process (screw speed, moisture content, die and barrel temperature).

The bioaccessibility of all elements varied among extrudates and unprocessed flours (Table 3.3). Fe and Cd had the widest ranges among extruded samples. The Fe

bioaccessibility for extruded samples ranged from 1.45 to 5.04%. This result was within the range of other studies (Hemalatha, Platel, & Srinivasan, 2007; Lombardi-Boccia, Lullo, & Carnovale, 1991). In general, the bioaccessibility of all elements in sample R8 was significantly lower than the rest of the runs. This could be related to less cooking subjected to sample R8, as it was extruded at positive axial point of moisture content (25.6%).

Nevertheless, the overall bioaccessibility data did not shown any common trend (apart from sample R8) among the elements, which is in accordance with the insignificant effects of extrusion parameters. Thus, the effect of extrusion on element bioaccessibility was assessed by the mean of all extruded samples compared with unextruded pinto bean flour (Fig 3.1B). There was an increase in the mean bioaccessiblity of all elements after extrusion processing, excpet for Pb. Our results were similar to Alonso et al. (2001), who reported an increase in bioaccessibility of Fe, Mg, and P after extrusion of pea and kidney bean flour. The average increase in bioaccessible Fe, Mg, P, and K after extrusion was 1.3%, 4.5%, 3.3%, and 1.5%, respectively, compared to an average of only 0.4% for Cd and no change for Pb. Thus, extrusion increase the bioaccessibility of the essential elements without a proportional increase in bioaccessibility of the toxic heavy metals.

While extrusion processing increased the mean bioaccessibility of all selected elements except Pb, extrusion had differential effects on the bio-inaccessible fractions (Fig. 3.1B). Fe and Pb showed significant increases in the soluble bio-inaccessible fraction, while Mg, P, and K showed significant reductions and Cd did not change. The insoluble bio-inaccessible fraction decreased significantly and dramatically in some cases for all elements except K. The soluble bio-inaccessible fraction ranged from 13 to 28% for Fe, Mg, P, and K, whereas this fraction only accounted for 1.2 and 1.5% of Cd and Pb,

respectively (Table 3.3). Although the insoluble bio-inaccessible fractions of the elements were decreased by extrusion (except for K), the insoluble fraction still accounted for 56-86% of the total Fe, Mg, K and P and almost 98% of Cd and Pb.

3.5.3. Change in dietary fiber composition

Total dietary fiber (TDF) of the extrudates ranged from 147 to 183 mg/g, whereas flour was 197 mg/g (Table 3.4). Soluble dietary fiber (SDF) of extrudates ranged from 28.1 to 49.7 mg/g compared to only 22.3 mg/g at flour. Insoluble dietary fibers (IDF) of extrudates decreased from 172 mg/g in flour to an average of 163 mg/g. These results were similar to those reported from extruding hard to cook bean, where a 3-4% of reduction in IDF with an increase in SDF was reported (Martín-Cabrejas et al., 1999).

While dietary fiber fractions varied among extrudates, these changes were not functions of the extrusion parameters (Table 3.5). Thus, the average change in dietary fiber fraction was compared with unprocessed flour as was done for the element bioavailability data. Extrusion clearly induced significant changes in the distribution of soluble and insoluble dietary fiber components, with an average increase of 15.7 mg/g for soluble dietary fiber and 46 mg/g for insoluble dietary fiber after extrusion. The increase in soluble dietary fiber has been reported previously (Arcila et al, 2015; Martín-Cabrejas et al., 1999). The decrease in insoluble dietary fiber was greater than the increase in soluble dietary fiber, suggesting some degradation of dietary fiber in addition to repartitioning. This was due to the substantial degradation of the lignin fraction of IDF, which decreased by an average of 27 mg/g upon extrusion. A significant reduction of lignin fraction was also reported in extruded hard-to-cook bean flour (Martín-Cabrejas et al., 1999).

3.5.4. Degradation of phytic acid and Tannins

Phytic acid concentration in the extrudates ranged from 1.5 mg/g to 2.1 mg/g whereas that of the unprocessed flour was 4.1 mg/g (Table 4). Tannins in flour were 6.4 mg/g and reduced to a range of 0.89 mg/g to 1.31 mg/g upon extrusion. The significant reduction of phytic acid and tannins has been widely reported by other studies (Anounye, Onuh, Egwin, & Adeyemo, 2010; Alonso, Augirre, et al., 2000; Batista, Prudencio, & Fernandes, 2010; Simon et al., 2015). In general, it is believed that during extrusion phytic acid is dephosphorylated by thermal or mechanical processing into smaller isomers with less affinity for mineral cations (A. S. Sandberg, 2002).

As with the element bioaccessibility and dietary fiber data, the extrusion parameters did not significantly influence the concentration of these components, except for the quadratic effect of moisture on tannin concentration (Supplementary Table 2). When data from extruded samples were averaged, phytic acid decreased by 2.3 mg/g, or 56%, upon extrusion (Fig 2B). This result was similar to other studies showed a reduction of 30% on extruded fava bean flour (Alonso et al., 2000), and a reduction of 20% on extruded hard-to-cook common bean flour (Batista et al., 2010). Although a recent study found no significant differences in phytic acid after extrusion of navy, black and pinto bean flours (Simons et al., 2015). Nwabueze (2007) indicated phytic acid was hydrolyzed mainly by mechanical shear exerted during extrusion cooking, while others have shown more effective reduction of phytic acid as barrel temperature increased (Batista et al., 2010). Thus the effect of extrusion parameters to the reduction of phytic acid is complex and specific to the material being extruded.

Tannins were reduced by an average of 5.3 mg/g, or 86%, after extrusion (Fig. 3.2B). A significant reduction in tannins upon extrusion was also reported by Alonso et al (2000), who showed a 70% reduction upon extrusion of kidney bean flour. Another study conducted on extruded soybean flour reported a reduction in tannins to a trace amount after extrusion (Anounye et al, 2010).

3.5.5. Correlation among *in vitro* element bioaccessibility and chemical components

Although the effects of extrusion parameters on element bioaccessibility remains unclear, previous studies have agreed that element bioaccessibility is related to the degree of phytic acid degradation and dietary fiber composition (Drago et al., 2007; Ummandi et al., 1995). Therefore, the variation in bioaccessibility among extrudates (Table 3.3) may be related to the changes in phytic acid concentration, tannin concentration, or dietary fiber partitioning, which were all largely independent of the extrusion parameters in this study.

Correlations between bioaccessible element concentrations and chemical composition indicated that four elements (Fe, Mg, K, and Cd) were correlated to the chemical composition by extrusion (Fig. 3.3), with two additional correlations for Fe and Pb that were nearly significant ($p<0.1$). Fe bioaccessibility showed a significant negative correlation with phytic acid concentration (Fig. 3.3), which was in agreement with a study showing a negative correlation of phytic acid to Fe bioaccessibility of green and black gram (Hemalatha et al., 2007). The strong association between Fe and phytic acid is well known (A.S. Sandberg & Svanberg, 1991).

Mg bioaccessibility was negatively associated with tannin concentration (Fig. 3). The same trend was apparent for Fe bioaccessibility ($p=0.056$). Tannins have been found

to bind to divalent elements in the gastrointestinal tract and reduce bioaccessibility (Alonso et al., 2001).

Soluble dietary fiber was significantly negatively correlated with bioaccessible Cd (Fig. 3.3) and nearly significant for bioaccessible Pb, (p=0.069). In addition, our study also indicated a significant correlation of soluble xyloglucans (r=-0.72, -0.52) and soluble pectic substances (r=-0.74, -0.54) with both Cd and Pb respectively. The exact inhibition mechanism of hemicellulose (xyloglucans) and pectic substances to the bioaccessibility of elements is still unclear, however several studies have indicated there are physical retention and chemical binding of free hydroxyl group with divalent cations (Behall, Scolfield, Lee,Powell, & Moser, 1987; Claye, Idouraine, & Weber, 1998). Due to its anionic nature, pectin can bind with divalent cations (Khon, 1987). Due to an increasing concern for heavy metals absorption recently, this result indicated a desirable effect of extrusion on element bioaccessibility, since extrusion was associated with significant increases in soluble dietary fiber.

The correlation can be also suggested that the effect of extrusion on dietary fiber component was due a significant degradation of lignin. Lignin represents the intercellular substances found on the cell wall of plant and it has two binding site to form an insoluble complex with divalent cations (Lestienne, Caporiccio, Besancon, Rochette, & Tréche, 2005). The inhibitory mechanism of element bioavailability by dietary fiber is through the formation of large complexes from carboxyl group of uronic acid or hydroxyl group from lignin. The binding site for this complex formation is depend on the element and pH of the matrix. Most of the fiber fractions have a high affinity to divalent elements (Lee & Garcia-Lopez, 1985).

The involvement of dietary fibers, tannins, and phytic acid in binding metals and inhibition bioaccessibility has been observed (Helmalatha et al., 2007; Lestienne et al., 2005; Porres et al., 2003). Nevertheless, the correlation analysis observed in our study suggested that the interactions of each element with these components is unique, with Fe mainly affected phytic acid, Mg affected by tannins, and heavy metals affected by soluble dietary fiber.

Insoluble dietary fiber (mainly cellulose) was positively correlated to K (Fig. 3.3A & D). The positive trend between K and insoluble dietary fiber was unexpected (Fig. 3.3). The element K in our study was the only monovalent cation, which would be expected to behave differently from the divalent cations. Additionally, the positive correlation between IDF and K may be related to other covariates not taken into consideration in the correlation analysis.

3.6. Conclusion

The extrusion parameters of moisture content, screw speed, and temperature had significant effects on the physical properties of extruded pinto bean flour, producing extrudates with differing physical properties, element bioaccessibility, and chemical composition. However, element bioaccessibility and chemical composition (phytic acid and dietary fiber) were not, although significant differences were noted among extrudates. Extrusion significantly reduced the phytic acid and tannins while increasing SDF and decreasing IDF. Extrusion significantly increased all essential elements (Fe, Mg, K, and P) without a comparable increased in bioaccessible of toxic elements (Cd and Pb). The correlation analysis suggested that each metal has unique binding mechanism to anti-nutritional factors, Fe mainly affected by phytic acid, Mg affected by tannins, and heavy

metals mainly affected by soluble dietary fibers. The non-significant effect of extrusion parameters to element bioaccessibility as well as significant correlation of bioaccessible elements with chemical contents suggested that element bioaccessibility is more influenced by the reduction of anti-nutritional factors rather than severity of extrusion processing.

3.7. References

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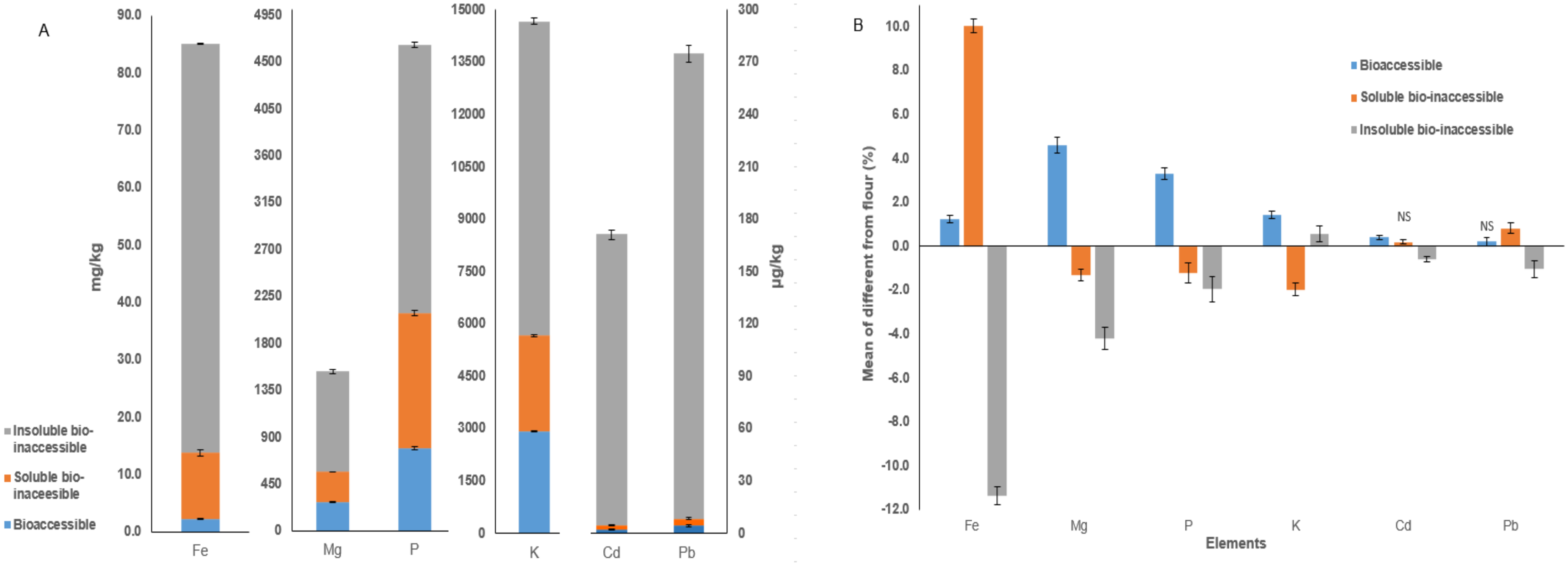

Figure 3.1. Concentration of selected elements in pinto bean extrudates (A) and change in element bioaccessibility fractions upon extrusion compared with flour (B); all bars in (B) are significantly different from flour except those marked not significant (NS; error bars represent standard error (n=17).

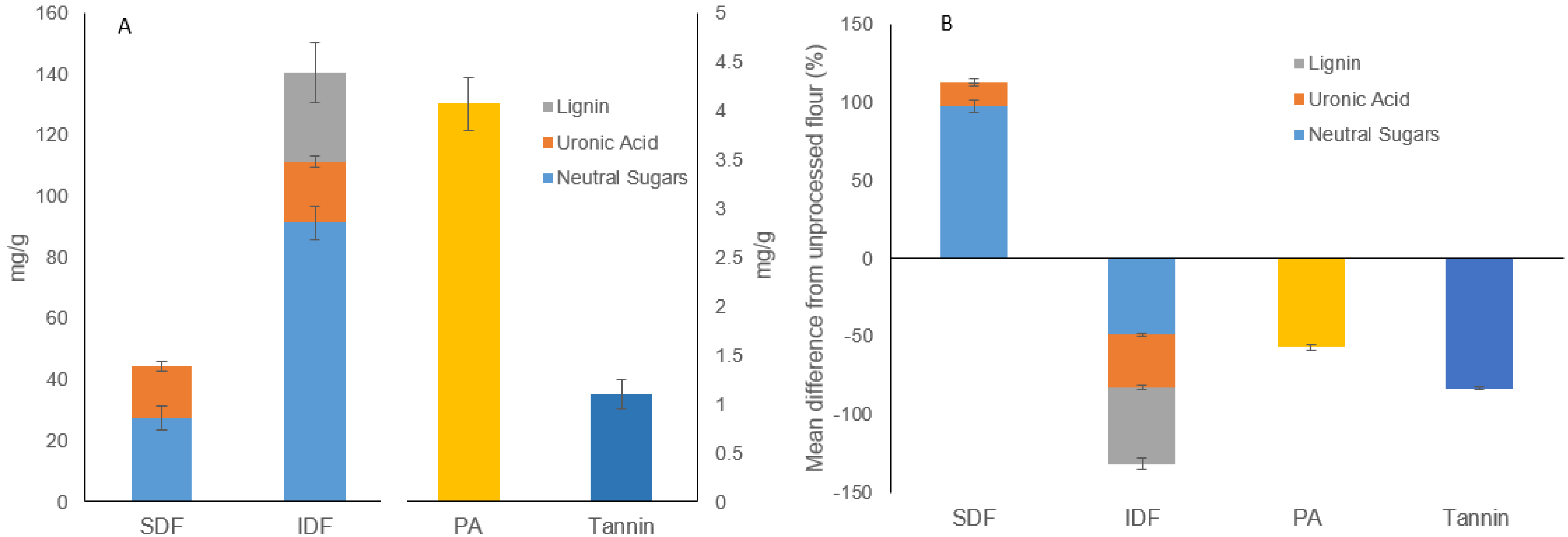

Figure 3.2. Concentration of chemical composition in pinto bean extrudates (A) and change in chemical compositions upon extrusion compared with flour (B);error bars represent standard error (n=17); PA, Phytic Acid; SDF, Soluble dietary fibers; IDF, Insoluble dietary fibers

A

Chemical properties	Fe	Mg	P	K	Cd	Pb
PA	-0.51	0.10	0.38	-0.12	0.06	-0.16
SDF	0.33	0.23	0.07	0.14	-0.68	-0.45
IDF	-0.03	-0.07	0.05	0.64	-0.27	0.25
Tannin	-0.47	-0.62	-0.15	-0.19	0.01	0.01
UA -Soluble	-0.24	-0.14	0.26	0.46	-0.41	0.18
NS -Soluble	0.20	0.38	0.08	-0.10	-0.63	-0.47
NS-Insoluble	-0.14	-0.41	0.00	0.38	0.21	0.32
UA-Insoluble	-0.29	0.36	-0.13	0.49	-0.06	0.14
Lignin	-0.02	-0.20	0.42	0.47	-0.42	0.10
XG (soluble)	0.22	0.39	0.23	-0.02	-0.72	-0.51
Pectin (soluble)	0.15	0.33	0.39	0.14	-0.74	-0.58
Cellulose	-0.30	0.22	-0.11	0.52	0.17	0.33
Non Cellulose (insoluble)	-0.29	0.36	-0.13	0.46	0.21	0.32

Elements

r	p
-1	<0.05
	<0.1
0	>0.1
1	<0.05
	<0.1

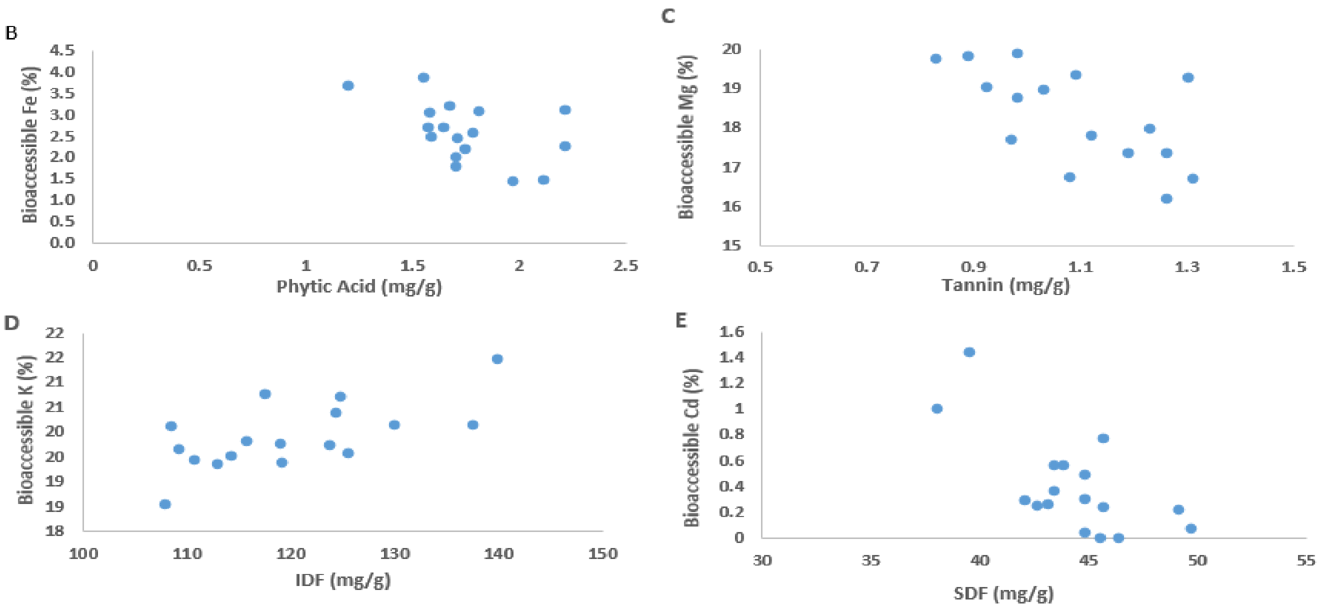

Figure 3.3. Correlations between element bioaccessibility and chemical composition of extruded pinto bean flour; SDF, soluble dietary fiber; IDF, insoluble dietary fiber;

Table 3.1. Design of experiments of coded and actual levels of extruded pinto bean

No of experiments	Samples Codes	Coded levels			Actual levels		
		X_1	X_2	X_1	X_1 (°C)	X_2 (rpm)	X_1 (%)
1	R1	-1	-0.7	0.7	90	179.8	23.1
2	R2	-1	-0.7	-0.7	90	179.8	18.9
3	R3	-1	0.7	0.7	90	225.3	23.1
4	R4	-1	0.7	-0.7	90	225.3	18.9
5	R5	0	1.43	0	115	248.9	21
6	CP	0	0	0	115	220	21
7	CP	0	0	0	115	220	21
8	R6	0	0	-1.43	115	220	16.71
9	CP	0	0	0	115	220	21
10	R7	0	-1.43	0	115	156.1	21
11	CP	0	0	0	115	220	21
12	CP	0	0	0	115	220	21
13	R8	0	0	1.43	115	220	25.29
14	R9	1	-0.7	-0.7	140	179.7	18.9
15	R10	1	0.7	0.7	140	225.3	23.1
16	R11	1	0.7	-0.7	140	225.3	18.9
17	R12	1	-0.7	0.7	140	179.7	23.1

X_1 is coded for barrel temperature, X_2 is coded for screw speed, X_3 is coded for moisture content. Code ‘0’ is for the central point value, ‘±1’ is for the factorial points, ‘±1.43’ is for α points, and ‘±0.7’ is for β points; CP, Centre point.

Table 3.2. Regression coefficient of the response surface model for physical responses

Parameters	Physical responses			
	UD	RER	WAI	WSI
Intercept	-0.25	4.81	2.01	58.6
temp	0.0005*[a]	-0.008*	0.005*	-0.03
SS	-0.0002*	0.0006	-0.003*	0.07*
MC	0.02*	-0.07*	0.12*	-1.87*
temp*temp	0.00003*	-0.0001*	-0.00034*	0.0008
SS*temp	2.16E-6	-3.6E-4	8.53E-6	-0.0002
SS*SS	4.19E-7	-5.26E-6	-8.77E-5*	0.0008*
MC*temp	-2.28E-5	0.00078*	0.0001	0.04
MC*SS	-1.13E-5	-0.0002	0.001*	0.003*
MC*MC	0.001*	-0.008*	-0.009*	-0.06
Adjusted R^2	0.98	0.98	0.95	0.97
F-value	124.5*	144.4*	35.5*	59.3*
MRE (%)	-4.3,4.4	-1.1,1.0	-1.2,2.1	-2.2,3.1

[a]= significant at $P<0.05$; UD = unit density; RER = radial expansion ratio; WAI = water solubility index; WSI = Water Absorption index;

SS= screw speed (rpm); temp= temperature (°C); and MC =moisture content (%)

Table 3.3. *In vitro* elements bioaccessibility of selected elements at different extrusion processing conditions

Sample codes	Bioaccessible (%)						Soluble bio-inaccessible (%)						Insoluble bio-inaccessible (%)					
	Fe	Mg	P	K	Cd	Pb	Fe	Mg	P	K	Cd	Pb	Fe	Mg	P	K	Cd	Pb
R1	1.49	17.8	15.9	19.8	2.09	3.00	14.2	18.7	25.6	17.3	1.70	3.19	84.3	63.5	58.5	62.8	96.2	93.8
R2	3.22	19.9	17.3	19.5	0.91	2.10	13.6	20.8	28.0	18.4	1.03	2.2	83.1	59.3	54.7	62.1	98.1	95.7
R3	2.21	19.0	16.1	20.1	1.42	1.36	15.3	20.6	27.5	18.4	1.42	1.83	82.5	60.4	56.3	61.5	97.2	96.8
R4	3.06	19.7	17.9	20.8	0.88	1.39	15.0	20.1	26.8	18.6	1.49	2.79	80.0	60.1	55.3	60.6	97.6	95.8
R5	3.67	17.7	17.0	19.3	1.14	1.36	13.7	16.7	22.5	15.9	1.23	1.26	82.7	65.6	60.5	64.8	97.6	97.4
R6	2.49	19.3	17.5	19.7	0.89	1.18	10.2	18.4	28.6	18.8	1.01	1.94	87.3	62.3	53.9	61.5	98.1	96.9
R7	2.45	17.4	15.5	19.4	0.73	1.09	13.5	18.7	27.7	18.9	1.44	0.93	84.0	64.0	56.8	61.7	97.8	98.0
R8	1.45	14.3	16.2	18.5	0.94	0.77	15.9	18.6	28.6	18.4	1.35	0.4	82.7	67.1	55.2	63.1	97.7	98.8
R9	3.85	17.4	16.6	19.7	1.65	1.84	15.0	17.9	26.8	18.5	1.36	1.94	81.1	64.7	56.6	61.8	97.0	96.2
R10	2.02	16.2	16.4	19.6	0.65	0.54	13.1	19.5	29.2	19.2	2.03	0.57	85.4	64.3	54.4	61.2	97.3	98.9
R11	3.06	18.0	17.8	20.1	0.65	1.20	12.1	20.8	31.9	20.9	1.20	2.06	84.8	61.2	50.3	59.0	98.1	96.7
R12	1.78	16.7	17.0	21.5	1.22	2.58	13.2	19.9	29.7	21.0	1.92	3.86	85.1	63.4	53.3	57.6	96.9	93.6
CP	2.71	18.8	17.2	20.7	0.92	1.87	13.2	18.5	28.0	18.4	1.47	1.17	84.1	62.8	54.7	60.9	97.6	97.0
CP	2.71	16.8	16.2	20.1	1.22	3.00	14.6	18.3	27.3	18.3	1.05	1.47	82.7	65.0	56.5	61.6	97.7	95.5
CP	2.59	19.8	16.4	20.4	0.95	0.96	13.0	18.3	28.6	18.1	1.55	1.15	84.4	61.8	55.0	61.4	97.5	97.9
CP	3.10	19.0	17.0	19.4	0.70	0.90	14.2	18.7	28.0	19.7	0.93	0.84	82.7	62.3	53.0	60.8	98.4	98.3
CP	2.25	19.3	16.6	19.8	1.02	0.75	13.5	18.4	27.9	19.0	0.68	0.31	84.3	62.2	52.4	61.3	98.3	98.9
Flour	1.29	13.2	13.7	18.4	0.67	1.29	3.10	20.4	29.1	20.8	1.14	0.78	95.6	67.4	57.2	60.8	98.2	97.9
Standard error [a]	0.43	1.62	0.58	0.55	0.15	1.34	0.67	0.16	0.46	0.92	0.51	0.62	1.23	1.26	2.32	0.47	0.59	1.87
LSD	1.19	4.50	1.62	1.99	0.73	NS[b]	2.63	0.65	1.81	2.55	NS	1.72	3.39	4.95	6.45	1.33	1.64	5.10

Each value represents the mean of two replications; [a] Standard error used a pooled estimated of error variance from CP (center point, N=5); LSD, least significant difference, the value of mean difference greater than LSD value is consider significant ($p=0.05$). [b] not significantly different ($p<0.05$)

Table 3.4. Chemical properties of at different extrusion processing conditions

Sample codes	SDF		IDF			TDF	PA	Tannins
	Chemical properties (mg/g) dry basis							
	NS	UA	NS	UA	Lignin			
R1	23.9	15.6	83.7	12.5	19.5	155.2	2.11	1.12
R2	28.0	14.6	77.8	11.4	25.2	157.0	1.67	0.98
R3	28.4	17.3	77.1	11.6	19.7	154.1	1.75	0.93
R4	29.8	19.3	74.7	12.4	30.4	166.6	1.81	0.83
R5	27.5	17.3	78.3	12.4	22.2	157.7	1.20	0.97
R6	29.1	16.6	77.9	13.0	32.7	169.3	1.59	1.30
R7	32.7	16.9	80.7	14.0	24.5	168.8	1.71	1.19
R8	24.9	17.2	71.5	13.7	22.9	150.2	1.97	1.23
R9	23.7	14.3	70.3	14.3	24.6	147.2	1.55	1.26
R10	27.7	17.8	71.9	13.2	40.4	171.0	1.70	1.26
R11	28.4	18.0	78.0	15.4	44.3	184.1	1.58	1.23
R12	24.2	19.2	82.8	15.4	41.7	183.3	1.70	1.31
CP	27.3	17.5	84.3	13.9	26.1	169.1	1.78	0.89
CP	26.2	16.9	79.4	14.4	30.8	167.7	1.65	0.98
CP	26.7	17.1	76.2	14.1	39.7	173.8	1.57	1.08
CP	27.6	17.2	73.2	11.0	26.4	155.4	2.22	1.03
CP	27.4	16.0	80.4	13.6	25.0	162.4	2.22	1.09
Flour	14.7	13.8	91.4	20.1	56.9	196.9	4.07	6.44
Standard error [a]	0.56	0.55	4.23	1.37	6.07	10.1	0.31	0.08
LSD	2.21	2.17	16.7	5.41	23.7	14.2	1.22	0.32

Each value represents the mean of two replications; [a] Standard error used a pooled estimated of error variance from CP (center point, N=5); LSD, least significant difference, the value of mean difference greater than LSD value is consider significant (p=0.05); PA, Phytic Acid; SDF, Soluble dietary fibers; IDF, Insoluble dietary fibers; UA, Uronic acid; NS, Neutral sugars

Table 3.5. Regression coefficient of response surface model for *in vitro* element bioaccessibility (%)

Parameters	Mineral bioaccessibility (%)					
	Fe	Mg	P	K	Cd	Pb
Intercept	26.8	-62.7	-24.8	-60.2	-18.9	-44.0
temp	-0.002	0.08	-0.27	-0.07	0.08	-0.05
SS	-0.22	0.35	0.13	0.44	0.06	0.21
MC	0.042	4.60	-0.53	3.68	0.99	2.66
temp*temp	2.E-04	-3E-04	6E-04	0.001	2E-04	2E-04
SS*temp	-1E-04	-2E-04	-1E-05	-0.001	-1E-04	5E-05
SS*SS	2E-04	-6E-04	-1E-03	-4E-04	-5E-05	-2E-04
MC*temp	-8E-04	-4E-05	5E-03	3E-03	-0.01	-1E-03
MC*SS	0.006	-0.003	-3E-03	-9E-03	-1E-03	-0.01
MC*MC	-0.04	-0.10	0.009	-0.05	-2E-03	-0.03
Adjusted R^2	NS	NS	NS	NS	NS	NS

Parameters	Soluble bio-inaccessibility (%)					
	Fe	Mg	P	K	Cd	Pb
Intercept	24.1	48.5	-12.5	-33.6	5.46	-35.5
temp	0.16	-0.67	-0.31	-0.19	-0.16	-0.35
SS	-0.12	0.16	0.70	0.51	0.01	0.34
MC	-0.79	-0.69	-1.23	1.09	0.23	2.24
temp*temp	4E-04	0.002	6E-04	7E-04	6E-04	0.0017*
SS*temp	-1E-03	9E-05	4E-04	-3E-04	-6E-05	-4E-04
SS*SS	-9E-05	-4E-03	-1E-03	-8E-04	9E-05	-3.E-05
MC*temp	-2E-03	5E-03	5E-03	5E-03	1E-03	1E-03
MC*SS	0.01	5E-04	-4E-03	-7E-03	-2E-03	-0.01*
MC*MC	-0.04	-0.002	0.03	-0.0068	2E-03	0.01
Adjusted R^2	NS	NS	NS	NS	NS	0.61

Parameters	Insoluble bio-inaccessibility (%)					
	Fe	Mg	P	K	Cd	Pb
Intercept	59.3	114	150	194	114	
temp	-0.11	0.59	0.32	0.27	0.08	
SS	0.22	-0.51	-1.07	-0.95*	-0.07	
MC	0.66	-3.90	-0.60	-4.78	-1.20	
temp*temp	-9E-04	-1E-03	-2E-04	-1E-03	-8E-04	-
SS*temp	1E-03	1E-04	-4E-04	9E-04	1E-04	
SS*SS	-2E-04	1E-03	2E-03	1E-03	-4E-05	
MC*temp	-9E-04	-5E-03	-1E-02	-8E-03	3E-03	
MC*SS	-1E-02	3E-03	7E-03	1E-02	3E-03	
MC*MC	7E-02	1E-01	1E-02	5E-02	-1E-04	
Adjusted R^2	NS	NS	NS	NS	NS	

[*] = significant at P<0.05; SS= screw speed (rpm); temp= temperature (°C); and MC =moisture content (%)

Table 3.6. Regression coefficient of the response surface model for chemicals analysis

Parameters	Chemical responses (mg/g)				
	PA	Tannins	SDF	IDF	TDF
Intercept	-20.6	6.12	16.2	-40.8	-24.6
temp	0.09	-6.9E-03	-0.09	-0.28	-0.37
SS	0.12	-5.1E-03	-0.05	0.34	0.29
MC	0.55	-0.42*[a]	-0.13	3.07	2.95
temp*temp	-4.1E-04	4.6E-05	3.4E-05	1.9E-05	5.3E-05
SS*temp	9.6E-05	5.7E-05	6.5E-06	6.1E-04	6.2E-04
SS*SS	-2.7E-04	1.4E-05	2.1E-04	-3.2E-04	-1.1E-04
MC*temp	-4.7E-04	-4.3E-04	3.7E-03	0.009	0.01
MC*SS	-0.001	-4.3E-04	-0.002	-0.01	-0.02
MC*MC	-0.006	1.0E-02*	-4.2E-04	-0.03	-0.03
Adjusted R^2	NS	0.79	NS	NS	NS

[a] = significant at $P<0.05$; PA = Phytic acids; SDF = soluble dietary fiber; IDF = Insoluble dietary fiber; TDF = total dietary fiber.

SS= screw speed (rpm); temp= temperature (°C); and MC =moisture content (%)

GENERAL CONCLUSIONS

The present thesis has reported that extrusion can be used as a promising technology to increase the utilization of dry edible beans especially in production of a nutritionally improved ready-to-eat snack products. The first objective was to incorporate pinto bean flour into an extrudate and to manipulate extrusion feed moisture in order to modify the physical characteristic and *in vitro* starch and protein digestibility of rice-bean extrudates. The second objective was to modify of extrusion parameters, specifically barrel temperature (^{o}C), screw speed (rpm), and moisture content (%), to alter both the physical and chemical properties of bean extrudates to change *in vitro* mineral element bioaccessibility.

For the first objective, results showed that the use of dry bean flour (pinto bean) in a brown rice and bean composite flour substantially improved the nutritional quality of the extrudates by not only increasing the protein and dietary fibers content, but also improving the *in vitro* protein digestibility and increasing resistant starch. Although some desirable physical properties including hardness, densities, and overall expansions were negatively affected, reducing the feed moisture within the extrusion barrel partially improved the negative effects of bean flour. Also, reduction of feed moisture increased digestible protein but decreased resistant starch.

For the second objective, it was demonstrated that extrusion parameters, barrel temperature, screw speed, and moisture content, had significant effects on the physical properties of bean extrudates. However, these parameters did not affect *in vitro* element bioaccessibility and chemical properties (phytic acid and dietary fibers component) except in few isolated cases. Nevertheless, extrusion processing significantly improved *in vitro*

bioaccessibility of Fe, Mg, K, P and Cd. In addition, it was also found that the improvements of essential element bioaccessibility (Fe, Mg, K, and P) were relatively higher than bioaccessible of "toxic" element (Cd and Pb). The change in bioaccessible element concentrations found in our study were correlated with chemical properties (phytic acid, tannins, and insoluble dietary fibers). Correlations suggested that each element was unique regarding the binding to different chemical components. Fe mainly associated with phytic acid, Mg associated with tannins, and toxic elements mainly associated with soluble dietary fiber.

The results from this study however, can be further improved by conducting an extension of conformation studies. The future direction for the first study can be focusing on the investigation of bean properties in affecting the expansion properties of rice-bean extrudates. Whereas, validation of correlation analysis (element binds differently to chemical component) by *in vivo* study from second study can be an important direction for future study.

APPENDICES

Appendix A. Selected SAS Code

A.1. Regression analysis for first study. SAS software (version 9.4, SAS Institute, Cary, NC) was used to determine the significant terms of each factor and their interactions up to cubic trends, which were defined as P<0.05, between feed moistures and bean compositions. Data were analyzed using a generalized mixed model analysis of variance (PROC GLIMMIX). Regression analysis was used.

Example ;UD=unit density; m= feed moisture; b= bean composition.

```
data UD;
input x m y;
m2=m*m; b2=b*b; mb=m*b; m2b=m*m*b;
mb2=m*b*b; m3=m*m*m; b4=b*b*b*b; m3b=m*m*m*b;mb3=m*b*b*b;
m2b2=m*m*b*b;
datalines;
.
.
.
;
proc glimmix data=UD;
model y=m|m|m|x|x|x/htype=1;
run;
proc glimmix data=UD;
model y=m m*m x m*x x*x x*x*x m*m*x/ htype=1;
run;
```

A.2.SAS was used to determine regression coefficient and statistical adequacy for the generated model

```
proc glimmix data=UD;
model y=m m*m x m*x x*x/solution;
run;
proc reg data=UD;
model y=m m*m x m*x x*x;
run;
```

Appendix B. Detailed Methodology

B.1. Total dietary fiber / carbohydrate assay [1]

1. Weigh up to 300 mg of sample to contain about 10-20 mg total dietary fiber and not more than 180 mg starch into a 15 ml screw cap test tube.
2. Add 3 ml of acetate buffer (0.1 M, pH 5, containing 5 mM $CaCl_2$) and 25 µl of thermostable α-amylase (Sigma A-9505 or Megazyme); cap; mix; heat to boiling for 1 h.
3. Cool; add 0.140 ml of amyloglucosidase (Sigma A-7095, 300 U/ml); incubate at 60 °C overnight.
4. Cool; add 12 ml absolute ethanol; mix; leave for 1 h in an ice bath.
5. Centrifuge at 800*g* for 10 min; discard supernatant; wash pellet by suspending and re-centrifuging with 80% ethanol (2 × 20 ml) and then with acetone (2 × 15 ml).
6. Insert glass rod in test tube and mix occasionally while pellet dries; allow pellet to dry completely overnight in the hood;
7. Add 0.3 ml of 12 M sulfuric acid; let stand at 30 °C for 1 h; turn water bath to 40 °C.
8. Add 1 ml of myo-inositol (amount of inositol added should approximate the average of the individual sugar contents, about 3 mg/ml) and 7.4 ml of water; add water in such a way as to wash the glass rod of any remaining residue and remove glass rod; make sure there is no residue clinging to the side of the test tube.
9. Pressure cook tubes on HIGH for 1 h.
10. Filter hot hydrolysate through a dry, tared, frittered glass crucible into a 25 ml volumetric flask thoroughly rinse the tube and crucible with water; make sure to transfer all of the solids to the glass crucible; this is the hydrolysate syrup (H).

11. Dry crucible in oven at 105 °C for 16 h (or overnight); re-weigh *(Wi)*; ash in furnace at 500 °C for 1 h (place crucible in cold furnace, heat to 500 °C at 10 °C/min, hold at 500 °C for 1 h, cool to 100 °C at 3 °C/min); remove from oven and cool completely in a dessicator before weighing *(Wf)*.

a. $KL = \frac{(W_i - W_f) \times 100\%}{S}$

b. All weights are in mg

12. Add 0.357 ml of hydrolysate syrup (H) to a 35 ml tube; add 71 µl of 12 M ammonium hydroxide (8 ml of 30% ammonium hydroxide diluted to 10 ml); mix; add 36 µl of freshly prepared 3 M ammonium hydroxide (2 ml of 30% ammonium hydroxide diluted to 10 ml) containing 150 mg/ml sodium borohydride; add 5 µl 2-Octanol; incubate at 40 °C for 1.5 h.
13. Add 36 µl of glacial acetic acid; mix; add 0.5 ml of 1-methylimidazole; mix; add 5 ml of acetic anhydride; mix; let stand 10 min at room temperature.
14. Add 1 ml absolute ethanol; let stand 10 min at room temperature.
15. Move tubes to a cooler with ice up to shoulder of tube.
16. *Slowly* add 5 ml of well mixed 7.5 M sodium hydroxide; mix; add another 5 ml of 7.5 M sodium hydroxide.
17. Transfer ethyl acetate (top) layer to a fresh tube; make sure not to transfer any aqueous phase to tube; if so, dry with anhydrous sodium sulfate and transfer to another tube.
18. Separate alditol acetates using the following conditions:
    a. Injection volume: 1 µl
    b. Inlet temperature: 240 °C

c. Carrier gas: He @ 2 ml/min

d. Split ratio: 1:20

e. Column: Elite 225,PerkinElmer N9316177, 30m×0.25mm×0.25µm

f. Temperature program: Isocratic @ 220 °C for 25 min

g. Detector temperature: 240 °C

19. Quantify alditol acetates relative to inositol peak using correction factors obtained from known standards (arabinose, xylose, mannose, glucose, galactose)

a. $CF_{MS} = \frac{A_{Std} \times W_{MS}}{A_{MS} \times W_{Std}}$

b. $MR = \frac{CF_{MS} \times A_{MS} \times W_{Std} \times F_m \times 100\%}{A_{Std} \times S}$

c. F_m= 0.88 for pentoses; 0.9 for hexoses; *S* = sample weight in mg

20. Add 125 µl of hydrolysate syrup (H) or 125 µl of galacturonic acid monohydrate (150 mg/ml; Std) to a 5 ml screw cap tube; add 125 µl of NaCl/boric acid (2 g NaCl and 3 g boric acid/100 ml); add 2 ml of concentrated sulfuric acid; mix *immediately*; cap; incubate at 70 °C for 40 min.

21. Cool; add 100 µl of freshly prepared 3,5-dimethylphenol (100 mg/100 ml in glacial acetic acid); mix several times over 5 min.

22. Read absorbance at 400 and 450 nm.

23. Calculate uronic acid content

a. $UA = \frac{A_H \times 150}{A_{Std}}$

b. $UR = \frac{UA \times F_u \times F_c \times 100\%}{S}$

c. F_u = 0.83 (monohydrate to residue); F_c = 0.81 (adjustment for absorbance of free uronic acid vs polyuronic acid)

24. TDF = UA + UR + KL

Notes: Total carbohydrate content (except Inulin): start from step 1(weigh 50mg samples), then add 0.3 ml of 12 M sulfuric acid, continue with step 8.

B.2. *In vitro* starch digestion

The *in vitro* digestion was followed by [2] with some modifications.

1. Whole grain flours and extrudates (250 mg) were dispersed in 2 mL of distilled water. Flour was boiled for 20 min and stirred at first 5 minutes. Extrudates was kept at 37 ºC water bath.

2. After cooling to room temperature. Four ml of 3.6% (w/v) pepsin (P-700, Sigma, St. Louis, MO USA) dissolved in 0.05M HCl was added and the mixture placed on an orbital shaker (150 rpm) at 37 ℃ for 30 min to achieve the gastric phase.

3. The small intestinal phase was initiated with the addition of 2 mL 0.5 M sodium acetate buffer (pH=5.2, containing 5 mM $CaCl_2$). Two mL of 15% (w/v) pancreatin (P-7545, Sigma) dissolved in sodium maleate buffer and 0.02 ml of amyloglucosidase (3260 U/ml; Magazyme) were then added and samples were incubated in shaking water bath at 37 °C.

4. Starch was digested for over 2 hours at 37ºC with horizontal shaking at 200 rpm, 50 μL of aliquots of slurry were removed from the tube after exactly 20 min and 120 min of digestion and mixed with 0.95 mL of absolute ethanol to stop the enzymatic reaction. The mixtures were then centrifuged at 5000g for 5 min. Glucose was quantified in the supernatant using the glucose oxidase-peroxidase method (Megazyme) and was converted to starch content by multiplying by a factor of 0.9.

B.3. *In vitro* protein digestion

1. The method was according to [2]. 200 mg of flour was weghted into erlemenyer flask

and mixed 5 mL of porcine pepsin (Sigma P-7000, activity: 890 U/mg) solution (1.5 g of pepsin/L in 0.1M KH_2PO_4, pH 2.0)

2. Sample was digested for 2 hours, by incubating at shaking water bath 37ºC

3. Digestion was stopped by addition of 2 mL of 2N NaOH

4. Samples were centrifuged (4,900 × g, 4°C) for 20 min, and the supernatants discarded. The residues were washed and centrifuged twice with 20 mL of buffer (0.1M KH2PO4, pH 7.0).

5. The collected pellets were dried overnight at 80ºC and analyzed for Nitrogen content (6.25 as nitrogen factor was used).

6. Digestibility was calculated as % digestible protein = (N in sample-undigested N)/N in sample x 100%

B.5.References

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Appendix C. Project Proposal

Improving the utilization of dry edible beans in ready-to-eat snack product by extrusion cooking.

Summary

The current trend of ready-to-eat snack production is marked by an increasing demand for healthy snacks. It has been reported that the growth of healthy snack production is 3.6% annually (IBIS market research, 2014). In order to meet the market demand, this study is aimed to create a healthy snack product through the incorporation of dry edible bean flour to improve the nutritional value of an extruded cereal snack. The study will investigate the effect of an extrusion parameters specially feed moisture, on the physical properties, *in vitro* digestibility and mineral bioavailability of the extruded rice-bean snack. This study will also determine the highest feasible bean flour substitution for extruded rice-bean snack without negatively affecting its desirability. The outcomes of this study are expected to promote utilization of dry edible beans in production of healthy ready-to-eat snack.

Justification and hypotheses

The growing demand for healthy snack food has been increasing over the past five years. According to IBIS world's market research (2014) North America's healthy snack production grew 3.6% from 2009 to 2014. However, most of the ready to eat snack production in the market is made from cereal grains, which are low in protein and dietary fibers and may negatively affect the nutritional value (Anton et al. 2009; Singh et al. 2007). With the addition of dry bean flour, a snack product made from composite flour substantially improve its nutritional quality. Dry bean flours are promising functional ingredients that will improve the nutritional quality as legumes are generally considered as a good source of protein, rich in dietary fiber, minerals and vitamins (Siddiq et al. 2010).

However, the utilization of dry bean flour has been limited due to the extended cooking time and the presence of anti-nutritional substances especially trypsin inhibitors and phytate (Borejszo and Khan 1992). Although dry bean flours are generally high in mineral content, the bioavailability however is relatively poor due to the present of these anti-nutritional substances (Thompson 1993).

One of the most versatile technologies for the production of healthy snacks is extrusion cooking which combines high temperature and high shear to transform raw ingredients into a ready-to-eat snack product (Anton and Luciano 2007; Linko et al. 1983). Extrusion cooking also offers several advantages over others processing methods for cereal-legume snack by shorten the cooking time and by reducing trypsin inhibitors and phytate (Alonso et al. 2001; BalandrÁN-Quintana et al. 1998). Therefore, we hypothesize that extrusion cooking can be used as a promising processing technology to improve the mineral bioavailability of cereal-legume snack product.

Several studies have demonstrated that substitution of cereal flour with high fiber and protein, for instance bean flour can negatively affect the texture, expansion, and overall acceptability of extruded product (Anton et al. 2009; Pastor-Cavada et al. 2011). The interaction between water and ingredient, mainly starch in extrusion cooking play an essential role in controlling the physical characteristic of final product (Stojceska et al. 2009). Therefore we further hypothesize that modification of feed moisture in extrusion cooking can be used to improve the overall acceptability of cereal-bean snack.

Objectives

The overall objective of this study is to use extrusion to improve the utilization of dry edible bean flour for production of a healthy snack. The type of cereal chosen for this experiment was brown rice flour as it is high in starch and one of the most common ingredients used in extrusion cooking. Pinto bean flour was used for this study because of its availability in Nebraska. The specific objectives consists of two parts. The First objective is to determine the highest level of bean flour substitution without significantly affecting the physical desirability of the extruded rice-bean snack under different moisture content. The second objective is to determine the relationship between extrusion parameter mainly feed moisture with mineral bioavailability on the rice-bean snack.

Literature review

Dry-edible bean (*Phaseoulus vulgaris* L) is one of the most important crops in North America with Nebraska being one of the largest producer in United States (Nebraska Bean Commission, 2015). A diet high in beans has been associated with many health benefits including prevention of various metabolic diseases for instances type II diabetes, coronary heart disease and colon cancer. Besides from being high in dietary fiber, dry bean

also contain polyphenolic compounds that may act as antioxidants to prevent the formation of free radicals (Siddiq et al. 2010; Tharanathan and Mahadevamma 2003). Additionally, brown rice flour is considered a relatively good base ingredient for extrusion because it has a high starch content, bland taste, and is hypoallergenic. The combination of rice and bean flour is also known for its complementary effect on the quality of the protein (Supat Chaiyakul 2009).

Extrusion cooking has been continuously gaining in popularity of ready to eat snack production since it was developed in the 1940's owing to the versatility, productivity and economical value (Harper 1989). Extrusion cooking is a combination of several unit operations including mixing, heating, conveying, drying and puffing into a single process (P.Colonna 1989). The intense conditions inside the extrusion barrel cause the starch to undergo a complex molecular transformation into an expanded ready-to-eat snack product (Castle et al 2005, Sigh 2007). The basic structure of the extruded snack is established by transforming and manipulating the natural biopolymers, mostly starch into a puffed product (P.Colonna 1989).

There are many extrusion parameters that may significantly affect the characteristics of final product. Feed moisture inside an extrusion barrel is one of the most important aspects in manipulating the physical desirability of the extruded snack (Thymi et al. 2005). Moisture content acts through modification of thermal and flow behavior. The starch polymer melt under high temperature and pressure, and the rheology of this molten material is highly depend on water content. When the viscosity of this molten material decreases inside the barrel (caused by increase in moisture content), the pressure drop through the die will also decrease and hence generating less expanded products. However,

when the moisture content inside barrel is too low, the molten viscosity may substantially increase causing a significant mechanical damage to starch and hence disrupt the expansion index (P.Colonna 1989).

Nutritional aspects of extrudate snack is one of the major advantages of extrusion cooking over others processing technology (Singh et al. 2007). The benefits of extrusion cooking including increase *in vitro* protein digestibility by denaturation of anti-nutritional substances (Arêas 1992), increase in the digestibility of starch by gelatinization process under lower moisture content (Qu and Wang 1994), and increase in mineral bioavailability by inhibition of anti-nutritional factor (Alonso et al. 2001).

Extrusion cooking normally does not affect composition of smaller molecules including minerals. It generally affects macromolecules which in turns alter the presence of other components. Fiber components, such as cellulose, lignin and some hemicellulose can slow gastrointestinal movement, which reduces mineral absorption. Extrusion cooking can change the characteristics of dietary component by reorganizing its chelating properties and hence improving the mineral bioavailability (Alonso et al. 2001).

A study by (Ummadi et al. 1995) compared the dialyzable iron in boiled legumes with extrusion cooking and the study concluded that low shear extrusion cooking had higher dialyzable iron than the sample cooked with conventional boiling process.

Experimental design and procedures

The experimental design for this experiment was a completely randomized design (CRD) with 2 factors (feed moisture and composite flours) organized into full factorial design. The feed moistures had 5 levels whereas composite flours had 4 levels; each experimental unit was a single combination of moisture and composite flours.

The brown rice flour was obtained from Sage Foods, California. Pinto bean (variety Poncho) was obtained from Stateline Bean, Nebraska. Whole seeds were ground in pilot plant scale hammer milled with 1 mm screen size and were sieved to pass a 1 mm opening (US standard mesh no 20). The composite flours were prepared at three different levels; 15%, 30%, and 45%.the control was prepared with solely brown rice flour. Salt was added to the flours at 1% (by total weight after addition of bean flour). The composite flours were stored at 4°C until extruded.

- Extrusion process

The composite flours were extruded using a pilot plant scale twin screw extruder (TX-57, Wenger). The barrel temperature was established into three different temperature zones; 80/90/110 °C. The barrel screw speed was set at a constant speed of 300 rpm and the diameter of die opening was 4.08 mm. the flours were fed from into the extrusion barrel with constant rate at 1.3 kg/min. Deionized water was injected into the screw zone at different rates, resulting with five different final moisture contents; 17.2%, 18.1%, 18.3%, 19.5% and 20.1%. Extrudates were dried in the oven at 103°C for 10 minutes and stored for further analyses.

- Chemical analysis

Total starch, crude fat, total protein, ash, and total dietary fibers content were performed on composites flour. Moisture content was measured by air oven drying method (AACC 44-19 method). Total starch was determined by Megazyme Amyloglucosidase method (AACC 76-21 method). Crude protein was determined by combustion method (AACC 46-30 method). Crude fat was measured by Soxtec extraction with hexane as

extraction solvent. Ash content was measured according to AACC method 32-23. Finally, Dietary fiber was measured according Uppsala method (AACC 32-25 method).

- Physical analysis.

Three expansion characteristics were measured including radial expansion, axial expansion, and overall expansion. Radial expansion was calculated by the ratio of cross-sectional diameter to the diameter of die opening (4.08 mm). The axial expansion was calculated as the ratio of overall expansion to radial expansion, suggested by (Ali et al. 1996). The overall expansion was determined as the ratio of true solid density and bulk density.

The density (g/cm^3) characteristics (bulk, apparent and true solid density) were measured according to method by (Őzer et al. 2004). Bulk density was determined by rapeseed displacement method. Apparent density was determined using a multi-pycnometer model MVP-6DC. The canister was filled completely with extrudates (6-8 g), and the true volume of extrudates was calculated by nitrogen air that penetrated inside the pores. True solid density was determined by measuring the volume of completely filled canister with ground extrudates (US mesh no 70) after tapping 10 times. Specific volume (cm^3/ piece) was measured by rapeseed displacement method to determine the volume of single extrudate.

Water solubility index (WAI) and water solubility index (WSI) of extrudates were measured according to method by (Anderson et al. 1970). Color was analyzed by Hunter calorimeter and was expressed as L* (whiteness), a* (redness+/greenness-) and b* (yellowness+/ blueness-). The texture of extrudates was characterized in terms of hardness (N) and jaggedness (Nsec). The analysis was performed using texture analyzer TA XT plus

with Kramer shear cell 5 blades as the probe. The samples were filled to height of 4 cm the canister, and were cut with blades at 2 mm/s. The hardness is characterized by maximum force (N) whereas the parameter for jaggedness is linier distance (Gumul et al. 2011).

- *In vitro* digestion

In vitro digestion (starch and protein) was performed on extrudates. In vitro starch digestion was determined according to method by (Mkandawire et al. 2013). The fraction of digested starch after 20 minutes of incubation was expressed as rapidly digestible starch (RDS), after 120 minutes was total digestible starch (TDS), and the digested starch fraction between 20 and 120 minutes was defined as slowly digestible starch (SDS). Resistance starch (RS) was defined as the remaining fraction of undigested starch after 120 minutes of digestion. *In vitro* protein digestion by pepsin was determined according to (Aboubacar et al. 2001).

Results

- Properties of the composites flours

Table 1
Physicochemical compositions (on a dry basis) of composites flours

sample	% starch (db)	% protein (db)	% fat(db)	% ash	% fiber (db)	
					soluble	insoluble
0% bean	88.3^{a}	9.25^{a}	1.46^{a}	2.34^{a}	0.90^{a}	5.91^{a}
15% bean	76.7^{ab}	9.96^{b}	2.14^{b}	2.83^{b}	1.08^{a}	7.13^{b}
30% bean	72.4^{b}	12.4^{c}	2.82^{c}	3.37^{c}	2.22^{b}	8.77^{bc}
45% bean	68.6^{b}	13.7^{d}	3.02^{c}	3.59^{d}	3.24^{c}	14.7^{c}

*means with the same superscript letters within same column are not significantly different ($p>0.05$), the number of replications were three (n=3) for each analysis.

The physicochemical compositions of composites flours have indicated a similar patterns. All of the compositions except for the starch content have significantly increased

as higher bean flour was added. In general, bean flours have higher protein, fat, and dietary fiber content and significantly lower starch content compared to brown rice flour.

- Effect of addition of bean flour on the physical properties of rice-bean extrudates.

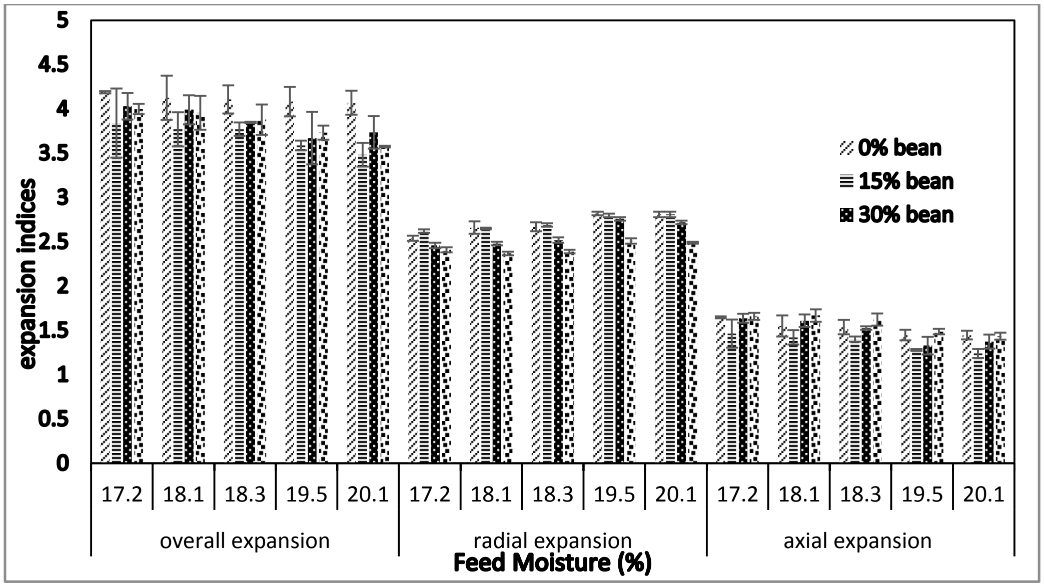

*Figure 1-Expansion Characteristics of extruded composite flours under different moisture contents.

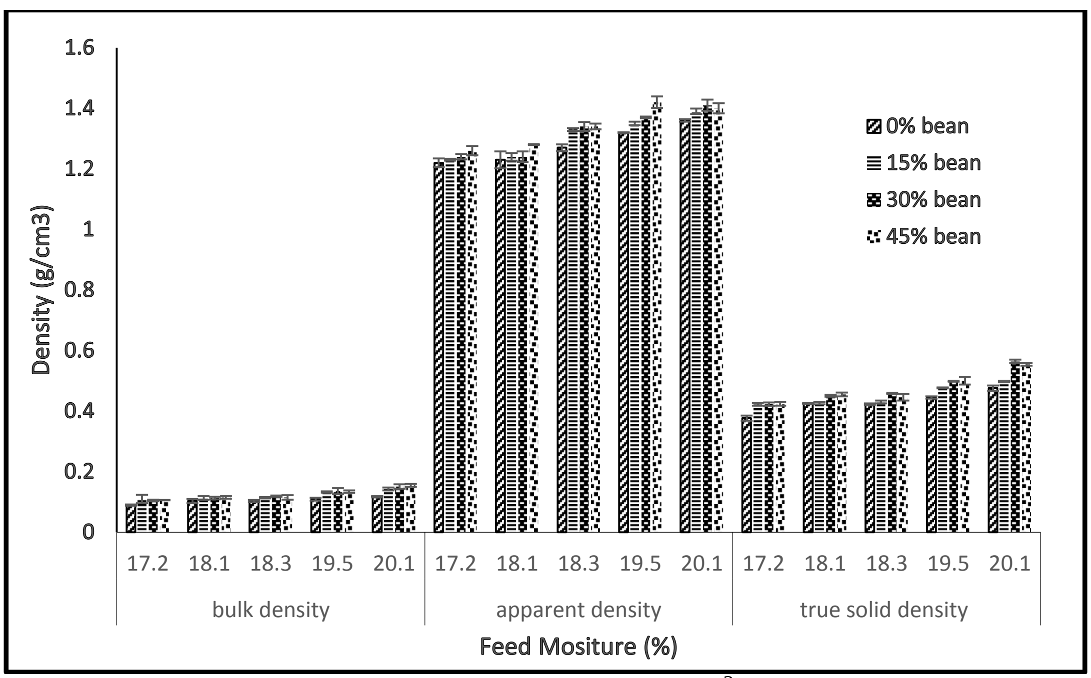

*Figure 2-Density Characteristics measured in gram/cm^3 of extruded composite flours.

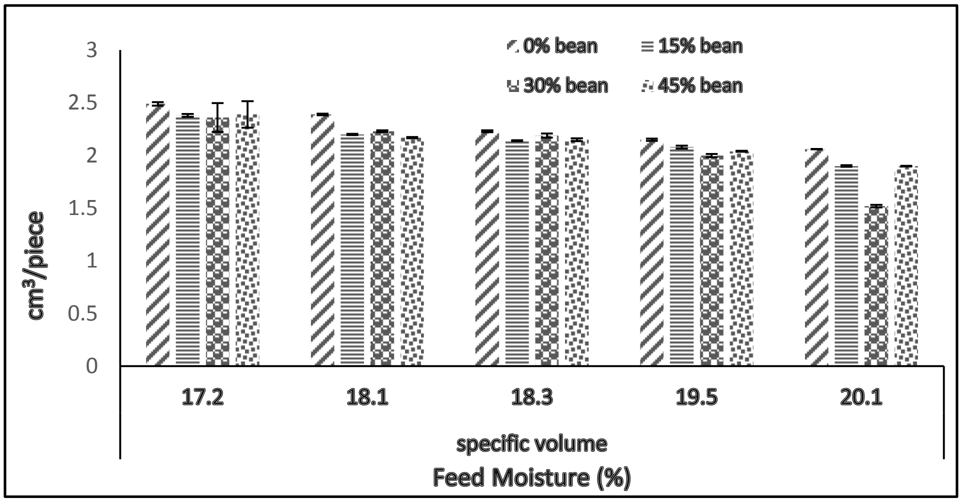

*Figure 3-Specific volume of extrudate per piece (cm3/piece).

Addition of bean flour negatively affected all of the expansion characteristics, as shown in figure 1 above. This may be due to a decrease in starch content and increase in fiber content (indicated at table 1) on composite flour. Insoluble fiber tends to disrupt the formation of air cell during extrusion (Pérez-Navarrete et al. 2006). Increase in feed moisture significantly decreased the overall expansion of extrudates. Radial expansion however is significantly higher for higher moisture content extrudates. The radial expansion indicated an inverse relationship with axial expansion, this is supported by others studies (Ali et al. 1996; Őzer et al. 2004). Expansion of extrudates is not only occur radially, but also at longitudinally and thus overall expansion is more appropriate to describe the expansion. Similar pattern was also seen on specific volume. The higher bean samples were not expanded well radially, instead they expanded axially giving it a comparable overall expansion to 0% bean sample. All density characteristics shown a similar pattern across the composite flours and feed moisture which are increase as more bean flour was added. This suggests that although the addition of bean flour may negatively impact the physical characteristics of extrudates, lowering the moisture content (17.2%) can significantly improve the physical desirability of the product.

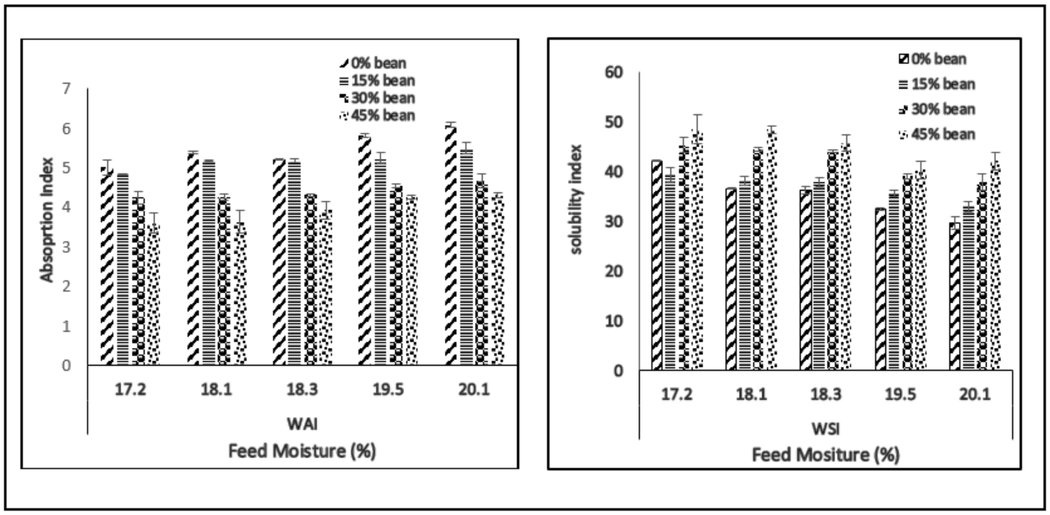

*Figure 4-Water Solubility index (WSI) and water absorption index (WAI) of extruded composite flours under different moisture contents.

Water solubility and absorption characteristic are inversely correlated and followed expected patterns. The solubility index significantly increased at more severe extrusion condition, in this case lower feed moisture (Anderson et al. 1970; P.Colonna 1989). Higher bean content significantly decreased the solubility which meant that the accessibility of moisture to the starch in the extrudate was restricted. This contributed to compactness of the extrudate (P.Colonna 1989). Water solubility is related to the amount of soluble molecules which affect the stickiness of the product. Water solubility significantly increased as more bean flour was added, however it decreased as feed moisture was lowered.

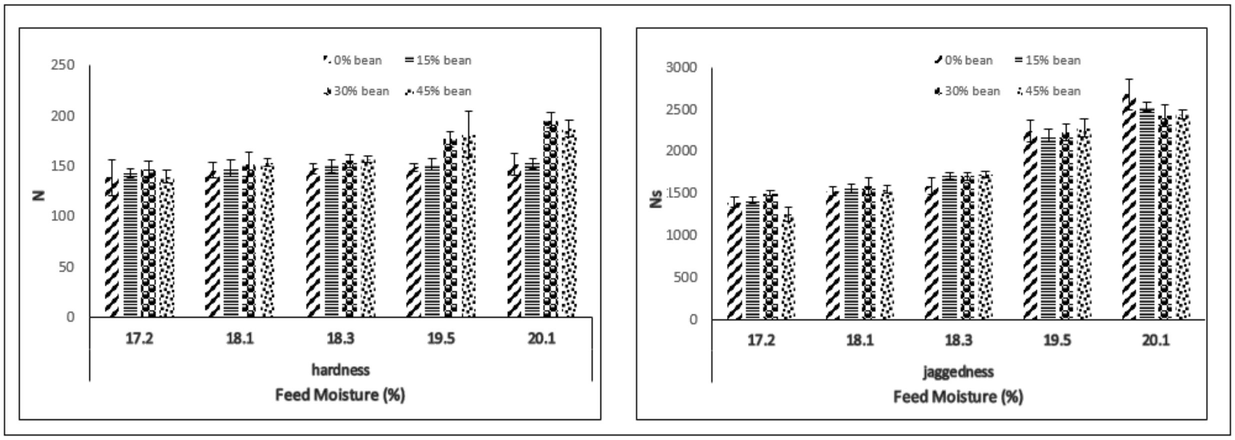

*Figure 5-Texture Characteristics of extruded composite flours under different feed moistures

Texture analysis indicated a similar pattern to density characteristic. This is reasonable, because denser products would also increase the hardness. Also addition of fiber tend to rupture the formation of air cells which eventually results in cell wall thickening (Gumul et al. 2011). Jaggedness measured the sustainability of product hardness(Duizer et al. 1998). Addition of bean flour was seen to increase jaggedness which meant the hardness of the product is not easily weakened.

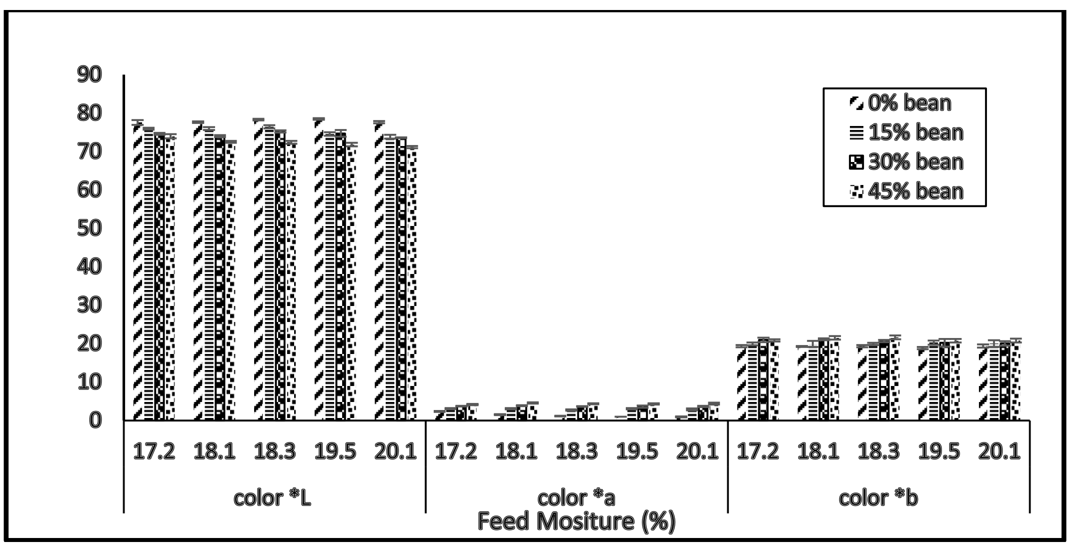

*Figure 6- color Analysis of extruded composite flours under different moisture contents. *L represents brightness, *a represents greenness -/ redness+, *b represents blueness-/ yellowness+.

The brightness of the product (represented by *L value) significantly decreased with the addition of bean flour, while both redness and yellowness also indicated a similar pattern. This was probably due to higher protein in bean flour which may participate in maillard reaction during extrusion cooking.

- **Effect of addition of bean flour on the *in vitro* digestion of rice-bean extrudates.**

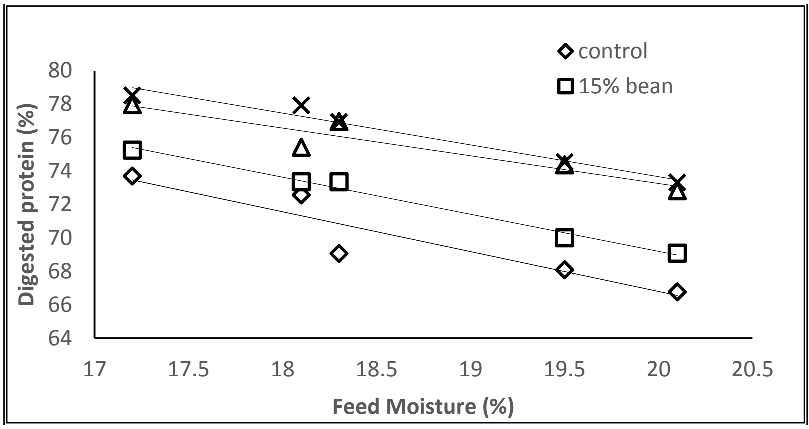

*Figure 7- *in vitro* protein digestibility of extruded composite flours under different moisture conditions expressed in percentage dry basis.

Protein digestibility significantly increased as feed moisture and bean composition decreased. The reduction in feed moisture resulted in more severe conditions which resulted in more deactivation of inhibitory enzymes, also denatured protein more efficiently and therefore facilitated enzymes hydrolysis (Bhattacharya et al. 1988).

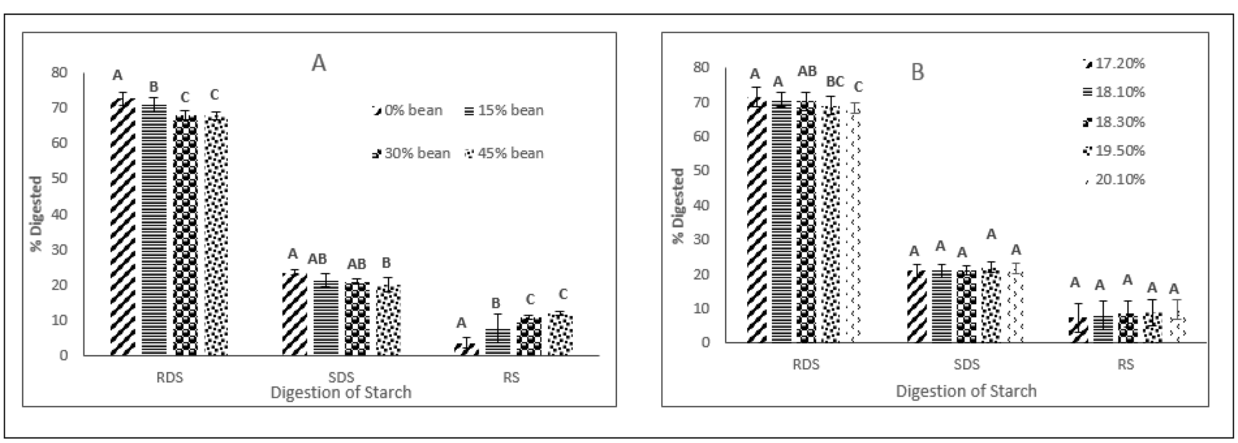

*Figure 8- *in vitro* starch digestibility of extruded composite flours under different moisture content. RDS- readily digestible starch, SDS-slowly digestible starch, RS-

Resistance starch. A; Main effects of starch digestion by bean composition. B; main effects of starch digestion by moisture contents. Same letter within category are not significantly different ($p>0.05$).

Addition of bean flour significantly decreased readily digestible starch (RDS), this is likely due to increase in higher fiber content on the bean flour. Slowly digestible starch only significantly decreased at higher level of bean flour substitution (45%). Interestingly, resistance starch significantly increased as the bean composition increased. The effect of feed moisture however, was only significantly pronounced on RDS.

Future work

The future work for this study will be evaluating *in vitro* mineral bioaccessibility of the rice-bean extruded snack. The study will investigate both the effect of bean flour addition and as well as the effect extrusion on *in vitro* mineral bioaccessibility of the extruded snack. The analysis will be performed on the same extruded samples. The method will utilize dialysis tubes to mimic the *in vitro* absorption of mineral.

Expected Outcomes

According to the preliminary results presented above, this study is expected to have several outcomes. Substitution of bean flour is feasible at 30% level. Although some physical desirability namely density, radial expansion, and hardness may be negatively affected by the substitution, extruding the composite flours under lower feed moisture (17-18%) can significantly improve its desirability. Improvement in nutritional value of extruded rice-bean snack is also expected by significantly increasing the *in vitro* digestibility of both starch and protein. Lastly, *in vitro* mineral bioavailability is expected to significantly increase in the extruded rice-bean snack. Thus, the proposed study will improve the utilization of bean flour to create a healthy ready to eat snack product

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