EFFECT OF EXTRUSION TEMPERATURE AND MOISTURE ON PHYSICAL, FUNCTIONAL AND NUTRITIONAL PROPERTIES OF KABULI CHICKPEA, SORGHUM, MAIZE AND THEIR BLENDS

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ABSTRACT

The overall goal of this research was to investigate extrusion and the effect of extrusion temperature (120 and 150^{o}C) and moisture (20 and 24%) on the physical, functional and nutritional properties of raw and/or extruded Kabuli chickpea, sorghum and maize flours in the first study, as well as their blends (chickpea-cereal) in the second study. The extrudates were analyzed for physical properties—expansion ratio, bulk density and hardness, and were then milled into flours for functional properties including water hydration capacity, oil holding capacity, foaming capacity and stability, emulsion activity and stability, and pasting property; *in vitro* protein digestibility and *in vitro* protein digestibility corrected amino acid score were analyzed for nutritional properties, and were compared to the WHO/FAO requirement for protein quality to determine the feasibility of the blends to be used as a food aid product or other potential applications. Nitrogen solubility and thermal properties were analyzed for some samples to demonstrate the effect of extrusion on protein solubility and starch gelatinization respectively.

The effect of extrusion temperature and moisture on different properties varied among samples. Generally, higher temperature and lower moisture content resulted in greater expansion, less hardness and bulk density. Extrusion reduced protein solubility and gelatinized/melted all detectable starch, which affected the functional and nutritional properties of the flours. Extrusion significantly increased (2-3 times) water hydration capacity, whereas decreased pasting viscosities (8-40 times) due to shear and gelatinization of starch. Oil holding capacity slightly decreased for the blends but remained relatively unchanged at the same level for the individual flours. None of the extruded samples showed foaming activity. Emulsion properties varied for the individual flours but showed a general decrease for the blends. Extrusion did not improve protein quality (*in vitro* protein digestibility corrected amino acid score) of the blends by much due to the loss of limiting amino acid lysine. Only chickpea-maize blend reached the 70% requirement by WHO to be used as food aid for the moderately malnourished. The great hydration property of the extrudates indicates the potential use as instant cold/hot beverage or porridge.

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LIST OF SYMBOLS AND ABBREVIATIONS

AA	Amino acid(s)
AACC	American Association of Cereal Chemists
AAS	Amino acid score
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BCAAs	Branched-chain amino acids
BD	Bulk density
BV	Breakdown viscosity
CFIA	Canadian Food Inspection Agency
CIDA	Canadian International Development Agency
cP	Centipoise
CSB	Corn soy blend
d.b.	Dry basis
DSC	Differential scanning calorimetry
EA	Emulsion activity
EAA	Essential amino acid
ER	Expansion ratio
ES	Emulsion stability
FAO	Food and Agriculture Organization of the United Nations
FBFs	Fortified Blended Foods
FA	Foaming activity
FFP	Food for Peace program
FS	Foaming stability
FV	Final viscosity
GMO	Genetically modified organism
HD	Hardness

HPLC	High performance liquid chromatography
HTST	High temperature short time
IVPD	*In vitro* protein digestibility
IV-PDCAAS	*In vitro* protein digestibility corrected amino acid score
kDa	Kilodalton
L/D	Extruder length-to-diameter
N	Newton
NGO	Non-governmental organization
OHC	Oil holding capacity
PDCAAS	Protein digestibility corrected amino acid score
PER	Protein efficiency ratio
pH	Acidity in logarithmic scale
PV	Peak viscosity
RDI	Reasonably Daily Intake
RTD	Residence time distribution
RUF	Ready-to-use food
RVA	Rapid viscosity analyzer
SAA	Sulphur amino acid(s)
SFIDC	Saskatchewan Food Industry Development Centre Inc.
SME	Specific mechanical energy
SV	Setback viscosity
TPD	True protein digestibility
TV	Trough viscosity
UN	United Nations
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
WFP	World Food Programme
WHC	Water hydration capacity
WHO	World Health Organization

1. INTRODUCTION

1.1 Overview

The demand for food, especially for the people in the least-developed countries, is becoming urgent as the global population grow towards 9 billion by the middle of this century (Godfray et al., 2010). The Food and Agriculture Organization (FAO) of the United Nation (UN) estimated in 2013 that 843 million people (about a seventh of the global population) are chronically in hunger, and even more are suffering from nutrient deficiencies (FAO, 2013). Protein, specifically, is seemingly the most lacking macronutrient. About one billion people worldwide have inadequate protein intake (Ghosh et al., 2012), with 10% to 30% children in central Africa and South Asia are protein malnourished (Grover and Ee, 2009). To alleviate the existing competition for energy, land and water between livestock and human beings, alternative food choices with innovative, plant-based, protein-rich foods should be made possible.

Epidemiological studies over the years have shown consistently that consumption of whole grains, the fruit or seed of plants in the *Gramineae* family of grasses, such as wheat, rice, barley, corn, oats, millets can lower the risk of cardiovascular disease, diabetes (He et al., 2010), metabolic syndrome (Sahyoun et al., 2006) and certain types of cancer (Jacobs, 1998). Similar benefits are also observed in consumption of pulses which are dried seeds from legume family such as bean, pea, lentil, and chickpea (Rebello et al., 2014). Both cereals and pulses are important sources of starch, dietary fibers and protein in human diet and animal feed. However, compare to cereal, pulse consumption is only limited to certain region and culture (Alizadeh and da Silva, 2013). Traditionally, cereal products can be consumed along with pulses to obtain a synergistic effect on protein quality (Rebello et al., 2014). When pulses, high in lysine and low in Sulphur containing amino acid (cysteine and methionine), are consumed with cereals, which are higher in Cys and Met but lack lysine, a complete amino acid profile is achieved.

Extrusion is a continuous high-temperature-short-time (HTST) process, during which the material is pushed by a piston or a screw under pressure and shear through a die with a given shape (Brnčić et al., 2006). This homogeneous and consistent heating process allows efficient production

of high quality final products with minimum waste. Since the process involves a combination of pumping, mixing, kneading, heating and cutting all in one process, and can result in a preferred appearance and texture, extrusion technology has been widely used in the food industry in cereals, snacks, pet food, feed, confectionery products, modified starches, baby food, instant foods and more. Extrusion is also a good way of processing pulses because of its versatility and flexibility as well as the ability to reduce and inactivate bioactive factors that are naturally present in pulses, and reduce cooking time when incorporated into products.

As the need and interest in the use of pulses in food grows in many developed countries (Boye et al., 2010), more and more pulse-based foods are being introduced to the current market (Asif et al., 2013), and therefore additional studies in this area are required. Although there are some studies on the effects of extrusion conditions on the nutritional and functional values of pulses, few are found which compare those values of pulse-cereal mixture before and after extrusion. In this study, two mixtures (kabuli chickpea and maize, kabuli chickpea and sorghum) were extruded, and the nutritional value and functional properties of the raw and pre-cooked flours were examined under different extrusion conditions.

1.2 Hypotheses

The following hypotheses will be tested in this research:

- Blending pulse flour with a cereal flour will improve the nutrional value of the mixture compared to a single type of flour.
- Extrusion conditions, specifically temperature and moisture, have interactive effect on physical properties (hardness, expansion ratio, bulk density) of extrudates.
- Extrusion process will improve the nutrition of flours by increasing their bioavailablity as a result of the cooking effect.
- Extrusion process will improve functionalities of flours due to the cooking effect.

1.3 Objectives

The following objectives will be included in this research:

- To study the nutrional properties of chickpea, sorghum, maize flours and their blends.
- To study the effect of extrusion conditions on physical properties of chickpea, sorghum, maize extrudates and their blends.

- To study the effect of extrusion conditions on nutritional properties of chickpea, sorghum, maize flours and their blends.
- To study the effect of extrusion conditions on functional properties of chickpea, sorghum, maize flours and their blends.

2. LITERATURE REVIEW

2.1 Food security and food assistance products

In 2050, the United Nation's Food and Agriculture Organization estimates the global population to reach 9 billion people (Godfray et al., 2010). As a result, supplying the world's population with high quality nutritious protein sources will represent significant challenges to overcome to ensure a secure food supply. There have been several proposals put forth to tackle some of these challenges, including increasing the agricultural land mass; increasing food production limits through genetic modification; reducing food waste; improving food-chain infrastructure and storage technologies in developing countries (Nellemann, 2009); and changing diets (Steinfeld et al., 2006).

Canada and other G-7 (+1) leaders pledged $20 billion to support a global effort to strengthen agriculture in developing countries at a 2009 Summit (Clinton, 2009). Canada has always been a strong partner to the United Nations (UN) in battling global food insecurity. Efforts have been made in the areas of research, sustainable agriculture development, and various food programs (David, 2012). At the G-8 Summit in L'Aquila in 2009, Canada contributed $600 million to the New Alliance for Food and Nutrition Security through the bilateral and multilateral programs of Canadian International Development Agency (CIDA), which elevated its total support to 1.18 billion, and became the first country to meet its commitment later in 2011 (David, 2012). Later in 2012, CIDA contributed another $219 million to the New Alliance, with focuses on bilateral food security programs in Ghana and Ethiopia, innovative nutrition research and technologies and food security programs (David, 2012).

Programs like the World Food Programme (WFP) of UN (WFP, 2017) and Title II or Food for Peace (FFP) program of the U.S. Agency for International Development (USAID) have been working to feed vulnerable populations in the underdeveloped countries since 1960s (USAID, 2017). In 2008, the U.S. committed $2.3 billion to the FFP, through which distributed 2.3 million metric tons of food to 50 million people in 49 countries (USAID, 2008). Title II commodities currently used as food assistance include Fortified Blended Foods (FBFs) such as corn-soy blend

(CSB), wheat-soy blend (WSB), pulses and legumes enriched cereal blends (e.g. soy fortified bulgur), and staple grains coupled with fortified vegetable oil, all of which are fortified with micronutrients (Webb, 2011). FBFs were first developed in the 1960s by the U.S. government to serve as nutrient-dense food supplement for preschool-aged children in developing countries (Senti, 1974). FBFs based on cereal are the most nutrient-dense products developed and distributed by the FFP program. They are typically used to supplement the overall diet of the most food insecure population such as infants, children, lactating and pregnant women, and HIV affected individuals (Fleige et al., 2010). Corn and wheat were the basic cereal components, and skim milk powder and soy flour were used as protein supplements. Corn soy milk, used for the domestic young children in the food program, was the original U.S. FBF that aimed to provide 25% of the energy requirement of young children and supplement vitamins and minerals with the exception of vitamin C. In the 1980s, the use of a corn-soy blend (CSB) largely replaced the use of corn-soy milk due to the shortage in skim milk powder. Today, the CSB is still the most widely used FBF product (Marchione, 2002; Webb, 2011). CSBs contain gelatinized (partially cooked) cornmeal prepared from de-hulled, degermed and shelled yellow corn, defatted (toasted) soy flour, soybean oil (refined, deodorized and stabilized) and supplement of minerals and vitamin antioxidant premix. The proportions of the ingredients are listed in Table 2.1 (USDA, 2008). CSB13 from 2008 and an upgrade, CSBP2, since 2014 are two of the CSBs used for exported programs by the USDA. CSBs are usually consumed as porridge or gruel by mixing an indicated proportion of flour and clean water followed by 10 to 15 min of boiling (USDA, 2014). Uncooked/partially cooked CSB or CWB are prone to spoilage, oxidation and segregation, and particle size variation resulted in poor mineral distribution, which decrease the nutritional value of the blend for the already malnourished. Bliss (2011) reported in a USDA document that Onwulata worked on the development of instant corn soy blend (ICSB) to enhance the nutritional value of the blends, which is cooked by extrusion, and can be stirred with potable drinking water (Bliss, 2011).

Table 2. 1 Proportion of ingredients in a corn-soy blend (CSB13) (USDA, 2008).

Ingredients	**% by weight**
Gelatinized cornmeal	69.6
Defatted and toasted soy flour	21.9
Refined soybean oil	5.5
Minerals	3.0
Vitamin antioxidant premix	0.1

CSBs has been used as a "one-size-fit-all" product for different age groups with significant variation in nutrition requirements, USAID commissioned a 2-year assessment of quality issues of Title II food aid products and gave recommendation as following: upgrade the macro- and micronutrient contents, increase protein quality (by adding whey protein concentrate), fat content, introduce new products that are nutritionally and culturally available in local area, introduce lipid-based ready-to-use food (RUF), encourage the development of new cereal-based FBFs with the use of more accessible crops in terms of location and price in Africa (e.g. sorghum, pea or other pulses), and more recommendations to improve food assistance products (Rosenberg et al., 2011).

Apart from being potentially capable of replacing CSB using other pulses and cereals, blending different crops may bring forth more applications than FBFs in food products due to the protein quality and functionality change, as well as the non-GMO and hypoallergenic property by avoiding corn and/or soy.

2.2 Pulses

Pulses, originated from the Latin "puls" which means thick soup or potage, and are the edible dry seeds within the legume family (*Fabaceae* or *Leguminosae*). The most common pulses are pea, lentils, chickpeas, faba bean and edible beans. Pulses are known for their nutritional value: a rising from their high protein, fibre and vitamin/mineral contents, and low levels of fat. Protein levels in pulses ranges from 18% to 30% (Chibbar et al., 2010). Pulse proteins are high in lysine

and contain all the essential amino acids at levels needed to support growth and development except for methionine (and cysteine) which are considered deficient. Although pulses do not have a complete set of essential amino acids, they are considered as an important source of protein in certain Asian, South American and African countries such as India, China, Myanmar, Brazil and Nigeria (Akibode and Maredia, 2012), and are also widely used in combination with cereal grains (Alizadeh and da Silva, 2013). Since the 1980s, Canada has gradually become the one of the leading producers to export pulses to 129 countries, mainly to Turkey (for lentils and chickpea), India and China (for dry peas), and the U.S. (for dry beans) (Statistics Canada, 2015).

Within the pulses, proteins are dominated by salt-soluble globulin proteins (60-80%) and water-soluble albumin proteins (15-25%) (Tiwari and Singh, 2012). For the globulin proteins, they can be divided into two types. Legumin, which is an 11 S (S, sedimentation coefficient) hexameric protein with a molecular mass of 300-400 kDa, with six subunits held together by non-covalent interactions. Each subunit (molecular mass of 60 kDa) is comprised of a large acidic α-chain (molecular mass of 40 kDa) and a small basic β-chain (molecular mass of 20 kDa) linked together by covalent bonds; vicilin, is a 7 S trimeric protein with a molecular mass of ~150 kDa, whose subunits lack covalent bonds, and are also held together by non-covalent interactions; a third globulin protein, known as convicilin, is in minor amounts with a molecular mass of ~270 kDa (Fouques et al., 1998). Pulses contain approximately 55-65% carbohydrates, mainly starch (FAO, 1994). They are high in soluble and insoluble fibre, with a total fibre content ranging from 8% to 27.5% and, soluble fibre from 3.3% to 13.8% (Guillon and Champ, 2002). There is also a significant amount of the B-vitamins and minerals such as calcium, folate, potassium and iron (Lebiedzińska and Szefer, 2006). Moreover, consumption of pulses has been shown to have beneficial effects on weight management, obesity, coronary heart disease and diabetes (Jenkins et al., 2012).

2.3 Cereals

Cereal grains are from the grass family (*Poaceae*) and are major dietary source of proteins around the world for both humans and animals. The most significant agricultural cereal species are wheat, triticale, rye, barley, oats, maize, rice, sorghum and millets by volume produced (Wrigley, 2010). Among all these species, cereal, maize and rice represent about 90% of the cereal grain production (Wrigley, 2010). The protein content in cereals ranges between 6-15% (Goldberg,

2008). Cereals are dominated by alcohol-soluble prolamin-type proteins, however still contain small amounts of albumin and globulin-type proteins (Giuberti et al., 2011). In contrast to pulses, cereals are deficient in lysine, but higher in the sulfur-containing amino acids, methionine and cysteine (De Lumen et al., 1986). Cereals contain 66-76% carbohydrates, which is by far the most abundant constituents (Koehler and Wieser, 2013). Vitamins and minerals are also condensed in the aleurone layer, pericarp and germ of cereals. Although cereals do not provide vitamin A, C and B_{12}, they are an important source of other micronutrients such as vitamin E and some of the B vitamins (McKevith, 2004).

2.4 Starches in pulses and cereals

As a major calorie source for human and animals, starch is the predominant carbon reserve in cereal and legumes. Starch is comprised of two polysaccharides: (a) amylose, which is a linear chain of α-(1→4)-linked D-glucopyranosyl units. Its degree of polymerization ranges from 500 to 6,000 glucose units, giving the molecular mass of 10^4 to 10^6 kDa (Buléon et al., 1998); and (b) amylopectin, which is a highly branched (every 20-30 glucose units) tree-like polysaccharide with both α-(1→4) and α-(1→6) linked D-glucopyranosyl units, giving a molecular mass of 10^7- 10^9 kDa (Chibbar et al., 2010). The unbranched outer chains are recognized as A-chain, whereas the inter chains as B-chain. A single C-chain contains reducing glucose residue and "terminates" the molecule (Peat et al., 1956). The partially crystalline structures in the native starch granules have birefringence and a 'maltese cross' under the polarization microscope. The degree of crystallinity of native starch is about 20 to 40% and is mainly contributed by the structural features of amylopectin (Hizukuri, 1996). The amylopectin branching sites are represented by amorphous regions, which sometimes contains a few amylose molecules. Amylopectin double helices can be packed into three different crystal types: A-type is found in most cereal starches and is densely packed, whereas the tube-like B-type is more hydrated and found in some tuber starches, high amylose cereal starches, retrograded starch and pulses (Hizukuri, 1996). C-type is a mixture of A and B in various proportions and is found in pulses (Hizukuri et al., 1983).

In pulses, starch accounts for 22-45% on a dry basis (Hoover et al, 2010). The amylose content ranges between 30-40%, which is ~5-10% more than cereals, and the amylopectin content 60-70% (Thorne et al., 1983). Their high amylose starch and high protein-starch interactions result in a low glycemic index after consumption, which is beneficial for human health (Jenkins et al.,

2012). In chickpea, the total amount of starch is ~29-35%, with ~27-34% and ~66-73% being amylose and amylopectin, respectively (Singh et al., 2004). However, pulse starches are not as widely used within the food industry as cereal starches due to the costly isolation process, high retrogradation rates and limited information on the exact structure of their amylose and amylopectin (Hoover et al., 2010). For example, the U.S., the largest producer of starch in the world, produces 98% of starch from maize and only 2% from wheat and potatoes; in the E.U. approximately 82% of its starch is derived from maize, followed by wheat and root crops such as potatoes and cassava (De Bragança and Fowler, 2004).

In cereals, the starch content ranges between 56 to 75% (Koehler and Wieser, 2013). Amylose accounts for 25-27% of the total starch, whereas amylopectin accounts for 72-75% (Colonna et al., 1992). In waxy-types almost 100% of the starch is amylopectin (Gunaratne and Corke, 2004). A portion of the starch is also considered to be resistant starch, which acts like soluble fiber but is not digested and absorbed in the gastrointestinal tract (Baghurst et al., 1996). Sorghum and other millets have high starch content upwards of 75% (Moorthy, 2004). Maize kernel consists 72 to 73% of starch (Boyer and Shannon, 1987).

The different ratio of amylose and amylopectin largely dictates the final product characteristics in food processing involving heat and moisture such as extrusion. When starch is mixed with excess water at room temperature, it can absorb upwards of 50% of its dry weight as water moves into void spaces between granules and as the granules swell. As temperature increases, gelatinization initiates as amorphous regions are hydrated, and crystallites melt due to dissociation of amylopectin double helices, which is irreversible and results in the loss of birefringence (Tester and Debon, 2000). A rapid visco analyzer can monitor the gelatinization process by recording the viscosity of starch suspension. During gelatinization, the less branched amylose chains leak out of starch granules to increase the viscosity of the starch suspension. With further heating, a starch paste is formed comprised of solubilized amylose and swollen granules. Retrogradation happens during cooling when amorphous regions re-associate to become more ordered and crystalline outside of the confines of the granule. Amylose is responsible for the initial retrogradation that occurs in minutes to hours upon cooling and contributes to initial gel structure and hardness, while amylopectin “trapped” in the starch granules that are embedded in the amylose gel mainly contributes to long-term gel structure as it happens in hours or days (Miles et al., 1985). A stable crystalline structure that cannot be melt again by heating will be formed as amylose

retrogradation proceeds. Whereas the amylopectin crystallites can melt at 60^{o}C. Also, the retrogradation of amylopectin is strongly influenced by pH and low-molecular-weight (LMW) compounds like salts, sugars and lipids (Eliasson and Gudmundsson, 1996).

Starch and protein can form gels through electrostatic interaction between the positively charged groups of protein and hydroxyl groups of starch (Jamilah et al., 2009), hydrogen bonding, van der Waals forces and entanglement (Morris, 1991). Three equilibrium status can occur in an aqueous solution of protein-starch mixture: (1) thermodynamic incompatibility, (2) miscibility and (3) complexation (De Kruif and Tuinier, 2001; Martínez et al., 2005; Martínez et al., 2007). When a mixed system including starch is heated, a competition between gelation and phase separation starts; the basic gel structure will be formed once the gelation occurs, and phase separation will be retarded (Owen and Jones, 1998).

2.5 Extrusion cooking

Extrusion is a high temperature short time process in which the feed material is cooked by combining multiple unit operations (e.g., mixing, cooking, forming/kneading and shearing) into one (Bordoloi et al., 2014). The extrusion process works by feeding the material (e.g., cereal or pulse flours) into a feeding unit (or hopper) that can feed directly into the extruder inlet or a pre-conditioner where both temperature and moisture adjustment occurs (based on your material and intended final product application). Preconditioners are assembled between the feeder and the extruder; they can precook and adjust moisture and the raw materials to improve product quality, reduce energy consumption and extruder wear (Fang et al., 2003). As the material is conveyed through the barrel of the extruder, where the material is heated, mixed, sheared and pushed forward to a die by either a single screw or twin screws under pressure. The die plate shapes the product into its final geometry as it leaves the extruder. After leaving the die, the material undergoes rapid expansion as pressure is released that leads to a honeycomb structure, which is shaped by bundles of molten protein fibers (Moscicki, 2011). Because of the high temperature short time processing, the nutritional loss of heat sensitive compositions such as protein, vitamins and enzymes in the extruded products is held to a minimum, however anti-nutritional factor can be reduced significantly during processing (Singh et al., 2007).

Screw extruders are typically divided into two major categories: single and twin screw. There are generally four types of single screw extruders based on their degree of shear, which

include: a) a cold forming extruder, which is usually used to form pasta and compact pastry dough; b) a high pressure forming extruder, which are used to produce pre-gelatinized flour and pellets; c) a low-shear cooking extruder, which usually is involved in pasteurization, enzyme inactivation, protein denaturation and starch gelatinization through external heating; and d) a collet extruder, often used to produce puffed snacks from corn grits (Riaz, 2000). Single-screw extruders typically have less mixing ability than twin-screw extruders (Connelly et al., 2007)

For twin screw extruders, there are four basic designs: counter-rotating and co-rotating twin-screw extruders, which could be either intermeshing or non-intermeshing as shown in Figure 2.1 (Guy, 2001). Between the non-intermeshing twin-screw extruder, the counter-rotating type is more effective at pumping materials than the co-rotating type, which is usually used to gently push a low viscosity non-cooked feed material (Guy, 2001). Intermeshing screws are better at pumping and mixing. However, this type of design generates more wear and tear on the screws and barrels, and extruders with an intermeshing design usually have very short barrels (Guy, 2001). The counter-rotating and intermeshing extruders are commonly used in the rubber industry and plastic processing. In a co-rotating twin-screw extruder, the screws can be distinguished into three individual elements (Fig. 2.2) (Teixeira et al., 2006): (1) right-handed elements that have conveying capacity, (2) kneading blocks that have staggered disks with various angles and induce barrel filling and pressure development, and (3) left-handed elements which induce intensive shear upon the material. The co-rotating, intermeshing and self-wiping twin extruders are the most used extruder-type as they have the most advanced control panels that are designed to protect extruder and operator from dangerous conditions. They can process the most varieties of food materials, from high fat, sugar, starch and protein, to food low in these, from a high viscous food to a very low viscosity material (Kazemzadeh, 2011). Therefore, this type of extruder was used in the current study to handle pulse ingredients like chickpea that are high in protein and lipid.

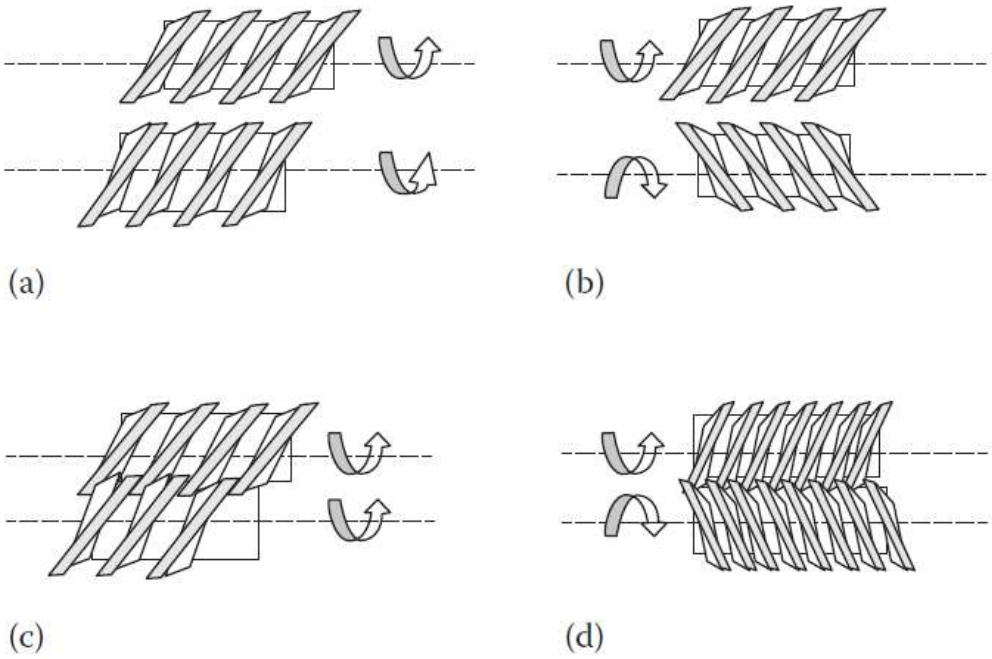

Figure 2.1 Screw design for twin-screw extruder: (a) Co-rotating and non-intermeshing, (b) counter-rotating and non-intermeshing, (c) co-rotating and intermeshing, and (d) counter-rotating and intermeshing (taken from Guy, 2001).

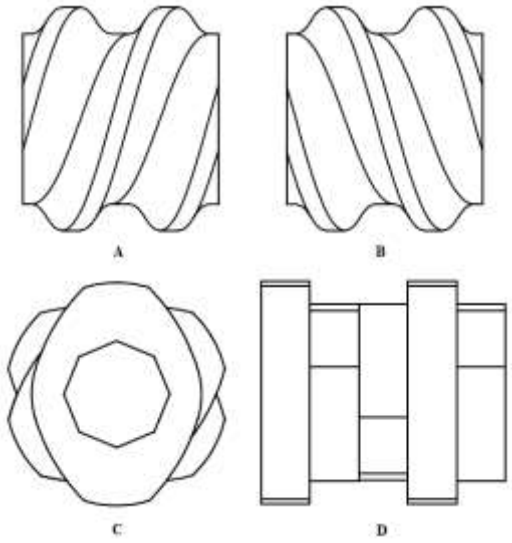

Figure 2.2 Typical screw elements: (A) right-handed element, (B) left-handed element, (C) kneading block front view, and (D) side view (Teixeira et al., 2006 with some modifications).

A typical single-screw extruder has three zones as shown in Figure 2.3: a feed conveying zone (Zone 1), compression zone (Zone 2) and metering zone (Zone 3) (Middleman, 1977). In a typical co-rotating, intermeshing twin-screw extruder, there are also three zones but located differently. The corresponding zones are: (1) one solid conveying zone with only right-handed element that functions the same as Zone 1, (2) two melting zones that work as Zone 2, and are signitured by the presence of left-handed element and kneading block (restrictive elements); they are each followed by a melt conveying zone, and (3) a metering zone that functions as Zone 3; although the pressure is significantly higher in the melting zones, but is zero in most conveying zones (Teixeira et al., 2006). The extruder barrel is composed of a jacketed head and screws. The jacket allows modification of temperature along the length of the barrel, which is typically heated by steam but sometimes hot water or oil. It can be also cooled with water or other cooling agents (e.g., glycol, air, and liquid nitrogen). The changes of feed material undergo in the corresponding zones of a twin-screw extruder are similar to those in a single-screw extruder, only that in twin-screw extruder the heat and shear effect on a sample is more intensive.

The feed matetrial in Zone 1 is usually low in denstiy due to trapped air within the materieal and its granular nature. Water is also injected in the feeding zone of the barrel to assist textural and viscosity development, as well as to enhance heat transfer (Planttner, 2007). The feed is then carried by the rotating screw into the Zone 2, where temperature rises and starts to melt the feed due to dissipatation of machanical energy (Godavarti et al., 1997) (Figure 2.3). In Zone 2, the melt starts to lose some of its granular integrity due to heat and shear (kneading) that contribute to starch gelatinization and/or melting, protein denaturization and other chemical reactions in the feed. Steam can be injected in the earlier part of the Zone 2 where pressure is not too high for the injection. The steam carries both thermal energy and moisture into the melt. As the melt moves forward this zone, a more integral flowing melt will form and eventually reach its maximum compaction. The shear in this middle section is usually moderate and extrudate temperature will continue to increase (Planttner, 2007). As the melt moves to the final Zone 3, temperature and pressure increase most rapidly and and shear rates are highest. As a result, a melted “fluid” will be expelled from the die capped at the end of the extruder. Die design can have effects on the final product quality and its functionality. The degree of barrel fill increases upon switching a die with one opening to one with multiple opening. Dies with higher shear rates can increase starch damage and denaturation of protein thus increased starch water solubility, decreased protein solubility and

other functional changes (Planttner, 2007). Temperature in the barrel can be controlled by altering the amount of steam injected, and residence time is determined by the speed of the shaft. In general, increase in shaft speed result in shorter residence time (Zhou, 2016). The single screw extruder is not typically used for transporting sticky, oily, or very wet materials feed material due to the slipping (Riaz, 2000).

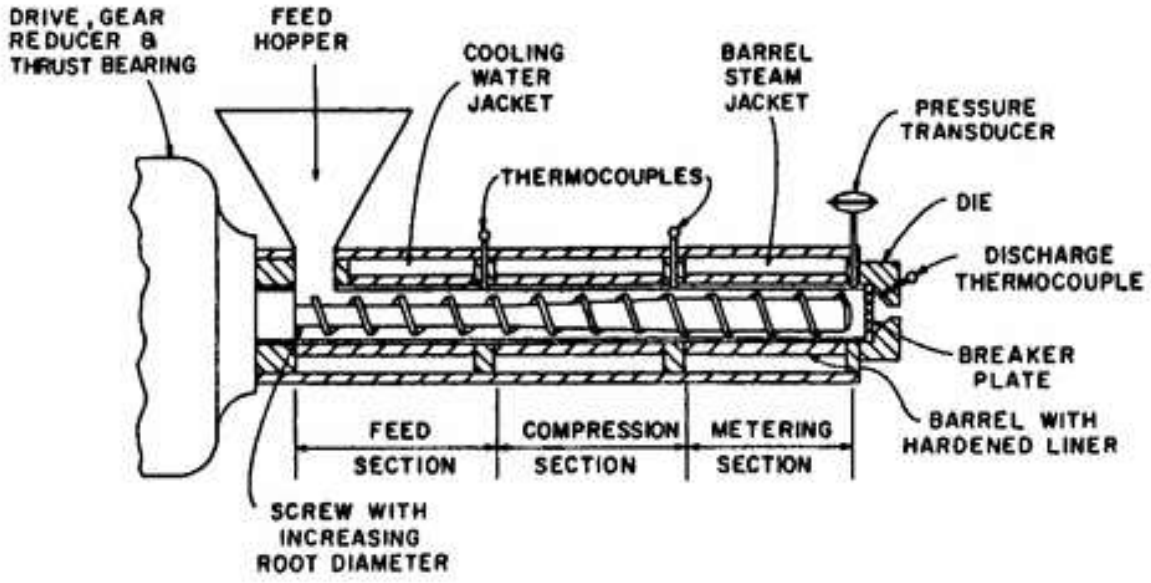

Figure 2.3 Cross-section of a typical single-screw food extruder (taken from Middleman, 1977).

Extrusion was first applied commercially in the late 1870's in England and early 1880's in the United States in the rubber industry. The first application of the single extruder in the food processing industry was for pasta making, starting in the 1920's and remains as a standard production process (Riaz et al., 2000). Corn snacks were the first extruded snacks in the mid to late 1940's. Later on the dry-expanded pet food market has grown into the largest commercial application of extrusion (Planttner, 2007). The total retail of extruded pet food sale in the U.S. skyrocketed from $3.62 (Huber, 2000) billion in 1998 to $5.3 billion by 2004 (Kvamme, 2005). To date, extrusion technology is not only widely used in the food and feed industries to produce breads, cereals, pet foods, aquatic feeds, pasta, snacks, starches and numerous other products, but also used by the pharmaceutical and nutraceutical industries for their products (Planttner, 2007).

2.6 Factors impacting extrusion

The feed material (e.g., source, moisture and particles size) and extrusion parameters (e.g., feed rate, barrel temparature, level of shearing, screw speed and screw profile) can greatly influence the physicochemical characteristics of the final extrudates or milled pre-cooked flours (Purwanti et al., 2010). In terms of the feed material, macomolecules play a very important role in the profile of extruded products.

(a) Starch: The ability of starch to gelatinize at temperatures above 60 to 85^{o}C in the presence of water can drastically change the viscosity of the melt and the expansive nature and structure of the extradate product (Okechukwu and Rao, 1996; Riaz et al., 2011). It was found that optimal expansion for corn starch containing 0 to 70% amylose could be obtained at 130 to 160^{o}C and 13 to 14% moisture (Chinnaswamy and Hanna, 1988a,b). When there is sufficient energy present during extrusion for intermolecular bondbreaking, gelatinization is greater at higher moisture content (Riaz et al., 2011). In general, increases in temperature, percentage of amylose (within 0-50%) (Chinnaswamy and Hanna, 1988b), friction, shear and decreases in moisture (which lead to increase in friction and shear) result in a greater amount of expansion of the starches; however, at a moisture content <20%, there is an increased production of dextrin due to inadequate hydration of starch, as well as wear in extruder screw and barrel (Riaz et al., 2011). Therefore, extrudates with higher starch contents require greater moisture content to obtain sufficient gelatinization and thus good expansion (Owusu-Ansah et al., 1984). This was supported by Rodriguez-Miranda et al. (2014) who confirmed that for bean flour extrudates, which has a high starch content, the greater moisture content led to the greater expansion. However, higher moisture levels (>~27%) tends cause a decrease in the melt viscosity to result in a denser extrudate.

(b) Protein: Protein generally has a negative effect on expansion due to its less viscoelastic nature compared to starch; therefore, increase in protein content tend to result in less expanded products and more rigid network (Chaiyakul et al., 2009). During extrusion, the heat and shear weakens or disrupts the tertiary and quaternary structure of protein molecules, resulting in the unfolding and aligning themselves with the melt flow (Harper, 1986). The exposed amino acid residues can react other food components. The hydrophobic amino acids (e.g. tryptophan and tyrosine) can associate with other hydrophobic residues of protein, starch and lipid, and influence the characteristics of extrudates (Zhou et al., 2016). Chaiyakul et al. (2009) reported that increasing protein content from 20 to 30% in a rice-based snack significantly increased hardness, crispness

but less sticky mouth feel. The effect of protein on extrusion also depends on other food components. For example, high moisture and fat content have protective effect agains protein denaturation by decreasing barrel temperature. Thus minimal denaturation of protein tend to occur in extrusion at high moisture (>25% w/w) content (Camire, 1991). Protein can also interact with lipid during extrusion mainly through electrostatic and hydrophobic interactions between the residues of these two macromolecules (Chapman, 1969). Nonpolar amino acid side chains and lipid side chains could associate by Van der Waals forces, which is strengthened by the hydrophobic interactions in the presence of water; hydroxyl groups of lipid can also form hydrogen bonds with carbonyl groups of protein (Izzo and Ho, 1989). In addition to these, the breakdown product of polyunsaturated fatty acid oxidation, malonaldehyde, has also been reported to interact with protein (Shin et al., 1972).

(c) Lipids: In general, lipid can work as a lubricant to reduce friction between the melt and screw/barrel, reduces mechanical energy input and starch gelatinization (Hu, 1994). Also it can lower shear stress to prevent breakdown of starch (Lin et al., 1997). However, small amount of lipid seems to be necessary strength development in extrudates (Bhattacharya and Hanna, 1988). It has been reported that due to high lipid and fiber content in oats, a highly expanded product is hard to achieve (Gordon et al., 1986). The lubricant effect of lipids was confirmed by Kumagai et al. (1987) where they observed a 50% increase in volume of the defatted (0.065% lipid content) dried rice flour extrudate compared to the untreated flour with 0.765% total lipids. For single screw extruder, it becomes increasingly difficult to have efficient mechanical energy transfer from screw into the melt as lipid content increases above 15%. However, by using a twin screw extruder, lipid level can be increased to ~25% while still maintaining high levels of mechanical energy input (Rokey and Plattner, n.d.). But such high level of lipids is not well received in a single screw extruder as it does not have a second screw to prevent slipping of the melt inside the extruder barrel. If lipid needs to be added during the extrusion process, it is critical to add it near the discharge of the preconditioner because the early addition will result in poorly hydrated and cooked starch from the lipid coating and interfere the heat transfer for gelatinization. Lipid-starch and lipid-protein complexes are likely to form under extrusion condition with low moisture (<20%) and high temperature (>150^{o}C) (Rokey et al., 2011).

(d) Fibre: The presence of fiber usually results in decreased expansion, because gas bubbles within the extrudates are ruptured by cell walls before they could fully expand (Jin et al.,

1994). Jones et al. (2000) investigated 36 branded ready-to-eat breakfast cereal and found that increasing fibre and protein content in feed decreased exapansion ratio. Reduced particle size of insoluble fibers like cellulose and corn bran only improves expansion to a limited extent (Blake, 2006). Therefore, it is common to see a compact, tough and not crisp high-fiber extruded product (Lue et al., 1991).

There are four critical parameters that can directly influence the final product characteristics: moisture, specific mechanical energy (SME, power over mass flow rate), thermal energy input (steam injection) and retention time (which can be controled by screw speed and feed rate). Consistent duplication of a product can be kept as long as all of these critical parameters are kept constant given the same raw material (Planttner, 2007).

(a) Feed rate: When feed rate increases, degree of fill and residence time for the feed is reduced, which means less degradation of the amylopectin networks (Fletcher et al., 1985), thus although the SME inputs may be unchanged, less expansion is uaually observed in the final product and piece density of the extrudate increase, not necessarily the bulk density (Planttner, 2005).

(b) Screw speed: Screw speed has a big influence in both single and twin screw extruders because it directly impact the SME, residence time for the feed and capacity of the extruder. Increased screw speed directly results in increasd SME due to more friction inside the extruder. Twin screw extrusion in general has a higher responsiveness to feed rate due to the advantage on feeding characteristics. A more precise product quality can be maintained by varying the screw speed (Planttner, 2007). When feed rate is fixed, increase in screw speed will decrease filling of the barrel, resulting in reduced mechanical energy and thus lower barrel and product temperature (Badrie and Mellowes., 1991).

(c) Barrel temperature: Barrel temperature is often easy to control. Most of the heat added to extrusion is controled via steam injection. The addition of steam can increase the capacity of the extruder and reduce the requirement for a large drive motors (Planttner, 2005). Cooling and heating the kneeding or melting section can ensure consitant product flow. Too hot or too cold (relative to the extrudates) at the head of the final section will cause material to stick to the inner barrel and interfere the viscous flow. In such case, the product will usually have a torn burnt apperance. Efficient heat transfer in general can be hard to obtain during extrusion because it requires a full barrel to accure. However, many extrusions only have the last section full of product, thus heat transfer is limitted by time availability. Also, extruder size plays a major role in heat

transfer. Many products experience failure or difficulties in scaling-up due to inefficient energy transfer as heat transfer increases only by the square but volume increases by the cubic (Planttner, 2007). Extruder barrel temperature has been reported as one of the most important parameters along with moisture to influence expansion and related characteristics of extrudates (Lawton et al., 1972; Holay and Harper, 1982). In general, increase in temperature would result in increased expansion ratio (Ding et al., 2006). Hagenimana et al. (2006) reported that expansion (1.61 to 3.94) increased with the increase in temperature (100-160^{o}C) when extruding rice flour. However, a plateau seems to exsit between 150 to 170^{o}C due to the starch degradation and air bubble rupture (Meng et al., 2010). Greater expansion means less hardness and bulk density (Yovchev et al., 2017).

(d) Moisture: Moisture can significantly affect the characteristics of extrudates. It can be alterted through preconditioning or water addtition. Badrie and Mellow (1991) found that cassava flour extruded at low moisture (~11%) had greater expansion of 2.7 compared to that at 16% moisture (1.8). Low moisture content typically favors expansion during extrusion (Faubion and Hoseney, 1982; Miller, 1985; Bhattacharya and Hanna, 1987). This is because foods with low moisture content is more viscous and thus experience greater pressure differential compared to those with higher moisture (Singh et al., 2007). Also, at low moisture content, melt flow will be restricted insided the barrel, which will increase shear and residence time, and in turn increase expansion due to greater starch gelatinization and/or melting (Chinnaswamy and Hanna, 1988a). However, since water is also critical in starch gelatinization, the effect of moisture content on expansion can sometimes seem conflicting (Miller, 1985), as water is required for starch gelatinization but too much water can act as a lubricant to reduce shear, pressure and temperature in the barrel.

(e) Specific mechanical energy (SME): The SME is dependent on other process variables such as the ones aforementioned, and the extrudate texture can be controled by chaning the SME input (Ryu and Ng, 2001). SME has been reported to decrease with the increase of temperature and moisture content due to reduced friction inside the barrel (Singh et al., 2007). Increase in expansion correlates with increase of SME (Ryu and Ng, 2001). The correlations of SME with density and texture has also been reported (Altan et al.,2008; Dogan and Karwe, 2003; Ilo et al., 1996). The effect of SME on starch gelatinization has also been reproted by reserchers (Gomez and Anuilera, 1983; Van Lengerich, 1990). Gropper et al. (2002) extruded a starch-protein mix

showed that the degree of gelatinization increased with the increase of SME (142-299 kJ/kg); starch granules became more swollen at SME 199 kJ/kg and seemed to achieve gelatinization completely at SME 299 kJ/kg. However, glass transition temperature (T_g) was reported to decrease with an increase in SME due to more fragmentation of starch during extrusion, which is more intensive at higher SME (Barrett and Kaletunc, 1998; Kaletunc and Breslauer, 1993; Davidson, 1984). Any increse in resistance to flow or anything disrupts coveying in the extruder will increase cook and SME. The impedance to flow will cause the barrel to fill up and thus increase retention time and heat transfer from the barrel heads. Shear locks, cut-flights or reverse flight screws can be added to increase the flow resistance (Planttner, 2007).

2.7 Effect of extrusion parameters on physical properties

Brnčić et al. (2006) studying the effect of twin-screw extrusion parameters on hardness of wheat extrudates found that hardness is influenced mainly by feed moisture, screw speed and temperature, while feed rate does not have significant impact. The authors reported that for the wheat extrudate, hardness increased with increased feed moisture content (18.3-24.5%), decreased screw speed (150-300 rpm) and barrel temperature (120-145^{o}C). They also found that feed moisture had the most significant effect on hardness (Brnčić et al., 2006). A similar conclusion was drawn by Liu et al. (2000) who extruded oat-corn puffs. The authors reported that hardness increased by increasing the oat concentration within the blend from 55 to 100%. Maxmimum hardness was found at high moisture (21%) and 85% oat flour. The authors hypothesized that the the higher moisture resulted in reduced expansion of the extrudate, leading to an increase in hardness in the final product. Köksel et al. (2004) studied the effects of extrusion variables on the properties of waxy hulless barley extrudates. The authors found that as the moisture content increased from 22.3 to 30.7%, the expansion index decreased, whereas the bulk density increase regardless of the shearing conditions and barrel temperature. The bulk density and expansion index provides a measures of puffiness, where an extrudate with a small bulk density and large sectional expansion index indicates a puffier product.

Köksel et al. (2004) reported a relationship between the shear rate and degree of gelatinization. The authors found that the maximum gelatinization for waxy hullless barley under high and low shear was 45% and 43%, respectively, which was lower than earlier studies that used corn as the feedstock. During gelatinization, a starch solution is heated to cause the starch granules

to swell, allowing the amlyose chains to leach out of the granular structure into the surrcounding solution (Lovegrove et al., 2017). Leaving the amplyeopectin chains to remain within the swollen granule (Morris, 1990). Köksel et al. (2004) found maximum gelatinization within barley flour to occur at the highest temperature (170 ^{o}C) tested and lowest moisture (22.3%) used, whereas gelatinization was minimum at the highest moisture (30.7%) used and lowest temperature (130 ^{o}C) tested. The authors noticed a positive relationship between the degree of gelatinization and expansion, which is negatively related to the moisture content. They hypothesized that the increased degree of gelatinization and expansion at low moisture might be attributed to the restricted flow within the barrel which increases the shear rate and residence time. The lower degree of gelatinization with higher moisture was hypothesized due to a reduction in friction between the dough and the barrel (Liu et al., 2000). Geetha et al. (2014) studied the effect of extrusion parameters on physicochemical and functional properties of kodo millet-chickpea blend (70:30) at temperature 80-150^{o}C, 20% moisture content, screw speed 250-300 rpm and feeder speed 15-30 rpm. They found that gelatinization index (or degree of gelatinization), which was essentially water absorption index, reached its maximum at highest temperature (150 ^{o}C). And expansion also increased with the increase in temperature.

The specific mechanical energy (SME) is the total work input from the driving motor into the raw material (dissipated as heat) in the extruder barrel (Harper, 1989), expressed per unit mass of the material (Godavarti and Karwe, 1997). SME directly affects final product quality (Godavarti and Karwe, 1997). De Mesa et al. (2009) found that SME is negatively correlated with bulk density of a corn starch-soy protein concentrate extrudate. And that a higher SME induces greater driving force for the expansion of extrudates. Rausch (2009) found that increased SME positively influences Maillard reaction, leading to darker extrudates (Fang et al., 2014). van Lengerich (1990) suggested that this correlation involving the Maillard reaction is likely the result of increased friction and temperature induced by the increased SME. Fang et al. (2014) also reported that an increased SME positivesly correlates to tensile strength, hardness and proportion of smaller fractions, whereas had a negative effect on melt viscosity at die. Wang et al (2013) studied the effects of feed moisture content (21.3-29.7%), screw speed (140-240 rpm) and extrusion temperature (133-167 ^{o}C) on SME using animal feed contained mainly soybean and wheat meal. They found a positive correlation between temperature and SME, while a negative correlation between screw speed, moisture content and SME.

Residence time distribution (RTD), a probability distribution function that describes the time raw materials can stay inside the barrel, is the parameter used mostly in scaling up and transferring processes to different extruder geometries due to its easiness for monitoring (Kumar et al., 2008). As expected, increased screw speed shortens the mean residence time (Altomare and Glossi, 1986). Kumar et al. (2008) reported the mean residence time increased with increased moisture content (16 $\rightarrow$ 28%) and decreased screw speed (80 $\rightarrow$ 160 rpm) for starch, when other variables such as moisture content, nozzle diameter and barrel temperature were kept constant. Altomare and Glossi (1986) studied the effect of extrusion parameters on RTD of rice flour in twin-screw co-rotating extruder. They found that screw profile and flow rate has the most effect on RTD, also noted the low fill levels in the extruders (<50%). Owing to the complete displacement and forward conveying motion, twin-screw extruders are known to have narrower RTD than single-screw extruders. Yeh et al. (1992) studied the effect of different screw profiles with forward flight element, reverse flight element and no-flight element on the extrusion of wheat flour in twin screw extruder (Figure 2.4). Their results show that of cooking time was the greatest with the forward flight, because the element accelarated the transportation of melt to the die nozzle, shorten the residence time, and thus result in lower degree of gelatinization. Lin and Armstrong (1990) studied the effects of extrusion temperature, screw speed and moisture content on RTD for a cereal mix (corn, wheat starch and bran) using a counter-rotating twin-screw extruder. Their results showed a positive effect of these three parameters on RTD: residence time increased from 45 – 32 s as moisture content increased from 20-30%; increased barrel temperature (100-140^{o}C) and screw speed (100-200 rpm) resulted in increase in RTD.

The length of the extruder or length-to-diameter (L/D) ratio is also an important parameter. Bhattacharya et al. (1994) studied the effect of L/D (16, 20 and 24) and temperature (75-185^{o}C) on extrusion of rice flour. The authors found that the barrel temperature had a greater effect on bulk density than L/D ratio. In agreement with the aforementioned studies, the increase in temperature had a negative result on bulk density. The effect of L/D ratio on bulk density is temperature dependent: at lower temperature, increase in L/D ratio (longer the barrel) resulted in decreased bulk density, whereas the opposite was observed at higher temperature. SME was found to increase with the increase of temperature and L/D ratio from 16-20, and decrease with L/D ratio above 20. They concluded that a barrel temperature greater than 150^{o}C and L/D ratio of 16-20 is sufficient enough to produce a crunchy and well expanded product (Bhattacharya et al., 1994).

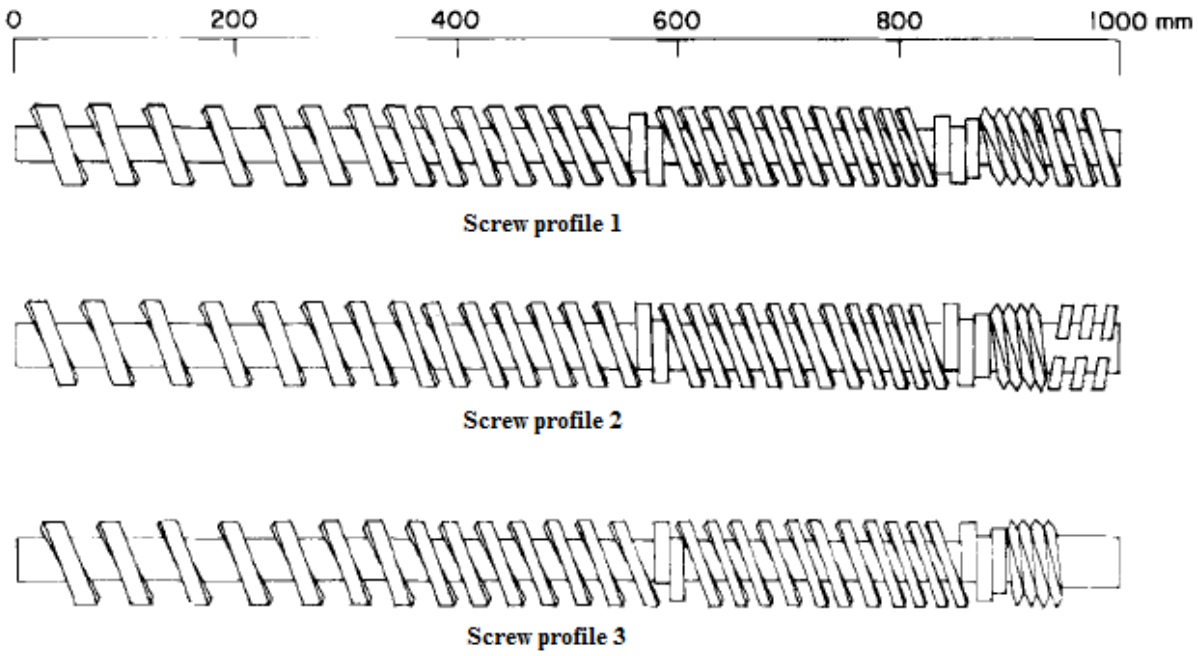

Figure 2.4 Three screw profiles. Screw elements at the end of the screw are different. Profile 1 has a forward flight; profile 2 has a reverse flight; profile 3 has a no-flight element (taken from Yeh et al., 1992).

2.8 Effect of extrusion on nutritional properties of extrudates

Extrusion has been shown to have both a positive and negative effect as it relates to its extrudates. For instance, HTST (high temperature short time) processing minimizes the cooking time, reduces the nutritional loss of heat sensitive vitamins, reduces or eliminates bioactive compounds such as protease and amylase inhibitors, improves starch gelatinization, and reduces lipid oxidation etc. However, extrusion also promotes the Maillard reaction between proteins and reducing sugars which decreases the nutritional value of protein and potentially produces non-nutritive intermediate compounds (e.g., acrylamide). Protein quality relates to its amino acid profile, availability of essential amino acids, and its digestibility (WHO, 1985).

The U.S. and Canada use different methods for determining protein quality. Health Canada (1981) evaluates protein quality based on the protein efficiency ratio (PER), which is the grams weight gain of male rat (20-23 days of age) per gram protein consumed with casein as a reference; protein rating of a certain food is the result of the PER adjusted against casein reference multiplied

by grams of protein in a Reasonably Daily Intake (RDI) of that food; foods can be claimed as a "Source of Protein" with protein ratings ranging from 20.0 to 39.9, and an "Excellent Source of Protein" from 40 or above. However, the use of PER has significant limitations as the rat is a poor model in determining amino acid requirements for adult humans; also, protein rating is dependent on RDIs, and it is impossible to combine PER values (Marinangeli et al., 2017). In the U.S., Protein-Digestibility-Corrected Amino Acid Score (PDCAAS) is used for labelling purposes towards non-infants (>1 year of age) (Marinangeli et al., 2017). A food is considered a quality source of protein with a PDCAAS (*in vivo*) above 70% to 80% (Michaelsen et al., 2009). Proximate and amino acid compositions and true fecal nitrogen digestibility are needed to calculate PDCAAS (WHO, 1991). The amino acid score is the ratio of the mg of essential amino acid in 1.0 g of test protein over mg of that amino acid in 1.0g of reference for the 9 essential amino acids and tyrosine and cysteine additionally; the suggested reference is based on the amino acid requirements for children of the age of 2-5 years. Multiplying this uncorrected amino acid score by true protein digestibility (TPD), determined by the rat balance method (Eggum, 1973), will give PDCAAS.

In many other regions of the world (e.g. China, Europe, Australia and New Zealand), protein claims are based on the protein (g) per serving, percentage of energy from protein per serving, and the proportion of protein per unit of energy (Marinangeli et al., 2017). It has been suggested that Canada should push to label and advertise food protein based on absolute protein content instead of protein quality to minimize the barrier to adapting the globally prominent trend in consuming more plant protein for the cause of human, animal health and environmental sustainability; or at least adopting the PDCAAS method, or a modified version such as *in vitro*-PDCAAS (IV-PDCAAS), to harmonize the regulatory system for protein claims between the two countries (Marinangeli et al., 2017). The assay involves multiplying the *in vitro* protein digestibility (IVPD) value by the limiting amino acid score for determination of the *in vitro* PDCAAS (Nosworthy et al., 2016).

Although the Canadian Food Inspection Agency (CFIA) permits only the estimation of PER using PDCAAS values, where PER is the ratio of PDCAAS of sample over PDCAAS of casein then multiplied by 2.5, *in vitro* PDCAAS is not recognized over *in vivo* (Marinangeli et al., 2017). Nevertheless, it was reported that a strong correlation (R2= 0.9971) exists between PDCAAS and IV-PDCAAS of extruded, cooked and baked red and green lentil flours (Nosworthy et al., 2018). In addition, such correlation (R2= 0.9898) between the two methods was also reported

for lentil, pea, faba bean and casein protein isolates (Nosworthy and House (2017). Although PDCAAS is the more preferred method by FAO and WHO to evaluate protein quality in human nutrition (WHO, 1991), IV-PDCAAS has its advantage in that it is more accurate and applicable in estimating the quality of potential food aid type products compared to PER; it avoids the lengthy and expensive bioassay as required by both PDCAAS and PER methods. The PDCAAS value above 70% (or 0.70) is the minimum requirement for food aid products for moderate malnourished children (WHO, 2012).

In contrast to conventional cooking or baking processes, extrusion seems to enhance the digestibility of plant proteins (Day and Swanson, 2013), possibly because of protein denaturation and the inactivation of enzyme inhibitors (Colonna et al., 1989). EI-Hady and Habiba (2003) found that extrusion at 140^{o}C and 18% moisture content increased IVPD of raw faba bean from 75% in to 80% after extrusion. The same pattern was found in kidney beans where IVPD increased from 71% in the raw to 79% in the extruded kidney beans under the same condition. Bhattacharya et al. (1988) extruded fish-wheat blend at 100-140^{o}C and 35% moisture; they found that increases in extrusion temperature led to increases in digestibility from 80 to 86% relative to the non-extruded form (77%). This increase in IVPD after extrusion was also reported in cereal-pulse blend. Patil et al. (2016) added different pulse (lentil, chickpea, green pea and yellow pea) flours at the level from 0 to 15% into wheat flour and found that extrusion (180^{o}C and 12% moisture) generally doubled the IVPD of the protein within the samples, which ranged from 29 to 38% for the raw and 60 to 66% for the extruded blends. Nosworthy et al. (2017) compared the protein quality (of buckwheat and buckwheat-pinto bean blend (50:50) after baking and extrusion using both *in vivo* and *in vitro* methods; they found that extruded products had greater digestibility and PDCAAS. In brief, the IVPD and TPD of the extruded samples range from 72 to 80% and 71 to 85% respectively; the IV-PDCAAS and PDCAAS of the samples range from 55 to 75% and 54 to 76% in order. The extruded buckwheat-pinto blend had the greatest PDCAAS (76%) among the diets investigated. Mosha and Bennink (2005) studied protein quality of bean meal and bean-sardine meal coupled with corn, sorghum and rice processed by extrusion, drum-processing and conventional cooking; the authors reported that extrusion yielded the greatest TPD and PDCAAS, ranging from 90 to 94% and 60 to 86% respectively.

Lysine is the most reactive among the essential amino acids (EAAs) owing to its two amino groups, making it highly susceptible to partake in the Maillard reaction (O'Brien and Morrissey,

1989). This chemical reaction happens during heating between free amino groups on the protein (not limited to Lys) and the carbonyl groups of reducing sugars. This reaction leads to browning, production of flavor compounds, decreased AAs availability and reduced protein digestibility due to cross-liking reactions involving proteins and intermediate compounds from the reaction itself. As such, Lys usually serves as an indicator of protein damage during extrusion and other cooking processes (Iwe et al., 2001), especially when extruding cereal-based products as Lys is the most limiting EAA. Bjorck and Asp (1983) found that Lys retention in wheat flour increased as feed rate increased, which is possibly due to decreased retention time and heat transfer in the extruder barrel. Pham and Del Rosario (1984) found that Lys retention decreased as feed moisture increased for cowpea and mung bean. Iwe et al. (2004) found that Lys content increased with increasing screw speed, however, decreased with decreasing die diameter in defatted soy flour and sweet potato flour. Other than Maillard reaction, Lys can also cross-link with alanine and threonine to form lysinoalanine and lanthionine, which results in reduced digestibility, loss in EAAs and decreased nutritional values (Camire et al., 1990). High barrel temperatures and low feed moistures are known to accelerate the Maillard reaction. Under these conditions, dextrin and free sugars might be produced from the high shear inside the extruder barrel and presents more substances that is favorable for the reaction. Hood-Niefer and Tyler (2010) extruded pea flour with protein content ranging from 6 to 18% at 100, 120 or 140^{o}C and 15, 18 or 21% moisture. They found that better lysine retention occurred at higher protein and moisture contents. For example, at 100^{o}C and 18% moisture, the available lysine in pea flour with 6% protein had a 53% decrease, whereas only 14% decrease for the flour containing 18% protein; when moisture content increased from 15 to 21%, lysine loss decreased from 60 to 45%. It was found that extrusion of a cereal mixture at 170^{o}C, 10-14% moisture and 60 rpm screw speed resulted in loss of Lys ranging from 32% to 80% (Harper, 1988). To minimize the loss, it was suggested to keep extrusion temperature below 180^{o}C and moisture content above 15% (Cheftel, 1986). In general, keeping moisture content between 15-25% can significantly increase Lys retention (Singh et al., 2007).

In the case of oil-soluble vitamins, vitamins D and K are quite stable during extrusion with only 15-20% loss depending on the conditions (Plattner, 2007). However, vitamins A, E and their derivatives such as carotenoids and tocopherols can change chemically with the presence of oxygen and heat (Killeit, 1994). In contrast, the water-soluble vitamins B and C (ascorbic acid) are less stable when heated. The loss of ascorbic acid can be as high as 90%, and as such, it is

common to have it applied to the final product after extrusion and drying (Soliman et al., 1987). Killeit and Wiedmann (1984) investigated B-complex vitamins in flat bread production using extrusion (150^{o}C, 13% moisture at 300 rpm screw speed) to find that increasing throughput improved the retention of B_1, B_6 and B_{12}, and that increase feed moisture by 3-11% also increased the above vitamins as well as folic acid. They presumed this improvement was due to less shearing brought by the added moisture. In general, increasing in temperature, screw speed and SME input as well as decreasing moisture, throughput/feed rate and die diameter will result in decreases in retentions of vitamins (Killeit, 1994). Furthermore, extrusion does not seem to have a big impact on small minerals as they usually have very high boiling points and are unlikely to be lost with water that evaporates at the die.

2.9 Effect of extrusion on functionalities of extrudates

During extrusion cooking the thermomechanical action results in gelatinization of starch, denaturation of protein, which in turn brings about changes in the functional properties of the pre-cooked flours, milled from the extrudates, such as water hydration capacity (WHC), oil holding capacity (OHC), emulsion activity/stability (EA/ES), foaming activity/stability (FA/FS), and pasting properties (Martínez et al., 2014).

(a) Water hydration capacity: WHC is the ability of a flour to hold water (own or added) during application of force, pressure, centrifugation, heating or from gravity (Sahni et al., 2014). Protein-water interactions, water-water interactions and physical capillary actions influences WHC the most (Dahl and Villota, 1991). Alonso et al (2000) studied the effect of extrusion (150^{o}C, 25% moisture) on the functional properties of pea and kidney bean proteins. The authors reported that extrusion increased the WHC from 1.2 to 2.9 g/g for peas and 2.0 to 2.9 g/g for kidney beans after extrusion. Similar results were found in another study where heat processed winged bean flour had higher WHC (3.1 g/g) compared to its raw counterpart (2.1 g/g) (Narayana and Narasinga Rao, 1982). Martínez et al (2014) also found a progressive increase in the hydration properties (water binding capacity and swelling) as extrusion intensity increased. It was suspected that in extruded samples, physical retention of water by capillary action in the new structure formed by aggregation of proteins probably plays the major role in the increased WHC (Alonso et al., 2000). Camire et al. (1990) also proposed that disruption of the starch granule integrity can lead to a poorly ordered molecular phase with hydroxyl groups to readily bind water molecules.

(b) Oil holding capacity: OHC is defined similarly as WHC, except it relates to how much oil the pre-cooked flour can hold. OHC is mainly contributed by the binding of lipid with the hydrophobic residues of proteins (Aguilera et al., 2009). When extruding pea and kidney bean at 148 to 156^{o}C and 25% moisture, extrusion was found to decrease the oil adsorption capacity only in kidney bean from 1.3 to 1.0 g/g, whereas the decrease observed in peas was not significant (Alonso el al., 2000). It has also been reported that the chemical structure of polysaccharides such as dietary fibre, its surface properties, overall charge density and hydrophobic nature of the polymer can also influence OHC; dietary fibers (DF) are grouped into water soluble (SDF) such as pectin and gums, and water insoluble (IDF) like cellulose, hemicellulose and lignin (Fernández-López et al., 2009). It has been reported that extrusion could transform some IDF into SDF (Vasanthan el al., 2002). Since IDF acts as an oil absorber, and its ability of oil holding may be adversely affected by treatment like cooking (Raghavendra et al., 2006). Huang and Ma (2016) studied the effect of extrusion on physicochemical properties of extruded orange pomace to find extrusion at 115 to 135^{o}C and 10 to 18% moisture decreased the OHC of orange pomace from 1.2 to 0.8 g/g. It is possible that the protein denaturation, aggregation and interactions with hydrophobic groups take place during extrusion can cause an overall decrease in hydrophobicity in samples, especially if they end up forming larger aggregates during mixing (Li and Lee, 1996). OHC plays an important role in flavor retention, especially when it comes to products (e.g. meat) that tend to lose fat during cooking (Thebaudin et al., 1997). On the other hand, samples with low OHC indicates the potential application in fried products due the non-greasy mouthfeel (Aguilera et al., 2009).

(c) Emulsifying properties: Food emulsions are macroemulsions representing a heterogeneous mixture of fat globules ranging from 0.2 to 50 μm in size. They can be of oil in water (O/W) such as milk, cream, mayonnaise, salad dressing and soups or water in oil (W/O) type like margarine and butter (Lam and Nickerson, 2013). Proteins are the components that works as emulsifier in most food emulsions (Zayas, 1997). The hydrophobic and hydrophilic parts of protein can be integrated at the interface of two immiscible phases and lower the interfacial tension (Bos and Vliet, 2001). Extrusion seems to have a positive effect in the emulsifying properties. Bueno et al. (2009) studied the effect of extrusion (135^{o}C, 15% moisture) on the emulsifying properties of soybean proteins and pectin mixtures, the extruded mixture showed an emulsifying capacity 41% higher than the non-extruded mixture. Martínez et al (2014) extruded wheat flour at extrusion

temperature ranging from 80 to 160°C. They found that emulsion capacity in general increased as extrusion temperature reached 120°C, from ~83 (raw) to 91% (at 160°C); whereas emulsion stability decreased as extrusion temperature reached 120°C from ~115% (raw) to 100% (at 160°C). Proteins are forced to unfold and aggregate due to crosslinking involving SH/SS interchange, oxidation, hydrophobic interactions (Rosell and Foegeding, 2007), and Maillard reaction during (Kato et al., 1990), along with starch gelatinization which increases number of hydroxyl groups, greater emulsion activity/capacity can be achieved (Mason, 2009). Recent studies have found that polysaccharide-protein complexes can stabilize O/W emulsions (Evans et al., 2013). Zhang et al. (2014) reported that the formation of protein-polysaccharide complex is important in preparation for an effective emulsifier due to the improved amphiphilic property. Such complex formation is commonly seen during Maillard reaction (Kato et al., 1990), as well as heating processing undergone such as during gelatinization (Evans et al., 2013). Essentially, an increased emulsion capacity is achieved by better adsorption of protein at the oil-water interface through increased protein hydrophobicity (Chen et al., 2011). However, emulsion stability depends mostly on oil droplet size and its interfacial tension, the unfolding and aggregation of protein could minimize the barrier effect against oil droplet coalescence, eventually leading to the separation of water and oil phases (Aluko et al., 2009).

(d) Foaming properties: Foams are like oil-in-water emulsions except foams have gas as their continuous phase instead of oil. Foaming properties are evaluated by foam capacity (FC) and foam stability (FS) (Ferreira et al., 1995). During foaming, air is introduced into the solution during whipping or homogenization; the hydrophobic regions of protein migrate and adsorb to the air-water interface; the proteins then undergo partial unfolding (surface denaturation) at the interface and form a stabilizing film around the air bubbles to create stable foam. In general, foams collapse due to the following reasons: 1) bubble disproportionation over time due to air diffusion from the higher pressure interior; 2) lamellae rupture from pushing and pulling between two bubbles and causes formation of holes; and 3) natural water drainage that removes of proteins around the bubbles, causing the film too thin to support the bubble eventually (Lomakina and Míková, 2006). According to Martínez et al (2014), extrusion (80 to 160°C) worsened FC for wheat flours extruded beyond 120°C, as well as FS. Extrusion decreased FC of wheat flour from ~52 (raw) to 31% (160°C), and FS from ~86 (raw) to 0% (160°C). Similar results were observed in a study by Onwulata et al. (2003) who extruded whey protein at 35-100°C at 38% moisture and found that

foam capacity and stability were significantly affected at and above 75°C. The foam capacity at 35°C, decreased from ~298%, to 173 and 77% at extrusion temperature of 75 and 100°C respectively; and the foam stability at 35°C (~30%) decreased to ~17 and 8% respectively; at pH 7, 85 and 95% of protein were found insoluble at temperature 75 and 100°C respectively, thus they concluded that the extrusion-induced protein insolubility was the main reason for the decrease observed.

(e) Pasting properties: Extrusion can result in great difference in the physicochemical properties of starch compared to the raw material, for example gelatinization/melting and dextrinization of starch generally happen during extrusion (Mitrus et al., 2017). These changes can be revealed in the pasting properties and based on which the effect of extrusion on starch could be better understood and entails different applications. In this study, the Rapid Visco Analyser (RVA) was used to test the degree of cook of the starch in the samples. RVA is a rotational viscometer that is used to describe the pasting properties of a flour or starch solution by measuring the viscosity of a sample continuously under controlled temperature and shear. Two standard testing profiles are provided by the manufacturer (Table 2.2) (Perten Instruments, 2015). Standard 1 is the AACC approved general method to analyze pasting properties of wheat or rye flour or starch (AACC, 2000b). Standard 2 increased the heating time for starch hydrolysis, thus is more accurate for samples like that are harder to hydrolyze due to their chemical composition. There are six key features collected from a pasting curve (Figure 2.5), which include:

- *Pasting temperature*, which is the temperature at which swelling of starch granule begins defined as an increase in viscosity of 25 centipoise (cP)/20 sec (Juhász et al., 2005).
- *Peak viscosity,* which is the maximum viscosity occurred during the heating or holding stage and is often correlated with final product quality. It is achieved when the rate of granule swelling (thus increase in viscosity) equals to the rate of breakdown of the granules. Thus, the difference in peak viscosity is related to the hydrating power and rate of disruption of the starch granules (Corke et al., 1997). Higher peak viscosity indicates greater thickening power of a material, thus maybe applied in foods requiring high gel strength and elasticity (Adebowale et al., 2005).
- *Trough viscosity*, which is the viscosity at the end of the holding temperature before cooling starts and relates to how well the material can withstand heating and shearing processes. Thus, a starch or flour with high trough viscosity could be potentially incorporated into a formulation

that requires better heat and shear resistance during food processing. Kaur et al. (2007) reported that starch, when swelled, with lower amylose content is more susceptible to shear.

- *Breakdown viscosity,* which is the difference between peak and trough viscosity, and is dependent on the nature of the sample, shear stress and temperature applied to it. The breakdown is the result of disruption of swollen starch granules during gelatinization (Kesarwani et al., 2016). It has been reported that protein content negatively correlates with breakdown viscosity (Champagne et al., 2007). Breakdown viscosity plays a significant role in estimation of the cooking quality of the test starch. For example, a higher peak and breakdown viscosity of cooked rice could indicate that the rice is soft and glutinous (Liu et al., 2007).
- *Setback viscosity*, which is the difference between final and trough viscosity, and describes the re-association between starch molecules upon cooling (known as retrogradation). Thus, it implies the degree of retrogradation (Kesarwani et al., 2016). It has been reported that protein content positively correlates with setback viscosity (Champagne et al., 2007). Higher setback viscosity indicates a stronger gel forming upon cooling, thus a harder final product.
- *Final viscosity,* which describes the final viscosity after gelation of amylose and some amylopectin polymers occurs (Perten Instruments, 2015). It indicates the ability of the starch to form viscous paste after heating and cooling (Li et al., 2014). The ratio of final viscosity over trough viscosity is setback ratio. A lower setback ratio indicates the potential application of the starch as a good thickener and stabilizer during food processing (Corke et al., 1997).

Table 2.2 Manufacturer standard pasting profiles (Perten Instruments, 2015).

Standard 1			Standard 2		
Time	Type	Value	Time	Type	Value
00:00:00	Temp	50ºC	00:00:00	Temp	50ºC
00:00:00	Speed	960 rpm	00:00:00	Speed	960 rpm
00:00:10	Speed	160 rpm	00:00:10	Speed	160 rpm
00:01:00	Temp	50ºC	00:01:00	Temp	50ºC
00:04:42	Temp	95ºC	00:08:30	Temp	95ºC
00:07:12	Temp	95ºC	00:13:30	Temp	95ºC
00:11:00	Temp	50ºC	00:21:00	Temp	50ºC
00:13:00	End		00:23:00	End	

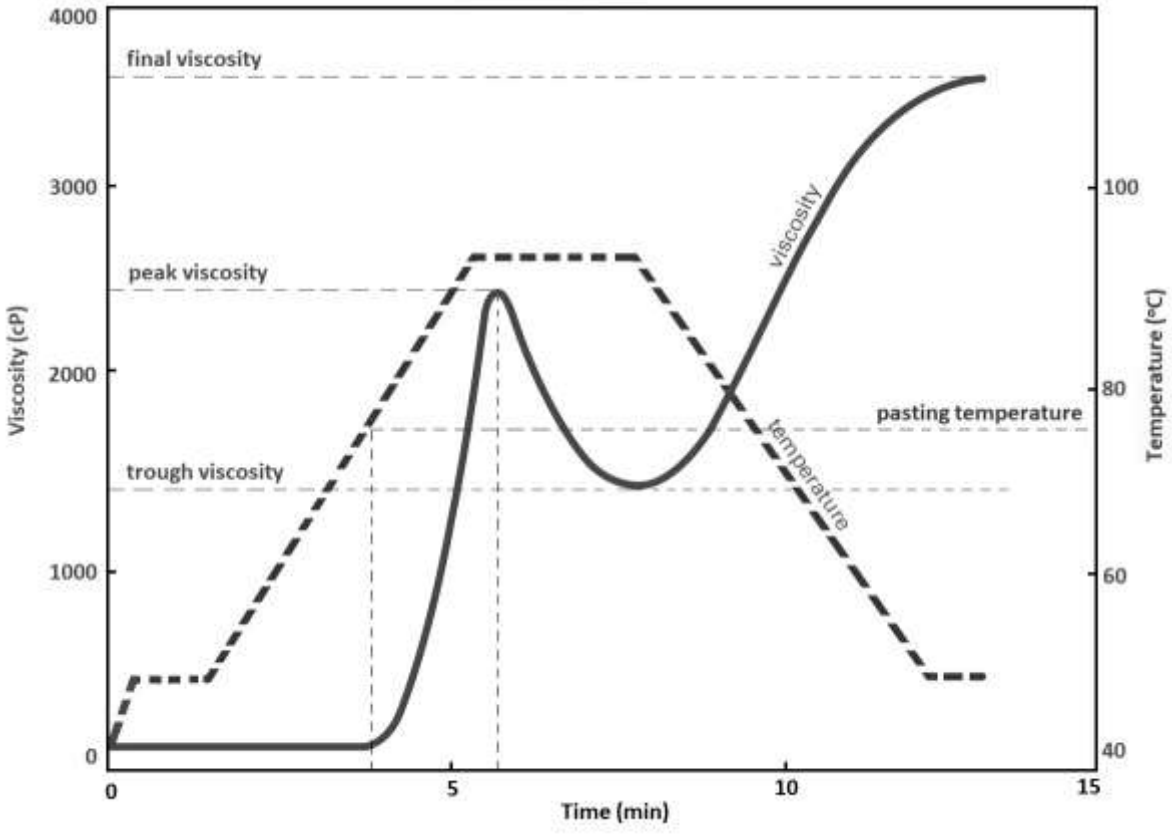

Figure 2.5 Typical pasting curve of starch measured by a rapid viscoelastic analyzer using Standard 1 profile.

Starch consumed by humans has mostly undergone some kind of processing (e.g. cooking), where native starch granules are gelatinized upon heating in water and retrograded during cooling. The degree of gelatinization and retrogradation are key to functional properties in food processing and could be demonstrated when performing pasting properties analysis by RVA. Botanical source of starch and processing conditions (time-temperature history) of the starch can determine the type and extent of changes in starch, resulting in different pasting properties (Wang and Copeland, 2013). Pasting properties assist product developers in ingredient selection given the data received on the starches ability to perform as thickeners, shear stabilizers, and form gels.

The analysis of pasting properties starts with hydration of a starch or flour sample. During hydration, water migrates into the starch granule to cause swelling, which is typically reversible if the temperature is <50^{o}C. As temperatures increases further, the starch granule starts to lose its crystalline structure as amylose polymers leach out of the granule leading to increases in viscosity and a phenomenon known as pasting. The test starch will reach peak viscosity when the rate of granule swelling (increase in viscosity) equals to the rate of breakdown of the granules, which may occur at any time during the heating phase or hold phase, depending on the starch. Peak viscosity of starch from different botanical source also vary. For example, Srichuwong et al. (2005) reported that the peak viscosity of 8% (w/w) starch suspension of corn, rice, and potato are 176, 211, and 791cP respectively. Whereas for pulse starch from black bean, chickpea, lentil and navy bean in 8% (w/w) solution, peak viscosity was found to be 1987, 1754, 1692 and 2796 cP respectively (Byars and Singh, 2016). In Standard 1 profile, a sample will be held at 50^{o}C for 1 min before heating starts. After 3 min 42 sec of heating, the sample is held at maximum temperature at 95^{o}C for 2 min 30 sec. The decrease of viscosity (disruption of starch granules) may start to occur before reaching the maximum temperature as mentioned above and continues during this stage. The cooling stage starts at 7 min 12 sec into the test and lasts for 3 min 48 sec to reach the final temperature 50^{o}C, during which an increase in viscosity occurs as retrogradation is observed. However, the initial viscosity increase is normally due to the drop of temperature. Then as cooling continues, the amylose polymers start to entangle with each other to form a gel (retrogradation). The entanglement, which can be indicated by the viscosity increase, is limited by high content of amylopectin in starch. Because unlike the longer unbranched amylose, the shorter and highly branched amylopectin does not associate with each other as efficiently as amylose. Therefore, the viscosity increases in the cooling stage for waxy starches (i.e. starches high in amylopectin) is

usually small. Upon cooling, the amylose leached out from starch granules would interact with each other through hydrogen bond and form "gel junction zones". Although amylopectin does not contribute much to the initial retrogradation, this highly branched starch that is embedded in the amylose gel is responsible for long-term gel structure (Miles et al., 1985) and vice versa. During the 2 min holding stage after the final temperature is reached, the viscosity will continue to increase possibly due to incomplete thermal mixing with a cooler and viscous outer part but warmer center of the mixture. However, given enough time, a plateau will eventually be reached, which usually takes 3-4 min at 50^{o}C (Crosbie and Ross, 2007).

As it relates to extrusion processing, starch degradation can have a big impact on the pasting properties of pre-cooked flours. Complete starch cooking when water content is low (<35%) could involve at least two mechanisms: (1) gelatinization in the presence of water (as previously described) and (2) starch melting under water-limiting conditions (Wang, 1993). In the case of the latter, when moisture content is <5% and temperature is high, a direct helix to coil transition occurs and starch crystallites melt into amorphous gels; when water is added up to 40%, starch gelatinizes in two steps: initial occurrence of disorder in double helices structure of amylopectin and melting (helix-coil transition) as amylopectin helices unwind to form amorphous gels (Waigh et al., 2000a,b). Starch will melt at 168^{o}C without the presence of water, and at 123^{o}C with 20% moisture (Tang and Ding, 1994). Ozcan and Jackson (2005) extruded corn starch at 165^{o}C at 20% moisture to find improved water absorption and solubility indices, and lower viscosity profiles compared with the native starch. Similar findings were reported by Hussain and Singh (2013), who investigated the pasting behavior of extruded rice grains at temperatures and moistures ranging between 59 - 110^{o}C and 31 - 45%, respectively. They found that all pasting properties (peak, hold, breakdown, final and setback viscosity) were significantly lowered for all conditions, with barrel temperature having the most significant effect. They gave three reasons for the reduction in the overall viscosity after extrusion: (1) disrupted starch granules could not swell like the native starch; (2) the partially bound starch structure from retrogradation following extrusion could inhibit swelling; and (3) the denaturation of the major water absorber— protein— could also reduce the viscosity.

(f) Thermal properties: Differential scanning calorimetry (DSC) has been widely used to study the thermal properties of food components, such as protein denaturation, starch gelatinization and melting and more. The rate of heat flow to the sample and a control material are

compared when heating or cooling them at the same rate; heat absorption or evolution of the sample changes the differential heat flow and a peak is recorded (Biliaderis, 1983). Two endothermic transitions are typically observed for native starches. The first peak at intermediate (45-50%) or higher water content and the temperature around 60 to 70oC is associated with gelatinization. Therefore, this peak is not exhibited by pre-gelatinised starches (Donovan, 1979). It has been reported by many that the endothermic peak is absent for pre-gelatinised starches (Davidson et al., 1984; Biliaderis et al., 1980; Gomez and Aguilera, 1983). The second peak at higher temperature is associated with "true melting" of the crystalline region of starch when heated under limited presence of water (Biliaderis et al., 1980). In addition to these two irreversible transitions, a reversible endotherm has been reported at even higher temperature (~100^{o} C), which is associated with disordering process of amylose-lipid complexes (Kugimiya et al., 1980).

3. MATERIAL AND METHODS

3.1 Materials

Kabuli chickpea (dehulled) flour (Best Cooking Pulses Inc., Portage-La-Parrire, MB, Canada), white whole grain sorghum flour (ADM Milling Co., Decatur, Illinois, USA) and whole grain maize meal (Bunge Ltd., White Plains, New York, USA) were purchased for extrusion at the Saskatchewan Food Industry Development Centre Inc. (SFIDC; Saskatoon, SK, Canada). All other chemicals used in this study are of reagent grade, purchased from Sigma-Aldrich Co. (Oakville, ON, Canada). All extrudates were milled into flours using a hammer mill (DAO6, Fitzpatrick, Elmhurst, IL, USA) with a 1mm diameter round hole perforated screen. Pre-cooked flours were composite flours combined from two extrusion runs.

A pre-study was set up to determine the best blending ratio of the chickpea and cereal flours to be used in extrusion based on the protein quality. Blends of chickpea: sorghum and chickpea: maize were then prepared through dry mixing the flours using vortex by weighing 10 g of total weight into a centrifuge tube at different ratios of 5:5, 6:4, 7:3 and 8:2 based on their dry weight (i.e., taking in account moisture). The composition and protein quality were then measured for all raw and blended flours. Based on the results, a blending ratio of 6:4 chickpea: cereals was selected for extrusion.

3.2 Extrusion

Extrusion were performed by a co-rotating twin-screw extruder (Clextral EV-32, Firminy, France) equipped with a volumetric feeder (Clextral VF/40/25-2) and a 2-blade die face cutter, at the SFIDC (Saskatoon, SK, Canada). The extruder barrel length: diameter ratio (L/D) and die diameter are 24:1 and 3 mm respectively. Screw speed and feed rate were kept constant respectively at 317 rpm and 14 kg/hr. There are six temperature zones within the extruder barrel: in zone 1,2 and 3 the temperature are set constant at 50, 80 and 100 ^{o}C, respectively; whereas zones 4 to 6 will all be kept at the same temperature and change according to experimental design. Extrusion temperature, which is the temperature from zone 4 to 6, were at 120^{o}C and 150^{o}C, and

moisture content were adjusted to 20% and 24% by water injection. The end product – extrudates, were dried for 5 min at 105ºC in a tunnel dryer (Chromalox, Pittsburgh, PA, USA).

3.3 Physicochemical properties

(a) Proximate analyses

Moisture, crude protein and ash contents were determined according to AOAC methods 925.10, 992.23 and 940.26 respectively (AOAC, 2000). Protein conversion factor was 6.25 for chickpea, sorghum, maize and the chickpea-cereal blends. Crude fat was determined gravimetrically by Swedish tube extraction using petroleum ether at POS Bio-Sciences (Saskatoon, SK, Canada), according to the method of Troeng (1955). Protein, ash and lipid contents are reported on percent dry weight basis (d.b.). All analyses were done in triplicate and reported as mean ± one standard deviation (n =3) except for crude lipid.

(b) Hardness (HD)

Hardness was measured as the maximum force (N) applied to break the extrudates. Hardness was measured using TMS-2000 Texture press (Food Technology Corporation, Sterling, VA, USA) equipped with a 1,334 N load cell and thin blade shear compression cell (Model CS-2) using a transducer speed is at 0.33 cm/s. Measurements were repeated six times for each moisture-temperature treatment, and reported as mean ± standard deviation (n = 2).

(c) Expansion ratio (ER)

Expansion ratio is the ratio between the diameter of the extrudates and the diameter of the extruder die orfice (3 mm). An electronic digital caliper (Model 62379-521, Traceable Products, TX, USA) was used. Measurements were repeated 40 times on extrudates from each moisture-temperature treatment, and reported as mean ± standard deviation (n = 2).

(d) Bulk density (BD)

Bulk density was determined by measuring the weight of extrudates required to fill a 1000 mL container, recorded in g/L. Extrudates are randomly added into the container and shaken few times during filling. Measurements were repeated 6 times for each moisture-temperature treatment, and reported as mean ± standard deviation (n = 2).

(e) Specific mechanical energy (SME)

Specific mechanical energy was determined by the twin-screw extruder computer control system and recorded during extrusion. Measurements were repeated twice for each moisture-temperature treatment and reported as mean ± standard deviation (n = 2).

3.4 Functional properties

(a) Water hydration capacity (WHC)

Water hydration capacity was determined by AACC method 56-20.01 (AACC, 1999). Briefly, 1 g of raw or pre-cooked flour was mixed with 20 mL of distilled water on a Vortex mixer for 10s at 0, 5 and 10 min followed by 15 min centrifugation (1,000 × g). Samples were analyzed in triplicate, and reported as the mean ± one standard deivation. WHC (g water/g flour) was determined according to Eq.1:

$$\boldsymbol{WHC = \frac{sediment\ weight - sample\ weight}{sample\ weight\ (dry\ basis)}} \quad [3.1]$$

(b) Oil holding capacity (OHC)

Oil holding capacity was measured according to Nidhina and Muthukumar (2015) with some modifications. In brief, 1 g of raw and pre-cooked flour (W_0) were wetted with 10 g of canola oil in a 50 mL centrifuge tube followed by 10 s vortex (VWR, Radnor, PA, USA) mixing every 5 min for 30 min; then centrifuged at 1,000 × *g* for 15 min at room temperature and weigh pellet (W′). Samples will be analyzed in triplicate, and reported as the mean ± one standard deivation. OHC was determined using Eq.2:

$$\boldsymbol{OHC = \frac{W' - W_0}{W_0}} \quad [3.2]$$

(c) Foaming capacity (FC) and stability (FS)

Foaming properties was determined according to Wilde and Clark (1996). In brief, 1% (w/w) raw and pre-cooked flour solutions, pH adjusted to 7.0 using 1 N NaOH, was prepared and stirred overnight (16-18 h). The pH of the solution will then be re-adjusted to 7.0 prior to analysis.

15 mL solution was transferred into a 400 mL beaker and homogenized with thev fixture blade at the water-air interface using a Omni Macro Homogenizer (Omni International, Marietta, GA, USA) at speed 4 for 5 min. Generated foam was transferred immediately into a 50 mL graduated cylinder to record the initial volume (V_i), and final volume (V_f) after 30 min of sitting. Samples were analyzed in triplicate, and reported as the mean ± one standard deivation. FC and FS were determined using Eq. 3 and 4:

$$\%\boldsymbol{FC} = \frac{\boldsymbol{V_i}}{\boldsymbol{15\ mL}} \times \boldsymbol{100}\% \quad [3.3]$$

$$\%\boldsymbol{FS} = \frac{\boldsymbol{V_i - V_f}}{\boldsymbol{V_i}} \times \boldsymbol{100}\% \quad [3.4]$$

(d) Emulsifying activity (EA) and stabilty (ES)

The emulsifying activity and emulsion stability were determined using the method from Kaur and Singh (2005). Emulsions were prepared by dispersing 3.5 g of raw or pre-cooked flour into 50 mL of water followed by 30 s of homoginization using Omni Macro Homogenizer (OMNI inc., Kennesaw, GA, USA) at speed 4. Then 25 ml canola oil was added and homoginized for another 30s. Another 25 ml of oil was added and homoginized for 90s. The homogenized mixture were then evenly devided and centrifuged in two 50 mL centrifuge tubes at 1,100 × *g* for 5 min. EA was measured as the percentage of the emulsified layer remaining after centrifugation. Calculation is shown in Eq. 5:

$$\%\boldsymbol{EA} = \frac{\boldsymbol{Height\ of\ the\ emulsified\ layer}}{\boldsymbol{Height\ of\ the\ entire\ solution\ in\ tube}} \times \boldsymbol{100}\% \quad [3.5]$$

To test emulsion stability, the same emulsion prepared for the EA measurements was heated in a water bath at 85ºC for 30 min, followed by 15 min cooling and centrifuged again at 1,100 × *g* for 5 min. ES was calculated as the percentage of the emulsified layer that remained after the heat treatment, shown in Eq. 6:

$$\%\boldsymbol{ES} = \frac{\boldsymbol{Height\ of\ the\ emulsified\ layer\ post\ heating}}{\boldsymbol{Height\ of\ the\ entire\ solution\ in\ tube}} \times \mathbf{100}\% \quad [3.6]$$

Samples were analyzed in triplicate, and reported as the mean ± one standard deivation.

(e) Pasting properties

Pasting properties of flours were determined by using a rapid visco analyser RVA4500 (Perten Instruments Inc., Stockholm, Sweden). Sample weight was fixed at 3.50 g with 14% moisture basis to give a constant dry solids content of 10.6%. Samples were stirred at 160 rpm under the 23 min analysis model. RVA parameters such as peak viscosity, trough, breakdown, final viscosity, setback, peak temperature and peak time were recorded by a PC with Thermocline for Windows (TCW3) software. Samples were analyzed in triplicate, and reported as the mean ± one standard deivation.

(f) Nitrogen Solubility Index (NSI)

Nitrogen solubility index was determined according to the method used by Rimamcwe et al (2017). In brief, 1 g of raw and pre-cooked flours were added into water at a ratio of 1: 60. The solution pH was adjusted to pH 7.0 using 0.1 N NaOH and HCl. After 2 h of extraction, the suspension was centrifuged at 3,000 × *g* for 20 min at room temperature (25 ± 2^{o}C) and nitrogen in the supernatant was determined by Micro-Kjeldahl method. The percent of nitrogen in flour samples was calculated as below:

$$\boldsymbol{Nitrogen}\ (\%) = \frac{\boldsymbol{Sample\ titre - Blank\ titre}}{\boldsymbol{Digest\ use\ for\ distillation}} \times \frac{\boldsymbol{Volume\ made}}{\boldsymbol{Weight\ of\ Sample}\ (\boldsymbol{g})} \times \boldsymbol{Normality\ of\ acid}\ \times \mathbf{14.007} \times \frac{\mathbf{100}}{\mathbf{1000}} \quad [3.7]$$

$$\boldsymbol{Soluble\ Nitrogen}\ (\%) = \frac{\boldsymbol{mg\ of\ nitrogen\ in\ extract}}{\boldsymbol{mg\ of\ nitrogen\ in\ sample}} \times \mathbf{100} \quad [3.8]$$

(g) Thermal properties

Differential scanning calorimeter (DSC, Model Q2000, TA Instruments Inc., New Castle, DE, U.S.A.) was used to test if complete gelatinization was achieved after exrusion, which is indicated by the absence of peak temperature. Method used was according to Ai et al. (2016) with slight modification. In brief, samples (~3 mg) were sealed in aluminum hermetic pan with 3× (w/w) of distilled water for 2 h of equilibration. The sample was then scanned from 10 to 105^{o}C at the rate of 10^{o}C/min. Test was done on extrudates treated at 120^{o}C, 20% moisture and 150^{o}C, 24% moisture. The analysis was performed once, and the result is listed in section 4.1.3 and 4.2.4.

3.5 Nutritional properties

(a) Amino acids

The amino acid composition of all raw and pre-cooked flours was performed at POS Bio-Sciences using a pico-tag amino acid analysis system (Waters Corporation, Milford, MA, USA) and high-performance liquid chromatography (HPLC). In general, 15 amino acid residues were quantified according to the method developed by Bidlingmeyer et al. (1987), which involves adding 15 mL of 6 N HCl to ~20 mg of samples and holding at 110^{o}C for 20 h to hydrolyze the proteins into individual amino acids prior to HPLC separation. The amount of sulfur-containing amino acids was determined according to Official Method 985.28 of AOAC International (AOAC, 2000) with some modifications, in which the addition of 1-octanol was omitted; 10 mL of cold performic acid was added to oxidize cysteine and methionine overnight at 4^{o}C, prior to protein hydrolysis with 15 mL of 6 N HCl at 110^{o}C for 16 h. The quantity of tryptophan was determined according to Official Method 988.15 of AOAC International (AOAC, 2000) with modifications, in which samples were hydrolyzed by treating with 10 M NaOH in a boiling water bath for 20 min, and then in an oven at 110^{o}C for 16 h prior to HPLC separation. All analyses were performed in duplicate.

An amino acid score was determined for each essential amino acid by taking the ratio of each essential amino acid within each raw and pre-cooked flour by the recommended standard levels (mg/g protein) putforth by the FAO: histidine (19), threonine (34), phenylalaine + tyrosine (63), valine (35), methionine + cysteine (25), isoleucine (28), leucine (66), lysine (58) and tryptophan (11) (WHO, 1991). The limiting amino acid was denoted by the lowest ratio.

(b) In vitro protein digestibility (IVPD)

IVPD of the raw and extrude flours were assessed using the pH-drop method described by Hsu et al. (1977). The pH of the protein suspension drops as a result of enzymatic digestion by freshly prepared and pH-adjusted enzyme solutions containing porcine trypsin (Sigma T0303), α-chymotrypsin from bovine pancreas (Sigma C4129) and protease from *Streptomyces griseus* (Sigma P5147). The pH drop (ΔpH) for each sample was recorded over a 10 min period using a pH meter. Samples were analyzed in triplicate and reported as mean ± standard deviation (n=3). IVPD is calculated using Eq. 9:

$$\boldsymbol{IVPD = 65.66 + 18.1 \times \Delta pH} \quad [3.9]$$

(c) In vitro protein digestibility corrected amino acid score (IV-PDCAAS)

IV-PDCAAS was determined as decribed by Nosworthy et al. (2016) by multiplying the amino acid score of the limiting amino acid by the IVPD value for each sample.

3.6 Statistical analysis

Statistical analysis was performed using SigmaStat 4.0 (San Jose, CA, USA). An Individual Degree Orthogonal Contrast analysis was performed in conjunction with the General Linear Model to test pre-determined questions. For instance, [1] raw vs. pre-cooked flours; and within the pre-cooked samples: [2] moisture - 20 vs 24%; [3] temperature 120 vs 150^{o}C; and [4] the moisture x temperature interaction. Also, a one-way ANOVA along with Tukey test was used to test the effect of blending ratio on protein quality in study 2 to choose a blend ratio. Significant difference will be considered at alpha (α) < 0.05.

4. RESULTS AND DISCUSSION

4.1 The impact of extrusion conditions on the physical properties of chickpea, sorghum and maize extrudates, and the functionality of their raw and pre-cooked flours

4.1.1 Composition of raw and precooked flours

The proximate composition for raw and pre-cooked chickpea, sorghum and maize flours as a function of barrel temperature and moisture during the extrusion process is given in Table 4.1. Although significant differences were noted in both protein and ash levels in Table 4.2 for all raw and precooked flours, they were not deemed to be substantial. Protein levels in raw/precooked flours were found to be 20.5-23.2% (d.b.), 10.2-10.5% (d.b.), and 7.6-9.1% (d.b.) for chickpea, sorghum, and maize, respectively (Table 4.1). Of note, chickpea flour showed a reduction in protein content by ~3% after extrusion (Table 4.1). Ash levels were found to be 2.7-2.9 % (d.b.), 1.4% (d.b.) and 1.6-1.7% (d.b.) for chickpea, sorghum and maize, respectively (Table 4.1). In the case of all flours, lipid contents were found to be decreased after extrusion. For instance, chickpea flour saw a decrease with extrusion (regardless of the temperature/moisture) from 6.7 to ~5.6% (d.b.), sorghum from 3.0 to 1.0% (d.b.), and maize from 3.9 to 3.0% (d.b.) (Table 4.1).

The protein content for chickpea is close to that reported by Nestares et al. (1996) (~21%), and within the range (21-25%) reported by Boye et al. (2010). The protein content for sorghum flour aligns with that reported by Jafari et al. (2018); also, within the range of low-protein varieties for maize (~7 to 9%) reported by Sauberlich et al. (1953). The decrease in protein content for chickpea after cooking was also observed by Clemente et al. (1998), where a 3.4% decline was observed. Izzo and Ho (1989) proposed this declined was likely caused by an increase in shear (SME) within the extruder which would lead to an increase in protein unfolding. This process would then lead to more binding sites to become exposed for other components in the melt such as starch, sugar, other proteins, or lipids to interact with. The authors also reported that an increase in temperature did not lead to increased protein-lipid interactions, where the indigenous oil was less effective in binding with protein compared to added oil. The limit of protein-lipid interactions

depends on the number of hydrophobic sites exposed during extrusion (Mitchell and Areas, 1992). In general, the compositional properties in the present study did not substantially change because of extrusion with the exception for protein in chickpea and crude lipid, which both decreased after extrusion. This could be the result of the Maillard reaction and the formation lipid-starch complexes during extrusion. This hypothesis was supported by De Pilli et al. (2012) who found that starch-lipid complexes occurred under various extrusion conditions, and the formation of the complex was only significantly influenced by barrel temperature.

Table 4.1 Proximate composition of raw and pre-cooked flour of chickpea, sorghum and maize. The pre-cooked flour represents a composite of two extrusion runs. Data represent the mean of triplicate measurements on the composite flour ± one standard deviation (n = 3). Data is reported on a dry weight basis, d.b. Only one measurement was made on the crude lipid.

Flour	Crude Protein (%, d.b.)	Crude Ash (%, d.b.)	Crude Lipid (%, d.b.)
Chickpea			
Raw flour	23.19 ± 0.06	2.94 ± 0.02	6.76
Pre-cooked flour			
120°C, 20%	20.87 ± 0.02	2.81 ± 0.02	5.52
120°C, 24%	20.54 ± 0.02	2.76 ± 0.02	5.41
150°C, 20%	20.65 ± 0.04	2.77 ± 0.00	5.83
150°C, 24%	20.79 ± 0.02	2.66 ± 0.09	5.93
Sorghum			
Raw flour	10.47 ± 0.02	1.43 ± 0.04	2.96
Pre-cooked flour			
120°C, 20%	10.52 ± 0.09	1.40 ± 0.03	0.84
120°C, 24%	10.19 ± 0.08	1.41 ± 0.02	1.06
150°C, 20%	10.24 ± 0.06	1.44 ± 0.02	0.90
150°C, 24%	10.16 ± 0.15	1.40 ± 0.01	1.07
Maize			
Raw flour	7.64 ± 0.02	1.75 ± 0.02	3.88
Pre-cooked flour			
120°C, 20%	8.12 ± 0.04	1.64 ± 0.01	2.37
120°C, 24%	9.07 ± 0.03	1.74 ± 0.01	3.41
150°C, 20%	8.07 ± 0.01	1.75 ± 0.01	2.83
150°C, 24%	8.05 ± 0.03	1.75 ± 0.02	3.47

Table 4.2a An individual degree of freedom (orthogonal) contrast analysis performed using the general linear model for chickpea, contrasting: raw vs. pre-cooked flours, 20% vs 24% moisture (within the extruder), 120°C vs. 150°C (within the extruder), and the moisture x temperature interaction.

Property	Raw vs. pre-cooked	Moisture during extrusion	Temperature during extrusion	Moisture x temperature interaction
a) Physical parameters				
Expansion index[1]	NT	$p<0.001$	$p<0.001$	$p<0.001$
Bulk density[2]	NT	NS^{+}	$p<0.001^{+}$	NS^{+}
Hardness[2]*	NT	NS	$p<0.001$	NS
SME[3]	NT	NS^{+}	$p<0.01^{+}$	NS^{+}
b) Composition[4,5]				
Protein[4]	$p<0.001$	$p<0.001$	NS	$p<0.01$
Ash[4]	$p<0.001^{+}$	$p<0.01^{+}$	$p<0.05^{+}$	NS^{+}
Lipid[3]	NT	NT	NT	NT
c) Functionality[4,5]				
Water hydration capacity	$p<0.001$	NS	NS	$p<0.01$
Oil holding capacity**	NS	NS	$p<0.001$	NS
Emulsion activity	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.05$
Emulsion stability	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA – peak viscosity	$p<0.001$	NS	NS	NS
RVA – Trough viscosity	$p<0.001$	$p<0.001$	NS	NS
RVA – Breakdown viscosity*	NS	$p<0.01$	NS	NS
RVA- Final viscosity*	$p<0.001$	$p<0.001$	NS	NS
RVA – Setback viscosity*	$p<0.001$	$p<0.001$	NS	NS
RVA – Pasting temperature*	$p<0.01$	NS	NS	NS
d) Protein quality[4]				
IVPD	$p<0.001$	$p<0.01$	$p<0.001$	NS
IV-PDCAAS	NS	NS	$p<0.001$	NS

Notes:

[1]Forty results for each temperature/moisture combination. (n = 160, df = 158)

[2]Six results for each temperature/moisture combination. (n = 24, df = 22)

[3]Only one crude lipid measurement was taken.

[4]Three results for each temperature/moisture combination using a composite flour blend from duplicate processing runs (n = 12, df = 10)

[5]Foaming was not tested, since all pre-cooked flours were found to be non-foaming.

Abbreviations: NT (Not tested); NS (Not significant, $p>0.05$) and SME (Specific mechanical energy)

(*) Indicates data transformed by once by log

(**) Indicates data transformed twice by log

($^{+}$) Indicates one of the two assumptions (normality or variance test) failed during analyses

Table 4.2b An individual degree of freedom (orthogonal) contrast analysis performed using the general linear model for sorghum, contrasting: raw vs. pre-cooked flours, 20% vs 24% moisture (within the extruder), 120°C vs. 150°C (within the extruder), and the moisture x temperature interaction.

Property	Raw vs. pre-cooked	Moisture during extrusion	Temperature during extrusion	Moisture x temperature interaction
a) Physical parameters				
Expansion index[1]*	NT	$p<0.01$	$p<0.001$	$p<0.01$
Bulk density[2]*	NT	$p<0.001$	$p<0.001$	$p<0.01$
Hardness[2]*	NT	$p<0.001$	$p<0.001$	NS
SME[3]	NT	$p<0.05^{+}$	NS^{+}	NS^{+}
b) Composition[4,5]				
Protein[4]	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Ash[4]	NS	NS	NS	NS
Lipid[3]	NT	NT	NT	NT
c) Functionality[4,5]				
Water hydration capacity	$p<0.001$	$p<0.01$	$p<0.001$	$p<0.001$
Oil holding capacity	$p<0.01$	NS	$p<0.01$	NS
Emulsion activity*	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.05$
Emulsion stability	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA – peak viscosity	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA – Trough viscosity**	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA – Breakdown viscosity*	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA- Final viscosity*	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA – Setback viscosity	$p<0.001^{+}$	NS^{+}	NS^{+}	NS^{+}
RVA – Pasting temperature	NT	NT	NT	NT
d) Protein quality[4]				
IVPD	NS	NS	NS	NS
IV-PDCAAS	NS	$p<0.001$	$p<0.001$	$p<0.001$

Notes:

[1]Forty results for each temperature/moisture combination. (n = 160, df = 156)

[2]Six results for each temperature/moisture combination. (n = 24, df = 22)

[3]Only one crude lipid measurement was taken.

[4]Three results for each temperature/moisture combination using a composite flour blend from duplicate processing runs (n = 12, df = 10)

[5]Foaming was not tested, since all pre-cooked flours were found to be non-foaming.

Abbreviations: NT (Not tested); NS (Not significant, $p>0.05$) and SME (Specific mechanical energy)

(*) Indicates data transformed by once by log

(**) Indicates data transformed twice by log

($^{+}$) Indicates one of the two assumptions (normality or variance test) failed during analyses

Table 4.2c An individual degree of freedom (orthogonal) contrast analysis performed using the general linear model for maize, contrasting: raw vs. pre-cooked flours, 20% vs 24% moisture (within the extruder), 120°C vs. 150°C (within the extruder), and the moisture x temperature interaction.

Property	Raw vs. pre-cooked	Moisture during extrusion	Temperature during extrusion	Moisture x temperature interaction
a) Physical parameters				
Expansion index[1]	NT	$p<0.001$	$p<0.001$	$p<0.001$
Bulk density[2]	NT	$p<0.001^{+}$	$p<0.001^{+}$	$p<0.001^{+}$
Hardness[2]	NT	$p<0.001$	$p<0.05$	NS
SME[3]	NT	$p<0.001$	NS	NS
b) Composition[4,5]				
Protein[4]	$p<0.001$	$p<0.01$	$p<0.01$	NS
Ash[4]	$p<0.01$	$p<0.001$	$p<0.001$	$p<0.001$
Lipid[3]	NT	NT	NT	NT
c) Functionality[4,5]				
Water hydration capacity	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Oil holding capacity	NS	$p<0.05$	NS	NS
Emulsion activity*	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Emulsion stability	$p<0.001$	$p<0.001$	NS	NS
RVA – peak viscosity	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.01$
RVA – Trough viscosity	$p<0.001^{+}$	$p<0.001^{+}$	$p<0.05^{+}$	$p<0.01^{+}$
RVA – Breakdown viscosity	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA- Final viscosity*	$p<0.001$	$p<0.05$	NS	NS
RVA – Setback viscosity**	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
RVA – Pasting temperature	NT	NT	NT	NT
d) Protein quality[4]				
IVPD	$p<0.001$	NS	NS	NS
IV-PDCAAS	NS	$p<0.001$	$p<0.001$	$p<0.001$

Notes:

[1]Forty results for each temperature/moisture combination. (n = 160, df = 156)

[2]Six results for each temperature/moisture combination. (n = 24, df = 22)

[3]Only one crude lipid measurement was taken.

[4]Three results for each temperature/moisture combination using a composite flour blend from duplicate processing runs (n = 12, df = 10)

[5]Foaming was not tested, since all pre-cooked flours were found to be non-foaming.

Abbreviations: NT (Not tested); NS (Not significant, $p>0.05$) and SME (Specific mechanical energy)

(*) Indicates data transformed by once by log

(**) Indicates data transformed twice by log

($^{+}$) Indicates one of the two assumptions (normality or variance test) failed during analyses

4.1.2 Physical properties of the extrudates

The SME, expansion ratio, bulk density, and hardness for chickpea, sorghum and maize fours as a function of barrel temperature and moisture is given in Table 4.3. An orthogonal individual degree of freedom analysis was used to delineate main the effects of temperature (120 vs 150^{o}C) and moisture (20 vs 24%) in the extruder, and their interactions in terms of the physical properties of the extrudates. In the case of SME, the effect of temperature was significant for chickpea, whereas sorghum and maize were only affected by moisture during extrusion (Table 4.2). SME values decreased from ~434 to 245 kJ/kg for chickpea as the barrel temperature increased from 120^{o}C to 150^{o}C, and from ~625 to ~432 kJ/kg, and ~447 to ~425 kJ/kg for sorghum and maize flours respectively as the extrusion moisture increased from 20 to 24%. Onwulata et al. (2001) reported the same reduction in SME when they incorporated 250 g/kg sweet whey solids and 500 g/kg whey protein concentrate to corn meal. The reduction in SME values with chickpea is thought to be associated with the elevated barrel temperature which acts to decrease the melt viscosity; thus, if moisture content, screw speed, and mass flow rate remain constant, SME would decrease (Akdogan, 1996). Planttner (2007) found that any increase in resistance to flow will increase SME. The lower starch and, higher protein and lipid content found within the chickpea was thought to cause the reduced melt viscosity relative to the cereal flours in the present study, which in turn lead to lower SMEs. Additionally, Ryu et al. (2001) reported that higher viscosity of melt due to lower melt temperature and moisture can also result in higher SME. Balasubramanian et al. (2012) found that incorporating of different legume blends into sorghum flour decreased the pasting properties of the extrudates; specifically, peak, trough, breakdown, final and setback viscosities of sorghum decreased from 455 to 320 cP, 226 to 214 cP, 229 to 106 cP, 281 to 259 cP and 55 to 45 cP respectively upon incorporating 15% of legume blends.

Table 4.3 Physical properties of raw flour and extrudates of chickpea, sorghum and maize.

Barrel Temperature	SME[1] (kJ/kg)	Expansion ratio[2]	Hardness[3] (N)	Bulk density[3] (g/L)
Chickpea				
120°C, 20%	445.0 ± 0.5	2.5 ± 0.2	449.1 ± 33.9	280.8 ± 10.7
120°C, 24%	423.8 ± 0.5	2.5 ± 0.3	448.1 ± 38.1	296.0 ± 6.5
150°C, 20%	222.1 ± 0.0	3.0 ± 0.3	248.5 ± 18.7	160.2 ± 27.7
150°C, 24%	266.9 ± 76.2	3.5 ± 0.3	271.3 ± 11.0	134.9 ± 30.9
Sorghum				
120°C, 20%	681.4 ± 0.0	3.2 ± 0.4	194.4 ± 10.0	115.3 ± 14.9
120°C, 24%	431.8 ± 0.5	2.9 ± 0.3	211.0 ± 8.3	168.1 ± 8.2
150°C, 20%	567.7 ± 161.4	3.2 ± 0.2	168.4 ± 3.9	82.7 ± 4.3
150°C, 24%	432.7 ± 0.8	3.2 ± 0.3	191.3 ± 9.4	99.9 ± 6.5
Maize				
120°C, 20%	446.5 ± 0.0	4.6 ± 0.4	186.1 ± 13.9	44.3 ± 0.7
120°C, 24%	425.4 ± 0.0	3.6 ± 0.3	205.8 ± 8.8	100.7 ± 2.5
150°C, 20%	446.9 ± 0.5	3.5 ± 0.3	159.0 ± 11.0	57.7 ± 0.5
150°C, 24%	425.4 ± 0.0	3.1 ± 0.3	194.4 ± 27.4	82.0 ± 0.8

Notes:

[1]Data represent the mean from duplicate extrusion processing runs ± one standard deviation (n = 2).

[2]Data represent the mean values of 20 measurements for each duplicate extrusion processing runs ± one standard deviation (n = 40).

[3]Data represent the mean values of 3 measurements for each duplicate extrusion processing runs ± one standard deviation (n = 6).

Abbreviations: SME (Specific mechanical energy)

The expansion ratio (ER) of all three flours was found to be significantly affected by barrel temperature and moisture, as well as their interaction (Table 4.2). The significant effect of temperature-moisture interaction on expansion ratio was also reported by Yovchev et al. (2017), who extruded barley and chickpea flours at 120 and 150°C at 20 to 24% moisture content. In the case of chickpea flour in the current study, expansion ratios were found to be similar at 120°C (2.5) regardless of the moisture content, but then increased overall as the barrel temperatures increased to 150°C. At this temperature, the expansion ratio was found to increase from ~3.0 to ~3.5 as the moisture content increased from 20 to 24% (Table 4.3). However, a different trend in the ER data was observed for the two cereals than for the chickpea. For sorghum, at 120°C the expansion ratio was found to decrease from ~3.2 to ~2.9 as the moisture increased from 20 to 24%, whereas at 150°C no effect of moisture was evident (3.2). In the case of maize, ER was found to be greater at 120°C (~4.9) than at 150°C (~3.3), however the amount of decline with increased moisture at each

temperature was different. At 120°C, expansion ratios declined from 4.6 to 3.6, whereas at 150°C, expansion ratios decline less from 3.5 to 3.1 (Table 4.3). In general, under the same extrusion temperature, the increase in moisture content had a positive effect on ER of chickpea, but negative on the two cereals. This difference in ER in chickpea and cereals was also reported by Yovchev et al. (2017) who found an increase in the expansion of chickpea extrudates under higher temperature and moisture, whereas higher expansion of their cereal sample barley was obtained at lower moisture only. The authors assumed that this was the result of differences within the protein content in the flours. Onwulata and Konstance (2006) proposed that higher protein content is responsible for lower melt viscosity and thus can result in less expansion. It is also possible that chickpea with high protein content would require higher moisture and temperature to overcome the interaction between protein and starch which opposes expansion. In general, among the various reported on ER, barrel temperature, moisture, and screw speed are considered the most significant parameters (Ding et al., 2006; Meng et al., 2010; Waramboi et al., 2014). In general, increasing melt viscosity increases the ER (Ding et al., 2006). However, Meng et al. (2010) reported that at very high temperatures (>168°C), ER starts to decline with the increase of temperature. Along with Ilo et al. (1996) and Doğan and Karwe (2003), they hypothesized that there is a temperature plateau between 150 to 170°C where air bubbles within the extrudate start to rupture due to starch degradation. Also, starch composition can also affect expansion. Chinnaswamy and Hanna (1988b) reported that the higher the amylose content the greater the expansion but the highest expansion was at 50% amylose for corn starch, and beyond that expansion decreased.

In the case of hardness (HD), only the barrel temperature was found to be significant for chickpea flour, where HD decreased from ~449 to ~260 N as temperatures increased from 120 to 150°C (Table 4.3, 4.2a). In the case of both sorghum and maize flours, both temperature and moisture were found to be significant, but not their interaction (Table 4.2b,c). For both flours, HD decreased as the barrel temperatures increased from 120 to 150°C, from ~203 to ~180 N, and ~196 to ~178 N for sorghum and maize respectively (Table 4.1). HD was also found to increase as moisture increased from 20 to 24%, going from ~181 to ~201 N, and ~173 to ~200 N for sorghum and maize flours, respectively (Table 4.3). Similar results were reported by Yovchev et al. (2017), where temperature, moisture or screw speed had no significant effect on hardness of chickpea (Desi) extrudates but exit-die temperature was marginally significant ($p<0.1$). However, all three extrusion parameters had a significant effect on hardness for barley, where lower hardness was

observed under low moisture, high temperature and moderate to high screw speed. Brnčić et al. (2006) also drew the same conclusion in terms of the effects of extrusion conditions on the hardness of wheat extrudates. In general, high barrel temperature and low feed moisture led to decreased HD. This is hypothesized to be the result of greater expansion that occurs at higher temperature, where extrudates become more puffed and less dense, and therefore less hard. In this study, the drop in HD for cereals because of increased moisture is probably due to reduced elasticity of the dough through plasticization, which resulting in reduced SME and gelatinization, thus less expansion and greater HD (Ding et al., 2006). HD of chickpea was less sensitive to this than the cereal flours possibly because the chickpea flour contains more protein and crude lipid, while less starch, which is most responsible in expansion characteristics.

For bulk density (BD), the effect of temperature was found to be significant (Table 4.2a) for chickpea where density was higher at the 120^{o}C (~288 g/L) than at 150^{o}C (~148 g/L) (Table 4.3). For both sorghum and maize flours, BD was found to be significantly affected by barrel temperature and moisture, as well as their interaction (Table 4.2b,c). Overall, BD for sorghum flours was found to be greater at 120^{o}C (~142 g/L) than at 150^{o}C (~91 g/L), however at the 120^{o}C barrel temperature the magnitude of increase was greater with increases in moisture than at the 150^{o}C temperature. In the case of the former, BD increased from ~115 to ~168 g/L as moisture increased from 20 to 24%, whereas at 150^{o}C BD increased to a lesser degree raising from ~83 to ~100 g/L (Table 4.3). Such increase in bulk density was also reported by Sacchetti et al. (2004). The authors found that BD decreased from ~238 to 130 g/L as temperature increased from 85 to 125^{o}C for their chestnut (42%) and rice (58%) flour blend. A similar trend was evident in the case of maize flour in the current study, where bulk density was higher at the 120^{o}C (~73 g/L) than at the 150^{o}C temperature (~69 g/L). And that bulk density increased from ~44 to ~101 g/L at 120^{o}C as the moisture increased from 20 to 24%, whereas at 150^{o}C the increase in bulk density with moisture was less (~58 to ~ 82 g/L) (Table 4.3). In general, an increase in temperature would result in less BD due to the greater expansion occurring during extrusion. The lack of correlation between BD and ER for sorghum and maize in this study could be the difference in packing of the extrudates. At higher temperatures, water instantaneous evaporates at the die more dramatically due to the temperature differential leading to expansion. Cereals experienced a greater amount of expansion (therefore lower BD values) than the chickpea flour due to their higher content of starch which is responsible for the expansion. The negative correlation of bulk density and expansion,

and positive correlation of bulk density and hardness was also reported by Yovchev et al. (2017). The authors found that temperature and screw speed (which is constant in the current study) had a significant effect on bulk density for chickpea, whereas all three factors (temperature, moisture and screw speed) and their interactions had significant effects on barley. It has been reported by many that extrusion conditions with high barrel temperature and low feed moisture in general would produce extrudates that are highly expanded with lower bulk density and hardness (Meng et al., 2010; Hagenimana et al., 2006; Lazou and Krokida, 2010).

4.1.3 Functional properties

The functional properties for the chickpea, sorghum and maize for the raw and pre-cooked flours, and as a function of barrel temperature and moisture is given in Table 4.4 and Table 4.5. An orthogonal individual degree of freedom analysis was used to delineate differences between raw and pre-cooked flours, and among extrusion conditions [i.e., effects of temperature (120^{o}C vs 150^{o}C) and moisture (20 vs 24%) along with their interactions] for the pre-cooked flours, as it relates to the functional properties of their flours (Table 4.2).

Water hydration capacity

The water hydration capacity (WHC) for all pre-cooked flours were found to significantly increase relative to raw flours (Table 4.2). For instance, WHC increased from ~2.1 to ~4.2 g/g for chickpea flour, from ~2.1 to ~5.4 g/g for sorghum flour, and from ~1.8 to ~6.2 g/g for maize (Table 4.4). WHC for all pre-cooked flours were found to be significantly affected by barrel temperature, moisture and their interaction (Table 4.2). For all flours cooked at the 120^{o}C barrel temperatures, WHC was found to decrease slightly as the moisture values increased. For instance, WHC decreased from ~4.3 to ~4.1 g/g for chickpea, from ~5.4 to ~5.0 g/g for sorghum and from ~6.0 to ~5.6 g/g for maize as moisture increased from 20 to 24% (Table 4.4). As barrel temperature increased to 150^{o}C, the opposite trend with increasing moisture was observed for all flours. For instance, WHC was found to increase from ~4.0 to ~4.3 g/g for chickpea, from ~5.6 to ~5.7 g/g for sorghum, and from ~6.6 to ~6.7 g/g for maize with the increase of moisture (Table 4.4). A similar phenomenon has been reported by Alonso et al. (2000) and Martínez et al. (2014). For instance, Alonso et al. (2000) reported that extrusion (148^{o}C to 150^{o}C, 25% moisture) increased the WHC from 1.24 to 2.86 g/g for peas and 2.00 to 2.93 g/g for kidney beans. And, Martínez et

al. (2014) reported progressive increase in water binding capacity and swelling with the increase of extrusion intensity, specifically the increase of temperature and decrease in moisture content. The increase in WHC after extrusion is thought to be associated with both protein and starch. Alonso et al. (2000) suspected that physical retention of water through capillary action in the new structure formed by aggregation of proteins probably plays the major role in the increased WHC. Camire et al. (1990) suggested that a poorly ordered molecular phase with hydroxy groups in the disrupted starch granules can bind water readily. Therefore, the gelatinization and disruption of starch granules during extrusion would allow water to bind easily upon rehydration. It was also postulated that protein with high molecular weight such as 7S and 11S (Naismith, 1955), which are the main constituents in chickpea, can dissociate upon heating, and possibly resulting in protein subunits with more water binding sites than the oligomeric protein (Narayana and Narasinga Rao., 1982). Although protein have an important role in WHC, its contribution could be hampered by extrusion processing. Since extrusion exposes hydrophobic protein residues, it is likely that pre-cooked starch is more hydrophilic in comparison. The pre-cooked cereal flours showed a greater increase in WHC than that of the chickpea flour in the current study, possibly because the latter has less starch than the cereals, and more protein and fat. Water and temperature play important roles in starch gelatinization, which affects WHC. Typically, higher moisture results in reduced viscosity and less energy input into the melt, thus lower temperatures. In return, starch gelatinization might be interfered at a high moisture and low temperature condition and results in reduced WHC. However, the negative effect of increased moisture content could be reversed under high temperature, which in return assists starch gelatinization.

Table 4.4 Functional properties of raw and pre-cooked flour of chickpea, sorghum and maize. The pre-cooked flour represents a composite of two extrusion runs. Data represent the mean of triplicate measurements on the composite flour ± one standard deviation (n = 3).

Flour	WHC (g/g)	OHC (g/g)	Emulsion Activity (%)	Emulsion Stability (%)	Foaming Activity (%)	Foaming Stability (%)	NSI (%)
Chickpea							
Raw flour	2.13 ± 0.17	1.37 ± 0.06	50 ± 0	55 ± 0	250 ± 16	3 ± 0.0	16.2 ± 0.2
Pre-cooked flour							
120ºC, 20%	4.26 ± 0.09	1.28 ± 0.05	44 ± 0	45 ± 0	ND	ND	NT
120ºC, 24%	4.10 ± 0.02	1.33 ± 0.03	46 ± 0	40 ± 0	ND	ND	NT
150ºC, 20%	4.02 ± 0.11	1.17 ± 0.03	45 ± 0	41 ± 0	ND	ND	NT
150ºC, 24%	4.31 ± 0.04	1.19 ± 0.03	47 ± 0	41 ± 0	ND	ND	2.3 ± 0.0
Sorghum							
Raw flour	2.13 ± 0.03	1.45 ± 0.07	38 ± 0	48± 0	113 ± 1	7 ± 0.0	2.7 ± 0.1
Pre-cooked flour							
120ºC, 20%	5.35 ± 0.06	1.39 ± 0.03	46 ± 0	40 ± 0	ND	ND	NT
120ºC, 24%	4.99 ± 0.04	1.45 ± 0.01	48 ± 0	38 ± 0	ND	ND	NT
150ºC, 20%	5.56 ± 0.01	1.51 ± 0.05	44 ± 0	42 ± 0	ND	ND	NT
150ºC, 24%	5.74 ± 0.05	1.51 ± 0.01	47 ± 0	39 ± 0	ND	ND	0.5 ± 0.0
Maize							
Raw flour	1.78 ± 0.01	1.51 ± 0.07	45 ± 1	40 ± 0	ND	ND	2.8 ± 0.1
Pre-cooked flour							
120ºC, 20%	5.98 ± 0.04	1.40 ± 0.07	51 ± 0	51 ± 0	ND	ND	NT
120ºC, 24%	5.62 ± 0.08	1.34 ± 0.06	43 ± 0	44 ± 1	ND	ND	NT
150ºC, 20%	6.58 ± 0.02	1.44 ± 0.04	51 ± 1	52 ± 0	ND	ND	NT
150ºC, 24%	6.65 ± 0.03	1.34 ± 0.07	48 ± 0	44 ± 0	ND	ND	1.5 ± 0.1

Notes:

Abbreviations: WHC (water hydration capacity), OHC (oil holding capacity), NSI (Nitrogen solubility index), ND (not detected), and NT (Not tested).

Table 4.5 Pasting properties of raw and pre-cooked flours of chickpea, sorghum and maize. The pre-cooked flour represents a composite of two extrusion runs. Data represent the mean of triplicate measurements on the composite flour ± one standard deviation (n = 3).

Flour	Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting Temperature (ºC)
Chickpea						
Raw flour	879.0 ± 25.2	836.0 ± 13.1	43.0 ± 13.1	1163.0 ± 26.1	327.0 ± 15.6	85.0 ± 0.3
Pre-cooked flour						
120ºC, 20%	130.0 ± 22.6	75.3 ± 1.5	54.7 ± 22.4	113.0 ± 1.0	37.7 ± 1.5	57.6 ± 8.1
120ºC, 24%	124.3 ± 12.7	102.0 ± 8.7	22.3 ± 4.5	169.3 ± 9.3	67.3 ± 0.6	54.8 ± 1.5
150ºC, 20%	133.0 ± 20.8	72.0 ± 1.0	61.0 ± 19.9	113.0 ± 41.0	41.0 ± 2.6	54.0 ± 2.7
150ºC, 24%	122.7 ± 15.0	94.0 ± 1.7	28.7 ± 13.3	161.0 ± 1.7	67.0 ± 0.0	60.2 ± 1.4
Sorghum						
Raw flour	2,286.7 ± 4.0	1,450.0 ± 60.7	836.7 ± 60.9	4,377.3 ± 156.5	2,927.7 ± 149.4	87.1 ± 0.1
Pre-cooked flour						
120ºC, 20%	206.0 ± 0.0	142.0 ± 0.0	64.0 ± 0.0	153.7 ± 1.5	11.7 ± 1.5	ND
120ºC, 24%	142.0 ± 1.0	135.3 ± 1.5	6.7 ± 0.6	138.3 ± 0.6	3.0 ± 1.0	ND
150ºC, 20%	200.3 ± 1.5	121.0 ± 1.0	79.3 ± 0.6	158.0 ± 2.0	37.7 ± 2.1	ND
150ºC, 24%	289.0 ± 2.0	179.7 ± 1.2	109.3 ± 1.2	229.0 ± 1.0	49.3 ± 0.6	ND
Maize						
Raw flour	2,170.0 ± 15.5	1,287.7 ± 11.7	882.3 ± 17.2	3,840.7 ± 57.6	2,553.0 ± 51.4	76.7 ± 0.0
Pre-cooked flour						
120ºC, 20%	377.7 ± 4.9	51.3 ± 0.6	326.3 ± 4.7	85.3 ± 1.5	34.0 ± 1.0	ND
120ºC, 24%	243.3 ± 2.1	81.3 ± 0.6	162.0 ± 1.7	122.0 ± 1.7	40.7 ± 1.5	ND
150ºC, 20%	438.7 ± 6.7	56.3 ± 0.6	382.3 ± 7.1	89.3 ± 0.6	33.0 ± 0.0	ND
150ºC, 24%	359.0 ± 24.3	62.3 ± 2.1	296.7 ± 22.4	120.3 ± 6.4	58.0 ± 4.4	ND

Notes:
Abbreviation: cP (centipoise = millipascal * second, mPa·s) and ND (not detected).

Oil holding capacity

The oil holding capacity (OHC) was found only significantly different for sorghum before and after extrusion (Table 4.2, 4.4). An orthogonal individual degree of contrast analysis found that for chickpea and sorghum flours, only the effect of barrel temperature was significant (Table 4.2a,b). OHC was found to decrease slightly as barrel temperatures were increased from 120 to 150^{o}C in the case of chickpea flour, whereas the opposite trend occurred for sorghum where OHC increased slightly as barrel temperatures increased (Table 4.4). In contrast, only the moisture content significantly impacted OHC of pre-cooked maize flours where OHC decreased slightly as moisture increased (Table 4.2c, 4.4). The overall negative effect of extrusion on OHC in this study was also observed by Alonso et al. (2000), who reported the decrease in oil absorption capacity in kidney bean from 1.34 to 1.00 g/g after extrusion at 115 to 135^{o}C and 10 to 18% moisture content. The decrease in OHC indicates the overall drop in hydrophobicity of samples after extrusion, during which protein denaturation, aggregation and hydrophobic group interaction could happen (Li and Lee, 1996). On the other hand, Narayana and Narasinga Rao (1982) reported that OHC of heated winged bean flour increased from 1.4 to 2.2 g/g. The contradictory results indicate that the more severe condition, especially the shear effect during extrusion, decreased the ability of protein to bind lipid.

Emulsification

Emulsification activity (EA) for all raw and pre-cooked flours were found to be significantly different (Table 4.2). In the case of chickpea flour, EA was lowered from 50% to ~46% after extrusion, whereas sorghum and maize both had an increase after extrusion from 38 to ~46%, and from ~45 to ~48%, respectively (Table 4.4). This increase in EA for cereal flours might be contributed to their lower crude lipid content and higher starch levels. Kasprzak et al. (2018) found that at least three types of non-chemically gelatinized starches (waxy rice, non-waxy rice and waxy maize starch) exhibited emulsifying capacity, and the ability for interfacial adsorption of these starches are independent from their crystallinity or amylose content. For all pre-cooked flours in the current study, EA was found to be significantly affected by moisture, barrel temperature and their interaction (Table 4.2). For pre-cooked chickpea flour, EA was found to increase at 120^{o}C from 44 to 46% as moisture increased from 20 to 24%, and at 150^{o}C from 45 to 47% with the same increase in moisture (Table 4.4). For pre-cooked sorghum flour, EA was found

to increase at 120^{o}C from 46 to 48% as moisture was increased from 20 to 24%, and at 150^{o}C from 44 to 47% (Table 4.4). In contrast for pre-cooked maize flour, EA was found to decrease at 120^{o}C from 51 to 43% and 51 to 48% at 150^{o}C as moisture increased from 20 to 24% (Table 4.4). This opposite trend in EA values in response to increased moisture in the case of maize relative to chickpea and sorghum flours may be the result of extrusion damage to natural gums present in the maize which act as emulsifiers like corn fiber gum (Singkhornart et al., 2013). The EA for chickpea in this study is similar to that was reported by Bai et al (2018) 44 to 47%, who studied the effect of infrared heating on functionality of Desi chickpea and hull-less barley. However, they reported an increase in EA of chickpea after heat treatment in contrary to the current result. Many related studies on emulsion were mostly focused on protein concentrates or isolates (Manoi and Rizvi, 2009; Lam et al., 2008; Liu et al., 2009). For example, Bueno et al. (2009) investigated the effect of extrusion on the emulsifying properties of soybean proteins and pectin mixtures, and they reported a 41% increase in emulsion capacity of the mixtures. Emulsion activity is mostly affected by protein solubility and hydrophobicity (Torrezan et al., 2007). A popular hypothesis describes that extrusion denatures proteins leading to improved adsorption of protein molecules at the interface resulting from more exposure of hydrophobic groups, resulting in improved emulsification properties. On the other hand, protein aggregation and decrease in protein solubility because of processing can negatively affect emulsion properties (Mirmoghtadaie et al., 2016). Wang et al. (2008) reported that protein aggregation can lead to reduced protein molecule flexibility. Karaca et al. (2011) found that emulsion activity of legume protein isolates was positively correlated with protein solubility, which increases with protein surface charge, but negatively with surface hydrophobicity as less protein can be solubilized. Therefore, it is reasonable to postulate that a certain balance should be met between surface hydrophobicity and protein solubility to obtain improvement in emulsion properties of different samples.

Emulsification stability (ES) for all raw and pre-cooked flours were found to be significantly different ($p<0.001$) (Table 4.2). In the case of chickpea and sorghum flours, ES was lowered from ~55% to 42%, and 48% to 46% respectively after extrusion, whereas maize flour showed the opposite trend where ES was found to be increased from 40% to 48% after extrusion (Table 4.4). An orthogonal individual degree of contrast analysis found that for pre-cooked chickpea and sorghum flours, ES was found to be significantly affected by moisture, barrel temperature and their interactions (Table 4.2a,b). Pre-cooked maize flour was only affected by the

moisture content (Table 4.2c). ES for pre-cooked chickpea flour at 120^{o}C decreased from 45% to 40% as the moisture increased from 20 to 24%, and ES was independent of moisture at 150^{o}C (41%) (Table 4.4). In the case of pre-cooked sorghum flour, ES at both 120^{o}C and 150^{o}C decreased from 40 to 38% and 42 to 39% respectively as moisture increased from 20 to 24% (Table 4.4). For pre-cooked maize flour, as the moisture content increased from 20 to 24% ES also decreased from ~52% to 44% (Table 4.4). Overall, increase in moisture had negative effects on ES for the cereal flours hypothesized because of reduced friction and interaction between molecules that help stabilize the emulsion. Little information was found on ES of extruded flours. Bai et al. (2018) mentioned above reported that ES of their infrared-heated chickpea and hull-less barley both increased, which is in contrary with the result in this study with the exception of maize. And the shear effect during extrusion is a possible cause of such difference. Since the protocol for ES in this study involves prolonged heating (85^{o}C) and cooling, it is likely that the starch in the emulsion layer formed a gel, which can undergo retrogradation upon cooling. This might explain why maize flours showed increased ES, as less retrogradation and syneresis can happen with the presence of corn fiber gum (Qiu et al., 2017), thus less decrease in the emulsion layer overall.

Foaming

Foaming activity and stability was measured for all raw and pre-cooked flours. Only raw chickpea and sorghum produced foams. Raw chickpea flour had a foaming activity and stability of 250% and 3%, respectively, whereas raw sorghum flour had values of 113% and 7%, respectively (Table 4.4). Maize flour and all its pre-cooked flours did not form foams. Foaming activity for unprocessed chickpea (~217%) and sorghum (~117%) reported by Bai et al. (2018) and Elbaloula et al. (2014), respectively is generally in agreement with the results from this study. Akubor and Onimawo (2003) reported that FA for maize flour was 4%, the small increase in FA compared to the current results could be due to the higher protein content (~9%) of their maize flour. Foaming stability in the current study was measured as percentage of loss in volume. The 3 and 7% of loss indicated that the foam of raw chickpea and sorghum, respectively, were relatively stable. Proteins can lower interfacial tension and help stabilize the foam. In order to have good foam formation, proteins must be highly soluble in water and able to migrate, unfold and rearrange themselves to form a cohesive film at the water-air interface (Wagner and Gueguen, 1999). However, if protein solubility is poor, less protein can migrate to the interface and no stable foam

can be formed and measured. Decreased foaming capacity has been reported for tempered chickpea (20% moisture) flour when heated at 115 and 135^{o}C (Bai et al., 2018). The absence in foaming activity and stability for all the pre-cooked flours in this study implies that extrusion likely rendered a combination of low protein solubility, protein denaturation and thus poor protein migration to the air-water interface. It was also reported that although there was a positive correlation between protein hydrophobicity and the emulsifying capacity of protein, such correlation was absent in foaming capacity of the protein (Townsend and Nakai, 1983). Since the interfacial tension in foam system (air-water) is much greater than that in emulsion (oil-water) system (Sengupta and Damodaran, 1998), proteins are denatured to greater extent to expose the buried hydrophobic residues for overcoming the higher free energy at air-water interface (Damodaran, 2005). We postulate that protein aggregation and protein complexes formed during extrusion made it more difficult for extensive protein exposure that is needed in foam formation, and thus the absence of foam in all the extruded samples.

Nitrogen solubility index

The nitrogen solubility index (NSI), an important indication for protein solubility, was tested for raw chickpea, sorghum and maize, as well as their extrudates treated at 150^{o}C and 24% moisture content, only (Table 4.4). Findings indicates that raw chickpea flour showed the greatest NSI (16.2%), followed by sorghum (2.7%) and maize (2.8%). The NSI for all pre-cooked flours decreased greatly, NSI decreasing to 2.3, 1.5 and 0.5 for pre-cooked chickpea, maize and sorghum flours, respectively (Table 4.4). This decrease in nitrogen solubility was also reported by Dahlin and Lorenz (1993) after extrusion with sorghum and corn, and Carbonaro et al. (1997) after cooking chickpea and other legumes. Nwabueze (2007) also reported more than a 50% decrease in NSI of corn-soy-African breadfruit blends after extrusion. The greater nitrogen solubility of the chickpea flours is expected as legume proteins are dominated by salt-soluble globulin (~70%) and water-soluble albumin (~10-20%) type proteins with only minor amounts of alcohol-soluble prolamin type proteins (Roy et al., 2010 and Papalamprou et al., 2010) which is the dominate protein in cereals (Giuberti et al., 2011). Electrostatic interaction between oppositely charged amino acids and hydrophobic interactions are two of the most important mechanisms responsible for low solubility in protein (Carbonaro et al., 1997). According to Bigelow (1967), greater solubility is generally obtained by proteins with lower average hydrophobicity and higher surface

charge. Extrusion cooking denatured protein and decreased NSI/protein solubility of the samples in this study, which in return affected functional properties by altering the surface properties of proteins (Schwenke, 2001).

Pasting properties

The pasting properties of all raw and pre-cooked flours were assessed in the Rapid Visco Analyser (RVA). During the test the following viscosities were measured: peak viscosity (PV; *the viscosity at full gelatinization*), trough/holding viscosity (TV; the *hot paste viscosity at the end of the temperature holding period*), breakdown viscosity (BV; the *viscosity difference between peak and trough viscosities*), final viscosity (FV; *the viscosity at the end of cooling period*) and setback viscosity (SV; *the difference between final and trough viscosity*).

All viscosities measured found that raw flours were significantly higher ($p<0.001$) than the pre-cooked flours except for the BV of chickpea ($p>0.05$) as a result of small difference in peak and trough viscosity (Tables 4.2a,c, 4.5; Figure 4.1). Whalen (2007) reported that the thermal effect in cooking such as extrusion generally results in lowering of the RVA profile, especially in the peak, through and final (setback) viscosities due to starch degradation. Figure 4.1 demonstrated the substantial decrease well. Unlike the raw flours that showed viscosity peaks, all the extruded flours, regardless of processing conditions, showed no obvious peak. Maize showed a higher cold viscosity in extruded products compared to unprocessed flours, which is commonly observed in extruded starch as they can hydrate and entangle more rapidly to increase viscosity (Whalen, 2007; Mitrus et al., 2017). In fact, although not shown clearly in Figure 4.1 according to the raw data the viscosities for all the extruded cereal flours before the heating stage were greater compared to the respective raw sample, which means just as the extruded maize flours, extruded chickpea and sorghum flours also had greater cold viscosity than their raw.

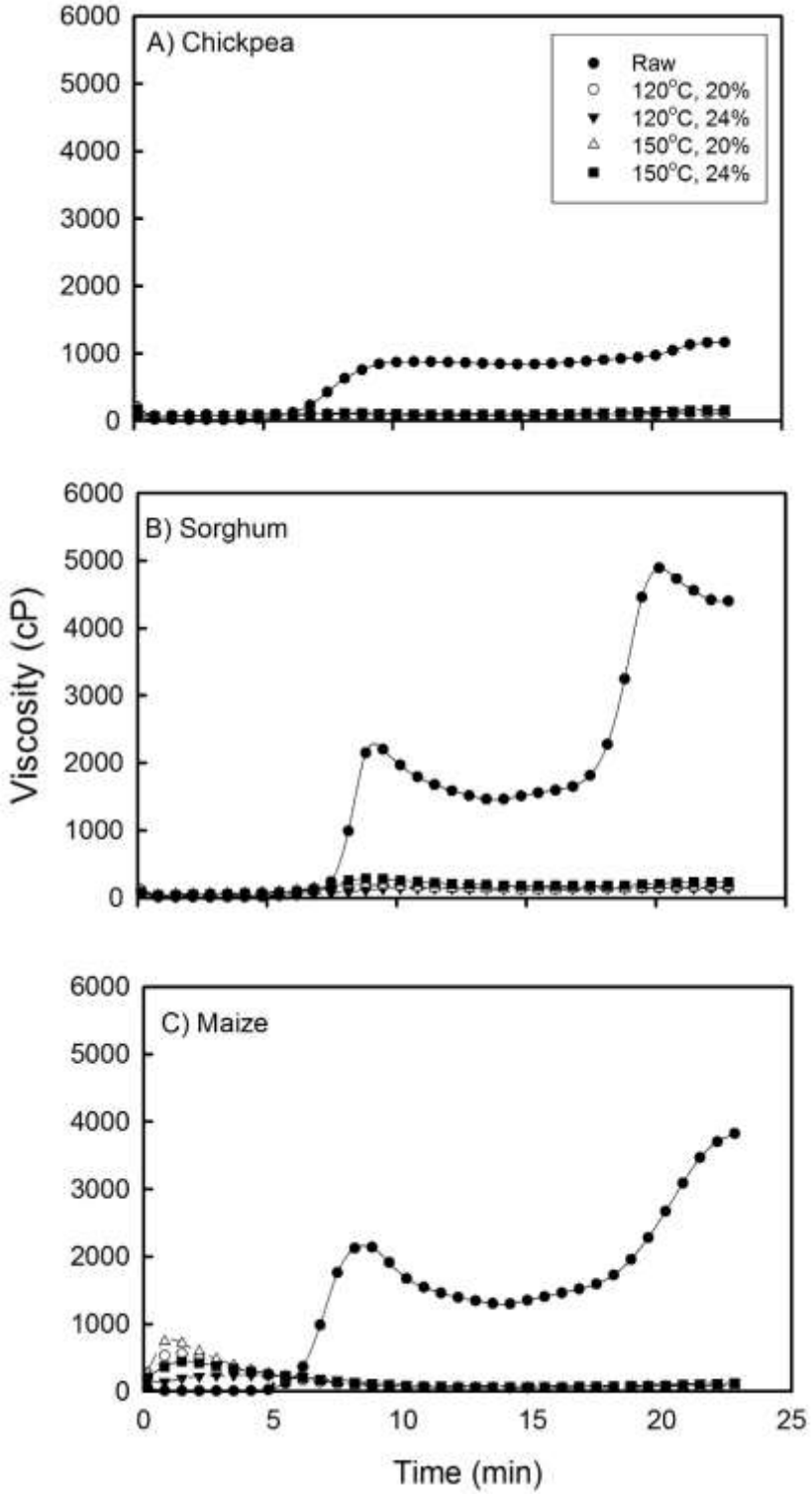

Figure 4.1 RVA profile for raw and extruded chickpea (A), sorghum (B) and maize (C) flours (screw speed: 317 rpm; feed rate: 14 kg/h).

For the raw flours, sorghum had the highest peak viscosity (2287 cP), followed by maize (2170 cP) and chickpea (879 cP). According to Figure 4.1, starch granules in cereals swelled faster and reached greater peak viscosity than that of raw chickpea. In the case of the pre-cooked flours, maize also had the highest PV (355 cP), followed by sorghum (209 cP) and chickpea (128 cP), but were lower than the raw flours. The much lower viscosities of chickpea compared to the cereal flours in this study is most likely caused by the higher protein and lower starch composition since the hydration and swelling of chickpea starch can be hindered as more starch granules are embedded in the protein matrices of the flour (Otto et al., 1997). There are two main mechanisms involved in the complete cooking of starch under limited water (<35%) condition such as extrusion: a) melting under water-limiting conditions and b) gelatinization in the presence of water, both of which convert starch structure from crystalline to amorphous (Wang, 1993). Waigh et al. (2000a,b) reported that when water was added from 5 to 40%, starch "cooking" proceeds in two steps: first, amylopectin helix dislocation (breaking), then melting (helix-coil transition) as helices of amylopectin unwind and gel formation. The higher PV of pre-cooked maize compared to sorghum flour could mean that extrusion cooking degraded more sorghum starch than that of maize. The effect of moisture, temperature, and their interaction were significant on PV for the pre-cooked cereal flours ($p<0.001$), but none of the extrusion conditions influenced PV for the pre-cooked chickpea flour ($p>0.05$) (Table 4.2). At 120^{o}C, PV decreased from 206 to 142 cP for sorghum, and 378 to 243 cP for maize as moisture increased from 20 to 24%. At 150^{o}C, PV increased from 200 to 289 cP for sorghum but decreased from ~439 to 359 cP for maize as moisture increased. It was reported that extruded corn starch could melt at 168^{o}C without the presence of water, and at 123^{o}C at 20% water content, and as low as 73^{o}C when moisture content is 60% (Souza and Andrade, 2002), which indicates that both melting and gelatinization happened during extrusion in the premise of the current study. Thus, the general decrease of PV at higher moisture content could be due to greater hydration and gelatinization with more water.

For the raw flours, trough viscosity was found highest for sorghum (1450 cP), followed by maize (1288 cP), and chickpea (836 cP). However, in the case of the pre-cooked flours, TV was found to be highest in sorghum (~145 cP), followed by chickpea (~86 cP) and maize (~63 cP). For chickpea, only the effect of temperature significantly ($p<0.001$) affected TV (Table 4.2a), where TV increased from ~139 to 150 cP as temperature increased (Table 4.5). The effect of moisture ($p<0.001$), temperature ($p<0.001$ for sorghum, and $p<0.05$ for maize) and their interaction

($p<0.001$ for sorghum, $p<0.01$ for maize) were significant on TV for both cereals (Table 4.2b,c). At 120^{o}C, TV decreased from ~142 to 135cP for sorghum but increased from ~51 to 81cP for maize as moisture increased. At 150^{o}C, TV increased for both sorghum from ~121 to 180 cP and maize from ~56 to 62 cP with the increase of moisture. In general, increases in moisture at each temperature increased TV with the exception of sorghum extruded at 120^{o}C. Since TV is usually the holding viscosity at the end of the holding stage at maximum temperature (Batey, 2007), raw chickpea flour is a better choice in application for food with consistent viscosity during heating.

In the case of breakdown viscosities, values decreased between the raw and pre-cooked flours from 882 to 292 cP, and 837 to 65 cP for maize and sorghum flours, respectively. For chickpea flour however, BV was similar between the raw and pre-cooked flours (~42 cP). Low BV of raw flours are often associated with lower hydration, swelling power, and high shear resistance (Shafie et al. 2016). The greater BV in cereal flours compared to chickpea is possibly due to the greater water hydration capacity (Table 4.4). Furthermore, on a molecular level, the higher protein and lower starch composition in chickpea may impede hydration and swelling (Otto et al., 1997). The effect of moisture, temperature, and their interaction had significant effects on the BV for the pre-cooked sorghum and maize ($p<0.001$) (Table 4.2b,c), whereas only moisture was significant for chickpea ($p<0.01$) (Table 4.2 a). At 120^{o}C, BV for chickpea, sorghum, and maize decreased from 55 to 22cP, ~64 to 7 cP and 326 to 162 cP, respectively as moisture increased from 20 to 24%. At 150^{o}C as moisture increased, BV decreased for both maize (from ~382 to 299cP) and chickpea (from ~61 to 29cP) but increased for sorghum from ~79 to 109cP. This increase in BV of sorghum aligns with PV under the same condition.

Final viscosity for the raw flours was found highest for sorghum (4377 cP), followed by maize (3840 cP) and chickpea (1163 cP). The higher FV of the raw flours compared to PV is the result of retrogradation due to gel formation, which involves the entanglement of glucan chains upon cooling. Normally greater entanglement occurs with higher amylose content, but in waxy starches (high in amylopectin) the relatively short and branched chains prevents such association that happens more readily between the unbranched long amylose (Batey, 2007). In the case of the pre-cooked flours, FV was found to be highest in sorghum (170 cP), followed by chickpea (139 cP) and maize (104 cP). The effect of moisture, temperature and their interaction were significant on FV of sorghum ($p<0.001$), but only moisture had a significant effect on chickpea ($p<0.001$) and maize ($p<0.05$) (Table 4.2a,c). For sorghum, FV decreased from ~154 to 138 cP at 120^{o}C, while

increased from ~158 to 229 cP at 150^{o} with the increase of moisture. For chickpea and maize, FV increased with the increase of moisture. FV increased from ~113 to 165 cP for chickpea and ~87.3 to 121 cP for maize as moisture increased from 20 to 24% (Table 4.5). The trend in FV agrees with that in TV.

Set back viscosity, the difference between TV and FV, for the raw flours was found highest for sorghum (2928 cP), followed by maize (2553 cP) and chickpea (327 cP). However, in the case of the pre-cooked flours, SV was found to be highest in chickpea (53 cP), followed by maize (41 cP) and sorghum (25 cP) (Table 4.5). Moisture, temperature and their interaction had a significant effect on SV for maize ($p<0.001$) (Table 4.2c); for chickpea, only the effect of moisture was significant ($p<0.001$) (Table 4.2a); the extrusion conditions did not affect SV for sorghum ($p>0.05$) (Table 4.2b). SV for chickpea increased from 38 to 67 cP at 120^{o}C, and from 41 to 67 cP at 150^{o}C as moisture increased from 20 to 24%. For maize, SV increased from 34cP at 20% moisture to 49 cP at 24% moisture. The higher moisture content seemed to result in higher setback in chickpea and maize flours. This could be the result of greater starch hydration and swelling, thus greater retrogradation. Since raw starch is the main reason for viscosity development during cooling (Ozcan and Jackson, 2005), and large SV was only observed in the raw flours and absent in the pre-cooked ones. As such, it was postulated that extrusion degraded most of the starch in the flours.

The pasting temperature for the raw flours is the highest in sorghum (~87^{o}C), followed by chickpea (~85^{o}C) and maize (~77^{o}C) (Table 4.5). In general, the lower the pasting temperature, the faster the starch hydration. Sorghum is usually grown in semi-arid area, which means starch granules in the flour would hydrate more slowly due to the thick protein matrix surrounding them (Griess et al., 2011). For the pre-cooked samples, pasting temperature was only detectable for chickpea, but not for the pre-cooked cereal flours (Table 4.5). Extrusion significantly lowered pasting temperature for chickpea compared to the raw ($p<0.01$) from ~85^{o}C to 57^{o}C, and extrusion conditions were not significant ($p>0.05$) (Table 4.2a). The reason for the undetectable pasting temperature for the cereal flours could be that the degraded cereal starch developed instant viscosity at the very beginning and at very low temperature (<20^{o}C) that is below the detection limit of the instrument, thus the inability to identify. The higher pasting temperature in the pre-cooked chickpea could be the result of higher amylose and lipid content (Jane et al., 1999). It is only reasonable that the pasting temperature were lowered more significantly for the cereal flours compared to that of chickpea because of the greater degradation of starch, reflected by their

viscosity profiles, according to Figure 4.1. Despite of the statistical significance of extrusion conditions on viscosities reported in this study, a general trend in Figure 4.1 shows that all the pre-cooked flours exhibit almost no viscosity compared to their raw counterparts. Such pasting properties of the pre-cooked flours enable them to be in instantized hot or cold beverages or porridges or be incorporated into raw flours at different ratios to achieve the desired product functionality. The production of Tortilla chips, for example, incorporated both raw and degraded starch for better expansion and bubble formation (Lanner et al., 2003).

Thermal properties

The thermal properties of raw and pre-cooked flours extruded under the most minimally processed conditions used in this study (120^{o}C and 20% moisture) were analyzed in the differential scanning calorimeter (DSC). Results in Figure 4.2 showed peaks for all the raw flours in the temperature range associated with gelatinization, with maize flour taking up more heat, followed by sorghum and chickpea. No peak was observed for the pre-cooked flours. Since starch is the major contributor to heat capacity increment (Noel and Ring, 1992), the flour containing more starch is expected to take up more heat during gelatinization. The complete absence of peak for the pre-cooked flours in Figure 4.2 indicates that extrusion processing pre-gelatinized/melted all starch that is detectable by rupturing the crystalline regions of the starch granules through shear and heat (Davidson et al., 1984; Gomez and Aguilera, 1983).

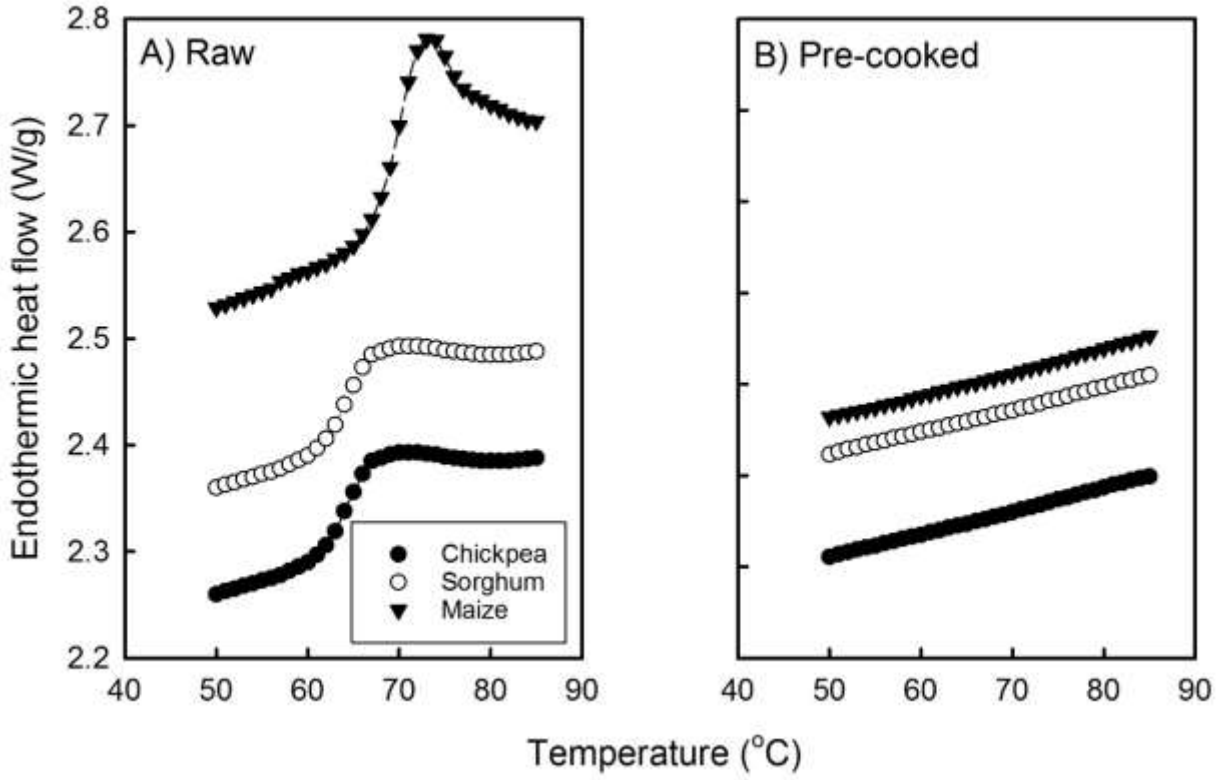

Figure 4.2 DSC thermograms of (A) raw and (B) pre-cooked chickpea, sorghum and maize flours (Extrusion temperature: 120°C; screw speed: 317 rpm; moisture content: 20%; feed rate: 14 kg/h).

4.1.4 Protein quality of raw chickpea, sorghum and maize flours

Protein quality for the chickpea, sorghum and maize for the raw and pre-cooked materials, and as a function of extrusion temperature and moisture is given in Table 4.6. *In vitro* protein digestibility (IVPD) of pre-cooked chickpea ($p<0.001$) and maize ($p<0.05$) flours were significantly higher compared to their raw counter parts, which increased from 77 to 81%, and from 73 to 76%, respectively; IVPD for sorghum did not change significantly ($p>0.05$) before and after extrusion, remaining similar at 73% (Tables 4.2 and 4.6). Temperature ($p<0.001$) and moisture ($p<0.01$) both had significant effects on IVPD for pre-cooked chickpea flours, but neither on sorghum and maize. The IVPD for chickpea was slightly higher at 150°C (82%) than 120°C (80%), and higher at 20% moisture (81%) than 24% (80%) (Table 4.6). The true protein digestibility (85%) of cooked Kabuli chickpea reported by Nosworthy et al. (2017) was also above

80%, but slightly higher than the extruded chickpea in this study. However, different result was reported for the effect of extrusion conditions on IVPD of the cereals compared to this research.

Table 4.6 Amino acid scores and protein quality data of raw and pre-cooked flours of chickpea, sorghum and maize. The pre-cooked flour represents a composite of two extrusion runs.

Flour	Limiting amino acid	Limiting amino acid score[1]	IVPD[2]	IV-PDCAAS[3] (%)
Chickpea				
Raw flour	THR	0.90	76.88 ± 0.31	69.38 ± 0.28
Pre-cooked flour				
120°C, 20%	VAL	0.84	80.38 ± 0.73	67.14 ± 0.61
120°C, 24%	VAL	0.84	79.05 ± 0.54	66.70 ± 0.46
150°C, 20%	VAL	0.86	82.86 ± 0.54	71.09 ± 0.47
150°C, 24%	VAL	0.87	81.53 ± 0.42	71.10 ± 0.36
Sorghum				
Raw flour	LYS	0.29	73.56 ± 0.46	21.33 ± 0.13
Pre-cooked flour				
120°C, 20%	LYS	0.31	73.08 ± 0.31	22.66 ± 0.10
120°C, 24%	LYS	0.30	73.56 ± 0.21	22.07 ± 0.06
150°C, 20%	LYS	0.26	73.74 ± 0.82	19.17 ± 0.21
150°C, 24%	LYS	0.30	74.11 ± 1.06	22.23 ± 0.32
Maize				
Raw flour	LYS	0.48	72.66 ± 1.56	34.88 ± 0.75
Pre-cooked flour				
120°C, 20%	LYS	0.47	76.10 ± 0.46	35.77 ± 0.21
120°C, 24%	LYS	0.51	75.98 ± 0.36	38.75 ± 0.18
150°C, 20%	LYS	0.38	76.04 ± 0.55	28.89 ± 0.21
150°C, 24%	LYS	0.49	75.55 ± 0.38	37.02 ± 0.18

Notes:

[1]Measurements were preformed once on the composite blend from two extrusion processing runs.

[2]Measurements were performed in triplicate on the composite blend from two extrusion processing runs. Data represent the mean ± one standard deviation (n = 3).

[3]Data represents the product of the limiting amino acid score and IVPD (measured in triplicate). Data represent the mean ± one standard deviation (n = 3).

Abbreviations: THR (Threonine), VAL (Valine) and LYS (Lysine), IVPD (*In vitro* protein digestibility), and IV-PDCAAS (*In vitro* protein digestibility corrected amino acid score)

Fapojuwo et al. (1987) reported that temperature was the key extrusion parameter that influenced sorghum IVPD. But the three temperature conditions (50, 125 and 200^{o}C) they chose had a much bigger gap compared to ours (120 and 150^{o}C). In addition, they only used one enzyme, pepsin to evaluate IVPD, which is comparatively less accurate than the trypsin-chymotrypsin-protease system in determining protein digestibility. The insignificant change in IVPD observed in sorghum in the current study could be explained by the greater reduction in protein solubility according to the NSI results in section 4.1.3.

Improvement in IVPD after extrusion have been reported by Day and Swanson (2013), El-Hady and Habiba (2003), Milán-Carrillo et al. (2002), Colonna et al. (1989) and Bhattacharya et al. (1988). The increase of IVPD in chickpea and maize in this study is likely contributed by the inactivation of bioactive compounds (polyphenols), enzyme inhibitors (trypsin and chymotrypsin inhibitors) and better protein exposure after extrusion, which created easier access for enzyme digestion (Bai et al., 2018; Colonna et al., 1989). Bai et al. (2018) found that even with treatment like infrared heating that has no shearing effect, trypsin inhibitor activity of chickpea flour declined from ~16.3 to 9.3 TIU/mg of flour (d.b.) when heated at 135^{o}C; and chymotrypsin inhibitor in chickpea was also lower significantly from 11.2 to 4.38 CIU/mg of flour under the same condition. Sharma et al. (2012) also reported decrease in total phenolic and condensed tannins after extrusion at 150 and 180^{o}C at 15 and 20% moisture. Tannins have been reported to form less digestible complexes with protein and is capable of precipitating more than 12 times its own weight of protein (Butler et al., 1984), and phenolic acids and flavonoids may be oxidized to quinones and form peroxides that could bring about polymerization of proteins due to oxidation of amino acids (Damodaran, 1996). Therefore, it is reasonable to see improved protein digestibility after extrusion cooking. According to the results in this study, extrusion temperature and moisture did not have an effect on protein digestibility of sorghum and maize flours but did show slight advantage at more severe extrusion condition (150^{o}C and 20% moisture) for chickpea flour.

The full amino acid composition of raw and pre-cooked flours reported in grams per 100 g flour is given in Appendix A (Table A.1); the essential amino acid concentration in milligram per gram protein, along with the FAO reference pattern is given in Table A.2; and the amino acid scores for each essential amino acid is given in Table A.3. According to Table A.2, the limiting amino acid for raw chickpea flour was threonine. Although the sulfur containing amino acids (methionine and cysteine) were the second limited, they are only 2 mg/g of protein short compared

to the FAO reference. All the other essential amino acids of chickpea were equal or much above the reference level. Threonine being the limiting amino acid with the amino acid score (AAS) of 0.90 in chickpea was not typical (Table 4.6), as pulses are known for their shortage in sulfur containing amino acids (Alizadeh and da Silva, 2013). A similar limiting amino acid was reported by Bai et al. (2018) for Canadian grown chickpeas of the same year. The use of sulfur containing fertilizers in Manitoba farms could be part of the reason for this abundance in sulfur containing amino acids such as cysteine and methionine (Manitoba Agriculture, 2018; Järvan et al., 2012). The metabolism of amino acids in plant could be the other part of the answer for the lower threonine level. The branched-chain amino acids (BCAAs), which include leucine, isoleucine and valine are essential for the development of bacteroids and thus symbiotic nitrogen fixation in legumes generally (Prell et al., 2009). BCAAs are accumulated by many-fold during osmotic stress such as drought. Isoleucine, specifically, is synthesized from threonine and methionine; the biosynthesis of these two in plants is competitive through their affinity to threonine synthase and cystathionin γ-synthase (for methionine synthesis) (Joshi et al., 2010). It was found that during drought season, the synthesis of methionine synthase commonly outcompetes that of threonine which is regulated by the same enzyme synthase (cystathionine γ-synthase) (Galili et al., 2005), and thus can result in extra low synthesis of threonine. For the two raw cereal samples, the only obviously lacking essential amino acid compared to the reference value is lysine, thus the limiting amino acid as expected; the limiting AAS for raw sorghum and maize were 0.29 and 0.48, respectively (Table 4.6); all the other essential amino acids concentration are higher than the FAO reference with the exception of threonine in sorghum (33mg/g protein), which is only one unit lower than the reference.

In the case of pre-cooked flours, the limiting amino acid of chickpea changed from threonine to valine but remained lysine for sorghum and maize flours. The AAS for pre-cooked chickpea ranged from 0.84-0.87, and that for sorghum and maize were found to be similar to their raw values ranging between 0.29-0.31, and 0.38-0.51 respectively, except for of the two lower scores (0.26 and 0.38) under extrusion condition at 150^{o}C barrel temperature and 20% moisture content (Table A.3). This low AAS indicates that high temperature and low moisture condition is detrimental for lysine retention as more dextrin and free sugar could be produced for Maillard reaction (Harper, 1988), and that low temperature and high moisture condition should be chosen if protein quality is priority. Similar results were reported by Singh et al. (2007), Chaiyakul et al.

(2009) and Meuser et al. (1987). It was suggested that to minimize the loss of lysine, extrusion temperature and moisture should be kept below 180^{o}C and between 15-25% respectively (Cheftel, 1986). For chickpea, the big decrease of AAS from 1.16 to ~0.85 for valine and the increase of that in the sulfur containing amino acids from 0.94 to 1.06 after extrusion explains the switch in limiting amino acid. The concentration of the corresponding essential amino acids for chickpea in Table A.2 aligns with their amino acid scores, where valine decreased from 41 to ~30 mg/protein and Met + Cys increased from 23 to ~27 mg/g protein. Among the essential amino acids, these sulfurs containing amino acids for pre-cooked chickpea were the only two that were higher in amino acid concentration and AAS (1.06) compared to the raw (Table A.2 and A.3). Protein digestibility corrected amino acid scores of cooked Kabuli chickpea (1.08), reported by Nosworthy et al. (2017) is almost identical to the AAS of our extruded chickpea. This increase in the sulfur containing amino acids could be the result of exposure of proteins after extrusion (Colonna et al., 1989). Unlike the other amino acids, cysteine and methionine residues can be involved in disulfide bridges formation (Deiana et al. 2010), thus the more stable structure enabled them to "survived" the heating and shearing during extrusion.

In vitro protein digestibility corrected amino acid score (IV-PDCAAS) was not significantly affected by extrusion for all three flours compared to their raw ($p>0.05$) (Table 4.2). The IV-PDCAAS for both raw and pre-cooked chickpea, sorghum and maize is 69, 21 and 35% respectively. Temperature only had a significant effect ($p<0.001$) (Table 4.2a) for chickpea, where IV-PDCAAS was higher at 150^{o}C (71%) compared to that at 120^{o}C (67%) (Table 4.6). Moisture, temperature and their interaction had significant effect on IV-PDCAAS for both sorghum and maize ($p<0.001$) (Table 4.2b,c). At 120^{o}C, the IV-PDCAAS decreased slightly from 23 to 22% as moisture increased from 20 to 24% for sorghum but increased from 36 to 39% for maize. It is worth mentioning that both cereals had the lowest IV-PDCAAS at higher extrusion temperature and lower moisture, which agrees with the PDCAAS results. The reason for this as mentioned above is that the limiting amino acid Lys is more susceptible to dry heat (Harper, 1988). At 150^{o}C, IV-PDCAAS both increased from 20 to 22% for sorghum and 29 to 37% for maize with the increase of moisture (Table 4.6). This seeming increase is likely due to the more severe loss of lysine at 20% moisture than 24%.

The United States Agency for International Development (USAID) recommended the development of new cereal-based blends that focus on culturally available and nutritionally

appropriate grains to improve the nutritional quality of Fortified Blended Foods (FBFs) (Webb. 2011). Compare to the current FBF in use, CSB13 with a PDCAAS value of 85% is much higher than the IV-PDCAAS (69%) for our chickpea sample. Such difference is because of the very high PDCAAS (93%) of soy (Hoppe et al., 2008). Nosworthy et al. (2017) investigated the PDCAAS of series of Canadian pulses including split green and yellow pea, whole green lentil, split red lentil, kabuli chickpea, navy bean, pinto bean, light red kidney bean and black bean. They found that PDCAAS of these pulses were all above 50%, with the value ~52% for chickpea. This lower PDCAAS for chickpea compared to our value (69%) could be from the different processing method. IV-PDCAAS for raw maize (38%) is close to PDCAAS reported by Hoppe et al. (2008) (35%) and Pires et al. (2006) (37%). For raw sorghum, the IV-PDCAAS (24%) falls within the range of 6 to 46% (Mokrane et al., 2010; Moraes et al., 2012). Although only the extruded chickpea sample (~71%) had IV-PDCAAS above the 70%, it is still feasible to develop them into a new type of FBF by adding dairy-based source of protein as recommended by USAID (Webb. 2011). It is also worth noting that the *in vitro* PDCAAS likely underestimated the real value as other enzymes such as lipase and amylase that are present in animal digestive system were not present in our case, protein that were entrapped in starch/lipid complex were not accessible to the digestive enzymes we used. Hoppe et al. (2008) reported that when milk was added to blends of soy (20%) with maize and wheat, the PDCAAS of the two types of blends increased from 65% to 81% and 64% to 76% respectively. Also, since the 70% PDCAAS minimum level is set for vulnerable population to treat moderate malnutrition, any protein with lower value close to 70% would still be great a choice for the healthy majority.

4.2 Effect of blending ratio on the composition and protein quality of raw chickpea-cereal flours

4.2.1 Selection of blend ratio based on composition and nutritional properties of the raw chickpea-cereal blends

The proximate composition of raw individual chickpea, sorghum and maize flours were determined to have protein values of 23.2, 10.5 and 7.6%, respectively, ash values of 2.9 1.4 and 1.7%, respectively and, crude lipid values of 6.7, 3.0 and 3.9%, respectively (Table 4.7). Blending the raw flours at varying ratios ranging from 5:5 to 8:2 chickpea: sorghum or chickpea: maize resulted in an increase in protein, ash and crude lipid as the chickpea content increased within the blend (Table 4.7). The corresponding protein quality data is also given in Table 4.7, with the amino acid composition in gram per 100 g flour for the individual and blended flours given in Appendix B (Table B.1), and both the essential amino acid concentration (mg/g protein) and amino acid scores for the individual and blended flours in Table B.2. For chickpea flour, the limiting amino acid was found to be threonine, whereas both cereal flours were deficient in lysine. The limiting amino acid score for the individual chickpea, sorghum and maize flours was found to be 0.90, 0.29 and 0.48, respectively (Table 4.7). In the case of the blended flours, the limiting amino acid switched from lysine at the 5:5 chickpea: cereal ratio to threonine when the chickpea flour became more prominent (i.e., 6:4 blending ratio) (Table 4.7). The limiting amino acid score for chickpea: sorghum and chickpea: maize blends ranged from 0.77-0.88 and 0.87-0.91, respectively (Table 4.7). *In vitro* protein digestibility was reported for individual chickpea, sorghum and maize flours to be 76, 74 and 73%, respectively (Table 4.7). In contrast, chickpea: sorghum and chickpea: maize flours showed limiting IVPD values ranging from 74-76% and 74-75%, respectively (Table 4.7). With the calculations of *in vitro* protein digestibility corrected amino acid scores (IV-PDCAAS), the individual chickpea, sorghum and maize were found to be 69, 21, and 35%, respectively. In the case of the chickpea: sorghum blend, IV-PDCAAS increased from 57% to 64 % as the blending ratio went from 5:5 to 6:4, and then it began to level off with similar values for the 7:3 and 8.2 blending ratios (67%) (Table 4.7). In the case of the chickpea: maize blend, IV-PDCAAS increased from 64% to 66% as the blending ratio increased from 5:5 to 6.4, afterwards it behaved similarly as to the other blends, leveling off at the 7:3 and 8:2 ratios (68%) (Table 4.7). Based on the protein quality (IV-PDCAAS) results in Table 4.7, the significant increase in IV-PDCAAS for both blends

at the lowest chickpea ratio (for better expansion) was observed at the blending ratio of 6: 4, from which point on chickpea: maize blend ratio no longer had significant impact on protein quality. Therefore, chickpea: cereal blends at ratio 6: 4 was chosen for comparative purposes in the study on blends.

4.2.2 Physical properties of extrudates from blended chickpea-cereal flours

The specific mechanical energy (SME) and, expansion ratio, hardness and bulk density for extrudates prepared from chickpea: cereal blend blended flours at a 6:4 blending ratio, as a function of barrel temperature and moisture is given in Table 4.8. An individual degree of freedom contrast analysis was preformed to test for differences within the main effects of temperature and moisture, and their interaction for SME data and all physical properties for both chickpea-sorghum (CS) (Table 4.9a) and chickpea-maize (CM) blends (Table 4.9b). Based on the analysis, all main effects and interactions were found to be significant for all data for both blends. At 120^{o}C, the SME decreased from 451 to 430 kJ/kg for CS and from 448 to 427 kJ/kg for CM, as moisture increased from 20 to 24%. A similar decline was found at 150^{o}C, where the SME data decreased from 226 to 216 kJ/kg for CS and from 224 to 213 kJ/kg for CM as moisture increased (Table 4.8). Akdogan (1996) and Planttner (2007) both indicated that a decrease in viscosity would result in a drop in SME. Whereas, Singh et al. (2007) reported that an increase in extrusion temperature and moisture content would result in less friction within the barrel to lower the SEM value. In the present study, it was hypothesized that the small increase in moisture within the extruder resulted in a lower melt viscosity and a corresponding lower SME. Blending of the chickpea flour with cereal flours acted to lower the SME in the blends compared to the individual sorghum and maize (Table 4.3), since torque increased with the increase of starch content, which contributes to the restriction to flow inside the barrel by increased viscosity due to starch swelling and gelatinization (Iwe et al., 2001; Bhattacharya and Prakash, 1994). Filli et al. (2013) extruded blends of Bambara groundnut and millet to find that the lowest SME was obtained when the starch content was the lowest, and when both the moisture and screw speed were higher. Overall, in the current study SME was reduced in half as the barrel temperature increased from 120 to 150^{o}C due to the decrease in melt viscosity with increasing temperature.

Table 4.7 Proximate composition and protein quality for raw chickpea, sorghum and maize flours, along with chickpea-cereal blends at blending ratios of 5:5, 6:4, 7:3 and 8:2.

Flour	Proximate composition			Protein quality			
	Crude Protein (%, d.b.)[1]	Crude Ash (%, d.b.)[1]	Crude lipid (%, d.b.)[2]	Limiting amino acid	Limiting amino acid score	IVPD[1] (%)	IV-PDCAAS[3] (%)
Chickpea	23.19 ± 0.06[a]	2.94 ± 0.02	6.76	THR	0.90	76.88 ± 0.31[a]	69.38 ± 0.28[a]
Sorghum	10.47 ± 0.02[b]	1.43 ± 0.04	2.96	LYS	0.29	73.56 ± 0.46[b]	21.33 ± 0.13[b]
Maize	7.64 ± 0.02[c]	1.75 ± 0.02	3.88	LYS	0.48	72.66 ± 1.56[b]	34.88 ± 0.32[c]
Chickpea: Sorghum							
5:5	16.89 ± 0.05[d]	2.18 ± 0.13	4.78	LYS	0.77	74.41 ± 0.75[c]	57.47 ± 0.58[d]
6:4	18.11 ± 0.04[e]	2.35 ± 0.13	5.26	THR	0.85	75.37 ± 0.28[a]	63.94 ± 0.23[e]
7:3	19.13 ± 0.13[f]	2.46 ± 0.22	5.63	THR	0.89	75.62 ± 0.31[a]	66.99 ± 0.28[f]
8:2	20.41 ± 0.07[g]	2.63 ± 0.30	5.97	THR	0.88	76.46 ± 0.91[a]	67.26 ± 0.80[f]
Chickpea: Maize							
5:5	15.33 ± 0.08[h]	2.07 ± 0.22	5.30	LYS	0.87	73.99 ± 0.00[b, d]	64.04 ± 0.00[e]
6:4	16.87 ± 0.09[d]	2.22 ± 0.16	5.54	THR	0.89	74.11 ± 0.52[b, d]	66.07 ± 0.47[f]
7:3	18.26 ± 0.17[e]	2.35 ± 0.22	5.86	THR	0.91	74.59 ± 0.55[b, d]	67.96 ± 0.59[a, f]
8:2	19.72 ± 0.13[i]	2.73 ± 0.15	6.07	THR	0.91	74.83 ± 0.73[d]	68.08 ± 0.67[a, f]

Notes:

[1]Data represent the mean of triplicate measurements ± one standard deviation (n = 3).

[2]Only one crude lipid measurement was made on the flours.

[3]Data represents the product of the limiting amino acid score and IVPD (measured in triplicate). Data represent the mean ± one standard deviation (n = 3).

Letter a-i represents significant difference of data in each column.

Abbreviations: THR (Threonine); LYS (Lysine), d.b. (dry basis), IVPD (*In vitro* protein digestibility), IV-PDCAAS (*In vitro* protein digestibility corrected amino acid score)

Table 4.8 Physical properties of chickpea: sorghum and chickpea: maize extrudates blended at a 6: 4 ratio, as a function of moisture and barrel temperature.

Barrel Temperature	SME[1] (kJ/kg)	Expansion ratio[2]	Hardness (N)[3]	Bulk density (g/L)[3]
Chickpea: Sorghum				
120°C, 20%	451.1 ± 0.3	2.2 ± 0.2	762.5 ± 29.7	317.3 ± 15.5
120°C, 24%	430.0 ± 0.6	2.1 ± 0.2	617.7 ± 37.9	323.01 ± 53.2
150°C, 20%	225.6 ± 0.0	3.0 ± 0.3	271.6 ± 25.0	168.4 ± 1.1
150°C, 24%	216.0 ± 1.1	3.6 ± 0.3	195.0 ± 9.8	97.3 ± 7.7
Chickpea: Maize				
120°C, 20%	448.5 ± 1.1	3.0 ± 0.3	641.8 ± 107.7	201.0 ± 31.4
120°C, 24%	427.4 ± 0.0	2.5 ± 0.3	782.4 ± 32.0	298.3 ± 5.7
150°C, 20%	224.1 ± 0.0	3.5 ± 0.3	202.4 ± 4.7	91.8 ± 0.7
150°C, 24%	213.7 ± 0.0	3.5 ± 0.3	204.5 ± 17.7	105.9 ± 3.2

Notes:

[1]Data represent the mean from duplicate extrusion processing runs ± one standard deviation (n = 2).

[2]Data represent the mean values of 20 measurements for each duplicate extrusion processing runs ± one standard deviation (n = 40).

[3]Data represent the mean values of 3 measurements for each duplicate extrusion processing runs ± one standard deviation (n = 6).

Abbreviations: SME (Specific mechanical energy)

Table 4.9a An individual degree of freedom (orthogonal) contrast analysis performed using the general linear model for a chickpea: sorghum flour blends at a 6: 4 ratio, contrasting: raw vs. pre-cooked flours, 20% vs 24% moisture (within the extruder), 120^{o}C vs. 150^{o}C (within the extruder), and the moisture × temperature interaction.

Property	Raw vs. Pre-cooked	Moisture during extrusion	Temperature during extrusion	Moisture × temperature interaction
a) Physical parameters				
Expansion index[1]*	NT	p<0.001	p<0.001	p<0.001
Bulk density[2]**	NT	p<0.001	p<0.001	p<0.01
Hardness[2]*	NT	p<0.001	p<0.001	p<0.05
SME[3]	NT	p<0.001	p<0.001	p<0.001
b) Composition				
Protein[4]*	p<0.001	p<0.01	p<0.01	NS
Ash[4]	p<0.05	NS	NS	NS
Lipid[3]	NT	NT	NT	NT
c) Functionality[4,5]				
Water hydration capacity	p<0.001	p<0.001	p<0.001	p<0.01
Oil holding capacity	p<0.001	NS	NS	NS
Emulsion activity	NS^{+}	NS^{+}	p<0.05^{+}	NS^{+}
Emulsion stability	p<0.001	p<0.001	p<0.001	NS
RVA – peak viscosity*	p<0.001	p<0.001	p<0.001	p<0.001
RVA – Trough viscosity*	p<0.001	p<0.001	p<0.001	p<0.001
RVA – Breakdown viscosity*	p<0.001	p<0.001	p<0.001	p<0.001
RVA- Final viscosity*	p<0.001	p<0.001	p<0.001	NS
RVA – Setback viscosity*	p<0.001	p<0.001	p<0.001	p<0.001
RVA – Pasting temperature	NT	NT	NT	NT
d) Protein quality[4]				
IVPD	p<0.001	NS	p<0.001	p<0.01
IV-PDCAAS	p<0.001	p<0.001	p<0.001	p<0.001

Notes:

[1]Forty results for each temperature/moisture combination. (n = 160, df = 156)

[2]Six results for each temperature/moisture combination. (n = 24, df = 22)

[3]Only one crude lipid measurement was taken.[4]Three results for each temperature/moisture combination using a composite flour blend from duplicate processing runs (n = 12, df = 10)

[5]Foaming was not tested, since all extruded flours were found to be non-foaming.

Abbreviations: NT (Not tested); NS (Not significant, p>0.05) and SME (Specific mechanical energy)

(*) Indicates data transformed once by log

(**) Indicates data transformed twice by log

($^{+}$) Indicates one of the two assumptions (normality or variance test) failed during analyses

Table 4.9b An individual degree of freedom (orthogonal) contrast analysis performed using the general linear model for a chickpea: maize flour blends at a 6: 4 ratio, contrasting: raw vs. pre-cooked flours, 20% vs 24% moisture (within the extruder), 120^{o}C vs. 150^{o}C (within the extruder), and the moisture × temperature interaction.

Property	Raw vs. Pre-cooked	Moisture during extrusion	Temperature during extrusion	Moisture × temperature interaction
a) Physical parameters				
Expansion index[1]	NT	p<0.001	p<0.001	p<0.001
Bulk density[2]	NT	p<0.001^{+}	p<0.001^{+}	p<0.001^{+}
Hardness[2]*	NT	p<0.05	p<0.001	p<0.05
SME[3]	NT	p<0.001^{+}	p<0.001^{+}	p<0.001^{+}
b) Composition				
Protein[4]	p<0.001	p<0.001	p<0.001	p<0.01
Ash[4]	p<0.001^{+}	NS^{+}	p<0.01^{+}	NS^{+}
Lipid[3]	NT	NT	NT	NT
c) Functionality[4,5]				
Water hydration capacity	p<0.001	p<0.001	p<0.001	p<0.001
Oil holding capacity	p<0.001	NS	NS	NS
Emulsion activity	p<0.001	p<0.001	p<0.001	p<0.001
Emulsion stability	p<0.001^{+}	NS^{+}	NS^{+}	p<0.01^{+}
RVA – peak viscosity	p<0.001	p<0.05	p<0.001	p<0.001
RVA – Trough viscosity**	p<0.001	p<0.001	p<0.001	p<0.01
RVA – Breakdown viscosity**	p<0.001	p<0.001	NS	p<0.001
RVA- Final viscosity	p<0.001^{+}	p<0.05^{+}	NS^{+}	NS^{+}
RVA – Setback viscosity*	p<0.001	p<0.001	p<0.001	p<0.001
RVA – Pasting temperature	NT	NT	NT	NT
d) Protein quality[4]				
IVPD	p<0.001	NS	p<0.01	NS
IV-PDCAAS	p<0.01	p<0.05	p<0.001	p<0.001

Notes:

[1]Forty results for each temperature/moisture combination. (n = 160, df = 156)

[2]Six results for each temperature/moisture combination. (n = 24, df = 22)

[3]Only one crude lipid measurement was taken.[4]Three results for each temperature/moisture combination using a composite flour blend from duplicate processing runs (n = 12, df = 10)

[5]Foaming was not tested, since all extruded flours were found to be non-foaming.

Abbreviations: NT (Not tested); NS (Not significant, p>0.05) and SME (Specific mechanical energy)

(*) Indicates data transformed by once by log

(**) Indicates data transformed twice by log

($^{+}$) Indicates one of the two assumptions (normality or variance test) failed during analyses

At 120^{o}C, the expansion ratio decreased slightly from 2.2 to 2.1 for CS and from 3.0 to 2.5 for CM as the moisture content increased from 20 to 24%, whereas at 150^{o}C, the expansion ratio increased from 3.0 to 3.6 for CS but was relatively unchanged for CM blends (~3.5) as moisture increased from 20 to 24% (Table 4.8). In general, the expansion ratio was lowered by blending the chickpea into the cereal flours relative to that of the cereal flours alone. Deshpande and Poshadri (2011) also found that blending protein rich flours such as chickpea or cow pea with rice flour, decreased the expansion ratio of the extrudates. The authors indicated that the reduced expansion was caused by the macromolecular structure of the proteins as well as its ability to influence water distribution within the melt, which influences the complexation/crosslinking of protein and hydration of starch thus varied expansion. In the present study, the lowest expansion occurred at lower temperature (120^{o}C) and higher moisture (24%) content. This is typical for extrusion of feed containing high levels of starch such as cereals (Seth et al., 2015; Singh et al., 2007). It is because at high barrel temperature, better expansion can occur due to greater starch gelatinization/melting (Ainsworth et al., 2007) while lower moisture would increase the dragging and thus more pressure as the melt exited the die, which in return result in greater expansion (Oke et al., 2013). Overall, greater expansion of both blends occurred at the higher temperature because of the greater temperature differential at the die, which led to greater expansion as the moisture evaporated more rapidly. In the case of bulk density at 120^{o}C, an increase from 317 to 323 g/L for the CS blend and from 201 to 298 g/L for CM was observed as moisture increased from 20 to 24% (Table 4.8). In contrast, at 150^{o}C bulk density decreased from 168 to 97 g/L for CS but increased from ~92 to 106 g/L for CM as moisture increased from 20 to 24%. The CS blend followed the same trend in the expansion properties and bulk densities and hardness of the individual chickpea flour, whereas for CM blend, other than the expansion behavior, which is like the individual chickpea, both bulk density and hardness followed a similar trend as the individual maize flour (Table 4.3). The greater influence from chickpea to physical properties is possibly due to its higher fat and protein content, which is able to impede expansion especially under low temperature condition and affect bulk density and hardness. Whereas the high content of starch (especially amylose) in maize contributes greatly to air bubble structures inside the extrudates, and thus have greater influence on hardness and bulk density. In the case of hardness, values at the 120^{o}C were found to decrease from 762 to 618 N for CS but increased from ~642 to 782 N for the CM blend, whereas at 150^{o}C, hardness decreased from 271 to 195 N for CS and remained unchanged for the CM blend at 203 N (Table

4.8). Overall, hardness values were 2-3x lower at the higher temperature. The change in trend and magnitude (specifically at 120ºC) in hardness values between the two blends is thought to be associated with differences in starch and protein composition and expansion profile of individual flours. Matthey and Hanna (1997) proposed that starch-protein complex could inhibit starch gelatinization and degradation. To maximise the stability and reduce shrinking/collapsing of the extrudate, it is important to have intermediate-sized starch granules that are not further degraded (Gomez and Aguilera, 1984). However, this process is impeded by the formation of starch-protein complex, thus not only is the extrudate less expanded, but also more susceptible to elastic recoil (Allen et al., 2007). Upon blending chickpea with cereal flours, more protein is available for the complex formation. Also, extrudates containing 20 to 30% protein were found to have much smaller, more non-uniform and wrinkled air bubble cell walls compared to those containing starch mainly (Gujska and Khan, 1991). This may explain the reduced expansion than their individual flours at lower temperature. Also, at lower temperature, there is more shrinking due to the more elastic nature than at higher temperature, which made the final extrudate dense and hard. The results in this study agree with previous reports from Matthey and Hanna (1997), Onwulata et al. (1998) and, Gujska and Khan (1991) who all found that higher protein extrudates are generally less expanded, but denser and harder. Hardness and bulk density of the blends negatively correlates with the expansion ratio. This phenomenon is also reported by Allen et al. (2007) and, Sebio and Chang (2000).

4.2.3 Composition of raw and pre-cooked chickpea-cereal flours

The proximate composition for the raw and pre-cooked CS and CM blended flours as a function of extrusion temperature and moisture is given in Table 4.10. An individual degree of freedom orthogonal contrast was performed on the proximate data to determined differences between raw and precooked, and for the pre-cooked, differences between moisture, temperature and their associated interactions. Statistical data are presented in Tables 4.9a and 4.9b for the CS and CM blend, respectively. Although there was some statistical differences found between the treatments for both blends (Table 4.9), the magnitude of those differences were not substantial for both protein and ash. For the CS and CM blends, protein levels were between 17-18% (d.b.) and 16-17% (d.b.), respectively, whereas ash levels ranged between 2.3-2.4% (d.b.) and 2.2-2.4% (d.b.), respectively (Table 4.10). In the case of crude lipid, a decrease was observed from the raw

CS flour (5.3%, d.b.) to that of pre-cooked (2.8-3.4%, d.b.), and for the raw CM flour (5.5%, d.b.) to that of the pre-cooked (3.5-4.3%, d.b.) (Table 4.10). The proximate values are close to the estimated values calculated based on blending ratio, which range from 16-18%, 2.2-2.3%, for CS and 15-17%, 2.3-2.5% for CM in the case of protein and ash respectively; the estimated lipid content for raw CS (5.2%) and CM (5.6%) are also close to the experimental results, but for the precooked CS and CM, ranging from 3.7-4.0% and 4.3-5.0% respectively, this estimation in lipid content are higher than the actual results. This means that blending chickpea and cereal flours for extrusion increased the formation of lipid-starch complex.

4.2.4 Functionality of raw and precooked chickpea-cereal flours

The functional properties for the raw and pre-cooked CS and CM blended flours as a function of extrusion temperature and moisture are given in Table 4.10 and 4.11. An individual degree of freedom orthogonal contrast was also performed with results given in Tables 4.9a and 4.9b for the CS and CM blend, respectively.

Water hydration capacity

Water hydration capacity was found to be significantly higher for both pre-cooked blends compared to the raw blended flours ($p<0.001$) (Table 4.9). WHC increased from 2.0 to 4.6 g/g for CS and from 2.0 to 4.9 g/g for CM. The effect of moisture, temperature and their interaction were also all significant factors influencing the WHC for both pre-cooked blends (Table 4.9). At 120^{o}C, WHC values increased slightly but by and large remained unchanged (~4.6 g/g) for CS and increased from 4.4 to 5.2 g/g for CM as the moisture content increased from 20 to 24% (Table 4.10). At 150^{o}C, WHC increased slightly but remained relatively unchanged for CM (~5.1 g/g) and increased from 4.5 to 4.8 g/g for CS as the moisture increased from 20 to 24% (Table 4.10). The greater increase in WHC seen at 120^{o}C for CM and 150^{o}C for CS might be the result of greater starch gelatinization at higher moisture content. Gujska and Khan (1990) reported nearly three times of an increase in WHC of extruded bean flours (navy, pinto and chickpea), ranging from 1.2 to 4.0 g/g relative to non-extruded flours. The increase in WHC with increasing temperature and moisture was also reported by Kumar et al. (2010), Seth et al. (2015), Ding et al. (2005), and Chakraborty et al. (2011) for rice-carrot pomace blend, yam-corn-rice blend, rice flour and millet-legume blend, respectively. After extrusion, starch granules are more disrupted and therefore can

bind more water (Seth et al., 2015). Viscosity of melt will also be lower at higher moisture contents, which allows more movement of starch molecules for heat transfer, and then a greater level of gelatinization (Sobukola et al., 2013). Blending chickpea with cereal flours improved the WHC compared to chickpea flours alone, whereas relative to the sorghum and maize flours, the WHC of the blend decreased. It is hypothesized that the extruded starches could bind more water than the protein. Improved WHC properties in the extruded flours could have applications in meat products as a binder (Mehta, 2016).

Oil holding capacity

Pre-cooking of the blends by extrusion led to significantly poorer OHC of the flours relative to the raw (Table 4.9), where OHC was found to decrease from 1.5 to 1.2 g/g for CS and from 1.6 to 1.1 g/g for CM (Table 4.10). Even though for both blends, extrusion conditions were found to not significantly effect OHC ($p>0.05$) (Table 4.9). The observed reduction in values upon extrusion could be the result of the formation of starch-lipid complexes during heating making the flours less available to abide oils. In general, the OHC for the two raw blends had similar values as the raw sorghum (1.5 g/g) and maize (1.5 g/g) flours and was higher than that of raw chickpea flour (1.4 g/g) (Table 4.10). In general, compared to the individual flours (Table 4.10), blending chickpea with cereal flours seemed to have a negative impact on OHC. Anuonye et al. (2012) also reported a decrease in OHC upon blending pigeon pea into unripe banana flour. Gujska and Khan (1991) studied the functionalities of extruded blends of high protein fractions of pinto and navy beans with their high starch fraction or corn meal. They also found that OHC decreased with increasing protein content in these blends. The authors proposed that the physical entrapment of oil by disrupted starch granules seemed to be the driving mechanism of lipid absorption since the highest values are seen in blends containing more starch and less protein. The decrease in OHC after blending means that extruded blends would have a less greasy mouthfeel and could potentially be used in fried products (Aguilera et al., 2009).

Table 4.10 Proximate composition and functional properties of raw and pre-cooked chickpea: sorghum and chickpea: maize flour blends at a 6: 4 ratio as a function of moisture and barrel temperature. The extruded flour represents a composite of two extrusion runs.

Flour	Proximate composition			Functional properties[1]					
	Crude protein[1] (%, d.b.)	**Crude ash[1] (%, d.b.)**	**Crude lipid[2] (%, d.b).**	**WHC (g water/g flour, d.b.)**	**OHC (g oil/g flour, d.b.)**	**Emulsion Activity (%)**	**Emulsion Stability (%)**	**Foaming Activity (%)**	**Foaming Stability (%)**
Chickpea: Sorghum									
Raw flour	18.1 ± 0.0	2.4 ± 0.1	5.3	1.97 ± 0.02	1.46 ± 0.04	47 ± 0	56 ± 0	203 ± 4	0.0 ± 0.0
Pre-cooked flour									
120ºC, 20%	17.1 ± 0.0	2.3 ± 0.1	3.1	4.57 ± 0.07	1.16 ± 0.05	48 ± 2	40 ± 0	ND	ND
120ºC, 24%	16.9 ± 0.0	2.3 ± 0.0	3.0	4.63 ± 0.02	1.21 ± 0.02	47 ± 1	41 ± 0	ND	ND
150ºC, 20%	17.2 ± 0.1	2.3 ± 0.0	2.8	4.50 ± 0.01	1.19 ± 0.07	45 ± 0	42 ± 0	ND	ND
150ºC, 24%	17.0 ± 0.0	2.3 ± 0.0	3.4	4.76 ± 0.05	1.18 ± 0.07	45 ± 0	43 ± 0	ND	ND
Chickpea: Maize									
Raw flour	16.9 ± 0.1	2.2 ± 0.0	5.5	1.98 ± 0.09	1.56 ± 0.10	46 ± 0	54 ± 0	169 ± 4	2 ± 0
Pre-cooked flour									
120ºC, 20%	16.3 ± 0.0	2.4 ± 0.0	3.5	4.41 ± 0.14	1.10 ± 0.03	42 ± 0	45 ± 1	ND	ND
120ºC, 24%	15.8 ± 0.0	2.4 ± 0.0	4.2	5.24 ± 0.07	1.17 ± 0.04	43 ± 0	42 ± 1	ND	ND
150ºC, 20%	16.4 ± 0.0	2.3 ± 0.0	4.3	4.97 ± 0.10	1.17 ± 0.01	43 ± 0	43 ± 0	ND	ND
150ºC, 24%	16.2 ± 0.1	2.3 ± 0.0	3.8	5.10 ± 0.06	1.11 ± 0.03	45 ± 0	44 ± 0	ND	ND

Notes:

[1]Data represent the mean of triplicate measurements on the composite flour ± one standard deviation (n = 3).

[2]Data represent the mean of triplicate measurements on the composite flour ± one standard deviation (n = 3).

Abbreviations: WHC (water hydration capacity), OHC (oil holding capacity), ND (not detected), and d.b. (dry basis)

Table 4.11 Pasting properties of raw and pre-cooked chickpea: sorghum and chickpea: maize flour blends at a 6: 4 ratio as a function of moisture and barrel temperature. The extruded flour represents a composite of two extrusion runs. Data represent the mean of triplicate measurements on the composite flour ± one standard deviation (n = 3).

Flour	Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Pasting Temperature (ºC)
Chickpea: Sorghum						
Raw flour	1162.3 ± 7.2	893.7 ± 9.1	268.7 ± 12.7	2110.3 ± 21.1	1216.7 ± 26.1	86.4 ± 0.0
Extruded flour						
120ºC, 20%	107.3 ± 1.2	93.3 ± 1.2	14.0 ± 0.0	112.0 ± 1.7	18.7 ± 0.6	ND
120ºC, 24%	110.0 ± 2.6	101.3 ± 2.1	8.7 ± 0.6	124.3 ± 3.1	23.0 ± 1.0	ND
150ºC, 20%	127.3 ± 0.6	100.7 ± 0.6	26.7 ± 1.2	149.3 ± 1.5	48.7 ± 1.5	ND
150ºC, 24%	151.0 ± 2.6	120.3 ± 2.1	30.7 ± 0.6	170.7 ± 3.2	50.3 ± 1.2	ND
Chickpea: Maize						
Raw flour	1081.7 ± 10.0	981.7 ± 24.2	100.0 ± 17.8	2009.7 ± 78.9	1028.0 ± 55.0	81.5 ± 0.0
Extruded flour						
120ºC, 20%	115.0 ± 3.5	52.0 ± 0.0	63.0 ± 3.5	72.0 ± 0.0	20.0 ± 0.0	ND
120ºC, 24%	137.5 ± 2.1	80.5 ± 2.1	57.0 ± 0.0	115.0 ± 2.8	34.5 ± 0.7	ND
150ºC, 20%	156.3 ± 2.1	67.7 ± 1.2	88.7 ± 2.1	91.3 ± 0.6	23.7 ± 1.5	ND
150ºC, 24%	150.0 ± 3.5	101.3 ± 2.1	48.7 ± 1.5	154.3 ± 2.5	53.0 ± 1.0	ND

Notes:
Abbreviations: cP (centipoise = millipascal-second, mPa·s) and ND (not detected).

Emulsification

Emulsion activity (EA) for the pre-cooked CM blend was found to be significantly reduced relative to its raw flour, where EA values decreased from 46 to 43% (Table 4.9b, 4.10). In contrast, EA for the CS blend remained unchanged after extrusion (47%) (Table 4.9a, 4.10). Although there were significant differences in all extrusion conditions for the EA values for the CM blend, and for temperature (only) for the CS blend (Table 4.9), the magnitude of changes was not substantial (Table 4.10). In terms of emulsion stability (ES), both pre-cooked blended flours had significantly lower ES relative to the raw flours where ES was reduced from 56 to 41% for CS and from 54 to 43% for CM (Table 4.9, 4.10). Similar to the EA data, some statistical significance between extrusion conditions were noted (Table 4.9), however the magnitude of those changes was not substantial (Table 4.10). Since protein is the key contributor to emulsifying properties, it is natural to speculate that such properties would be improved with an increase in protein content. Gujska and Khan (1991) reported a positive correlation between emulsion capacity and protein content in their blended extrudates. For example, the emulsion capacity of pre-cooked high starch fraction of pinto bean increased from 19.2 mL/g to 42.5 mL/g upon blending in 30% (w/w) of its high protein fraction. However, this is not the case in the current study except for the raw blends, which showed some improvement: upon blending in 60% (w/w) of chickpea into individual sorghum and maize flours, the raw protein content increased from 9.5 to 18.1% and 7.0 to 16.9% respectively. The magnitude of increase in EA and ES does not match that seen in protein content, where EA increased from 38% for raw sorghum to 47% for CS, and from 45% for raw maize to 46% for raw CM; and ES increased from 48% for raw sorghum to 56% for raw CS, and from 40% for raw maize to 54% for raw CM (Table 4.4, 4.10). Unlike the raw blends, the extruded ones showed a general decrease in average EA and ES. In brief, EA remained 46% for both sorghum and CS extrudates, and decreased from 48 to 43% for maize and CM respectively; ES remained around 40% for both sorghum and CS and decreased from 48 to 44% for maize and CM respectively. This contradictory result from the other study indicates that the effect of protein on extrudate functionality is dependent on both type and concentration of protein (Gujska and Khan, 1991). Continuous phase viscosity can lead to enhanced stability by resisting gravitational separation (Meybodi et al., 2014). Extrusion process led to denaturation of the proteins and losses in solubility (Table 4.4). Therefore, mobility of the proteins to the interface would be less than unprocessed flours, and after starch gelatinization the continuous phase viscosity decreased significantly as

shown in pasting property (Table 4.11), ultimately leading to poorer emulsifying properties. The blends in the current study regardless of processing conditions did not seem to exhibit desirable emulsifying function compared to the commonly used commercial surfactants such as Tween 20, 80, and soy lecithin, which showed ES over 80% and EA of 93, 87, and 88% respectively (Lee and Choo, 2015). However, this is likely because of the low protein content in the emulsion (<1% w/w) and does not mean that they are not applicable as potential emulsifiers. For example, Gumus et al. (2017) found that lentil protein concentrate added at 5% (w/w) exhibited stable emulsifying activity under different stresses such as pH, ionic strength and temperature changes. Therefore, application of different pulse proteins could be of important value in the production of clean-label fortified foods, beverages and emulsion-based products such as cosmetic products.

Foaming

Foaming activity and stability for the raw and pre-cooked flour blends were examined, however only the raw flours were able to form foams (FA = 203% for CS; FA = 169% for CM), which were inherently very unstable (Table 4.10). Although blending chickpea flour into the cereals did improve the foaming performance of the individual raw cereal flours, the extruded blends behaved the same as the pre-cooked chickpea, sorghum, and maize flours, which produced no foam after homogenization. The presence of foam for raw CM versus its absence for raw maize indicates that chickpea is the main contributor to foaming in our case. The poor FA of blends containing maize flour has also been reported by Bhise et al. (2015). They substituted 0-40% extruded defatted sunflower seeds with maize flour, and found low FA ranging from 6 to 20%. The difference in the magnitude of the values compared to this study is likely the due to different ingredients. In general, the ingredients, either extruded or not, in the current study are not suitable to be used as foaming agent.

Pasting properties

The pasting properties of all raw and pre-cooked blended flours were assessed in the Rapid Visco Analyser (RVA). Just at the individual chickpea, sorghum and maize flours, during the test all viscosities [peak viscosity (PV), trough/holding viscosity (TV), breakdown viscosity (BV), final viscosity (FV) and setback viscosity (SV)] were found to be significantly lower after extrusion relative to the raw blends (Table 4.9, 4.11). In general, the effect of extrusion conditions

significantly affected all viscosities, with few exceptions (Table 4.9) which are likely due to blending in chickpea flour. During the extrusion process, starch gelatinization increases due to the combination of moisture, heat and shear leading to a large reduction in viscosity. During extrusion, starch granules are hydrated with the added moisture and heated as it is mixed within the barrel. Starch granules then swell, and amylose chains and some amylopectin within the granule become amorphous and migrate outside of the granule (known as pasting) (Mitrus et al., 2017). The process is expedited by shearing which induces further damage to the starch granules. The amylose chains then re-orient during cooling outside of the broken granules (Perten Instruments, 2015). In general, as the moisture levels and temperatures increase in the barrel, a greater amount of starch gelatinization occurs. The differences between the blends, reflects differences in the composition of the starch fraction for both sorghum and maize. Pasting temperature was also only found for the raw blends, with temperatures of 86.4 and 81.5^{o}C for the CS and CM blends, respectively (Table 4.11). Temperatures were not detected for the pre-cooked samples since gelatinization of the starch likely had already occurred during the extrusion process, prior to running the rapid visco analysis. According to Figure 4.3, extruded blends showed no viscosity peak compared to their raw. This result aligns with what was demonstrated in Figure 4.1 for the individual flours. Compared to the pre-cooked maize, there is no substantial cold viscosity in the extruded chickpea-maize blend. This decrease might be contributed by the increased (chickpea) protein content in the blend, which was denatured and more hydrophobic and thus decreased swelling and viscosity (Zhou et al., 2016). The low viscosity of the pre-cooked blends means that they could also be used as cold or hot beverages, and other formulations that require a consistent low viscosity upon heating.

Figure 4.4 shows the thermal properties of raw and pre-cooked blends extruded under mild condition at 120^{o}C and 20% moisture content using the DSC. The raw blends both displayed endothermic peaks associated with starch gelatinization whereas no detectable heat flow related to gelatinization was seen for the pre-cooked blends. This result verified our postulation and agrees with that obtained from Study 1, which implies the complete gelatinization and/or melting of starch during extrusion. The complete starch gelatinization during extrusion has also been reported by Ai et al. (2016).

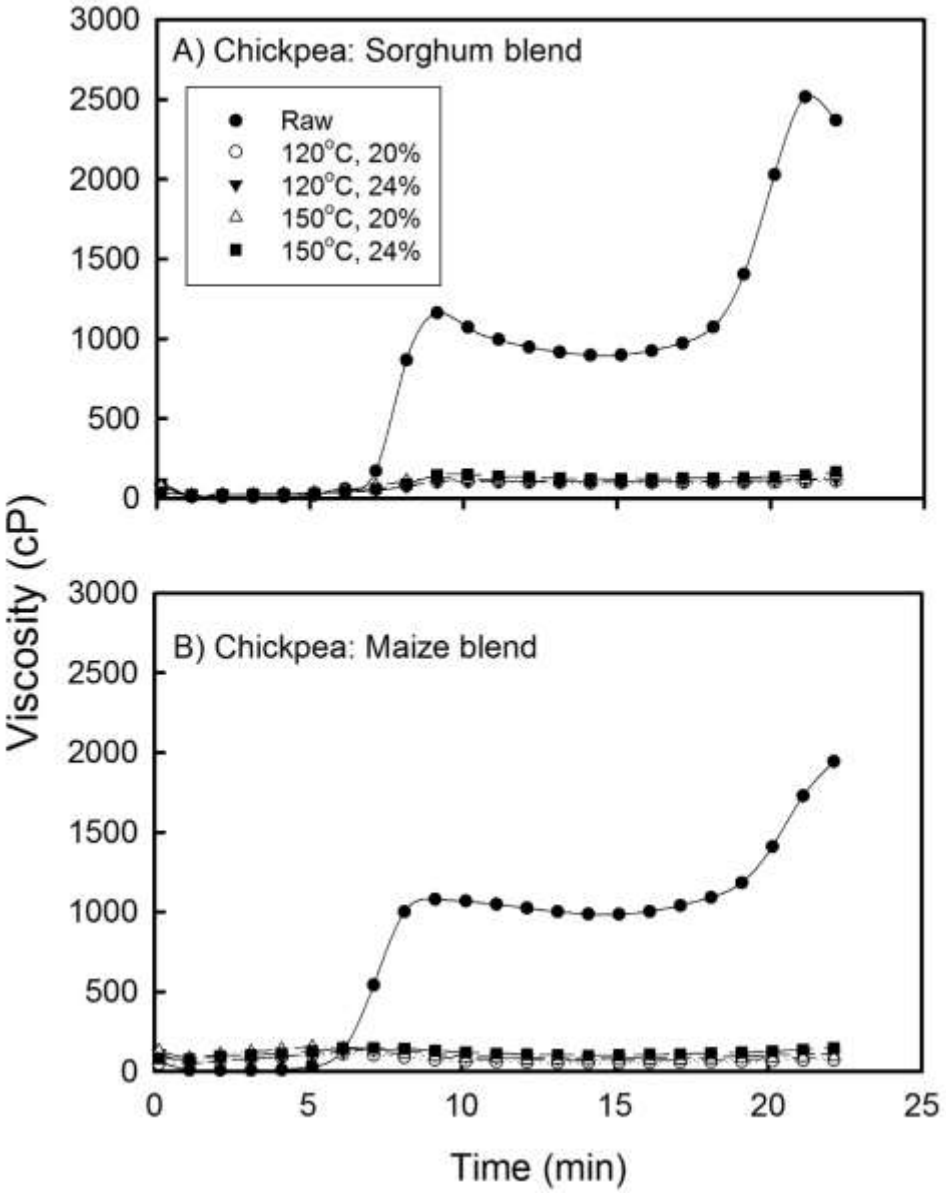

Figure 4.3 RVA profile for raw and extruded (A) chickpea-sorghum and (B) chickpea-maize flours (screw speed: 317 rpm; feed rate: 14 kg/h).

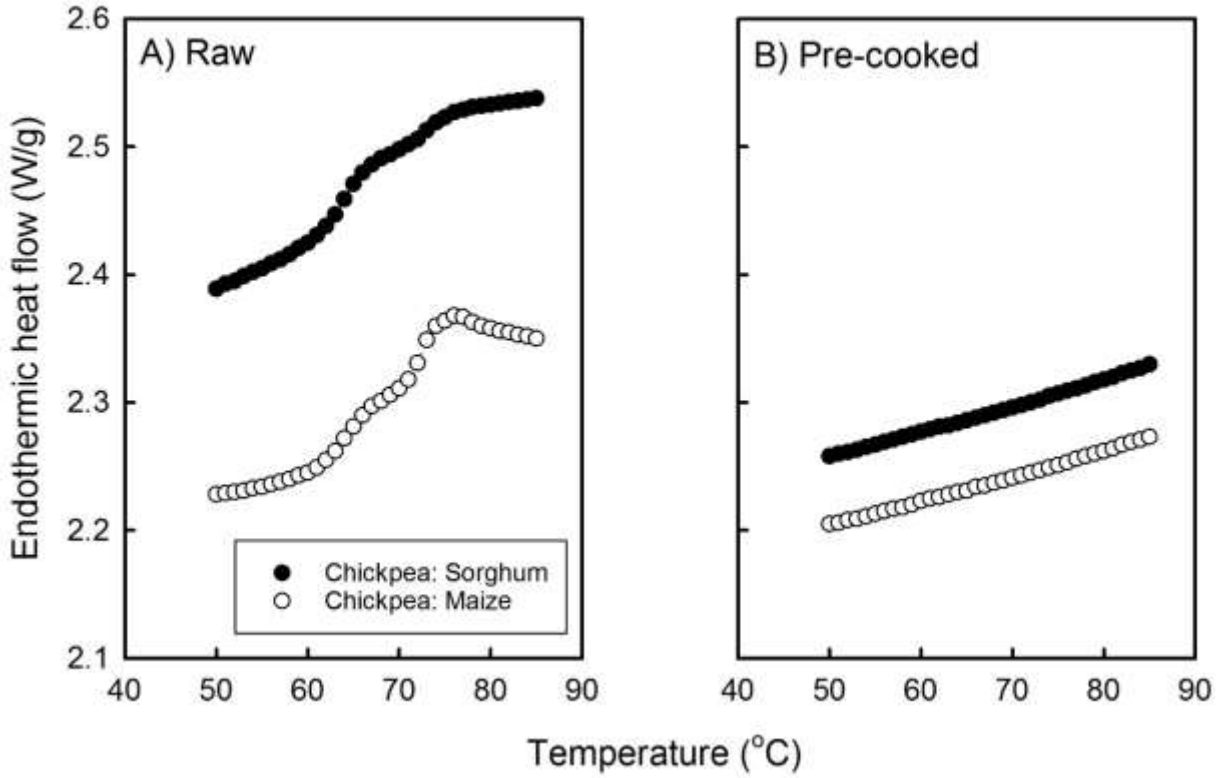

Figure 4.4 DSC thermograms of (A) raw and (B) extruded chickpea-sorghum and chickpea-maize flours (Heating rate=10°C/min. Extrusion temperature: 120°C; screw speed: 317 rpm; moisture content: 20%; feed rate: 14 kg/h).

4.2.5 Protein quality of raw and precooked chickpea-cereal flours

Protein quality for the CS and CM blends for the raw and pre-cooked materials, and as a function of extrusion temperature and moisture is given in Table 4.12. *In vitro* protein digestibility (IVPD) of pre-cooked CS and CM blends were found to be significantly higher compared to their raw counter parts, which increased from 75 to 80%, and from 74 to 82%, respectively (Tables 4.12). Similar results in IVPD after extrusion were reported by El-Hady and Habiba (2003), Alonso et al (2000), and Wang et al. (2008) for four kinds of legumes (peas, chickpea, faba and kidney beans), beans (faba and kidney) and flaxseed, respectively. Although there were some significant effects of moisture, temperature and their interaction within the orthogonal individual degree of freedom contrast analysis (Table 4.9), changes in the magnitude of the IVPD within the pre-cooked blends were not substantial (Table 4.12). Compared to the average IVPD of individual chickpea (81%) and maize (76%), the protein digestibility of the CM (82%) blend was noticeably higher,

and that of CS blend (80%) did not have substantial change. When compared to the IVPD of sorghum (74%), however, there was a 6% increase. This result is expected as the protein content was significantly increased upon addition of chickpea flour. The improved IVPD upon blending different pulses (yellow and green pea, lentil, and chickpea) into cereal (wheat) flour was also reported by Patil et al. (2016). By added legumes to wheat up to a level of 15%, the authors found that IVPD increased from 32 to 38% for the raw blends, and 59 to 66% for the extruded blends. Nosworthy et al. (2017) also reported increase in IVPD from raw to extruded (77 to 80%) buckwheat-pinto bean blends (50:50). Although the negative effect of bioactive compounds (e.g. phenolics and enzyme inhibitors) on protein digestibility is widely known, extrusion seems to play a greater role than the limiting effects from these compounds due to the physicochemical changes that happens during processing in the extruder barrel (Patil et al., 2016). During extrusion, the combination of moisture, heat and shear is thought to induce the partial unraveling of the protein's conformation to allow for greater exposure of sites for digestive enzymes to attack (Fontana et al., 1997). In addition to the increased bioavailability, the bioactive compounds are inactivated during the heating process (Marquardt et al., 1974).

The full amino acid composition of raw and pre-cooked flours reported in grams per 100 g flour is given in Appendix B (Table B.3); the essential amino acid concentration in milligram per gram protein, along with the FAO reference pattern is given in Table B.4; and the amino acid scores for each essential amino acid is given in Table B.4. For both of raw blends, all the essential amino acid concentration is above the FAO reference except for Met+Cys for CS and threonine for both blends; after extrusion, a general decrease in the concentration was observed, whereas that of Met+Cys was increased in both blends (Table B.4). Both methionine and cystine are hydrophobic amino acids, they are often buried in the hydrophobic core (Brosnan et al., 2006). Therefore, it is expected to see an increase in them after extrusion where shear and heat denature protein and expose more of these amino acids. In both blends, the limiting amino acid was found to be threonine for the raw material, which is associated with the chickpea component of the blend, whereas in all pre-cooked blended flours lysine became limited. The limiting amino acid score for the raw CS and CM blends was 0.85 and 0.89, respectively, whereas for the pre-cooked CS and CM blends scores decreased and ranged between 0.72-0.78 and 0.78-0.87, respectively (Table 4.12). Because of the 6:4 chickpea: cereal blending ratio, the amino acids are dominated by the composition of the chickpea.

Table 4.12 Amino acid scores and protein quality data of raw and pre-cooked chickpea: sorghum and chickpea: maize blends at a 6: 4 ratio as a function of moisture and barrel temperature. The extruded flour represents a composite of two extrusion runs.

	Limiting amino acid	Limiting amino acid score[1]	IVPD[2] (%)	IV-PDCAAS[3] (%)
Chickpea: Sorghum				
Raw flour	THR	0.85	75.37 ± 0.28	63.94 ± 0.23
Pre-cooked flour				
120°C, 20%	LYS	0.78	79.72 ± 0.66	62.06 ± 0.49
120°C, 24%	LYS	0.74	78.81 ± 0.69	58.02 ± 0.50
150°C, 20%	LYS	0.74	80.44 ± 0.42	59.45 ± 0.31
150°C, 24%	LYS	0.72	81.35 ± 0.10	58.29 ± 0.07
Chickpea: Maize				
Raw flour	THR	0.89	74.11 ± 0.52	66.07 ± 0.47
Pre-cooked flour				
120°C, 20%	LYS	0.84	81.71 ± 0.73	68.51 ± 0.61
120°C, 24%	LYS	0.87	80.91 ± 0.44	70.28 ± 0.38
150°C, 20%	LYS	0.79	82.37 ± 0.38	64.89 ± 0.30
150°C, 24%	LYS	0.78	82.43 ± 0.38	64.24 ± 0.29

Notes:

[1]Measurements were performed once on the composite blend from two extrusion processing runs.

[2]Measurements were performed in triplicate on the composite blend from two extrusion processing runs. Data represent the mean ± one standard deviation (n = 3).

[3]Data represents the product of the limiting amino acid score and IVPD (measured in triplicate). Data represent the mean ± one standard deviation (n = 3).

Abbreviations: THR (Threonine), LYS (Lysine), IVPD (*In vitro* protein digestibility), and IV-PDCAAS (*In vitro* protein digestibility corrected amino acid score)

The atypical phenomenon in the limiting amino acid in chickpea is possible as the nutritional quality of chickpea is dependent on various factors such as environment, climate, soil nutrition, soil biology, agronomic practices and biotic and abiotic stress factors (Wood and Grusak, 2007). Galili et al. (2005) reported that the biosynthesis of threonine, methionine, (iso)leucine are interconnected. Environmental stress would result in increased accumulation of isoleucine, whose precursors are threonine, synthesized by threonine synthase, and methionine, catalyzed by cystathionin γ-synthase (Joshi et al., 2010). Different type of stress could lead to one synthase outcompete the other one, and drought seems to induce the accumulation of methionine and thus the reduction of threonine (Galili et al., 2005). The greater accumulation of methionine under

drought stress were reported by both Du et al. (2012) for Bermuda-grass and Shen et al. (1989) for flat pea.

During the extrusion process, the heat liability of lysine results in deficiencies. Unlike the individual cereal extrudates that had noticeable decrease in limiting amino acid score at high temperature and low moisture condition (Table 4.6), the blends did not show such a trend. We hypothesize that this is due to the protective effect of increased lipid, which can act as a lubricator during extrusion and lessen the shear and temperature (Hu, 1994). Compared to the other eight essential amino acids, lysine is the most reactive during processing because of its two amino groups (O'Brien and Morrissey, 1989). The Maillard reaction between free amino groups and carbonyl groups of reducing sugar leads to loss of lysine, especially under high temperature and low moisture conditions during extrusion (Singh et al., 2007). It was found that heat treatment only just above 100^{o}C was able to modify cystine (Klarenbeek, 1984), which is more stable compared to lysine (Higgs and Boland, 2014). Blending of chickpea greatly increased the lysine concentration of sorghum and maize from 20 to 50 mg/g protein and 32 to 53 mg/g protein respectively, although that of individual chickpea was as high as 60 mg/g protein, the decrease in chickpea is not comparable to the increase of lysine for the cereal blends. In general, greater lysine retention for the blends are obtained at lower temperature, where CS and CM retained 44 (88%) and 50 mg/g protein (94%) respectively. At higher temperature, the lysine retention for CS and CM were 42 (85%) and 45 (86%) mg/g protein. Similar results were reported by Hood-Niefer and Tyler (2010), who reported 46 mg/g protein (87%) retained lysine out of 53 mg/g protein in pea flour that contain 18% protein, which is similar to that of the blends, when extruding the flour at 100^{o}C and 18% moisture. Another study investigating the retention of essential amino acids during extrusion of protein blend (milk and egg protein with wheat flour) and reducing sugar solution (fructose, galactose and glucose) (110 and 125^{o}C, 19 and 23.5% moisture) showed only up to 40% of lysine retention, yet 80 to 100% retention of other essential amino acids (Singh et al., 2007). The very low retention in their study likely due to the carbohydrate substance they chose, fructose and galactose, which are both reducing sugar and much more readily to undergo Maillard reaction with protein. Overall, the lysine reduction during extrusion in this study falls within the range of 10 to 15% reported by Singh et al. (2000), who extruded rice and wheat bran blends. Albeit the reduction, extrusion still retained more lysine compared to other food processing methods that loses 20 to 40% (Singh et al., 2000).

In the case of the *in vitro* protein digestibility corrected amino acid score (IV-PDCAAS), significant decreased were found between the raw and pre-cooked blended flours, where for CS IV-PDCAAS was reduced from 64 to 59%, however for the CM blend (on average), the slight increase in scores are not substantial and remained relatively unchanged (66 - 67%) (Table 4.12). The reduced scores for the CS blends relative to the CM, reflects much lower lysine levels in the sorghum flour vs. the maize (Table 4.6). In contrast to the insignificant effect of extrusion on IV-PDCAAS for the individual flours, the significant reduction for CS indicates that blending chickpea with sorghum resulted in greater loss of lysine and is likely due to the intensified Maillard reaction because of increase in protein (Singh et al., 2000). Significant differences were found for all extrusion conditions and their interactions in the case of both blends (Table 4.9). For CS, IV-PDCAAS decreased from 62 to 58 at 120^{o}C and remained relatively unchanged at 150^{o}C (58-59%) as the moisture increased from 20 to 24%. For CM, IV-PDCAAS was more sensitive to temperature than moisture, where values were reduced from 68-70% at 120^{o}C to 64-65% at 150^{o}C (Table 4.12). The larger reduction associated with temperature is likely due to the chickpea because temperature was the only significant factor that affected the IV-PDCAAS whereas the interaction for both temperature and moisture was significant to that of the cereal extrudates (Table 4.6). This reduction is believed to be associated with the increased heat susceptibility of lysine which drove the limiting amino acid score used in the calculation of IV-PDCCAS lower.

IV-PDCAAS increased significantly upon the addition of chickpea flour compared to their cereal counterparts. It is worth noting that the limiting amino acid score and IV-PDCAAS calculated based on the blend ratio and results for individual flours in section 4.1 ranges from 0.63 to 0.67 and 50 to 53% for CS, and 0.69 to 0.75 and 56-59% for CM, respectively. These calculated values are lower than the experimental results (Table 4.12), which means that blending pulse with cereals improved protein quality likely through increased lysine retention. It was reported that higher protein content could result in better lysine retention (Hood-Niefer and Tyler, 2010). In general, the CM blend had the greater IV-PDCAAS. Among the pre-cooked blends, only CM extruded at 120^{o}C, 24% moisture content had IV-PDCAAS value reached the requirement (70%) for food aid products for moderate malnourished children (WHO, 2012). A significant gap in protein quality still exists compared to the FBFs currently in use with PDCAAS value of 85%. The USAID recommended the development of new cereal-based blends using locally cultivated grains with proper nutritional values, which encourages the addition of whey protein (Rosenberg et al.,

2011). Therefore, even though the blends by themselves are not enough to be used for the moderate malnourished population, the inclusion of dairy could significantly increase the protein quality to over 80% (Hoppe et al., 2008). Thus, potential future development for the blends could be in lipid-based ready-to-use food and weaning foods for infants (Mosha et al., 2005), as well as snack foods that typically have lower PDCAAS value than 70% (Brennan et al., 2013).

5. OVERALL CONCLUSIONS

Food security has been an ongoing focus for FAO and UN countries such as Canada and the United States. Since the 1960s, food aid products using corn and soy blends have been produced and distributed to many countries in need. In recent years, the USAID recommended the development of new blends using crops that are nutritionally and culturally available. Chickpea, sorghum and maize are the three important crops widely cultivated especially for semi-arid countries in Africa. This research examined the effect of extrusion temperature and moisture on the physicochemical, functional and nutritional properties of these three flours and their blends. The understanding of extrudate physical characteristics, and the composition and functionality of pre-cooked milled extrudate flours is important for determined their suitability in food aid products, and in future applications in the food industry.

In the first study, Kabuli chickpea was found to have the highest protein, ash and lipid contents relative to the two cereals. Extrusion was found to decrease the content of protein in chickpea and lipid in all flours due to the formation of lipid-protein and protein-starch complexes but did not substantially affect the ash content. Chickpea flour was also found to have the least specific mechanical energy during extrusion, and subsequently the higher bulk density and hardness, and less expansion compared to the cereal flours. SME generally decreased with the increase of barrel temperature for chickpea, whereas decreased with the increase of moisture content for sorghum and maize. In general, high temperature and moisture favors the expansion of chickpea, whereas lower moisture content favors the expansion of cereal flours. Oil holding capacity did not change substantially for the all flours after extrusion. However, extrusion significantly altered the water hydration capacity and pasting properties of each flour. All flours showed increase in WHC by ~2-3 times, and a decrease in pasting viscosities by ~8-40 times. The disappearance of the endothermic heat flow peak of the extruded flours indicates that extrusion processing pre-gelatinized and/or melted all detectable starch. Emulsion capacity for chickpea was found to decrease but increase for the maize flour after extrusion. In the case of emulsion stability, extruded maize was the only flour showing improved emulsion stability. Only raw chickpea

(~250%) and sorghum (~48%) flours were able to generate foams. However, none of the extruded samples showed foaming capability. Although there was a general increase in the *in vitro* protein digestibility after extrusion, the protein quality (IV-PDCAAS) was not remarkably improved possibly due to the disruption of limiting amino acid (Val and Lys) and lowered nitrogen solubility because of extrusion.

In the second study, the blending ratio of 60:40 for chickpea: cereal was chosen based on the better protein quality of the raw blends and extrudability; although the blend with highest chickpea flour has the highest protein quality, the high fat and protein content could result in jamming of the extruder and poor expansion (Gearhart and Rosentrater, 2014). Blending chickpea with cereal flours increased the protein, ash and fat content compared to the individual cereal flours. Protein and lipid content for the blends slightly decreased after extrusion, whereas ash content remained relatively unchanged. The specific mechanical energy decreased with the increase of temperature and moisture in both blends. And the magnitude of blends' SME (ranging from ~214 to 451 kJ/kg) was comparable to that of individual chickpea flour, ranging from ~222 to 445 kJ/kg. In general, higher temperature resulted in greater expansion, thus less hardness and bulk density. It is worth mentioning that blending chickpea with cereal noticeably increased the hardness at 120^{o}C for both blends (ranging from ~618 to 782 N) compared to those of the chickpea (~448 N) and cereal extrudates (ranging from 186 to 211 N) treated at the same temperature. In general, extrusion decreased oil holding capacity, emulsion activity and stability. For the raw flours, blending chickpea into cereals improved the foaming functionality compared to the individual cereal flours. However, none of the extruded samples showed foaming capability. Extrusion decreased the pasting viscosities of the blends by 8-37 times compared to their raw counterparts. In general, although protein digestibility increased for both blends because of extrusion, the overall protein quality (IV-PDCAAS) decreased possibly due to the loss of the limiting amino acid lysine, especially at higher extrusion temperature. Also, although the protein quality of the blends (both raw and extruded) are higher than their cereal counterparts, it did not show the complimentary effect as expected, which could be explained by the exceptional amino acid profile of the specific chickpea cultivar used in this study.

According to the results, blending in chickpea flour increased the protein quality of the cereal flours. Only the IV-PDCAAS for CM (70%) treated at 120^{o}C and 24% moisture reached the requirement (70%) by WHO to be used as food aid for the moderately malnourished. However,

the addition of dairy product such as whey protein into the blends, as recommended by the USAID, could significantly improve the protein quality. Due to the great hydration property after extrusion, the pre-cooked flour could be developed into instant cold/hot beverage or porridge. Other potential applications include lipid-based ready-to-use food, snack foods, and infant weaning foods.

6. FUTURE STUDIES

This research examined the effect of extrusion temperature and moisture on the physicochemical, functional and nutritional properties of Kabuli chickpea, sorghum, maize and chickpea-cereal blends. However, the temperature chosen for this work was relatively low compared to that used by the food industry for high protein ingredients (e.g. 130-180^{o}C for meat analogue) (Osen and Schweiggert-Weisz, 2015), and the moisture relatively high if the product were to be puffed snacks or breakfast cereals (e.g., 17-20%) (Reddy et al., 2014). Therefore, expanding the extrusion condition would be beneficial not only from the development perspective, but also could provide a clearer trend in the effect of these two variables. The application of scanning electron microscopy (SEM) would be useful for future analysis of the extrudate microstructure in terms of their cell wall thickness, shape and structure of the pores, which is closely related to the physical parameters such as expansion ratio, hardness and bulk density of extruded products. The use of SEM would also provide more insight of an emulsion system.

The functional properties such as emulsion and foaming are largely dependent on the surface property of protein, mainly the hydrophobicity. If measured, it would provide more fundamental reasons as to why emulsion and foaming properties are the way they are, also the extent of protein denaturation as the result of extrusion could be better revealed. The hydrophobicity also relates to protein solubility and thus affect protein digestibility. The sever processing condition in the extruder barrel is known for dextrinizing starch, which in turn affects the viscosity of the melt and thus downstream physical and functional properties of the extrudates. Thus, the degree of starch dextrinization after extrusion should be addressed.

In terms of the nutritional analysis, this research only focused on protein. However, carbohydrates are also an indispensable source of nutrition. Starch digestibility after extrusion should be investigated in the future study, and related to the type of starch (e.g., digestible, indigestible and resistance starch). The protein quality data reported in this research was evaluated using an *in vitro* protein digestibility assay. Although a good correlation between IV-PDCAAS and *in vivo* PDCAAS was reported by researchers, it would still be important to carry out the *in*

vivo assay to obtain more accurate results despite of the ethnic controversy and cost of using lab animals.

Based on the results of this research, further product development of the extrudates would also be of value. The addition of whey protein to the blends would probably render a good food aid product. It is also quite viable to develop an instant cold/hot beverage from the pre-cooked flours owing to its great water hydration capacity.

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APPENDIX A

Table A.1 Amino acid composition (g per 100 g of flour, on an *as is* basis) for raw and pre-cooked chickpea, maize and sorghum flours.

Amino acids	Raw flour	Pre-cooked flours			
		Moisture 20%		Moisture 24%	
		120ºC	150ºC	120ºC	150ºC
a) Chickpea					
% Protein[1]	20.86	20.14	20.00	19.64	20.00
% Moisture	10.04	3.5	3.16	4.36	3.62
Aspartic Acid	2.30	1.98	1.92	1.89	1.92
Glutamic Acid	3.43	2.86	2.82	2.79	2.83
Serine	1.17	0.90	0.88	0.88	0.89
Glycine	0.82	0.57	0.56	0.55	0.56
Histidine‡	0.46	0.36	0.36	0.38	0.37
Arginine	1.82	1.39	1.39	1.38	1.42
Threonine‡	0.64	0.62	0.61	0.61	0.62
Alanine	0.92	0.63	0.62	0.61	0.62
Proline	0.97	0.70	0.70	0.70	0.67
Tyrosine	0.55	0.49	0.50	0.50	0.49
Valine‡	0.85	0.59	0.60	0.58	0.61
Methionine*‡	0.20	0.28	0.28	0.28	0.29
Cysteine*	0.29	0.24	0.25	0.25	0.25
Isoleucine‡	0.78	0.62	0.63	0.60	0.64
Leucine‡	1.47	1.24	1.22	1.19	1.22
Phenylalanine‡	1.15	1.00	0.98	0.96	0.99
Lysine‡	1.20	1.09	1.08	1.09	1.08
Tryptophan‡	0.23	0.19	0.20	0.21	0.20
b) Sorghum					
% Protein[1]	9.42	10.05	9.81	9.70	9.72
% Moisture	10.00	4.47	4.23	4.78	4.33
Aspartic Acid	0.60	0.59	0.54	0.55	0.54
Glutamic Acid	1.94	1.81	1.68	1.68	1.69
Serine	0.46	0.37	0.37	0.39	0.37
Glycine	0.30	0.25	0.23	0.23	0.23
Histidine‡	0.17	0.16	0.15	0.15	0.15
Arginine	0.43	0.34	0.31	0.31	0.31
Threonine‡	0.28	0.28	0.27	0.27	0.27
Alanine	0.86	0.68	0.65	0.65	0.66
Proline	0.94	0.68	0.66	0.68	0.66
Tyrosine	0.30	0.34	0.34	0.33	0.33
Valine‡	0.43	0.40	0.36	0.33	0.36
Methionine*‡	0.12	0.17	0.17	0.17	0.16
Cysteine*	0.15	0.15	0.13	0.15	0.14
Isoleucine‡	0.33	0.35	0.30	0.29	0.31
Leucine‡	1.19	1.12	1.05	1.04	1.06
Phenylalanine‡	0.47	0.47	0.44	0.43	0.44
Lysine‡	0.16	0.18	0.15	0.17	0.17
Tryptophan‡	0.12	0.10	0.10	0.09	0.10

Table A.1 (cont.)

Amino acids	Raw flour	Pre-cooked flours			
		Moisture 20%		Moisture 24%	
		120°C	150°C	120°C	150°C
c) Maize					
% Protein[1]	7.64	7.82	7.84	7.78	7.81
% Moisture	10.16	3.62	2.80	3.64	2.95
Aspartic Acid	0.49	0.47	0.45	0.48	0.48
Glutamic Acid	1.15	1.08	1.07	1.09	1.10
Serine	0.35	0.33	0.32	0.32	0.30
Glycine	0.28	0.26	0.25	0.27	0.26
Histidine‡	0.17	0.16	0.15	0.16	0.17
Arginine	0.45	0.37	0.34	0.38	0.38
Threonine‡	0.23	0.23	0.23	0.23	0.23
Alanine	0.50	0.39	0.39	0.40	0.40
Proline	0.70	0.51	0.50	0.50	0.50
Tyrosine	0.20	0.26	0.24	0.25	0.24
Valine‡	0.30	0.26	0.24	0.30	0.32
Methionine*‡	0.11	0.16	0.15	0.16	0.16
Cysteine*	0.14	0.14	0.14	0.14	0.15
Isoleucine‡	0.20	0.19	0.18	0.22	0.23
Leucine‡	0.70	0.65	0.63	0.66	0.67
Phenylalanine‡	0.30	0.29	0.29	0.30	0.30
Lysine‡	0.19	0.21	0.18	0.23	0.22
Tryptophan‡	0.08	0.07	0.07	0.08	0.07

Notes:
(1) Percent protein on a wet basis; *, sulfur amino acid; ‡, essential amino acids.
Measurements were preformed once on the composite blend from two extrusion processing runs.

Table A.2 Essential amino acid concentration (mg/g protein) for raw and pre-cooked flours as a function of moisture and barrel temperature for chickpea, sorghum and maize.

Flours	Amino acids								
	THR	**VAL**	**MET + CYS**	**ILE**	**LEU**	**PHE + TYR**	**HIS**	**LYS**	**TRP**
Chickpea									
Raw flour	31	41	23	37	70	82	22	58	11
Pre-cooked flour									
120ºC, 20%	31	29	26	31	61	74	18	54	10
120ºC, 24%	31	30	27	31	61	75	19	56	10
150ºC, 20%	31	30	27	31	61	74	18	54	10
150ºC, 24%	31	31	27	32	61	74	18	54	10
Sorghum									
Raw flour	30	46	29	35	126	82	18	17	13
Pre-cooked flour									
120ºC, 20%	27	40	32	34	111	80	16	18	10
120ºC, 24%	27	34	33	30	107	77	15	17	10
150ºC, 20%	28	36	30	30	107	79	15	15	10
150ºC, 24%	27	37	31	32	110	80	16	17	10
Maize									
Raw flour	33	44	36	29	102	73	25	28	12
Pre-cooked flour									
120ºC, 20%	30	33	38	25	83	70	20	27	9
120ºC, 24%	30	39	38	28	85	71	21	30	10
150ºC, 20%	29	30	37	23	80	68	19	22	9
150ºC, 24%	30	40	39	29	86	70	21	28	9
FAO reference	34	35	25	28	66	63	19	58	11

Notes:

Abbreviations: THR (threonine); CYS (cysteine); VAL (valine); MET (methionine); ILE (isoleucine); LEU (leucine); TYR (tyrosine); PHE (phenylalanine); HIS (histidine); LYS (lysine); and TRP (tryptophan).

Measurements were preformed once on the composite blend from two extrusion processing runs.

Table A.3 Amino acid scores for raw and pre-cooked flours as a function of moisture and barrel temperature for chickpea, sorghum and maize.

Flours	Amino acids								
	THR	**VAL**	**MET + CYS**	**ILE**	**LEU**	**PHE + TYR**	**HIS**	**LYS**	**TRP**
Chickpea									
Raw flour	*0.90	1.16	0.94	1.34	1.07	1.29	1.16	0.99	1.00
Pre-cooked flour									
120ºC, 20%	0.91	*0.84	1.04	1.10	0.93	1.17	0.93	0.93	0.87
120ºC, 24%	0.91	*0.84	1.07	1.10	0.92	1.19	1.01	0.96	0.95
150ºC, 20%	0.90	*0.86	1.06	1.12	0.92	1.17	0.94	0.93	0.92
150ºC, 24%	0.91	*0.87	1.06	1.13	0.92	1.17	0.96	0.93	0.91
Sorghum									
Raw flour	0.87	1.30	1.15	1.25	1.91	1.30	0.95	*0.29	1.16
Pre-cooked flour									
120ºC, 20%	0.81	1.14	1.27	1.23	1.68	1.27	0.82	*0.31	0.89
120ºC, 24%	0.80	0.98	1.30	1.06	1.62	1.23	0.82	*0.30	0.87
150ºC, 20%	0.81	1.03	1.22	1.08	1.62	1.26	0.81	*0.26	0.92
150ºC, 24%	0.80	1.06	1.24	1.13	1.66	1.27	0.82	*0.30	0.91
Maize									
Raw flour	0.98	1.25	1.46	1.04	1.54	1.16	1.30	*0.48	1.06
Pre-cooked flour									
120ºC, 20%	0.88	0.95	1.54	0.88	1.26	1.22	1.04	*0.47	0.83
120ºC, 24%	0.88	1.11	1.54	1.01	1.29	1.24	1.09	*0.51	0.87
150ºC, 20%	0.87	0.86	1.48	0.81	1.34	1.21	0.99	*0.38	0.85
150ºC, 24%	0.88	1.15	1.56	1.03	1.43	1.30	1.13	*0.49	0.81

Notes:

Abbreviations: THR (threonine); CYS (cysteine); VAL (valine); MET (methionine); ILE (isoleucine); LEU (leucine); TYR (tyrosine); PHE (phenylalanine); HIS (histidine); LYS (lysine); and TRP (tryptophan).

Measurements were preformed once on the composite blend from two extrusion processing runs.

(*) Indicates the first limiting amino acid.

APPENDIX B

Table B.1 Amino acid composition (g per 100 g of flour, on an *as is* basis) for raw chickpea, sorghum and maize flours, and chickpea: sorghum and chickpea: maize blends as a function of blending ratio (by mass).

Amino acids	Flour			Chickpea: sorghum blend				Chickpea: maize blend			
	Chickpea	Sorghum	Maize	5:5	6:4	7:3	8:2	5:5	6:4	7:3	8:2
% Protein[1]	20.86	9.42	6.87	15.18	16.30	17.26	18.39	13.74	15.18	16.45	17.78
% Moisture	10.04	10.00	10.16	10.11	10.03	9.76	9.89	10.32	10.04	9.93	9.83
Aspartic Acid	2.30	0.60	0.49	1.41	1.68	1.79	1.96	1.39	1.56	1.78	2.00
Glutamic Acid	3.43	1.94	1.15	2.66	2.94	3.04	3.16	2.31	2.53	2.95	3.00
Serine	1.17	0.46	0.35	0.81	0.91	0.97	1.04	0.76	0.86	1.06	1.02
Glycine	0.82	0.30	0.28	0.56	0.65	0.69	0.75	0.56	0.62	0.65	0.72
Histidine‡	0.46	0.17	0.17	0.33	0.37	0.39	0.43	0.33	0.37	0.65	0.42
Arginine	1.82	0.43	0.45	1.07	1.23	1.32	1.53	1.09	1.22	1.39	1.49
Threonine‡	0.64	0.28	0.23	0.43	0.47	0.51	0.55	0.42	0.46	0.51	0.55
Alanine	0.92	0.86	0.50	0.87	0.90	0.92	0.91	0.70	0.73	0.72	0.81
Proline	0.97	0.94	0.70	0.94	0.98	0.98	1.05	0.82	0.87	1.07	0.84
Tyrosine	0.55	0.30	0.20	0.41	0.44	0.44	0.50	0.38	0.42	0.50	0.45
Valine‡	0.85	0.43	0.30	0.61	0.70	0.72	0.75	0.56	0.62	0.69	0.73
Methionine*‡	0.20	0.12	0.11	0.16	0.17	0.18	0.20	0.16	0.17	0.19	0.19
Cysteine*	0.29	0.15	0.14	0.18	0.19	0.20	0.23	0.22	0.23	0.25	0.22
Isoleucine‡	0.78	0.33	0.20	0.55	0.63	0.66	0.70	0.49	0.55	0.65	0.67
Leucine‡	1.47	1.19	0.70	1.34	1.40	1.43	1.44	1.11	1.18	1.29	1.32
Phenylalanine‡	1.15	0.47	0.30	0.81	0.91	0.95	1.02	0.73	0.82	0.94	0.94
Lysine‡	1.20	0.16	0.19	0.68	0.82	0.90	0.99	0.69	0.81	1.09	0.96
Tryptophan‡	0.23	0.12	0.08	0.18	0.19	0.20	0.21	0.16	0.17	0.20	0.20

Notes:

*, sulfur amino acid. ‡, essential amino acids.

Measurements were preformed once on flour or blend flour

Table B.2 Essential amino acid concentration (mg/g protein) and amino acid score for raw chickpea, sorghum and maize flours, and chickpea: sorghum and chickpea: maize blends as a function of blending ratio (by mass).

Flours	Amino acids								
	THR	**VAL**	**MET + CYS**	**ILE**	**LEU**	**PHE + TYR**	**HIS**	**LYS**	**TRP**
a) Essential amino acid concentration (mg/g protein)									
Chickpea	30.68	40.75	23.49	37.40	73.13	84.58	22.05	59.70	11.44
Sorghum	29.72	45.65	28.66	35.03	126.33	81.74	18.05	16.99	12.74
Maize	33.48	43.67	36.39	29.11	101.89	72.78	24.75	27.66	11.64
Chickpea: sorghum									
5: 5	28.33	40.18	22.40	36.23	95.71	87.14	21.74	48.57	12.86
6: 4	28.84	42.95	22.09	38.66	93.65	90.30	22.70	54.85	12.71
7: 3	30.12	41.71	26.07	40.55	87.50	91.67	38.23	63.69	12.50
8: 2	29.91	40.79	23.38	38.07	82.95	87.56	23.38	57.03	12.10
Chickpea: maize									
5: 5	30.56	40.74	27.65	35.65	87.13	87.13	24.01	54.16	12.56
6: 4	30.31	40.85	26.36	36.24	82.75	86.96	24.38	56.80	11.92
7: 3	31.00	41.94	26.75	39.51	81.59	91.08	39.51	68.94	12.65
8: 2	30.93	41.05	23.06	37.68	78.38	82.54	23.62	57.01	11.88
FAO reference	34	35	25	28	66	63	19	58	11
b) Amino acid score									
Chickpea	0.90*	1.16	0.94	1.34	1.07	1.29	1.16	0.99	1.00
Sorghum	0.87	1.30	1.15	1.25	1.91	1.30	0.95	0.29*	1.16
Maize	0.98	1.25	1.46	1.04	1.54	1.16	1.30	0.48*	1.06
Chickpea: sorghum									
5: 5	0.83	1.15	0.90	1.29	1.34	1.28	1.14	0.77*	1.08
6: 4	0.85*	1.23	0.88	1.38	1.30	1.31	1.19	0.87	1.06
7: 3	0.89*	1.19	1.04	1.45	1.29	1.42	2.01	1.07	1.11
8: 2	0.88*	1.17	0.94	1.36	1.19	1.31	1.23	0.93	1.04
Chickpea: maize									
5: 5	0.90	1.16	1.11	1.27	1.22	1.28	1.26	0.87*	1.06
6: 4	0.89*	1.17	1.05	1.29	1.18	1.30	1.28	0.92	1.02
7: 3	0.91*	1.20	1.07	1.41	1.19	1.39	2.08	1.14	1.11
8: 2	0.91*	1.17	0.92	1.35	1.12	1.24	1.24	0.93	1.02

Notes:

Abbreviations: THR (threonine); CYS (cysteine); VAL (valine); MET (methionine); ILE (isoleucine); LEU (leucine); TYR (tyrosine); PHE (phenylalanine); HIS (histidine); LYS (lysine); and TRP (tryptophan).

Measurements were preformed once on the composite blend from two extrusion processing runs.

(*) Indicates the first limiting amino acid.

Table B.3 Amino acid composition (g per 100 g of flour, on an *as is* basis) for raw and pre-cooked chickpea: sorghum and chickpea: maize flour blends at a 6: 4 ratio.

Amino acids	Raw flour	Pre-cooked flour			
		Moisture 20%		Moisture 24%	
		120°C	150°C	120°C	150°C
a) Chickpea: Sorghum					
% Protein[1]	16.30	16.37	16.81	16.20	16.59
% Moisture	10.03	4.12	2.27	4.33	2.62
Aspartic Acid	1.68	1.40	1.55	1.37	1.40
Glutamic Acid	2.94	2.40	2.47	2.32	2.43
Serine	0.91	0.68	0.69	0.70	0.72
Glycine	0.65	0.44	0.46	0.43	0.43
Histidine‡	0.37	0.30	0.31	0.27	0.29
Arginine	1.23	0.98	1.00	0.97	0.98
Threonine‡	0.47	0.48	0.49	0.47	0.48
Alanine	0.90	0.63	0.67	0.64	0.65
Proline	0.98	0.66	0.70	0.68	0.70
Tyrosine	0.44	0.40	0.43	0.42	0.43
Valine‡	0.70	0.57	0.59	0.47	0.49
Methionine*‡	0.17	0.24	0.24	0.23	0.25
Cysteine*	0.19	0.23	0.22	0.22	0.23
Isoleucine‡	0.63	0.57	0.60	0.47	0.49
Leucine‡	1.40	1.19	1.23	1.15	1.17
Phenylalanine‡	0.91	0.79	0.82	0.77	0.78
Lysine‡	0.82	0.74	0.72	0.69	0.69
Tryptophan‡	0.19	0.16	0.16	0.16	0.15
b) Chickpea: maize					
% Protein[1]	15.18	15.59	15.90	14.90	15.60
% Moisture	10.04	4.19	3.13	5.65	3.35
Aspartic Acid	1.56	1.43	1.42	1.36	1.39
Glutamic Acid	2.53	2.21	2.24	2.14	2.20
Serine	0.86	0.45	0.47	0.44	0.45
Glycine	0.62	0.45	0.47	0.44	0.45
Histidine‡	0.37	0.29	0.32	0.29	0.29
Arginine	1.22	1.02	1.03	1.02	1.01
Threonine‡	0.46	0.47	0.48	0.46	0.47
Alanine	0.73	0.55	0.56	0.53	0.54
Proline	0.87	0.64	0.64	0.62	0.65
Tyrosine	0.42	0.41	0.40	0.41	0.41
Valine‡	0.62	0.48	0.47	0.51	0.47
Methionine*‡	0.17	0.25	0.23	0.23	0.23
Cysteine*	0.23	0.22	0.21	0.21	0.22
Isoleucine‡	0.55	0.47	0.56	0.49	0.47
Leucine‡	1.18	1.03	1.09	1.02	1.02
Phenylalanine‡	0.82	0.73	0.78	0.72	0.74
Lysine‡	0.81	0.76	0.72	0.75	0.71
Tryptophan‡	0.17	0.16	0.15	0.15	0.14

Notes:

*, sulfur amino acid. ‡, essential amino acids.

Measurements were preformed once on the composite blend from two extrusion processing runs.

Table B.4 Essential amino acid concentration (mg/g protein) and amino acid score for raw and pre-cooked chickpea: sorghum and chickpea: maize flour blends at a 6: 4 ratio.

Flours	Amino acids								
	THR	VAL	MET + CYS	ILE	LEU	PHE + TYR	HIS	LYS	TRP
b) Essential amino acid concentration (mg/g protein)									
Chickpea: sorghum									
Raw flour	29	43	22	39	86	83	23	50	12
Pre-cooked flour									
120°C, 20%	29	35	29	35	73	73	18	45	10
120°C, 24%	29	29	28	29	71	73	17	43	10
150°C, 20%	29	35	27	35	73	74	18	43	9
150°C, 24%	29	30	29	30	71	73	17	42	9
Chickpea: Maize									
Raw flour	30	41	26	36	78	82	24	53	11
Pre-cooked flour									
120°C, 20%	30	31	30	30	66	74	19	49	10
120°C, 24%	31	34	30	33	68	75	20	50	10
150°C, 20%	30	30	28	35	68	74	20	46	10
150°C, 24%	30	30	29	30	65	74	19	45	9
FAO reference	34	35	25	28	66	63	19	58	11
b) Amino acid score									
Chickpea: sorghum									
Raw flour	*0.85	1.23	0.88	1.38	1.3	1.31	1.19	0.87	1.06
Pre-cooked flour									
120°C, 20%	0.86	1.00	1.14	1.24	1.11	1.15	0.96	*0.78	0.87
120°C, 24%	0.85	0.82	1.12	1.03	1.07	1.17	0.88	*0.74	0.88
150°C, 20%	0.86	1.01	1.08	1.27	1.11	1.18	0.96	*0.74	0.85
150°C, 24%	0.86	0.85	1.16	1.06	1.07	1.16	0.91	*0.72	0.85
Chickpea: Maize									
Raw flour	*0.89	1.17	1.05	1.29	1.18	1.3	1.28	0.92	1.02
Pre-cooked flour									
120°C, 20%	0.89	0.89	1.19	1.08	1.00	1.17	0.99	*0.84	0.91
120°C, 24%	0.90	0.97	1.19	1.17	1.03	1.20	1.03	*0.87	0.93
150°C, 20%	0.90	0.84	1.10	1.26	1.04	1.18	1.05	*0.79	0.87
150°C, 24%	0.89	0.86	1.16	1.08	0.99	1.17	0.99	*0.78	0.81

Notes:

Abbreviations: THR (threonine); CYS (cysteine); VAL (valine); MET (methionine); ILE (isoleucine); LEU (leucine); TYR (tyrosine); PHE (phenylalanine); HIS (histidine); LYS (lysine); and TRP (tryptophan).

Measurements were preformed once on the composite blend from two extrusion processing runs.

(*) Indicates the first limiting amino acid.