Effects of Extrusion Cooking on Physical and Nutritional Quality of Puffed Snacks Made from Blends of Barley and Green Lentil Flours

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PREFACE

This thesis is written in manuscript style, including 6 chapters. Chapter 1 is the introduction of the study and Chapter 2 is the literature review covering the basic knowledge of the elements studied throughout the thesis. Chapter 3 and 4 are research sections, which include two manuscript style written chapters. General discussions and conclusions for this study, and limitations and recommendations for future research are presented in Chapter 5 and 6, respectively.

Contributions of Authors:

Chapter 3, "Effects of Extrusion Cooking on Nutritional Quality of Puffed Snacks Made from Blends of Barley and Green Lentil Flours" was prepared for journal publication with authorship by Xiang Li, Adam Franczyk, James D. House and Filiz Koksel. X. Li was responsible for the conduction of the research and the draft of the manuscript. A. Franczyk and J. D. House were responsible for the analysis of protein tests related data and giving critical advice on protein related discussion in the manuscript. F. Koksel was the corresponding author and was in charge of the research design and the reviewing of the manuscript. All authors were agreed on the final version of the manuscript.

Chapter 4, "Effects of Extrusion Cooking on Physical Quality of Puffed Snacks Made from Blends of Barley and Green Lentil Flours" was prepared for journal publication with authorship by Xiang Li and Filiz Koksel. X. Li was responsible for the conduction of the research, data analysis and the draft of the manuscript. F. Koksel was the corresponding author and was responsible for the research design and the reviewing of the manuscript. All authors were agreed on the final version of the manuscript.

ABSTRACT

Consumer demand for healthy snack foods is increasing. Puffed snacks currently in the market are predominately high in starch but low in nutritious compounds such as dietary fiber and protein, and thus are usually recognized as "junk food" by consumers. This work explored the potential of using blends of a high dietary fiber cereal (i.e., barley) and a high protein pulse (i.e., green lentil) flours to produce nutritionally-dense puffed snacks using extrusion cooking. Five barley: green lentil blending ratios (100: 0, 75: 25, 60: 40, 45: 55, 0: 100, db), three feed moistures (15, 18, 21 g/100 g) and two barrel temperature profiles (60/80/100/120/130℃ and 70/90/110/130/140℃ from the feeder to the extruder die) were employed. Nutritional attributes of puffed snacks including protein content, amino acid composition, *in vitro* protein digestibility (IVPD), *in vitro* protein digestibility corrected amino acid score (IVPDCAAS), insoluble, soluble and total dietary fiber contents were tested to evaluate the nutritional value of extrudates. Physical attributes including density, expansion index, textural properties (i.e., hardness, crispness and crunchiness) and color were also investigated. Extrusion significantly ($p < 0.0001$) enhanced the IVPD values, especially at low feed moisture and high die temperature. Blending improved the limiting amino acid score of blended flours and their extrudates and hence increased the IVPDCAAS of extrudates, where 45: 55 barley: green lentil blend had the highest average IVPDCAAS. Extrusion ($p < 0.0001$) significantly decreased soluble and total dietary fiber contents of the blends 45: 55 and 60: 40. High die temperature improved the overall expansion of extrudates significantly ($p < 0.0001$). The effect of feed moisture on expansion varied with the blends. Extruded blend 45: 55 showed the lowest hardness and the highest crispness, followed by blend 60: 40. Compared to the flours, extrudates showed darker color, redder and yellower tones. Overall, barley and green lentil blends of 45: 55 and 60: 40 had better

nutritional and physical properties compared to the other blends in this study, showing promising application in the real market. The results of this study indicated that using blends of cereal and pulse flours to replace traditional starchy materials and producing nutritious puffed snacks with acceptable physical quality is achievable.

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LIST OF ABBREVIATIONS

AACC. . . . . American Association of Cereal Chemists

AAS. . . . . amino acid score

AOAC. . . . . Association of Official Analytical Chemists

Ala. . . . . alanine

Arg. . . . . arginine

Asp. . . . . aspartate

Bly. . . . . barley

BR. . . . . blending ratio

CAC. . . . . Codex Alimentarius Commission

CFIA. . . . . Canadian Food Inspection Agency

CGC. . . . . Canadian Grain Commission

CIE. . . . . International Commission on Illumination

db. . . . . dry basis

DP. . . . . Degree of polymerization

DT. . . . . die temperature

Eq. . . . . equation

FAO. . . . . Food and Agriculture Organization of the United Nations

FDA. . . . . Food and Drug Administration

FM. . . . . feed moisture

GC/MS. . . . . Gas Chromatography–Mass Spectrometry

GL. . . . . green lentil

Glu. . . . . glutamate

Gly. . . . . glycine

His. . . . . histidine

HMWDF. . . . . high molecular weight dietary fiber

IDF. . . . . insoluble dietary fiber

Ile. . . . . isoleucine

IRI. . . . . Information Resources Incorporated

IVPD. . . . . *in vitro* protein digestibility

IVPDCAAS. . . . . *in vitro* protein digestibility corrected amino acid score

LAA. . . . . limiting amino acid

LAAS. . . . . limiting amino acid score

Leu. . . . . leucine

LMWSDF. . . . . low molecular weight soluble dietary fiber

Lys. . . . . lysine

Met. . . . . methionine

PDCAAS. . . . . protein digestibility corrected amino acid score

Phe. . . . . phenylalanine

Pro. . . . . proline

RACC. . . . . reference amounts customarily consumed

SDF. . . . . soluble dietary fiber

SDFP. . . . . dietary fiber soluble in water but insoluble in 76 g/100 g aqueous ethanol

SDFS. . . . . dietary fiber soluble in both water and 76 g/100 g aqueous ethanol

Ser. . . . . serine

TDF. . . . . total dietary fiber

Thr. . . . . threonine

Trp. . . . . tryptophan

Tyr. . . . . tyrosine

UPLC. . . . . Ultra-Performance Liquid Chromatography

USDA. . . . . U.S. Department of Agriculture

Val. . . . . valine

WHO. . . . . World Health Organization

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CHAPTER 1 Introduction

Puffed snacks are an important part of human diet, usually consumed between meals to ease hunger. The features of high diversity and availability made puffed snacks grow continuously in sales in the last few decades (Meng et al., 2010; Parker, 2019). However, the ingredients of puffed snacks are mainly limited to refined cereal flours which have considerable amounts of highly digestible carbohydrates. These characteristics enroot the image of puffed snacks as junk foods in consumers' minds and are opposite to the healthy snacks trends that more and more consumers are pursuing recently.

Extrusion technology was invented in 3rd century BC and one of its stream, extrusion cooking, has been employed to produce puffed snacks for more than 60 years (Maskan & Altan, 2012; Fellows, 2009). During extrusion cooking, under high temperature, shear, pressure, and with the presence of water in the extruder barrel, starch melts and forms a soft doughy mass. When the product exits the barrel through the die opening, due to the pressure difference between the barrel and atmosphere, water evaporates and grows as bubbles within the soft starchy mass when the product solidifies after cooling down (Guy, 2001). Briefly, the highly soluble starch and the elastic mass that starch forms are the keys of the formation of cellular structure, which is the major feature of puffed snacks that attracts consumers. Other food components such as protein and dietary fiber do not favor the formation of bubbles during extrusion cooking the same way that starch does. Traditional ingredients (e.g., corn meal) of puffed snacks generally need to contain more than 60% starch to ensure desirable expansion (Guy, 2001).

Although most studies reported that nutritious ingredients (e.g., pea protein isolate, barley bran) affected expansion of puffed snacks negatively, few studies claimed that ingredients containing protein and dietary fiber at intermediate levels did not impair product expansion significantly (Philipp et al., 2017; Kumari et al., 2018). Modifying extrusion conditions including feed moisture and die temperature can also increase expansion, making up for the absence of starch (Sharma et al., 2016). These findings from the literature formed the core design adopted in this MSc project: to improve the nutritional quality of puffed snacks without compromising the physical quality.

Two nutrients can be considered important when developing nutritious puffed snacks: protein and dietary fiber. Protein provides structure for cells and organisms and is responsible for catalytic reactions, considered as an essential nutrient for humans (Watford & Wu, 2011). Dietary fiber cannot be digested by the enzymes present in the small intestine of humans, meaning zero contribution to calorie count (Turner & Lupton, 2011), but has physiological effects such as promoting gastrointestinal health and lowering cholesterol (Berrios et al., 2010). Pulses have relatively high protein content (20-30 g/100 g), while cereals have relatively low protein content (8-15 g/100 g) but are rich in sulfur-containing amino acids (i.e., methionine and cysteine) that pulses are short of. Moreover, pulses are rich in lysine, the specific amino acid that cereals generally lack (Asif et al., 2013; Robin et al., 2010; Schuchmann & Palzer, 2012). Accordingly, cereals and pulses are complementary to each other in terms of protein quality (Balasubramanian et al., 2012). Furthermore, both cereals and pulses are good sources of dietary fiber (10-30 g/100 g), having benefits on improving lipid metabolism, reducing the glycemic index of foods and lowering triglycerides (Sullivan et al., 2013; Boye et al., 2010; Ciudad-Mulero et al., 2018; Asif et al., 2013). Therefore, the blends of cereal and pulse flours, which have intermediate contents of both protein and dietary fiber, are perfect choices of ingredients to ensure desirable nutritional quality of puffed snacks.

In terms of physical quality, expansion and textural properties are vital attributes affecting consumer's sensory experience of puffed snacks. Based on the literature, feed moisture and die temperature during extrusion cooking had significant effects on expansion and hardness of puffed snacks. The effect of screw speed was consistent with the effect of die temperature in most of cases, but the degree of its impact was less than die temperature and feed moisture (Meng et al., 2010; Sharma et al., 2016; Dogan et al., 2013). Extrusion cooking conditions also have substantial effects on nutritional properties of puffed products. For example, intensive extrusion conditions (e.g., low feed moisture and high die temperature) helped to increase protein digestibility of products but less amino acids were retained (Rathod & Annapure, 2016; Bjórck et al., 1983). In general, low to medium feed moisture (10-25 g/100 g), medium die temperature (110-140℃) and medium screw speed (200-250 rpm) are possible conditions that can produce protein- and dietary fiber- rich puffed snacks with reasonable expansion and desirable textural properties (Meng et al., 2010; Seth et al., 2015; Schmid et al., 2005).

This study aimed to: (1) produce nutritionally-dense puffed snacks without impairing the physical quality by using blends of whole cereal and pulse flours and manipulating extrusion conditions, and (2) systematically investigate the effects of cereal to pulse blending ratio, feed moisture and extrusion temperature profile on the nutritional and physical quality of puffed snacks. In order to do achieve these objectives, blends of barley and green lentil flours with 5 blending ratios (100:0, 75:25, 60:40, 45:55, 0:100, barley: green lentil, db), 3 feed moistures (15, 18, 21 g/100 g) and 2 extrusion temperature profiles (60/80/100/120/130 and 70/90/110/130/140℃ from feeder to die end of the extruder) were employed. This study adventurously didn't use any additional starchy material but only used whole flours in the formula to produce novel healthy puffed snacks. Its results are expected to provide valuable insights to the puffed snacks industry and open new market opportunities for whole barley and lentil flours.

CHAPTER 2 Literature Review

The aim of this literature review was to cover the history and application of extrusion cooking technology in food production and provide up-to-date information of puffed snacks industry. The urgent challenge of puffed snacks industry that is improving nutritional value of snacks foods without compromising their physical quality, and its possible solutions by employing novel protein- and dietary fiber-rich materials with modifications of extrusion conditions were also outlined and discussed. In addition, the major nutritional and physical attributes of puffed snacks were introduced.

2.1 Extrusion cooking in puffed snacks industry

2.1.1 Principle

In the 3rd century BC, a wooden barrel chamber with a screw inside, invented by a Greek mathematician and physicist as a simple tool to lift water, opened the history of extrusion technology. Nowadays, the term "extrusion" refers to the process that a liquid or semi-liquid material is forced through a restricted opening (Maskan & Altan, 2012). Extrusion technology is applied in many fields, including but not limited to metal fabrication, ceramics, concrete, plastic and foods. The use of extrusion in food industry can be divided into two streams, forming and cooking. Extrusion forming generally uses low shear (screw speed < 50 rpm) to shape products requiring low energy input, such as pasta and unexpended precooked pellets. While extrusion cooking uses medium to high shear (screw speed > 100 rpm), providing a significant amount of thermal and mechanical energy, to make products such as texturized proteins and puffed snacks (Maskan & Altan, 2012).

Extrusion cooking has been used to produce puffed snack for more than 60 years. Compared to single-screw extrusion, twin-screw extrusion is more popular in puffed snack industry due to its greater flexibility, easier operation and ability to handle a wide range of food materials (e.g., viscous, oily, wet and sticky) (Fellows, 2009). Depending on whether the paired screws on the two shafts are touching each other or not while rotating, a twin-screw extruder is categorized as intermeshing and non-intermeshing, respectively. Depending on the direction of rotation, twin-screw extruders can also be divided into co-rotating and counter-rotating. Among these 2×2 combinations, the co-rotating intermeshing twin-screw extruder has the best performance, which not only can handle high viscosity food materials but also is self-wiping (i.e., one screw wipes clean the next one, providing better mixing of the material inside the extruder) (Navale et al., 2015; Fellows, 2009; Maskan & Altan, 2012).

An extruder is generally composed of 4 essential pieces: (1) drive, converting electrical or hydraulic power to rotating energy to drive shaft(s); (2) feeder, storing food materials and feeding them into the barrel; (3), barrel with screws inside, where food materials are deformed and reconstructed by shear and heat; and (4) die opening, where products come out (Maskan & Altan, 2012). Apart from these, a water pump is required for regulating moisture during extrusion. Well-equipped extruders may also have auxiliary parts such as pre-conditioner, control panel, cutting system, gas injector and etc.

During extrusion cooking process, food materials are dropped from the feeder into the barrel, mixed and delivered by the screws towards the die. When food materials are travelling in the barrel, due to the application of high temperature, high shear, presence of water and build up of pressure, big particles are broken down and flattened, dry polymers are hydrated, deformed and transformed into fluid-like materials. In addition to these physical changes, chemical reactions also take place, including protein denaturization and starch melting. With a series of changes, food materials form a soft doughy mass called the “melt” (Guy, 2001). When the melt exits the die, due to the sharp drop of pressure, water (in the melt) vaporizes and expands within the melt forming the initial bubbly structure of the extrudate. After reaching the expansibility limit of the melt, water vapor escapes, sometimes followed by the collapse of bubbles. After cooling down, the melt solidifies and the cellular structure of extrudate sets. This basic principle behind puffed snack production as well as the choice of food materials (e.g., corn starch and corn meal) and corresponding processing conditions have not changed over the last few decades. However, with the changing demands of the health conscious consumers, puffed snacks industry is seeking improvements, such as lower calories per serving size, higher protein and dietary fiber contents, and lower glycemic index.

2.1.2 Process variables

2.1.2.1 Ingredients

Guy (2001) categorizes food ingredients into 6 groups depending on their functional roles in extrusion cooking as (1) structure forming materials, (2) dispersed phase filling materials, (3) plasticising or lubricating materials, (4) soluble solids, (5) nucleating materials and (6) colouring and flavouring materials. Structure forming materials are those which melt and form a continuous soft fluid after they are heated over their glass transition temperature. Once above their glass transition temperature, these materials allow bubbles to expand in the melt after leaving the die, as superheated water is converted to vapor due to the pressure drop outside of the extruder barrel. When temperature drops below glass transition temperature, as the extrudate cools down, structure forming materials solidify, and the cellular structure of the end-product (i.e., the extrudate) is formed. Polymers with 2-10^5 DP (degree of polymerization) which have sufficient molecular weight to give the melt enough viscosity to hold the bubbles in addition to good flow properties to allow bubbles to grow due to relatively low molecular weight, are considered as typical structure forming materials (Guy, 2001). Starch is the perfect structure forming material in extrusion cooking. Maskan & Altan (2012) mentioned that food ingredients suitable for making puffed snacks should generally contain more than 60 % starch. Corn, wheat and potato starches are most common ingredients for puffed snacks.

Dispersed phase filling materials affect the structure of extrudates in two ways. Firstly, they may disrupt the starch phase due to their physical structure, impairing extrudate expansion. Secondly, they can affect the die swell of starch (die swell is the expansion of the extrudate at the die due to starch's released energy that was stored elastically in its molecular structure when molted starch enters the die). Dispersed phase filling materials include proteins and fibers. Their presence in the product formula reduces this die swell effect (Guy, 2001). Therefore, it is widely agreed that proteins and fibers have negative effects on extrudate expansion and therefore not commonly preferred by puffed snacks industry. Proteins can go through glass transition and form a separate phase lying within the starch phase (Guy, 2001). However, proteins are also considered as thermosetting materials (i.e., once they are heated over glass transition temperature and then cooled down, they tend to become hard and impair the mouthfeel of the final product) (Maskan & Altan, 2012). Fibers are generally stable during extrusion but may decompose under high thermal energy inputs (Guy, 2001).

Plasticising (e.g., water) or lubricating (e.g., oils and fats) materials help transform dry polymers to deformable plastic fluids and relive the friction between particles in the fluid and metal surfaces of barrel and screws. The concentration of water, oils and fats have significant effects on extrudate expansion from many aspects. For example, water level affects the viscosity of the melt while oils and fats level (> 2 g/100 g) reduce starch degradation and may hinder extrudate expansion (Guy, 2001). Soluble solids are low molecular weight materials, such as sugar and salt. Their effects on extrudates are dependent on their concentrations and chemical reactions with starch and proteins (Guy, 2001). Nucleating materials are fine powdery materials (e.g., calcium carbonate) that remain insoluble during extrusion and can reduce the energy required for bubble formation and thus increase the number of bubbles (Guy, 2001). More recently, physical blowing agents in the gaseous phase have also been used as nucleating materials during extrusion cooking of snack foods (Koksel & Masatcioglu, 2018). Finally, colouring and flavouring materials may naturally be present in the raw materials (e.g., anthocyanin), generated during chemical reactions induced by extrusion (e.g., Maillard reactions), or added to the recipe (Guy, 2001). All these different food ingredients affect the physical structure and the nutritional value of final products, and are therefore considered in the first step of developing new puffed snack products.

2.1.2.2 Feed moisture

Feed moisture is a crucially important variable affecting extrudate properties from many aspects, including expansion, texture, amino acid losses, etc. First of all, feed moisture directly affects the level of starch conversion and melting. Hence, it affects the viscosity of the melt, the intensity of the shear it is exposed to, and the mechanical energy input transferred to it. In addition, feed moisture has significant impacts on physical and chemical reactions undertaken in the barrel, e.g., protein degradation, transformation between insoluble and soluble fibers, Maillard reaction and the inactivation of antinutritional factors. In terms of producing puffed snacks, relatively low feed moisture contents are preferred (10-25 g/100 g) in order to obtain extrudates with desirable properties such as high expansion, low hardness, high protein digestibility, etc. (Meng et al., 2010; Seth et al., 2015).

2.1.2.3 Extrusion temperature profile

Increasing the temperature during extrusion increases the level of starch melting, reduces melt viscosity, lowers specific mechanical energy (i.e., the mechanical energy input (kW h) to the extruder per kg of raw material), and leads to more moisture vaporization when the extrudate leaves the die. Therefore, die temperature generally has a positive effect on extrudate expansion. However, in some cases, when die temperature is extremely high (e.g., > 210℃), the melt viscosity might drop to a value that is too low so that starch's swelling effect at the die will be impaired, resulting in decreased expansion (Maskan & Altan, 2012; Liang, 2008). Considering the physical quality (e.g., overall expansion and texture), the preferred die temperature for producing puffed snacks is around 140-180℃ (Schmid et al., 2005). Die temperature also have substantial effects on protein digestibility, inactivation of antinutritional factors, insoluble to soluble fiber transformation and other nutritional properties of extrudates. For example, an increase in die temperature generally results in enhanced protein digestibility but lower amino acid retention. The trend and extensity of these effects depend on the nature of the food ingredient.

2.2 Market trends and challenges for puffed snacks industry

2.2.1 Global

Puffed snacks are usually consumed between meals to ease hunger. The market size of puffed snacks has been continuously growing recently. Based on the data of Information Resources Incorporated (a market research company in Chicago), from September 2018 to September 2019, the market sale of Frito-Lay, the leading brand in puffed snacks industry increased 10.4% to $3.2 billion. Sales of Frito-Lay's top three products, Funyuns, Chester's and SunChips grew 10.1, 10.6 and 10.8% to $423.6, $327.1 and $292.4 million, respectively (Parker, 2019).

The high availability and diversity are the main reasons that ready-to-eat puffed snacks are attracting more and more people, especially children (Meng et al., 2010). Puffed snacks currently on the market are predominately made from refined cereal flours (e.g., corn and wheat), and they are generally rich in saturated fats and highly digestible carbohydrates, but low in proteins and fibers (Patil et al., 2016; Sumargo et al., 2016), making them calorie dense but nutritionally inferior. It is argued that the high consumption of puffed snacks might contribute to the increasing rate of obesity (Patil et al., 2016; Onwulata et al., 2010). Based on these concerns, consumers started seeking nutritionally-denser snack foods. For example, Mintel reports stated a 33% increase in consumers who demand healthier snacks (Sumargo et al., 2016).

Currently, there are two main solutions to improve the nutritional value of puffed snacks. The first solution is to employ protein- and dietary fiber-rich ingredients in the formulation of puffed snacks. Pulses, carrots and beets are the top three alternatives reported in the literature. Other options such as hemp, quinoa and even crickets are also popular. Second solution is to optimize the extrusion process conditions to maintain or enhance the nutritional value of extrudates. For example, increasing extrusion temperature to increase protein digestibility and using hot air expansion (i.e., further processing extrudates into 300-400℃ high velocity hot air for a short time for dehydration and enhancing their puffing) to replace frying (Kvidahl, 2018; Guraya & Toledo, 1994).

2.2.2 Canada

Based on Vatanparast et al. (2019), 80.4% of Canadians consumed more than one snack per day, where 10% reported consuming at least four per day. On average, snacks contributed to 23% of total daily energy intake of Canadians, varying with age. The highest total daily energy intake was for 2-5 years old children (27%). In terms of improving the nutritional value of puffed snacks, Canada has great advantages on exploring new ingredients in puffed snack formulae, such as whole cereal grains and pulses, considering its world-class leading production of these ingredients. For example, based on Roy et al. (2010), Canada was the second largest oat producer in the world in 2005 and produces more than 70% of world's green lentil over the years.

2.3 Potential ingredients for puffed snacks

2.3.1 Cereals

Cereals, which are defined as cultivated grasses belonging to the Graminae family, are the most important sources of food for humans since ancient times. Rice is the main food for Asia, sorghum and millet are the staples for Africa, and wheat, rye and barley are the traditional foods for Europe. Corn has become the new staple in the modern society, especially in Americas, due to the intensive use in feedstock and fuel ethanol. Based on the data of FAO (Food and Agriculture Organization of the United Nations), the worldwide planting area for cereal crops had increased to 700 million ha in 2007, accounting to more than 70% of total farmland, and yielded 2.3 billion ton grains (Barbosa-Cánovas, 2010). This yield was estimated to increase to 2.7 billion ton in 2019 (FAO, 2020).

Cereals are good sources of carbohydrates including dietary fiber (10-20 g/100 g), but generally low in proteins (8-15 g/100 g) compared with pulses (20-30 g/100 g) (Robin et al., 2012; Mickowska et al., 2012; Asif et al., 2013; Boye et al., 2010). Main types of dietary fiber present in cereals include cellulose, arabinoxylans, xyloglucans, beta glucans and glucomannans (Johnson, 2012). Cereals are known for lacking lysine, which is the major factor limiting the overall protein quality of cereals.

2.3.1.1 Barley

In 2007, the world's production of barley was 136 million ton, ranking the fourth in global production of cereals (Barbosa-Cánovas, 2010). Based on the data of CGC (Canadian Grain Commission), the estimated planting area of barley in Canada was 3.0 million ha and the estimated yield was 10.0 million ton in 2019 (CGC, 2020). Barley can survive in a wide range of climatic conditions, so it is a common crop grown by almost all countries. However, only about 2% of barley is used for human consumption and the rest of it is mostly used as brewing material and animal feed. The major reason for its low use for food consumption is that barley is low in gluten, so it is hardly used in baking applications (Sharma & Kotari, 2017; Kirjoranta et al., 2012).

Barley contains relatively high protein content (10-15 g/100 g) among cereals (Sullivan et al., 2013). It is one of the richest and cheapest protein sources, supporting millions of low-income people worldwide (Alka et al., 2017). In terms of amino acid composition, according to Cervantes-Pahm et al. (2014), on as-is basis, barley was high in glutamic acid (2.57 g/100 g), leucine (0.73 g/100 g), and aspartic acid (0.59 g/100 g). The lysine content of barley was 0.39 g/100 g, which is slightly higher than some other cereals such as white rice and wheat.

Barley contains 11-20 g/100 g of dietary fiber (Sullivan et al., 2013). The most important and unique type of dietary fiber present in barley is beta-glucan, which can account for 2-10% of barley weight alone. In some barley cultivars, it can even go up to 20% (Sullivan et al., 2013; Sharma & Gujral, 2013). It is believed that beta-glucan can lower plasma cholesterol, improve lipid metabolism and reduce the glycemic index of foods (Sullivan et al., 2013). The health benefits of consuming barley include but not limited to lowering the risk of coronary heart disease, type-2 diabetes, etc. (Sullivan et al., 2013). Health Canada (2020b) recognizes the effect of barley on lowering blood cholesterol and has approved qualified barley products to carry relevant health claims such as “barley fibre helps reduce/lower cholesterol”.

2.3.2 Pulses

Pulses are dried seeds of legumes (e.g., pea, chickpea, lentil, etc.), except few crops that are used for oil extraction (i.e., soybean and peanut). Pulses are the second most important source of food for humans following cereals, and the world production of pulses is around 57 million tons per year (Asif et al., 2013). Pulses contain well-balanced nutrients and have many health benefits, including preventing chronic diseases, cardio-vascular diseases, type-1 diabetes, etc. (Morales et al., 2015a). One of the major advantages of pulses is their high protein content. The typical ranges of protein contents of pea, chickpea and lentil are 20-30, 20-25 and 26-31 g/100 g, respectively (Day, 2013; Zhao et al., 2005; Asif et al., 2013; Boye et al., 2010), which are significantly higher than cereals (e.g., 7-9 g/100 g for rice and 9-12 g/100 g for corn) (Mickowska et al., 2012). In addition to the quantity, the amino acid compositions of pulse and cereal proteins are also dramatically different. Pulses are rich in lysine and limited in sulfur-containing amino acids (i.e., methionine and cysteine), while cereals are generally the opposite. It is believed that pulses and cereals can complement each other from a nutritional perspective (Balasubramanian et al., 2012; Lazou et al., 2010). The dietary fibers present in pulses contain more amylose than amylopectin, which are good for lowering cholesterol and triglycerides (Asif et al., 2013).

2.3.2.1 Lentil

Lentil, also known as dahl, masur, massar and masuri tillseed, is now produced by over 48 countries, growing on various soil types from sand to clay loam. India, Turkey and Canada are the main producers of lentil in the world (Roy et al., 2010). In 2011, Canada produced over a third of the world's lentils (1.5 million ton) and generated $873 million in exports (Bekkering, 2011). Lentils are generally categorized into 6 types, which are red, French green, Spanish brown, Laird, Eston, and Richlea. At the global scale, 70% of produced lentil belongs to the red type, 25% belongs to the green type and the rest of 5% belongs to brown and other types. However, Canada produces mainly the green type, which accounts for more than 70% of world's green lentil production (Bekkering, 2011).

The typical protein content range of lentil is 26-31 g/100 g, which is outstanding even among pulses (Zhao et al., 2005; Asif et al., 2013; Boye et al., 2010). In terms of amino acid composition (g/100 g), on as-is basis, green lentil is relatively rich in glutamate (3.86), aspartate (2.81), arginine (2.2) and lysine (1.61), but limited in methionine (0.19), cysteine (0.20) and tryptophan (0.67) (Nosworthy et al., 2018). The total dietary fiber content of lentil is in the range of 10-30 g/100 g, including around 5 g/100 g of arabinoxylans (Boye et al., 2010; Ciudad-Mulero et al., 2018; USDA, 2020).

2.4 Nutritional quality attributes of puffed snacks

2.4.1 Protein content and amino acid composition

Proteins, which are composed of amino acid chains, are responsible for catalytic reactions, DNA replications, and providing structure to cells and organisms. Proteins can be digested to small peptides and free amino acids in human gastrointestinal tract, then re-synthesized to proteins again in human cells for specific needs. There are 20 types of amino acids that humans need, and 9 of them that humans cannot synthesize or cannot synthesize enough to maintain growth. These 9 are called essential amino acids, which are leucine (Leu), valine (Val), isoleucine (Ile), histidine (His), lysine (Lys), methionine (Met), threonine (Thr), tryptophan (Trp), and phenylalanine (Phe) (Watford & Wu, 2011).

Kjeldahl is one of the most common methods used to determine crude protein content of foods, which is inexpensive, applicable to all types of foods and can measure proteins on microgram level (using modified micro Kjeldahl method) (Chang, 2010). The Kjeldahl method measures the nitrogen content in the food, considering nitrogen is the most distinguishing element in proteins. There are 5 steps in the Kjeldahl method, including digestion, neutralization, distillation, titration and calculation. Firstly, a sample is digested with concentrated sulfuric acid and catalysts (e.g., potassium permanganate) to break down all organic matter under high temperature (e.g., 380℃) until the sample solution becomes optically clear. During this digestion process, nitrogen (N) present in the sample is converted to non-volatile ammonium sulfate [$(NH_4)_2SO_2$] (Eq. 1). Secondly, sodium hydroxide (NaOH) is added to the digested sample solution to neutralize the sulfuric acid and convert the ammonium sulfate to volatile ammonia (NH_3) (Eq. 2). Thirdly, released ammonia gas is distilled and captured by boric acid (H_3BO_3) in the form of ammonium cation (NH_4^+) and borate anion ($H_2BO_3^-$) (Eq. 3). Then, borate anion is titrated with hydrochloric acid (HCl) (Eq. 4). Lastly, the nitrogen content is calculated based on the amount of titrated hydrochloric acid and the law of conservation of quantity. Obtained nitrogen content is then multiplied with nitrogen conversion factor to get the final protein content of the food (Chang, 2010; AACC, 1999). Nitrogen conversion factor is the factor used to convert g/100 g nitrogen to g/100 g protein, varying with the type of the food. Most of food proteins contain 16 g nitrogen/100 g of protein, therefore, the conversion factor is 100/16= 6.25 (Chang, 2010). The disadvantage of Kjeldahl method, as well as Dumas method (the other widely used protein determination method), is that they measure all organic nitrogen present in the food, including non-protein nitrogen, so they may sometimes overestimate the protein content of foods (Chang, 2010).

$$N \rightarrow (NH_4)_2SO_2 \quad \text{(Eq. 1)}$$

$$(NH_4)_2SO_2 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O \quad \text{(Eq. 2)}$$

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^- \quad \text{(Eq. 3)}$$

$$H_2BO_3^- + HCl \rightarrow H_3BO_3 + Cl^- \quad \text{(Eq. 4)}$$

The determination of amino acid composition is a complex procedure, which includes dividing amino acids into 3 groups (acid-stable, sulfuring-containing, and Trp) to quantify them separately (AOAC, 2000). Acid-stable amino acids include serine (Ser), arginine (Arg), glycine (Gly), aspartate (Asp), glutamate (Glu), alanine (Ala), proline (Pro), tyrosine (Tyr), Leu, Val, Ile, His, Lys, Thr and Phe. To determine these amino acids, the sample needs to be hydrolyzed with 6N HCl under vacuum to break down proteins so that free amino acids can be released. The hydrolyzed sample is then adjusted to pH 5.5-6.0 with NaOH, diluted and filtered for derivatization. The aim of derivatization is adding chromophore to amino acids, for example, mixing the amino acid filtrate with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, making them detectable in Ultra-Performance Liquid Chromatography (UPLC). The determination of sulfur-containing amino acids, which are Cys and Met, is same with acid-stable amino acids determination, except the sample needs to be oxidized with performic acid to form cysteic acid and methionine sulfone, respectively, before hydrolyzing with HCl. Trp cannot survive acidic hydrolysis, so it is hydrolyzed with 4.2N NaOH or 3.5 barium hydroxide [$Ba(OH)_2$]. Hydrolyzed sample is then adjusted to pH 3.0-3.2 with HCl, diluted and filtered. Trp filtrate does not require derivatization before loading to UPLC (Nosworthy et al., 2017; AOAC, 2000).

Extrusion cooking has significant effect on amino acid composition of foods. Nosworthy et al. (2018) who studied the effect of extrusion on lentil flour, found that 5 amino acids (e.g., aspartate, glutamate and isoleucine) in green lentil and 12 amino acids (e.g., threonine, serine and glutamate) in red lentil increased more than 0.1 g/100 g on as-is basis after extrusion, respectively. Based on Duranti (2006), the increase of amino acids in extrudates might be due to extrusion's ability at high temperatures to inactivate proteinous antinutritional factors (e.g., hydrolase inhibitors and lectins), releasing amino acids. However, extrusion has also been reported to cause great losses of some amino acids (e.g., lysine, methionine and cysteine) due to extensive heat applied during the process (Clemente et al., 1998). Lysine is the most limited essential amino acid in cereal based products. Therefore, lysine retention is an important parameter to evaluate the effect of extrusion on protein quality. Bjórck et al. (1983) studied the effect of extrusion cooking on wheat flour and corn starch-based products and reported that lysine retention increased with increasing feed moisture and decreasing die temperature. The trend of the retention of sulfur-containing amino acids (i.e., methionine and cysteine) was consistent with lysine retention. Frias et al. (2011) claimed that different amino acids had different tolerances for increase in temperature. For example, at 129°C die temperature, losses of valine, phenylalanine and lysine were in the range of 10-22%, while glycine only had 8% loss.

Overall, die temperature is the most critical factor affecting amino acid retention during extrusion. In general, most types of amino acids can survive ≤130°C. Feed moisture has a positive relationship with amino acid retention. In addition, theoretically, blending ingredients which have complementary amino acid composition (i.e., blending cereals and pulses) could be an efficient way to give extrudates a balanced amino acid composition and help make puffed snacks with high protein quality.

2.4.2 *In vitro* protein digestibility

In addition to protein content and its amino acid composition, the digestibility of a protein is also an important determinant of protein quality. Due to the differences of the nature of proteins (e.g., protein configuration), the digestion level of different proteins are not the same. Moreover, due to the presence of some constituents that can impair protein digestion (e.g., tannins and dietary fiber) in the food matrix, the digestibility of the same protein may be different in different food materials (FAO/WHO, 1991).

Compared with the determination of *in vivo* protein digestibility, requiring animal trials, extensive labour and experiment time, *in vitro* protein digestibility (IVPD) is a more practical method and is therefore studied by many researchers. The principle of IVPD method is using combination of 3-4 enzymes (trypsin, chymotrypsin, peptidase and bacterial protease) to digest a food sample, simulating the function of human's digestive system, and using the decrease of pH caused by enzymatic digestion to estimate the sample's protein digestibility (FAO/WHO, 1991). After years of development, IVPD method can be completed within an hour and is highly sensitive to detect the changes of digestibility caused by antinutritional factors (e.g., trypsin inhibitor) and heat treatments (e.g., extrusion cooking) (Hsu et al., 1977). Regression analyses conducted by Hsu et al. (1977) showed that IVPD results were highly correlated with *in vivo* protein digestibility of rats, having the correlation coefficient at 0.90.

Cereal and pulse proteins generally have low IVPD values compared with animal proteins due to substantial antinutritional factors present in plants (Boye et al., 2010). Fortunately, it has been widely approved that heat treatments, including extrusion cooking, can inactivate antinutritional factors and hence improve food materials' protein digestibility. For example, IVPD value of raw lentil was reported to be as low as 39.4% (Rathod & Annapure, 2016) but could be increased to

84-88% after extrusion (Nosworthy et al., 2018). Nosworthy et al. (2017) studied the effect of extrusion on IVPD values of pinto bean and buckwheat blends. Their results suggested that blending pulse with cereal flours can significantly improve blend's protein digestibility when compared to that of cereal flour alone. It has also been reported that IVPD values increase with decreasing feed moisture and with increasing die temperature. In general, low feed moisture (<20 g/100 g), high die temperature (>130°C) and high screw speed (200-300 rpm) are preferred (Sumargo et al., 2016; Rathod & Annapure, 2016) for achieving high IVPD values.

2.4.3 Amino acid score

To evaluate protein quality of food products systemically, FAO/WHO (1991) recommended that the pattern of amino acid requirement of pre-school children (2-5 years) be used as the basis of amino acid scoring system for all children and adults, except for infants. This reference pattern includes the requirements of 9 individual/groups of amino acids, which are His, 19 (mg/g protein); Iso, 28; Leu, 66; lysine, 58; Met+Cys, 25; Phe+Tyr, 63; Thr, 34; Typ, 11; and Val, 35. Amino acid score (AAS) is the ratio of the amino acid composition of a food product to the corresponding requirement in the pre-school children pattern (Eq. 5).

AAS= mg of an amino acid in 1.0 g of test protein / mg of the same amino acid in 1.0 g of preschool children (2-5 years) reference pattern (Eq. 5)

For example, if an oat sample contains 19 mg His per g protein, its His score would be 19/19=1.0. For food products that contain multiple ingredients, instead of measuring the AAS of the product as a whole, if the protein content and amino acid composition data of all individual ingredients is known, the AAS of the product can be calculated based on weight proportions of ingredients. For example, if a cereal product is composed of 70% oat and 30% rye by weight, while the oat and rye contain 8 and 10 g/100 g protein, 19 and 38 mg His per g protein, respectively, the His score of the cereal product would be calculated as follows:

$$\frac{\frac{19\times 8\times 70\%+38\times 10\times 30\%}{8\times 70\%+10\times 30\%}}{19}=1.3.$$

This example also indicates that blending can affect food products' AAS significantly. Among 9 of obtained scores, the lowest amino acid score is termed as the limiting amino acid score (LAAS) and the amino acid with the LAAS is referred as the limiting amino acid (LAA).

2.4.4 *In vitro* protein digestibility corrected amino acid score

In vitro protein digestibility corrected amino acid score (IVPDCAAS) is the easiest approach to assess the protein quality of a certain food product, which does not involve *in vivo* experiments, but takes both human amino acid requirements and the biological availability of the proteins into consideration (Nosworthy & House, 2017; FAO/WHO, 1991). It is the product of LAAS and IVPD (Eq. 6). The IVPD value of a complex food product can also be calculated based on the weight proportions of individual ingredients as the way to calculate its AAS, so the IVPDCAAS of the food product can then be obtained easily (FAO/WHO, 1991).

$$\text{IVPDCAAS} = \text{LAAS} \times \text{IVPD} \qquad \text{(Eq. 6)}$$

The calculation of IVPDCAAS uses the LAAS, not the highest or the average of 9 AASs, showing that a high protein quality food product must have a balanced amino acid composition. Therefore, blending materials that have complementary amino acid composition (i.e., cereals and pulses) can be a great way to improve products' protein quality. For example, Wang et al. (2019) found that blending chickpea and corn flours increased the LAAS of corn from 0.5 to 0.8-0.9 and the IVPDCAAS from 35 to 64-68.

2.4.5 Dietary fiber

Dietary fiber does not have a worldwide agreed formal definition. Based on the definition of Codex Alimentarius Commission (CAC), dietary fiber is "carbohydrate polymers with ten or more monomeric units and cannot be hydrolyzed by the endogenous enzymes in the small intestine of humans" (Codex Alimentarius, 2010). Some other definitions include the oligosaccharides in dietary fiber as well, which are composed of 3-9 monomeric units (McCleary, 2013). CAC categorizes dietary fiber into 3 groups, which are (1) natural carbohydrate polymers present in foods; (2) carbohydrate polymers from foods but obtained by physical, enzymatic or chemical means; (3) synthetic carbohydrate polymers (Codex Alimentarius, 2010). Dietary fiber can also be categorized based in its solubility in water and in ethanol, and molecular weight (McCleary, 2013). Although the definition and categorization of dietary fiber given by different authorities might be slightly different, it is commonly agreed that dietary fiber has physiological effects that are beneficial to human health (McCleary, 2013) such as promoting bowel and gastrointestinal health, lowering cholesterol and balancing blood glucose (Berrios et al., 2010). AACC method 32-07.01 is a widely used method to measure insoluble, soluble and total dietary fiber (IDF, SDF and TDF, receptively) of foods and food products, recommended by the CAC. The principle of this method is digesting a sample with heat-stable alpha-amylase, protease, and amyloglucosidase to hydrolyze starch and protein. Then the digested sample solution is filtered to obtain the IDF residue while the filtrate is saved and precipitated in 76 g/100 g aqueous ethanol to obtain the SDF residue. Protein and ash contents are later determined and subtracted from the two residues to get IDF and SDF contents. TDF content is the sum of IDF and SDF contents (AACC, 1999). It should be noted that the SDF measured using this method only includes the dietary fiber that is soluble in water but insoluble in 76 g/100 g aqueous ethanol

(SDFP), while it does not include the dietary fiber that is soluble in both water and 76 g/100 g aqueous ethanol (SDFS). Thus, the TDF measured using this method is also referred to as high molecular weight dietary fiber (HMWDF) (McCleary, 2013; CAC, 2010). AACC method 32-07.01 is inexpensive and has relatively low requirements for personnel, compared with other methods such as those that use high performance liquid chromatography (e.g., AACC method 32-45.01). However, due to the incubation temperature of heat stable alpha-amylase being as high as 95℃, this method may cause the hydrolysis and hence the loss of resistant starch (i.e., starch and its degradation products which cannot be digested by small intestine of humans). The SDF determined by this method also tends to be lower than other methods, which might because of the difficulty in retaining SDF during filtration (McCleary, 2013).

The presence of dietary fiber, especially IDF, in extrudates, often leads to reduced expansion index, harder texture, and lower crispness. SDF has been reported to have relatively less damage on extrudate expansion than IDF (Robin et al., 2012). During extrusion cooking, dietary fibers' properties (i.e., solubility and structure) can be modified and even result in physiochemical transformation (e.g., resistant starch can be transformed into in-vitro digestible starch) due to intensive shear and thermal energy input (Robin, Schuchmann & Palzer, 2012). Frias et al. (2011) found that extrusion slightly decreased the TDF content (2 g/100 g) of pea extrudates and reported that increasing die temperature had a negative quadratic effect on extrudates' TDF content. Sharma & Gujral (2013) reported that total beta-glucan content of barley was not affected by extrusion but increased soluble/insoluble beta-glucan ratio was observed after extrusion, especially under low feed moisture and high temperature extrusion conditions. Similarly, Morales et al. (2015b) observed increased SDF/IDF ratio of lentil based snacks after extrusion, with a decrease of TDF content. However, Ciudad-Mulero et al. (2018) reported an increase of TDF content of lentil flour after extrusion. Overall, extrusion cooking can have a significant effect on dietary fiber content and composition, and its effect can vary with the type of food materials and the severity of extrusion conditions used. Among extrusion conditions, feed moisture content and die temperature are the two major factors involved in the changes in dietary fiber.

2.5 Physical quality attributes of puffed snacks

2.5.1 Extrudate density

Extrudate density is calculated by dividing the mass of an extrudate piece by its volume, where the volume is commonly determined by a seed displacement method (Ryu & Ng, 2001). A high quality puffed snack is expected to have a low density, indicating a high gas volume fraction in the extrudate. Devi et al. (2013) studied sorghum based puffed snacks enriched with various protein sources and found that extrudate density was highly dependent on the chemical composition of the feed and the type of protein source. High protein and dietary fiber contents generally cause an increase in extrudate density (Philipp et al., 2017), while die temperature and screw speed usually have negative relationships with extrudate density (Sharma et al., 2016; Kirjoranta et al., 2016). The balance of die temperature and screw speed is important since they have the same effect on expansion. For example, high die temperature may only require medium to low screw speed to ensure reasonable density. Increasing feed moisture increases extrudate density in most of cases, so low feed moisture (<15 g/100 g) is preferred during extrusion cooking of puffed foods. Dogan et al. (2013) reported that the effect of screw speed had less impact on extrudate density, compared with other extrusion variables (e.g., feed moisture).

2.5.2 Expansion index

Expansion index (radial, sectional, or longitudinal) is a measure of how well an extrudate puffs. Radial expansion is calculated as the ratio of cross-sectional diameter of the extrudate to the diameter of the die opening (Sumargo et al. 2016). Sectional expansion is the ratio of squared extrudate diameter to squared die diameter (Devi et al., 2013). Longitudinal expansion is the ratio of the exiting velocity of the extrudate after expansion to its velocity in the die orifice (Patil et al., 2017). The term "expansion index" mentioned throughout the rest of this thesis without specification refers to radial expansion index.

Based on Lazou & Krokida (2010) and Navale et al. (2015), the effects of one particular extrusion variable on expansion index and extrudate density are opposite. For example, an increase in dietary fiber content generally causes an increase in extrudate density but a decrease in expansion index. While an increase in protein and feed moisture contents generally have negative effects on expansion index, increasing die temperature and screw speed have positive effects (Navale et al., 2015; Devi et al. 2013). However, Kumari et al. (2018) studied the expansion of barley flour supplemented rice based puffed snacks and found that low levels of barley flour addition increased expansion. In this study, the maximum expansion was achieved by the extrudate enriched with 20% hulled barley flour instead of pure rice extrudate which had the lowest protein and fiber contents. Yovchev et al. (2017) found the effect of screw speed on expansion index was less significant than feed moisture and die temperature.

2.5.3 Textural properties

Textural properties including hardness, crispness and crunchiness are critical sensory properties for puffed snacks. One common method to measure textural properties of puffed snacks is using a steady force to deform a sample while plotting a force vs. time graph during the deformation to observe the response of the snack food. Defined by Stable Micro Systems (2020), a British manufacturer of texture analyzers that are widely used to study textural properties of food products, hardness (N) is the mechanical force required to crush a sample completely, crispness is the number of observed positive peaks and crunchiness (N sec) is the linear distance under the test curve. In general, well-expanded puffed snacks have high crispness, but low hardness and crunchiness (Philipp et al., 2017).

Taverna, Leonel & Mischan (2012) who studied quinoa flour fortified cassava starch based extrudates at 0-20% quinoa addition levels reported that 10% of quinoa flour fortified extrudate had the lowest hardness, indicating that intermediate protein content benefited extrudate's hardness. However, Devi et al. (2013) and Kumari, Abhishek & Gupta (2018) reported that intermediate protein addition lowered extrudates' crispness, which is not desirable. Navale et al. (2015) reported that feed moisture and screw speed had negative effects on hardness, while die temperature had a positive effect on hardness. Extreme high die temperature (170-230°C) was also found to have a positive effect on crispness and negative effect on crunchiness. However, Singh et al. (2015) who studied extrudates made from blends of chickpea, broken rice and potato found that die temperature had negative relationship with hardness. Lazou et al. (2010) who studied lentil and corn blends suggested that increasing feed moisture (13-19 g/100 g) decreased crispness and crunchiness, but increased hardness. Yovchev et al. (2017) studied chickpea and barley blended puffed snacks and reported that the effect of screw speed on textural properties of extrudate was less significant than those of feed moisture and die temperature.

Overall, addition of protein and fiber at relatively low amounts are not harmful to extrudate hardness. Feed moisture has a positive relationship, while die temperature and screw speed have negative relationships with extrudate hardness in most of cases. Effects of feed composition, feed moisture, die temperature and screw speed on crispness and crunchiness of extrudate varies with the extrusion conditions and ingredients used, and are not as well documented as hardness is. The common preferred extrusion conditions, in terms of achieving desirable extrudate texture are low moisture, high die temperature and high screw speed.

2.5.4 Color

International Commission on Illumination (CIE) color measurement is widely used in textile, pharmacy and food industry, where parameter $L*$ describes lightness (luminosity), $a*$ measures the color component from green (-) to red (+), and $b*$ measures the color component from blue (-) to yellow (+). Compared with other color measurements (e.g., Hunter), CIE measurement is more sensitive at measuring yellowness and dark colors (Cheng et al., 2018).

There is no doubt that color is an important appearance attribute of puffed snacks, directly affecting customers' preferences. In addition, color is also an indicator of the nutritional quality of puffed snacks. There are two main reasons responsible for the color change of extrudates during extrusion processing, (1) Maillard reaction and (2) decompositions of ingredients' endogenous pigments. Maillard reaction involves sugars and amino acids, generally leading to the destruction of amino acids and giving products a darker color (Taverna et al., 2012; Friedman, 1996). The decompositions of natural pigments (e.g., flavonols) can commonly be observed on colored food materials such as corn, lentil, etc. (Lazou et al., 2010). The extent of Maillard reaction and pigment decomposition are associated with the intensity of extrusion conditions, which can be accelerated by increasing die temperature and decreasing feed moisture in most cases. Other than these, color properties of extrudates are dependent on the original color and composition (e.g., sugar and pigment level) of ingredients that go into the extrudate formula (Taverna et al., 2012; Kumari et al., 2018; Teba et al., 2017; Yu et al., 2012).

CHAPTER 3 Effects of Extrusion Cooking on Nutritional Quality of Puffed Snacks Made from Blends of Barley and Green Lentil Flours

3.1 Abstract

Increasing demand for nutritionally-dense foods warrants the investigation of high fiber and protein ingredients in snack food applications. When blended, cereals and pulses can complement each other and yield nutritious ingredients. Blends of barley (22.9 dietary fiber g/100 g, db) and green lentil (26.4 protein g/100 g, db) flours were extruded at five blending ratios (barley: green lentil, 100: 0, 75: 25, 60: 40, 45: 55, 0: 100, db), two barrel temperature profiles (60/80/100/120/130°C and 70/90/110/130/140°C from feeder to die) and three feed moisture contents (15, 18 and 21 g/100 g). Extrusion significantly improved *in vitro* protein digestibility (IVPD) of all blends by up to 10%. Decreasing feed moisture and increasing die temperature improved IVPD. Blending increased the limiting amino acid score and hence improved the *in vitro* protein digestibility corrected amino acid score (IVPDCAAS) of extrudates, where blend 45: 55 showed the highest average IVPDCAAS (68.62%). While extrusion negatively impacted soluble and total dietary fiber contents of blends, decreasing feed moisture increased soluble dietary fiber. All extrudates from blend 60: 40 met the requirement to be labelled as "good source of dietary fiber" in the US. Overall, extrudate blends 60: 40 and 45: 55 showed the greatest nutritional quality.

3.2 Introduction

Puffed snacks currently on the market are predominately made from refined cereal flours (e.g., corn and wheat), and they are generally rich in saturated fats, highly digestible carbohydrates, and low in proteins and fibers (Patil et al., 2016; Sumargo et al., 2016), making them calorie dense but nutritionally inferior. It is argued that frequent consumption of said snacks might contribute to health problem such as obesity (Patil et al., 2016). Using innovative ingredients (Peckenpaugh, 2017), such as those rich in proteins and dietary fibers, which refined cereal flours are short of, can address these concerns by improving product nutritional quality.

Among different plants, the outstanding protein content, especially when compared to cereals (e.g., 10-15 g/100 g for barley), is one of the major advantages of pulses (e.g., 26-31 g/100 g for lentil) (Boye et al., 2010; Sullivan et al., 2013). In addition to the protein quantity, the amino acid compositions of pulse and cereal proteins are also different. Pulses are rich in lysine and limited in sulfur-containing amino acids (i.e., methionine and cysteine), while cereals are generally the opposite. Thus, when blended together, pulses and cereals can complement each other's amino acid composition (Asif et al., 2013; Lazou et al., 2010).

Blending whole pulse and cereal flours to replace refined cereal flours in snack food formula can also enhance the content of dietary fibers. Both pulses (e.g., 9-17 g/100 g for lentil) and cereals (e.g., 11-20 g/100 g for barley) are major sources of dietary fibers (Chen et al., 2016; Morales et al., 2015b; Sullivan et al., 2013; Asif et al., 2013). It has been reported that the dietary fibers present in pulses, including lentil, have high insoluble: soluble ratio up to 8: 1 (Chen et al., 2016). However, insoluble dietary fiber was shown to impair the overall extrudate expansion more than soluble dietary fiber (Robin et al., 2012). Since, barley has a relatively low insoluble: soluble dietary fiber ratio (< 4: 1) (Arendt & Zannini, 2013; Robin et al., 2013), it can potentially offset the negative impact of lentil fiber on extrudate expansion when blended.

The objectives of this study were (1) to investigate the potential of using the blends of barley and green lentil as new protein- and fiber- rich ingredients that can potentially replace refined cereal flours in puffed snacks, and (2) to assess the effect of extrusion cooking (three feed moisture levels and two temperature profiles) on nutritional quality (i.e., protein content, amino acid composition, *in vitro* protein digestibility, and insoluble, soluble and total dietary fiber content) of puffed snacks made from blends of barley and green lentil flours.

3.3 Materials and methods

3.3.1 Materials

Hull-less *CD Ratan* barley was provided by Against the Grain Farms (Mountain, ON, Canada) and milled by Ottawa Valley Grain Products (Carp, ON, Canada) using a Ferkar mill (Velenje, Slovenia). The milled barley flour was not sifted so as to obtain whole grain barley flour. Dry milled commercial dehulled *CDC Richland* green lentil flour was provided by AGT Food and Ingredients (Regina, SK, Canada).

3.3.2 Proximate composition of materials

Crude protein (N×6.25) and moisture contents of barley and green lentil flours were determined in duplicate using a micro-Kjeldahl unit following AACC method 46-13 and 44-19.01, respectively (AACC, 1999). Crude fat and crude ash contents were measured in duplicate following Min & Ellefson (2010) and Marshall (2010), respectively. Carbohydrate contents were calculated by subtracting protein, moisture, fat and ash contents from 100%.

Insoluble and soluble dietary fiber contents were measured in duplicate following AACC method 32-07.01 (AACC, 1999) with slight modifications. Sample size was reduced to 0.5 g and the protease digestion time was extended to 1 hr. Enzymes were purchased from Megazyme (Total Dietary Fiber test kit, Ireland). Protein and ash contents were subtracted from both insoluble and soluble dietary fiber residues. Total dietary fiber content was calculated as the sum of insoluble and soluble dietary fiber content. Averages and standard deviations were calculated based on the two replications in duplicate dietary fiber measurements (n=2×2=4).

3.3.3 Amino acid composition

Methionine and cysteine contents were determined using AOAC method 45.4.05 (AOAC, 2000). Tryptophan content was determined following Nosworthy *et al.* (2017). Other amino acids were measured using AOAC method 982.30 (AOAC, 2000).

3.3.4 *In vitro* protein digestibility

In vitro protein digestibility (IVPD) was determined in triplicate using a pH drop method following Nosworthy et al. (2017) as modified from Tinus et al. (2012) and Hsu et al. (1977). Briefly, the equivalent of 62.5 mg of protein of each flour or extrudate was added to 10 ml Milli-Q water, heated to 37℃ in a beaker-made water bath and adjusted to pH 8.0. Then 1 mL enzyme solution containing trypsin, chymotrypsin and peptidase was added to the sample solution and incubated at 37℃. The pH drop of the initial 10 min of incubation (ΔpH_{10min}) was recorded to calculate IVPD using Eq. 7:

$$IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH_{10min} \quad \text{(Eq. 7)}$$

65.66 and 18.10 are the intercept and slope of the regression line, respectively (Tinus *et al.*, 2012). Casein (80 mesh, Dyets Inc., Bethlehem, PA, US) was also tested as the control.

3.3.5 Amino acid score and *in vitro* protein digestibility corrected amino acid score

Following FAO/WHO (1991), the amino acid score (AAS) and *in vitro* protein digestibility corrected amino acid score (IVPDCAAS, %) of flours and extrudates were calculated according to Eq. 8 and 9, respectively:

AAS= mg of an amino acid in 1.0 g of test protein / mg of the same amino acid in 1.0 g of preschool children (2-5 years) reference pattern (Eq. 8)

IVPDCAAS= the lowest amino acid score × IVPD (Eq. 9)

The pre-school children (2-5 years) reference pattern includes the requirements of 9 individual amino acids or groups of amino acids, which are as follows: histidine, 19 (mg/g protein); isoleucine, 28; leucine 66; lysine, 58; methionine+cysteine, 25; phenylalanine+tyrosine, 63; threonine, 34; tryptophan, 11; and valine, 35. The lowest amino acid score is termed as the limiting amino acid score (LAAS) and the amino acid with the LAAS is referred as the limiting amino acid (LAA).

3.3.6 Identifying the flour blending ratios for extrusion

Flour blending ratios for extrusion were identified base on the amino acid composition and protein quality of flours. Firstly, protein content, amino acid composition and IVPD of barley and green lentil flours were determined. Then, according to the blending ratios of barley and green lentil flours, the corresponding protein content, amino acid composition, IVPD, AAS and IVPDCAAS values for the proposed flour blends (80: 20, 75: 25, 70: 30, 60: 40, 50: 50, 45: 55, 40: 60, 30: 70, 25: 75, 20: 80, barley: green lentil, db) were compiled. Among proposed blends, the blend of 60: 40 barley: green lentil showed the most balanced AAS and the highest IVPDCAAS, indicating the best amino acid composition and protein quality, and was therefore selected for extrusion. In addition to the 60: 40 (60Bly40GL) blending ratio, 75: 25 (75Bly25GL) and 45: 55 (45Bly55GL) ratios were also selected for extrusion so that acceptable amino acid composition, protein quality and measurable differences between blends are ensured.

3.3.7 Extrusion cooking

Extrusion cooking was performed using a twin-screw extruder (MPF19, APV Baker Ltd., Peterborough, UK), with a constant feed rate of 2.0 kg/hr, screw speed of 250 rpm and a die orifice diameter of 5.5 mm. Three feed moisture (FM) levels (15, 18 and 21 g/100 g on flour weight basis) and two barrel temperature profiles (60/80/100/120/130℃ and 70/90/110/130/140℃

from the feeder to the die exit) were used. The lower and higher temperature profiles are referred to as die temperatures (DT) 130°C and 140°C, respectively, throughout the rest of this manuscript. All blends were extruded in duplicate for each extrusion treatment.

Extrudates were collected, cooled down to room temperature and then dried in an air oven (Heratherm OGS100, Thermo Scientific, Langeselbold, Germany) at 40°C for 15 hrs. Dried extrudates were milled (ZM 200 Ultra Centrifugal Mill, Retsch, Haan, USA) to pass through a 250 micron sieve (Ring sieve, Retsch, Haan, USA). A mixed sample was then created by blending ground extrudates produced from two extrusion runs equally by weight and stored at -40°C for all tests performed in this study.

3.3.8 Statistical analysis

IVPD, IVPDCAAS, IDF, SDF and TDF data was analyzed by SAS software (Version 9.2) using the GLIMMIX procedure with extrusion as a factor, and blending ratio, FM and DT as three fixed effects. Flour blends were included as controls. A three-way ANOVA along with Tukey's test were used to analyze the individual effects and interactions of fixed effects. Significant differences were considered at $p < 0.05$.

3.4 Results and discussion

3.4.1 Proximate composition of materials

The barley flour had relatively lower protein and ash, but higher fat and total carbohydrate contents compared to the green lentil flour (Table 3.1). The proximate compositions of barley and green lentil flours were similar to those reported in the literature (Arendt & Zannini, 2013; Farooq & Boye, 2011; Tosh & Yada, 2010).

Table 3.1 Proximate compositions of barley and green lentil flours. Data is expressed as the average± standard deviation (n=4 for dietary fiber tests, n=2 for other tests) on dry basis.

Composition (g/100 g)	**Barley**	**Green lentil**
Crude protein	14.91±0.02	26.42±0.12
Crude fat	3.35±0.25	1.80±0.19
Crude ash	1.95±0.01	2.61±0.01
Carbohydrate	80.39±0.25	69.52±0.30
Total dietary fiber	22.9±1.7	10.1±1.8
Insoluble dietary fiber	13.6±1.5	9.0±1.6
Soluble dietary fiber	9.3±0.7	1.1±0.2

3.4.2 Protein content and amino acid composition

Flour blends with lower barley to green lentil ratio had higher protein contents following the corresponding protein contents of barley and green lentil flours (Table 3.2). Protein contents of extrudates were similar to their respective flours. Protein contents and amino acid compositions of blended flours and their extrudates were presented in Table 3.2 as a function of DT and FM. Amino acid compositions of barley and green lentil were found to be complementary to each other, where barley protein is limited in lysine but relatively rich in sulfur-containing amino acids (cysteine and methionine), and green lentil protein generally showed the opposite trend, aligning with the findings of Asif et al. (2013). The lysine content of green lentil flour was 1.72 g/100 g, which was twice as high as that of barley flour. On the other hand, cysteine and methionine contents in barley flour were 0.03-0.05 g/100 g higher than those in green lentil flour. The amino acid composition of barley and green lentil flours agree with those reported by Arendt & Zannini (2013) and Nosworthy et al. (2018).

The amounts of several amino acids (e.g., cysteine, tyrosine, arginine, etc.) decreased after extrusion, which could be due to high temperatures reached during extrusion cooking. Clemente et al. (1998) had reported that heat can cause significant amino acid losses, especially for cysteine, methionine and tyrosine, which agrees with the results of this study. The increase in some amino acids (e.g., histidine, arginine, aspartate, etc.) after extrusion were mainly observed for extrudates of GL, which might be attributed to the inactivation of proteinous antinutritional factors (e.g., hydrolase inhibitors and lectins) in legumes, as proteinous antinutritional factors can release amino acids after heat denaturation (Duranti, 2006). Overall, Table 3.2 suggested that the amino acid compositions of blend 45Bly55GL were relatively more stable than those of other blends after extrusion, especially compared with Bly and GL. This higher stability might be because many amino acids of barley and green lentil reacted to extrusion cooking in opposing trends. For example, Bly extrudates lost 0.02-0.04 g/100 g threonine after extrusion, while the threonine content of GL extrudates increased by 0.01-0.04 g/100 g, so the higher stability in threonine content of blend 45Bly55GL was possibly a result of neutralization. FM and DT did not show substantial effects on amino acid composition in this study. This might be because the selected ranges of FM (15- 21 g/100 g) and DT (130 and 140℃) were not wide enough to cover detectable differences. Bjórck et al. (1983) also reported that increasing extrusion FM from 13 to 18 g/100 g did not have significant effect on retention of most amino acids for extrusion of protein enriched wheat biscuits.

Table 3.2 Protein contents (g/100 g), amino acid compositions (g/100 g) of blended flours and extrudates produced at different blending ratios, feed moistures (FM, g/100 g) and die temperatures (DT, °C). All data is expressed on dry basis and protein contents are expressed as the averages± standard deviations (n=2).

Blends	DT	FM	Protein	His	Ser	Arg	Gly	Asp	Glu	Thr	Ala	Pro	Cys	Lys	Tyr	Met	Val	Ile	Leu	Phe	Trp
	Before extrusion		14.91±0.02	0.31	0.77	0.69	0.60	0.95	3.84	0.52	0.56	1.82	0.34	0.49	0.53	0.24	0.71	0.54	1.02	0.82	0.17
		15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	130	**18**	14.74±0.03	0.27	0.66	0.65	0.57	0.95	3.89	0.48	0.58	1.85	0.30	0.51	0.41	0.22	0.76	0.54	1.07	0.84	0.18
Bly		**21**	14.84±0.02	0.29	0.69	0.71	0.56	1.02	3.94	0.50	0.57	1.89	0.29	0.51	0.44	0.23	0.75	0.55	1.05	0.84	0.18
		15	14.66±0.07	0.28	0.68	0.70	0.56	1.08	3.97	0.49	0.57	1.85	0.30	0.52	0.48	0.24	0.77	0.55	1.08	0.84	0.18
	140	**18**	14.82±0.40	0.29	0.68	0.69	0.58	1.03	3.97	0.49	0.57	1.87	0.31	0.53	0.47	0.23	0.77	0.55	1.06	0.84	0.18
		21	14.94±0.01	0.28	0.66	0.70	0.56	0.93	3.82	0.49	0.54	1.85	0.30	0.52	0.48	0.24	0.77	0.56	1.05	0.84	0.18
	Before extrusion		17.94±0.13	0.46	0.85	1.11	0.69	1.54	4.10	0.62	0.69	1.69	0.29	0.87	0.58	0.24	0.93	0.72	1.32	0.98	0.19
		15	17.90±0.17	0.48	0.86	1.06	0.71	1.60	4.18	0.62	0.71	1.71	0.27	0.87	0.51	0.22	0.93	0.72	1.34	1.00	0.19
	130	**18**	17.94±0.19	0.45	0.83	1.12	0.68	1.61	4.02	0.61	0.68	1.68	0.26	0.86	0.55	0.21	0.93	0.73	1.30	0.99	0.19
75Bly25GL		**21**	18.10±0.06	0.46	0.83	1.07	0.70	1.58	4.06	0.62	0.68	1.68	0.27	0.86	0.51	0.22	0.93	0.73	1.31	0.98	0.19
		15	17.72±0.08	0.49	0.86	1.07	0.71	1.56	4.16	0.63	0.71	1.71	0.32	0.87	0.56	0.26	0.93	0.72	1.35	1.00	0.18
	140	**18**	17.98±0.13	0.50	0.88	1.03	0.72	1.57	4.16	0.62	0.71	1.70	0.31	0.87	0.54	0.25	0.93	0.73	1.31	0.99	0.18
		21	17.89±0.16	0.47	0.89	1.04	0.73	1.55	4.17	0.62	0.71	1.69	0.26	0.88	0.54	0.22	0.93	0.72	1.33	1.00	0.17
	Before extrusion		19.83±0.11	0.61	1.00	1.31	0.79	2.02	4.28	0.71	0.79	1.61	0.33	1.09	0.63	0.28	1.04	0.83	1.51	1.11	0.19
		15	19.99±0.12	0.55	1.00	1.34	0.78	1.91	4.28	0.71	0.79	1.60	0.26	1.09	0.60	0.22	1.04	0.83	1.51	1.10	0.19
	130	**18**	19.80±0.05	0.62	0.98	1.23	0.79	1.85	4.20	0.70	0.78	1.59	0.28	1.08	0.61	0.23	1.03	0.83	1.52	1.11	0.18
60Bly40GL		**21**	20.01±0.15	0.61	0.96	1.24	0.74	1.81	4.05	0.67	0.76	1.54	0.22	1.06	0.55	0.18	1.00	0.82	1.48	1.09	0.19
		15	19.86±0.28	0.60	0.93	1.23	0.75	1.86	4.05	0.67	0.76	1.51	0.29	1.05	0.52	0.23	1.00	0.82	1.51	1.10	0.18
	140	**18**	20.12±0.22	0.62	0.97	1.36	0.73	1.90	4.20	0.68	0.78	1.47	0.24	1.10	0.56	0.19	1.04	0.84	1.55	1.11	0.19
		21	20.01±0.09	0.56	0.99	1.28	0.79	1.87	4.25	0.70	0.76	1.60	0.23	1.06	0.62	0.19	1.03	0.82	1.49	1.08	0.20
	Before extrusion		21.46±0.09	0.68	1.09	1.49	0.85	2.27	4.28	0.77	0.86	1.49	0.24	1.24	0.64	0.21	1.10	0.92	1.58	1.15	0.20
		15	21.68±0.07	0.68	1.11	1.56	0.83	2.28	4.33	0.78	0.87	1.51	0.25	1.26	0.61	0.21	1.11	0.90	1.63	1.16	0.20
	130	**18**	21.44±0.16	0.69	1.09	1.50	0.84	2.23	4.24	0.76	0.85	1.48	0.26	1.24	0.61	0.22	1.09	0.88	1.59	1.14	0.20
45Bly55GL		**21**	21.42±0.14	0.68	1.09	1.53	0.84	2.28	4.29	0.77	0.86	1.49	0.26	1.27	0.59	0.22	1.10	0.91	1.59	1.15	0.20
		15	21.23±0.02	0.68	1.11	1.49	0.85	2.28	4.32	0.77	0.88	1.50	0.26	1.27	0.58	0.22	1.11	0.90	1.61	1.16	0.20
	140	**18**	21.38±0.04	0.69	1.12	1.58	0.85	2.34	4.37	0.78	0.87	1.53	0.25	1.27	0.64	0.21	1.13	0.93	1.62	1.17	0.20
		21	21.28±0.32	0.68	1.09	1.56	0.83	2.29	4.28	0.77	0.86	1.50	0.25	1.25	0.65	0.21	1.10	0.90	1.60	1.14	0.20
	Before extrusion		26.42±0.12	0.94	1.46	2.12	1.07	3.07	4.27	1.01	1.05	1.13	0.29	1.72	0.90	0.21	1.20	1.14	1.89	1.35	0.20
		15	27.63±0.37	1.05	1.49	2.27	1.11	3.41	4.72	1.05	1.15	1.24	0.22	1.92	0.72	0.21	1.45	1.26	2.14	1.47	0.22
	130	**18**	27.86±0.26	1.05	1.47	2.34	1.08	3.41	4.69	1.03	1.14	1.22	0.21	1.92	0.80	0.19	1.43	1.23	2.12	1.45	0.22
GL		**21**	27.36±0.02	1.00	1.42	2.20	1.11	3.12	4.50	1.01	1.08	1.20	0.21	1.85	0.79	0.20	1.39	1.21	2.07	1.41	0.21
		15	27.61±0.01	1.04	1.51	2.23	1.12	3.44	4.76	1.05	1.16	1.22	0.22	1.94	0.74	0.21	1.44	1.24	2.13	1.46	0.22
	140	**18**	27.68±0.10	1.02	1.46	2.30	1.08	3.29	4.58	1.02	1.13	1.21	0.22	1.86	0.76	0.21	1.40	1.22	2.10	1.43	0.22
		21	27.69±0.23	1.03	1.48	2.25	1.10	3.42	4.64	1.04	1.16	1.23	0.22	1.92	0.76	0.20	1.42	1.25	2.12	1.46	0.22

Abbreviations: His, histidine; Ser, serine; Arg, arginine; Gly, glycine; Asp, aspartate; Glu, glutamate; Thr, threonine; Ala, alanine; Pro, proline; Cys, cysteine; Lys, lysine; Tyr, tyrosine; Met, methionine; Val, valine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine and Trp, tryptophan.

3.4.3 *In vitro* protein digestibility

IVPD values of barley and green lentil flours were 72.90 and 76.28%, respectively, which agree with Bai et al. (2018) and Han et al. (2007), respectively. IVPD values of 75Bly25GL, 60Bly40GL and 45Bly55GL flours were in the range of 72.36-73.68% and increased slightly with decreasing barley to green lentil ratio. After extrusion, IVPD values of extrudates increased significantly ($p < 0.0001$), up to 10%, except for higher FM treatments of Bly. This significant increase could be attributable to the applied heat, shear and pressure during extrusion, leading to changes in protein structure, possibly exposing originally buried sites to be more easily accessible for protease to attack. In addition, extrusion cooking can destroy antinutritional factors (e.g., trypsin and chymotrypsin inhibitors) and enhance IVPD (Ghumman et al., 2016). IVPD values for extruded GL were similar to Nosworthy et al. (2018) who studied slightly lower extrusion DT (120°C) and FM (11 g/100 g).

Decreasing FM increased IVPD values of extrudates (Fig. 3.1), which might be because low FM increased the intensity of extrusion (Chapter 4). Increasing DT showed a positive relationship with IVPD for blends 75Bly25GL, 60Bly40GL and 45Bly55GL, aligning with the findings of Ghumman et al. (2016). IVPD values of extrudates of Bly and GL at 140°C were slightly lower than those at 130°C, indicating that higher processing temperature did not enhance IVPD. The temperature dependence of (Muyonga et al., 2014) where dietary fibers were reported to adversely affect protein digestibility (Joehnke et al., 2018). This might explain why extrusion cooking had relatively limited beneficial effect on the IVPDs of Bly extrudates (the highest dietary fiber content) when compared to other extrudates. In addition, Omosebi et al. (2018) reported that phenolic compounds, which green lentil flour is rich in, could directly bind with trypsin and reduce its activity. It is possible that this reaction is accelerated at higher temperatures, and hence be responsible from the lower IVPD values of GL extrudates at DT 140℃. The three blends whose IVPD values were substantially improved by extrusion and high DT (75Bly25GL, 60Bly40GL and 45Bly55GL) had relatively low concentrations of dietary fiber and possibly phenolic compounds due to the effect of blending.

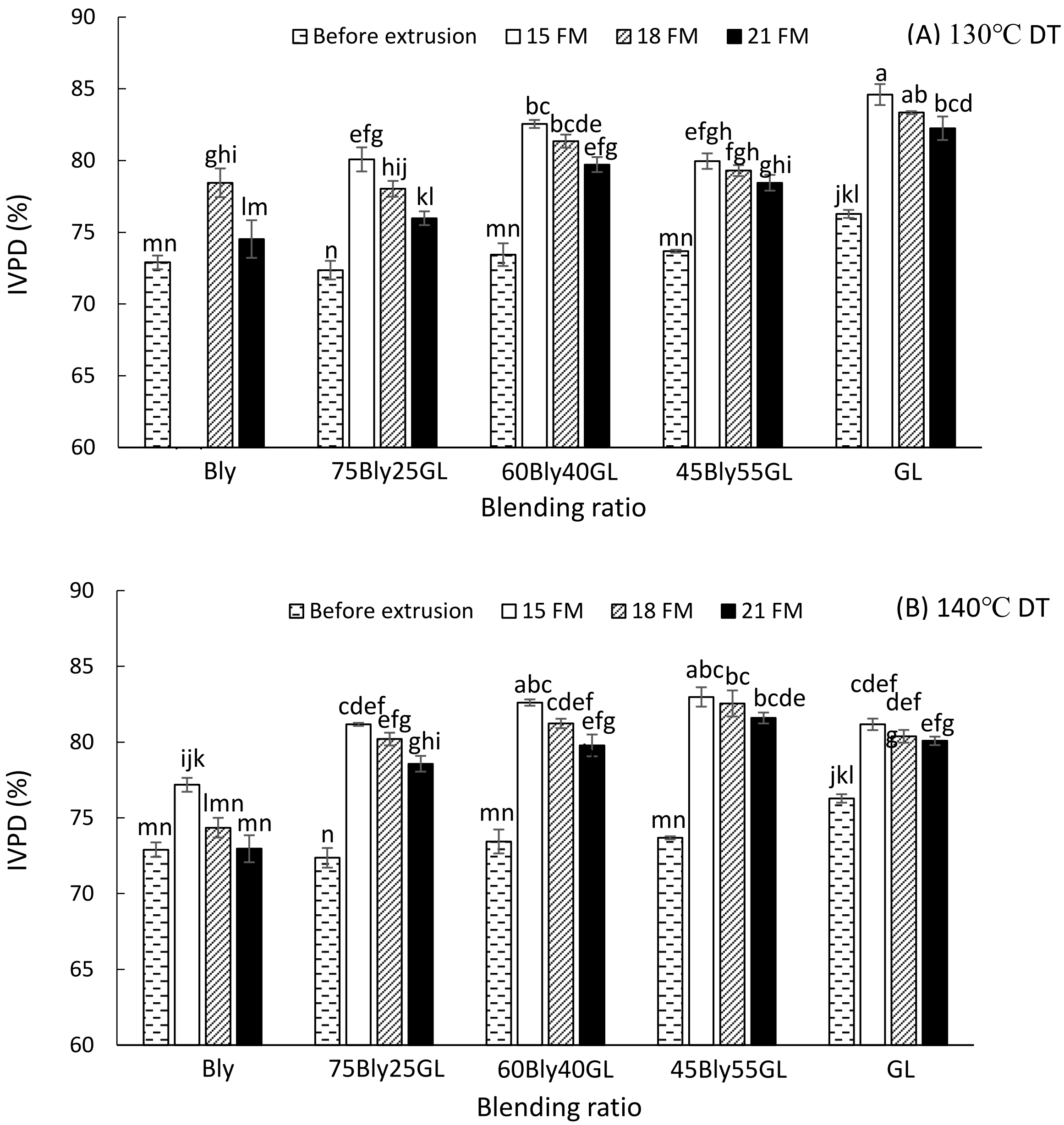

Fig. 3.1. In vitro protein digestibility (IVPD) values of blended flours and extrudates with different blending ratios and feed moistures (FM, g/100 g) produced at (A) 130℃ and (B) 140℃

die temperature (DT). Error bars represent ±1 standard deviations (n=3). Values of IVPD followed by different letters are significantly different ($p < 0.05$).

3.4.4 Amino acid score and *in vitro* protein digestibility corrected amino acid score

The AAS of blended flours and extrudates are presented in Table 3.3. The LAA of Bly and 75Bly25GL flours was lysine, while the LAA of 60Bly40GL, 45Bly55GL and GL flours was tryptophan. The AAS of blended flours followed the trend of blending (e.g., flours with lower barley to green lentil ratio had the higher AAS of lysine). Flour 60Bly40GL (0.86) showed the highest LAAS, followed closely by 75Bly25GL (0.84) and 45Bly55GL (0.84) flours, all of which were higher than Bly (0.56) and GL (0.69) flours. These results indicated that blending provided a more balanced amino acid profile. The LAA of extruded Bly, 75Bly25GL and 45Bly55GL remined the same as their flour blends. However, due to the significant loss of methionine and cysteine during extrusion at high FM (Table 3.2), the LAA of 60Bly40GL extrudates produced at 21 g/100 g FM switched to methionine+cysteine. Similarly, due to the loss of cysteine, the LAA of extruded GL changed to methionine+cysteine. Extrudates of 60Bly40GL showed higher LAAS among the five extruded blends, and the one produced at 130℃ and 15 g/100 g FM had the highest LAAS (0.87), indicating the most balanced amino acid composition using the reference pattern of pre-school children (2-5 years), as required by the FDA.

The IVPDCAAS values of barley and green lentil flour were 40.82 and 52.63%, respectively. Compared to them, blended flours 75Bly25GL, 60Bly40GL and 45Bly55GL had relatively higher IVPDCAAS values (60-64%). Flour blend 60Bly40GL showed the highest IVPDCAAS value (63.45%) because of its higher LAAS resulting from blending. Due to the significant ($p < 0.0001$) effect of extrusion cooking on IVPD, IVPDCAAS of extrudates were also significantly improved with extrusion. The IVPDCAAS of 60Bly40GL fell into the range of 63-72%, followed by 45Bly55GL (66-71%), 75Bly25GL (62-69%), GL (47-54%) and Bly (43-47%). Although, the extrudate which had the highest IVPDCAAS was 60Bly40GL produced at 130℃ and 15 g/100 g FM (71.99%), extrudates of 45Bly55GL showed less variability and the highest average IVPDCAAS (68.62%).

To carry any protein content claims in the US, snack foods need to have at least 5 g PDCAAS (*in vivo* protein digestibility corrected amino acid score)-corrected protein per 30 g reference amounts customarily consumed (RACC) (FDA, 2013). For example, a snack with 10 g/100 g crude protein and 70% PDCAAS value, it would have $10/100 \times 30 \times 0.7$ g = 2.1 g PDCAAS-corrected protein per RACC. While the official method for protein claims requires the use of *in vivo* digestibility assays for measuring PDCAAS and corrected protein levels, IVPDCAAS can be used as a proxy for formulation purposes (but not for regulatory approval) when *in vivo* PDCAAS values are not available. In the current study, extrudate 45Bly55GL produced at 140℃ DT and 15 g/100 g FM had the highest IVPDCAAS-corrected protein per RACC (4.2 g). As such, it fell just short of the 5 g mark that is needed for a "good source of protein" claim. Canada has recently permitted the use of PDCAAS values of a food for estimation of its protein efficiency ratio (PER), where estimated PER equals to PDCAAS×2.5 (CFIA, 2021). CFIA (2021) stipulates that the product of the protein content in a food per reference amount (50 g for snacks) and the PER of the food, referred as protein rating, is the key to determine if a food can carry protein claims. The extrudate from blend 45Bly55GL, having the highest IVPDCAAS-corrected protein per RACC, also had the highest protein rating (17.5) based on its IVPDCAAS and estimated PER values. This protein rating is only 2.5 points below the 20 points mark that qualifies to be labelled as "good source of protein" in Canada. The relatively small serving size and the need to balance the carbohydrate: protein ratio for both sensory and nutritional quality of snacks makes it difficult to obtain protein content claims for these foods. However, it should be noted that the IVPDCAAS has been shown to underestimate the PDCAAS value by 2-4% in absolute values (Nosworthy et al., 2018), so the true PDCAAS-corrected protein content of the optimized blends may be slightly higher than those observed with the IVPDCAAS method, and possibly qualify for protein claims.

Based on the superior physical quality (Chapter 4) and promising IVPDCAAS results of extruded 60Bly40GL and 45Bly55GL blends over other blends, 60Bly40GL and 45Bly55GL extrudates were selected to be tested (as a function of extrusion FM and DT) for their total, insoluble and soluble dietary fiber contents.

Table 3.3 Essential amino acid score (AAS) and in vitro protein digestibility corrected amino acid score (IVPDCAAS) values of blended flours and their extrudates produced from different blending ratios, feed moistures (FM, g/100 g) and die temperatures (DT, °C). The limiting amino acid score (LAAS) of each sample is bolded. Theoretical data of blended flours were calculated based on experimental data of Bly and GL flours, following FAO/WHO (1991). For abbreviations of amino acids, see Table 1. IVPDCAAS is expressed as the average± standard deviation (n=3) and values followed by different letters are significantly different ($p < 0.05$).

Blends	DT	FM	His	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Trp	Val	IVPDCAAS (%)
Bly	Before extrusion		1.10	1.29	1.03	**0.56**	1.57	1.43	1.02	1.05	1.35	40.82±0.27^{t}
	130	15	-	-	-	-	-	-	-	-	-	-
		18	0.97	1.31	1.10	**0.59**	1.40	1.35	0.96	1.13	1.47	46.55±0.59^{qr}
		21	1.03	1.33	1.07	**0.59**	1.40	1.37	0.98	1.08	1.45	43.99±0.95^{s}
	140	15	1.00	1.33	1.11	**0.61**	1.47	1.43	0.98	1.11	1.49	46.98±0.28^{pqr}
		18	1.04	1.33	1.08	**0.62**	1.47	1.40	0.97	1.09	1.48	46.23±0.41^{r}
		21	0.98	1.33	1.07	**0.60**	1.45	1.40	0.96	1.11	1.47	43.58±0.53^{s}
75Bly25GL	Before extrusion		1.36	1.43	1.12	**0.84**	1.17	1.38	1.02	0.94	1.48	60.78±0.55^{k}
	130	15	1.42	1.44	1.13	**0.84**	1.11	1.34	1.03	0.95	1.49	67.42±0.70^{fgh}
		18	1.31	1.45	1.09	**0.83**	1.07	1.36	1.01	0.95	1.48	64.59±0.46^{i}
		21	1.35	1.44	1.09	**0.82**	1.08	1.31	1.00	0.93	1.47	62.21±0.39^{jk}
	140	15	1.46	1.44	1.15	**0.85**	1.30	1.40	1.04	0.93	1.51	68.65±0.09^{def}
		18	1.47	1.46	1.11	**0.84**	1.25	1.35	1.02	0.92	1.47	67.11±0.35^{gh}
		21	1.37	1.44	1.13	**0.85**	1.07	1.36	1.03	0.89	1.49	66.42±0.44^{h}
	Theoretical		1.39	1.38	1.05	**0.77**	1.27	1.40	1.06	0.92	1.33	56.78±0.22
60Bly40GL	Before extrusion		1.63	1.50	1.15	0.95	1.22	1.39	1.05	**0.86**	1.49	63.16±0.68^{ij}
	130	15	1.45	1.48	1.15	0.94	0.95	1.35	1.04	**0.87**	1.49	71.99±0.24^{a}
		18	1.64	1.49	1.16	0.94	1.03	1.38	1.03	**0.84**	1.49	68.70±0.38^{def}
		21	1.60	1.45	1.12	0.92	**0.79**	1.30	0.99	0.85	1.43	62.98±0.41^{j}
	140	15	1.60	1.47	1.15	0.91	1.06	1.29	0.99	**0.84**	1.44	69.31±0.18^{cde}
		18	1.62	1.50	1.17	0.94	0.85	1.32	1.00	**0.84**	1.47	67.92±0.26^{efg}
		21	1.48	1.45	1.13	0.91	**0.83**	1.35	1.03	0.90	1.47	66.22±0.60^{h}
	Theoretical		1.52	1.43	1.06	0.87	1.13	1.39	1.07	**0.86**	1.32	63.86±0.15
45Bly55GL	Before extrusion		1.66	1.52	1.11	1.00	0.85	1.32	1.05	**0.84**	1.46	61.89±0.09^{jk}
	130	15	1.66	1.49	1.14	1.00	0.85	1.30	1.06	**0.84**	1.47	67.26±0.46^{fgh}
		18	1.69	1.47	1.12	1.00	0.90	1.30	1.04	**0.84**	1.45	66.69±0.32^{gh}
		21	1.67	1.51	1.13	1.02	0.90	1.29	1.06	**0.85**	1.47	66.80±0.47^{gh}
	140	15	1.69	1.52	1.15	1.03	0.90	1.30	1.07	**0.86**	1.49	71.01±0.54^{ab}
		18	1.70	1.55	1.15	1.02	0.86	1.34	1.08	**0.84**	1.51	69.61±0.72^{bcd}
		21	1.68	1.51	1.14	1.01	0.86	1.34	1.07	**0.86**	1.48	70.32±0.31^{bc}
	Theoretical		1.63	1.46	1.07	0.95	1.01	1.38	1.09	**0.80**	1.32	59.81±0.05
GL	Before extrusion		1.87	1.55	1.08	1.12	0.76	1.35	1.12	**0.69**	1.30	52.63±0.19^{ml}
	130	15	2.00	1.62	1.17	1.20	**0.63**	1.26	1.11	0.72	1.50	53.35±0.46^{l}

	18	1.98	1.58	1.15	1.19	**0.57**	1.28	1.09	0.71	1.47	47.89±0.06^{pq}
	21	1.92	1.58	1.15	1.17	**0.60**	1.27	1.08	0.71	1.46	49.56±0.49^{no}
	15	1.99	1.60	1.17	1.21	**0.63**	1.26	1.11	0.73	1.49	51.41±0.24^{m}
140	**18**	1.94	1.57	1.15	1.16	**0.62**	1.26	1.08	0.72	1.45	49.84±0.26^{n}
	21	1.96	1.61	1.16	1.20	**0.60**	1.27	1.10	0.71	1.47	48.26±0.17^{op}

3.4.5 Dietary fiber content

In Table 3.4, IDF and SDF contents of 60Bly40GL and 45Bly55GL extrudates were measured, while the IDF and SDF contents of 60Bly40GL and 45Bly55GL flours were calculated based on their blending ratios and the dietary fiber contents of the Bly and GL flours. Extrusion significantly affected SDF ($p < 0.0001$) and TDF ($p = 0.0013$) but did not substantially impact IDF ($p = 0.08999$). SDF contents of 60Bly40GL and 45Bly55GL flours decreased slightly after extrusion, in line with the results of Ralet et al. (1990). However, this decrease was significant only for some of the 60Bly40GL extrudates (DT 130℃ and FM 21 g/100 g, DT 140℃ and FM 18 g/100 g, and DT 140℃ and FM 21 g/100 g). DT did not significantly impact SDF ($p = 0.4495$), possibly due to the narrow DT range studied (130-140℃).

All extrudates from blends 60Bly40GL and 45Bly55GL contained more than 2.8 g dietary fiber per 30 g RACC, meeting the requirement of FDA (2013) for snacks to be labelled as “good source of dietary fiber” in the US. Four extrudates from blend 60Bly40GL (all three produced at 130℃ DT regardless of their FM, and 21 g/100 g FM extrudate produced at 140℃ DT) contained more than 6 g or more dietary fiber per 40 g serving size, which can be recognized as very high source of dietary fiber snacks and carry content labels such as "very high dietary fiber" in Canada (CFIA, 2021). Considering the increasing pursuit of consumers to dietary fiber worldwide, 60Bly40GL would be the optional blending ratio since it can achieve dietary fiber claims in both the US and Canada.

Table 3.4 Insoluble, soluble and total dietary fiber contents of 60Bly40GL and 45Bly55GL flours, and their extrudates produced at different die temperatures (DT, °C) and feed moistures (FM, g/100 g). Data is expressed as the average± standard deviation (n=4) on dry basis. Values followed by different letters in each column are significantly different ($p < 0.05$).

Blends	DT	FM	Dietary fiber (g/100 g)		
			Insoluble	Soluble	Total
60Bly40GL	Before extrusion		11.8±0.4^{ab}	6.0±0.4^{a}	17.8±0.3^{a}
	130	15	11.0±1.3^{ab}	5.6±0.4^{ab}	16.6±1.0^{abc}
		18	11.8±0.9^{ab}	5.5±0.6^{ab}	17.2±1.4^{ab}
		21	12.3±1.0^{a}	4.4±0.2^{cde}	16.7±0.9^{abc}
	140	15	10.7±0.7^{ab}	5.3±0.4^{abc}	15.9±0.8^{abcd}
		18	10.0±0.5^{ab}	5.0±0.2^{bcd}	14.9±0.3^{bcd}
		21	11.4±1.1^{ab}	4.9±0.7^{bcd}	16.3±1.6^{abc}
45Bly55GL	Before extrusion		11.1±0.3^{ab}	4.8±0.3^{bcde}	15.9±0.2^{abcd}
	130	15	10.6±1.5^{ab}	3.9±0.2^{e}	14.4±1.7^{cd}
		18	9.4±0.5^{b}	4.1±0.5^{de}	13.5±0.7^{d}
		21	11.4±0.9^{ab}	4.1±0.1^{de}	15.5±0.9^{abcd}
	140	15	10.8±0.4^{ab}	4.4±0.2^{cde}	15.3±0.3^{abcd}
		18	9.6±1.2^{b}	4.6±0.5^{bcde}	14.2±1.2^{cd}
		21	10.5±1.6^{ab}	3.9±0.1^{e}	14.4±1.6^{cd}

3.5 Conclusion

Barley flour with relatively lower protein and lysine, but higher dietary fiber and sulfur-containing amino acids contents, and green lentil flour with the opposite characteristics, were shown to complement each other's protein quality attributes. The effects of extrusion on amino acid composition varied with individual amino acids and blending ratio. Overall, amino acid compositions of extrudate 45Bly55GL were relatively more stable than others. Extrusion significantly improved IVPD of all blends by up to 10%. DT had a positive relationship with the IVPDs of blends 75Bly25GL, 60Bly40GL and 45Bly55GL, while FM showed a negative relationship with IVPD.

Blending showed significant effects on both flours and extrudates. The LAAS for Bly and GL blends were less than 0.65 and 0.70, respectively, while most LAAS for 75Bly25GL, 60Bly40GL and 45Bly55GL blends were greater than 0.80. The IVPDCAAS of Bly and GL blends were lower than 50 and 60%, respectively. IVPDCAAS of the rest of three blends fell into the range of 61-72%. Among extrudates, blend 45Bly55GL showed less variability and the highest average IVPDCAAS (68.62%). FM and DT affected IVPDCAAS of extrudates through their effect on extrudates' IVPD. Extrudate 45Bly55GL produced at 140°C DT and 15 g/100 g FM, having 4.2 g IVPDCAAS-corrected protein per RACC and a protein rating of 17.5, was the closest extrudate to carry "good source of protein" claim in the US and Canada, respectively. The effect of extrusion was significant on SDF and TDF but not on IDF. All extrudates from blend 60Bly40GL met FDA's requirement to be recognized as "good source of dietary fiber", and most extrudates from the same blend met CFIA's requirement to be recognized as "very high dietary fiber".

Overall, this study showed that barley and green lentil blends have great potential to produce protein- and fiber- rich puffed snacks, meeting consumers' demand for nutritionally-dense snacks. Among tested blends, 60Bly40GL and 45Bly55GL showed great and balanced nutritional quality. This study can also be generalized to other blends of cereals and pulses, bringing more innovative products and opportunities to puffed snacks industry.

CONNECTIONS between CHAPTER 3 and CHAPTER 4

Improving the nutritional quality of the extruded products is an important focus for developing appealing and novel puffed snacks, however, physical quality is also a primary factor affecting consumers' choice that cannot be ignored or compromised. In the following chapter the physical properties including extrudate density, expansion index, texture and color of barley and green lentil extrudates were investigated. The effects of blending ratio, feed moisture, die temperature and their interactions on those physical properties were discussed. The particle size distribution of materials and specific mechanical energy input during extrusion were also studied to help understand the performance of extrudates more comprehensively.

CHAPTER 4 Effects of Extrusion Cooking on Physical Quality of Puffed Snacks Made from Blends of Barley and Green Lentil Flours

4.1 Abstract

Increasing consumer demand for healthy snacks drives this industry to search for new ingredients and replace traditional refined cereal flours. In this study, flours of a cereal with high dietary fiber content, i.e., barley (22.9 g/100 g dietary fiber, db) and a pulse with high protein content, i.e., green lentil (26.4 g/100 g protein, db) were blended at five ratios (barley: green lentil, 100: 0, 75: 25, 60: 40, 45: 55, 0: 100, db), and extruded at two barrel temperature profiles (60/80/100/120/130°C and 70/90/110/130/140°C from feeder to die) and three feed moisture contents (15, 18 and 21 g/100 g). The effects of blending as a function of barrel temperature and feed moisture on the physical properties of barley and green lentil extrudates were investigated. High temperature improved extrudate overall expansion and textural properties significantly. The effects of feed moisture mainly depended on the blending ratio, through its effect on extrudates' total protein and dietary fiber contents. Extrudates with barley: green lentil of 45: 55 showed the optimum expansion and texture, followed by extrudates with barley: green lentil of 60: 40. Barley and green lentil showed great potential to be used as protein- and fiber-rich ingredients in puffed snacks without compromising extrudate physical quality.

4.2 Introduction

Being readily available and having ample diversity, ready-to-eat puffed snacks are an important part of our diet, attracting more and more consumers, especially children (Meng et al., 2010). Current puffed snacks on the market are made predominately from refined cereal flours (e.g., corn and wheat), which are generally rich in highly digestible carbohydrates, but low in proteins and fibers (Patil et al., 2016), making puffed snacks calorie dense but nutritionally inferior. Accordingly, it is argued that very frequent consumption of puffed snacks might contribute to the increasing rate of obesity (Patil et al., 2016).

During extrusion, raw materials are mixed with water under high temperature and pressure, forming a doughy mess, also known as a “melt”. When the melt is pushed out through the die at the end of the barrel, water vaporizes, expands within the melt and bubbles nucleate and grow due to the sharp drop in pressure. After reaching the expansibility limit of the melt, water vapor escapes, sometimes followed by collapse of bubbles. The melt solidifies as it cools down to ambient temperature and the cellular structure of extrudate forms (Guy, 2001). Starch plays an important role in the formation of this unique cellular structure. Accordingly, raw material selection for puffed snacks is mainly constrained by the changes that starch goes through during extrusion cooking (Seth et al., 2015; Guy, 2001). However, for puffed snacks that are enriched, the changes that proteins and dietary fibers go through with heat and pressure are also crucially important to extrudate physical quality. For example, protein and dietary fiber-rich materials have been reported to expand less during extrusion which is undesirable for puffed snacks (Kumari et al., 2018). Few reports (Kumari et al., 2018; Meng et al., 2010) also found that at a low addition level of proteins and dietary fibers, and with the help of modifying extrusion parameters (i.e., feed moisture, die temperature, etc.), extrudates may have acceptable expansion and texture properties.

Up to date, most studies on nutritionally enhanced puffed snacks focused on either higher protein (Lazou et al., 2011; Matthey & Hanna, 1997; Patil et al., 2016) or higher dietary fiber (Kumari et al., 2018; Sharma & Gujral, 2013) contents. In addition, a good portion of starchy raw materials (e.g., corn meal) in extrudate formula was retained in such studies to ensure reasonable expansion (Lazou et al., 2011; Matthey & Hanna, 1997; Ryu & Ng, 2001). In order to fill this research gap, in the current study, only hull-less barley (high dietary fiber) and dehulled green lentil (high protein) flours were used with no additional starchy ingredients in the formula. The objectives of this study were to systematically investigate the effects of extrusion barrel temperature profile and feed moisture content on physical properties of extrudates made from barley and green lentil, and to identify optimal formulations to produce nutritious puffed snacks with consumer acceptable physical properties. Accordingly, blends of barley (dietary fiber-rich) and green lentil (protein-rich) flours with five blending ratios were used to produce extrudates at different extrusion conditions (three feed moisture contents and two barrel temperature profiles), and extrudates were evaluated for their overall expansion, textural quality (hardness, crispness and crunchiness) and color.

4.3 Materials and methods

4.3.1 Materials

Hull-less barley (*CD Ratan*) flour (14.91 g/100 g protein and 22.9 g/100 g dietary fiber, db) was provided by Against the Grain Farms (Mountain, ON, Canada) and milled by Ottawa Valley Grain Products (Carp, ON, Canada) using a Ferkar mill (Velenje, Slovenia). The milled barley flour was not sifted so as to obtain whole grain barley flour. Dry milled commercial dehulled green lentil (*CDC Richland*) flour (26.42 g/100 g protein and 10.1 g/100 g dietary fiber, db) was provided by AGT Food and Ingredients (Regina, SK, Canada). The protein and dietary fiber contents of the barley and green lentil flours were measured according to AACC method 46-13 and AACC method 32-07.01, respectively (AACC, 1999).

4.3.2 Particle size distribution of materials

Particle size distributions of barley and green lentil flours were determined in triplicate using a particle size analyzer (Master Sizer 2000, Malvern Instruments, Worcestershire, UK). Volume weighted mean diameter, D[4,3], was calculated using Eq. 10:

$$D[4,3]= \sum_1^n D_i v_i \,/\, \sum_1^n v_i \qquad \text{(Eq. 10)}$$

where D_i is the geometric mean of the i^{th} size range (i.e., the square root of the product of upper and lower diameters of the i^{th} size range), n is the total number of size ranges and v_i is the g/100 g volume distribution of sample in the i^{th} size range.

4.3.3 Extrusion cooking

Extrusion cooking was performed using a twin-screw extruder (MPF19, APV Baker Ltd., Peterborough, UK), according to Koksel & Masatcioglu (2018) with minor modifications. Briefly, feed rate (2.0 kg/hr) and screw speed (250 rpm) were kept constant. A circular extruder die with a diameter of 5.5 mm, three feed moisture levels (15, 18 and 21 g/100 g) and two barrel temperature profiles (60/80/100/120/130°C and 70/90/110/130/140°C from the feeder to the extruder die) were used. The lower and higher temperature profiles are referred to as die temperatures 130°C and 140°C, respectively, throughout the rest of this manuscript.

Five blending ratios (barley: green lentil, db) were selected as 100: 0 (Bly), 75: 25 (75Bly25GL), 60: 40 (60Bly40GL), 45: 55 (45Bly55GL) and 0: 100 (GL) based on preliminary protein quality analysis of the blends. All blends were extruded in duplicate for each extrusion condition. The Bly extrudate produced at 15 g/100 g feed moisture and 130°C die temperature was not collected due to extrusion failure (torque value increased sharply over the limit of the lab scale extruder used in this study and caused the extruder to shut down).

Extrudates were collected, cooled down to room temperature and then dried in an air oven (Heratherm OGS100, Thermo Scientific, Langeselbold, Germany) at 40°C for 15 hr. A portion of dried extrudates was packed in zipped plastic bags and stored at room temperature for extrudate density, expansion index and texture analyses. The rest of the dried extrudates were milled (ZM 200 Ultra Centrifugal Mill, Retsch, Haan, USA) to pass through a 250 μm sieve (Ring sieve, Retsch, USA). A mixed sample was then created by blending ground extrudates produced from two extrusion runs equally by weight and stored at −40°C for color analysis. Blended flours were also milled to reduce the particle size to pass through a 250 μm sieve for color analysis and stored at the same condition as the extrudates.

4.3.4 Specific mechanical energy

Specific mechanical energy (SME) was calculated using Eq. 11 based on the method of Luo et al. (2020) as modified from Meng et al. (2010):

$$\text{SME}=\frac{\text{Actual screw speed}}{\text{Maximum screw speed of the extruder}}\times\frac{\text{Torque}}{100}\times\frac{\text{motor power}}{\text{mass flow rate}} \qquad \text{(Eq. 11)}$$

Actual screw speed was kept at 250 rpm for all extrusion runs. The maximum screw speed and motor power of the extruder were 500 rpm and 2.2 kW, respectively. Torque values were recorded as percentages during extrusion (100% torque of the extruder is 9.5 N·m). Mass flow rate was the sum of the dry feed rate (2.0 kg/hr) and the water injection rate (adjusted depending on the desired feed moisture content). Die pressure (kPa), torque (%) and SME (Whr/kg) values were presented as the averages of duplicate extrusion runs.

4.3.5 Extrudate density

Extrudate density (ED) (g/ml) was measured following Ryu and Ng (2001) as modified by Koksel & Masatcioglu (2018) using canola seeds according to Eq. 12:

$$ED = W_E / V_E = W_E / (W_s / \rho_s) \quad \text{(Eq. 12)}$$

where W_E, V_E, W_s and ρ_s are extrudate weight, extrudate equivalent volume, canola seed weight and density, respectively. Measurements were performed in quintuplicate for each extrusion run.

4.3.6 Expansion index

Expansion index (EI) was determined by dividing the radial diameter of an extrudate to the diameter of the extruder die (5.5 mm). To measure the radial diameter of an extrudate, a 10 cm long extrudate was randomly selected. Its diameter was measured along its length at the locations of 2, 4, 6 and 8 cm, using a digital caliper. The average of the four readings on one piece of extrudate was considered as one measurement. Measurements were carried out in triplicate for each extrusion run.

4.3.7 Textural properties

Textural properties were measured following Masatcioglu et al. (2014) using a texture analyzer (TA-XT-plus, Stable Micro Systems, Gudalming, UK) with a 10 kg load cell and a 1 mm thick Warner-Bratzler shear blade probe. Extrudates were pre-cut to 4 cm long pieces and then conditioned in an air oven (Heratherm OGS100, Thermo Scientific, Langeselbold, Germany) at 30°C for 40 hr to equilibrate moisture content to 6.0-6.5 g/100 g. Textural quality parameters (hardness, crispness and crunchiness) were measured by the associated software Exponent by plotting the time (sec, x-axis) vs. force (N, y-axis) graph. Hardness (N) is defined as the maximum peak force, crispness is the total number of positive peaks and crunchiness (N sec) is the linear distance of the test curve. For each textural quality parameter, the average of three measurements was considered as one replication. Tests were carried out in quintuplicate for each extrusion run.

4.3.8 Color

Color properties were measured using a colorimeter (CM-3500d, Minolta, Osaka, Japan) and analyzed by its associated software Spectra Magic (Version 3.60) according to CIE color system. Illuminant D65 was used and the observing angle was set at 10°. Measurements were carried out in triplicate for both extrudates and flours. Color difference (ΔE) between each extrudate and its corresponding flour blend was calculated using Eq. 13:

$$\Delta \mathrm{E} = \sqrt{(L_{\mathrm{E}}^{*} - L_{\mathrm{R}}^{*})^2 + (a_{\mathrm{E}}^{*} - a_{\mathrm{R}}^{*})^2 + (b_{\mathrm{E}}^{*} - b_{\mathrm{R}}^{*})^2} \quad \text{(Eq. 13)}$$

where L_{E}^{*}, a_{E}^{*} and b_{E}^{*} are color parameters (lightness, redness/greenness, yellowness/blueness, respectively) of the extrudates. L_{R}^{*}, a_{R}^{*} and b_{R}^{*} are color parameters of the corresponding flour blends.

4.3.9 Statistical analysis

Statistical analysis was performed by Proc GLIMMIX procedure of SAS software (Version 9.2). Significant differences were considered at $p < 0.05$ using Tukey's test.

4.4 Results and discussion

4.4.1 Particle size distribution

The D[4,3] of barley and green lentil flours were 187.1 and 16.8 μm, respectively. In general, materials with smaller particle size have higher mechanical resistance to shearing, requiring higher specific mechanical energy (SME) input during extrusion and leading to more intensive starch conversion inside the barrel, compared with materials with larger particle size (Al-Rabadi et al., 2011). This trend was also confirmed in our study, i.e., SME input values during extrusion of green lentil flour were substantially higher compared to that of barley flour (see SME results in Table 1). Due to the differences in particle size distributions of barley and green lentil flours

(Fig. 4.1), their blends would also have particle size distributions that follow the trend of their blending ratio, which may contribute to the mechanical resistance to shearing perceived by the particles in the extruder barrel, and thus the changes in physical properties of extrudates (Navale et al., 2015). However, it should be kept in mind that the effects of particle size on extrudate expansion and texture vary with materials and processing conditions (Al-Rabadi et al., 2011).

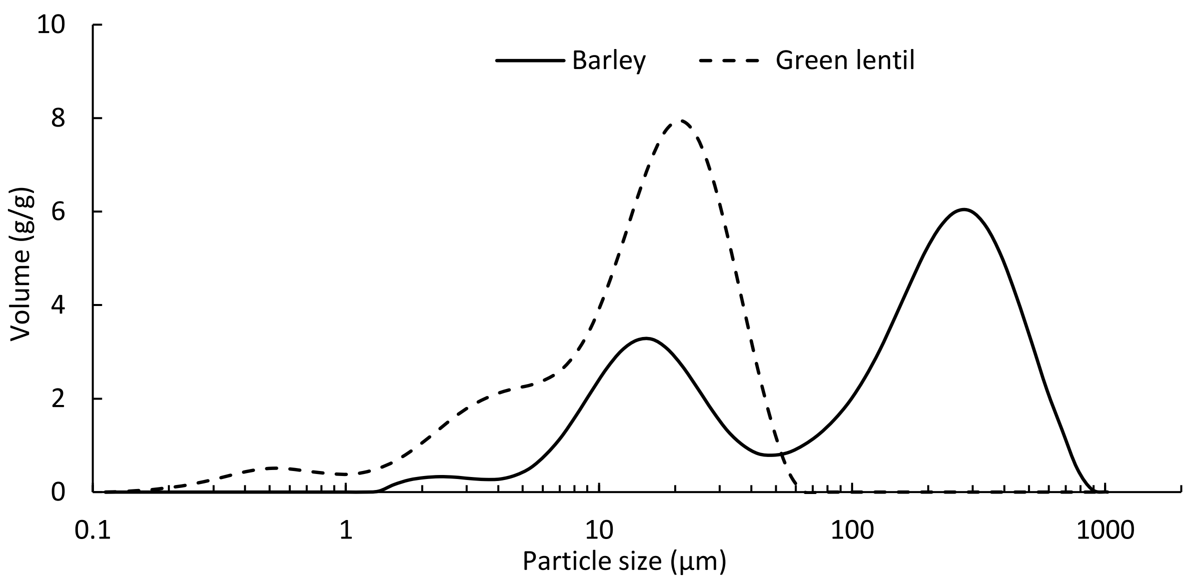

Fig. 4.1. Particle size distributions of barley and green lentil flours.

4.4.2 Specific mechanical energy

Based on Table 4.1, increasing the die temperature from 130°C to 140°C decreased the SME values by ~10-30 W hr/kg and reduced the torque values by ~2-7%. These effects might be because higher extrusion temperatures raise the degree of starch conversion and reduce the viscosity of the melt (Singh et al., 2015). Feed moisture also showed an overall negative relationship with torque and SME values: increasing feed moisture by 3 g/100 g caused the torque and SME values to decrease ~3-12% and ~30-100 W hr/kg, respectively. These findings are in line with the results of Ryu & Ng (2001) and Singh et al. (2015) who found that moisture acts as a lubricant in the barrel, reduces friction and energy use, leading to lower torque and SME. In terms of the effect of blending, increasing the green lentil ratio, i.e., the raw material with relatively higher protein content, increased the torque and SME values. This finding agrees with the results of Matthey & Hanna (1997) who reported that protein content had a positive relationship with SME, possibly due to an increase in the melt viscosity at higher protein concentration.

Table 4.1 Die pressure, torque and SME values of extrudates with different blending ratios and produced at different die temperatures (DT) and feed moistures (FM). Data is presented as the average of two extrusion runs.

Blend	DT, °C	FM, g/100 g	Die pressure, kPa	Torque, %	SME, W hr/kg
Bly	130	18	1450	31.5	159.9
		21	900	26.5	129.6
	140	15	2000	38.5	202.6
		18	1400	26.5	134.5
		21	950	22	107.6
75Bly25GL	130	15	2750	39	199.4
		18	1650	34	167.7
		21	1050	28.5	135.4
	140	15	2450	33	168.7
		18	1500	30	147.9
		21	1150	28.5	135.4
60Bly40GL	130	15	2400	47.5	241.2
		18	1650	40.5	198.4
		21	1250	31.5	148.7
	140	15	2100	45.5	231.1
		18	1300	44	215.6
		21	1350	30	141.6
45Bly55GL	130	15	2350	54	275.2
		18	1350	50	245.8
		21	1300	41	194.2
	140	15	1900	51.5	262.4
		18	1400	46.5	228.6
		21	1050	39	184.7
GL	130	15	2750	61.5	307.7
		18	1800	42	202.7
		21	1400	36.5	169.7
	140	15	2400	54.5	272.7
		18	1850	44	212.4
		21	1550	33	153.5

4.4.3 Extrudate physical properties

The results of extrudate physical properties as affected by the three main factors (barley to green lentil blending ratio, feed moisture and die temperature), as well as the statistical effects of these factors and their interactions are presented in Table 4.2. The interaction of the three main factors (i.e., the 3-way interaction) had significant effects on all tested properties except crunchiness. When 2-way interactions were considered, the interaction of blending ratio (BR) and feed moisture (FM) significantly affected on all extrudate properties, while the interaction of BR and die temperature (DT) did not significantly affect the crunchiness of extrudates, and the interaction of FM and DT did not significantly affect color properties except a^*. When individual effects of main factors were examined, it was found that BR had significant effects on all tested properties. The effects of FM and DT on extrudate density and crunchiness, respectively, were not as significant, but differences were detected based on the averages (Fig. 4.2 and Table 4.3).

Table 4.2 Extrudate density, expansion index, textural and color properties of extrudates as affected by blending ratio (BR), feed moisture (FM) and die temperature (DT). The significances of BR, FM, DT, and interactions of BR×FM, BR×DT, FM×DT, BR×FM×DT were analyzed by ANOVA and Tukey's test.

Factors	Extrudate density	Expansion index	Textural properties			Color properties			
	g/cm^3		Hardness N	Crispness	Crunchiness N sec	*L**	*a**	*b**	ΔE
BR									
Bly	0.73	1.37	68.01	7.15	250.92	80.87	2.10	14.40	9.60
75Bly25GL	0.64	1.37	58.07	7.17	229.74	81.29	1.65	16.83	10.10
60Bly40GL	0.41	1.65	38.02	8.42	133.89	80.31	1.64	19.82	12.33
45Bly55GL	0.31	1.87	28.80	13.39	125.55	80.52	1.31	21.47	12.61
GL	0.70	1.45	54.02	6.87	224.16	85.56	-0.05	24.75	9.73
FM, g/100 g									
15	0.53	1.69	50.80	9.62	198.83	81.72	1.47	20.28	11.28
18	0.56	1.50	48.96	8.53	192.06	81.67	1.31	19.45	10.91
21	0.57	1.46	46.67	7.90	182.45	81.82	1.15	19.22	10.60
DT, °C									
130	0.69	1.39	50.23	7.36	183.34	82.10	1.17	19.45	10.44
140	0.43	1.69	47.35	9.86	197.86	81.40	1.43	19.79	11.36
Effects				***P* value**					
BR	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
FM	0.7871	<.0001	<.0001	0.0002	0.0001	<.0001	<.0001	0.0002	<.0001
DT	<.0001	<.0001	<.0001	<.0001	0.0513	<.0001	<.0001	<.0001	<.0001
BR×FM	<.0001	<.0001	<.0001	<.0001	0.0006	<.0001	<.0001	<.0001	<.0001
BR×DT	<.0001	<.0001	<.0001	0.0138	0.2643	<.0001	<.0001	<.0001	<.0001
FM×DT	<.0001	<.0001	<.0001	0.0004	<.0001	0.2202	0.0005	0.1410	0.2020
BR×FM×DT	0.0004	<.0001	<.0001	0.0024	0.0568	<.0001	<.0001	0.0006	<.0001

4.4.3.1 Extrudate density

In Fig. 4.2A and 4.2B, the density of extrudates produced at 130 and 140℃, respectively, are presented. It is clear that increased DT lowered the density of extrudates significantly, a desirable attribute in puffed snacks, in agreement with the results of Meng et al. (2010). Variations in extrudate density, comparing the error bars in Fig. 4.2A and 4.2B, were lower at 140℃ because extrudates formed higher numbers of small cells with thin walls at higher temperature leading to higher extrudate uniformity (Fig. 4.3), in line with Lazou & Krokida (2010).

In terms of the effect of blending on extrudate density, blends Bly, 75Bly5GL and GL had significantly higher density than blends 60Bly40GL and 45Bly55GL, especially at the lower die temperature and higher feed moisture. It has been reported that some proteins and dietary fibers may form dispersed phases in the starchy melt. At low concentration levels, these dispersed phases may allow bubble growth upon die exit, and therefore cause no harm to extrudate expansion (Navale et al., 2015; Guy, 2001). However, at high concentration levels, proteins and dietary fibers could penetrate the continuous starch phase and disrupt bubbles. This is especially significant for dietary fiber due to its relatively larger particle size, leading to shrinkage (Guy, 2001). Moreover, the starch of the *CD Ratan* barley flour used in the study is known for exclusively high amylopectin content, which is around 95% (Yang et al., 2013). Due to its high branch chain length and relatively higher molecular weight than amylose, gelatinization behaviour of amylopectin-rich starches, and thus rheological properties of their pastes, result in relatively lower extrudate expansion followed by a higher level of extrudate shrinkage during extrusion cooking (Sarka & Dvoracek, 2017; Guy, 2001). These may explain high extrudate density values measured for blends Bly, 75Bly5GL (i.e., higher dietary fiber) and GL (i.e., highest protein), but lower density for blends 60Bly40GL and 45Bly55GL. For blends

60Bly40GL and 45Bly55GL, extrudate density increased with increasing feed moisture at both 130℃ and 140℃ die temperatures. Higher moisture levels may reduce the SME input during extrusion, as also evidenced in Table 4.1, leading to reduced starch conversion and impaired expansion correspondingly (Ajita & Jha, 2017).

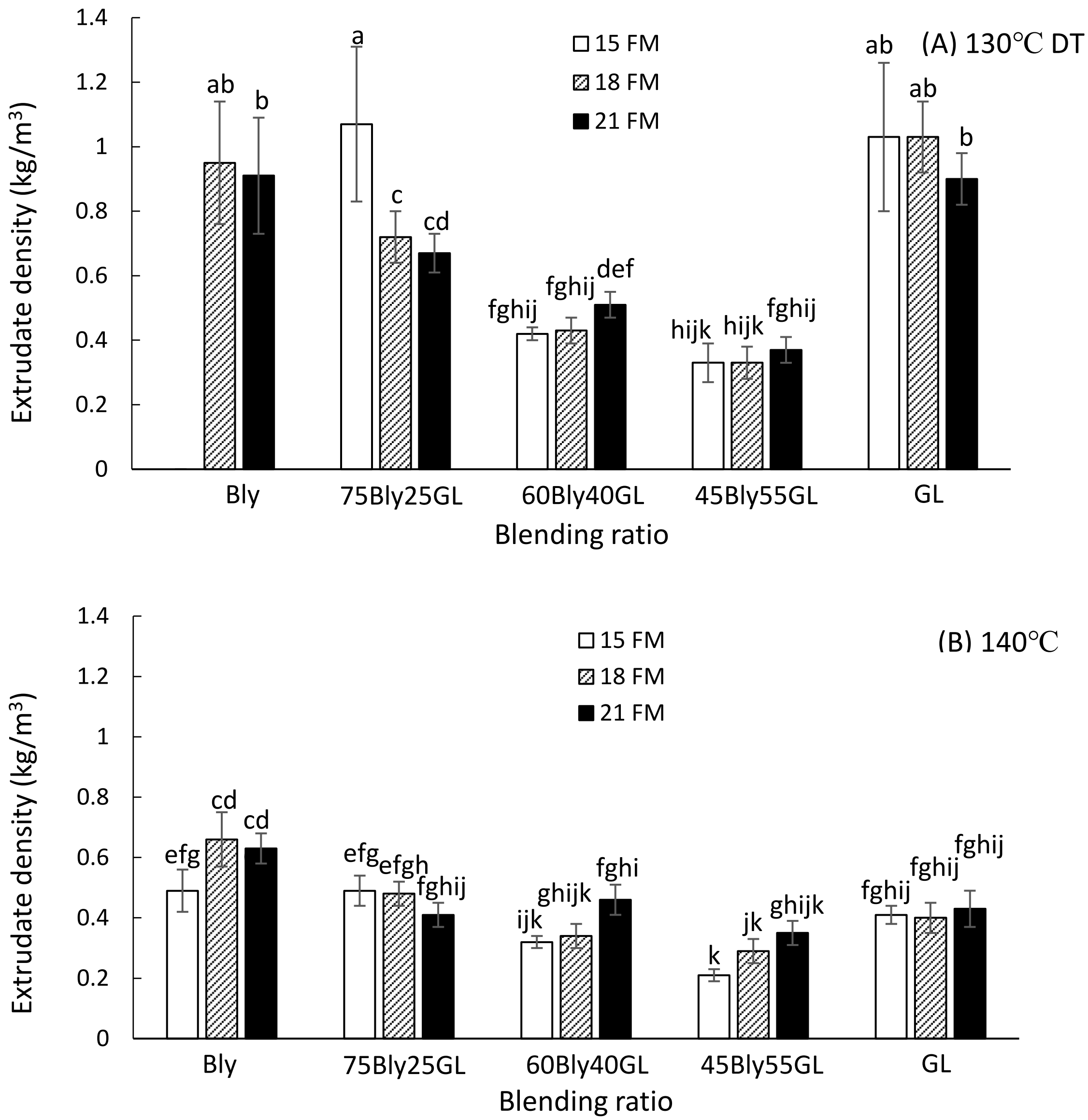

Fig. 4.2. Density of extrudates with different blending ratios and feed moistures (FM, g/100 g) produced at (A) 130℃ and (B) 140℃ die temperature (DT). Error bars represent standard deviations (n=2 for extrusion runs, n=5 for extrudate density measurements). Values of extrudate density followed by different letters are significantly different ($p < 0.05$).

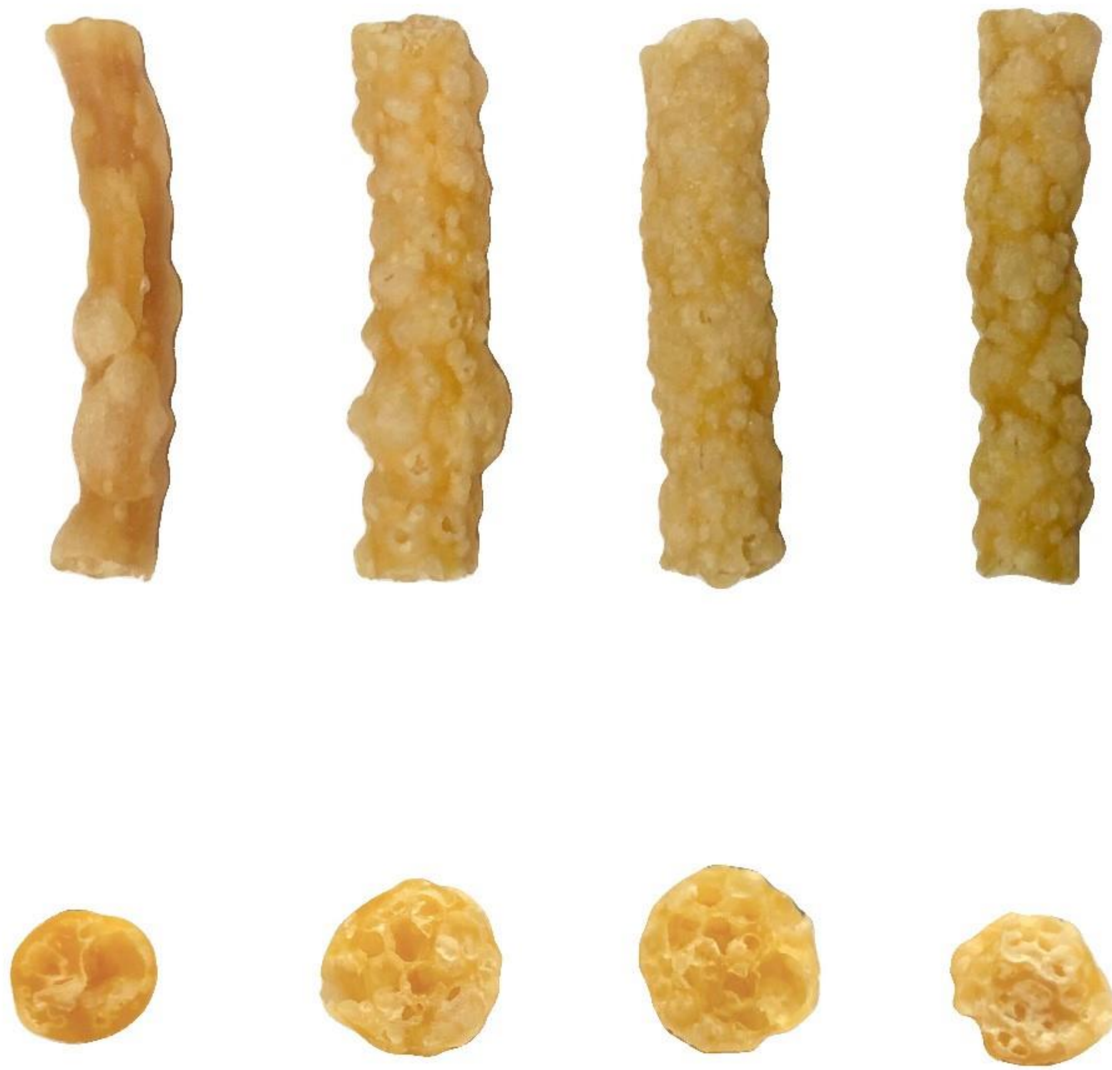

Fig. 4.3. Surface (top) and cross-sectional (bottom) images of GL extrudates produced at 15 g/100 g feed moisture (FM), 130°C die temperature (DT); 15 g/100 g FM, 140°C DT; 18 g/100 g FM, 140°C DT and 21 g/100 g FM, 140°C DT (from left to right).

4.4.3.2 Expansion index

Common extrusion variables (e.g. die temperature, feed moisture, screw speed, etc.) generally show opposite effects on expansion index and extrudate density (Navale et al., 2015), which aligns with the findings of this study. It is obvious that DT had a positive relationship with expansion index (Fig. 4.4), which might be because high DTs increase the degree of starch conversion (Navale et al., 2015). In terms of the effect of blending, blends 60Bly40GL and 45Bly55GL had significantly higher expansion index than other blends, especially at higher DT and lower FM. According to Table 4.2, blends 60Bly40GL and 45Bly55GL had the average expansion index of 1.65 and 1.87, respectively, which were 0.3-0.5 units higher than that of other blends. The reason behind this phenomenon might again be that controlled and balanced protein and dietary fiber contents do not impair expansion. For blends 60Bly40GL and 45Bly55GL, increasing FM lowered expansion index at both DTs, which agrees with the literature (Ajita & Jha, 2017).

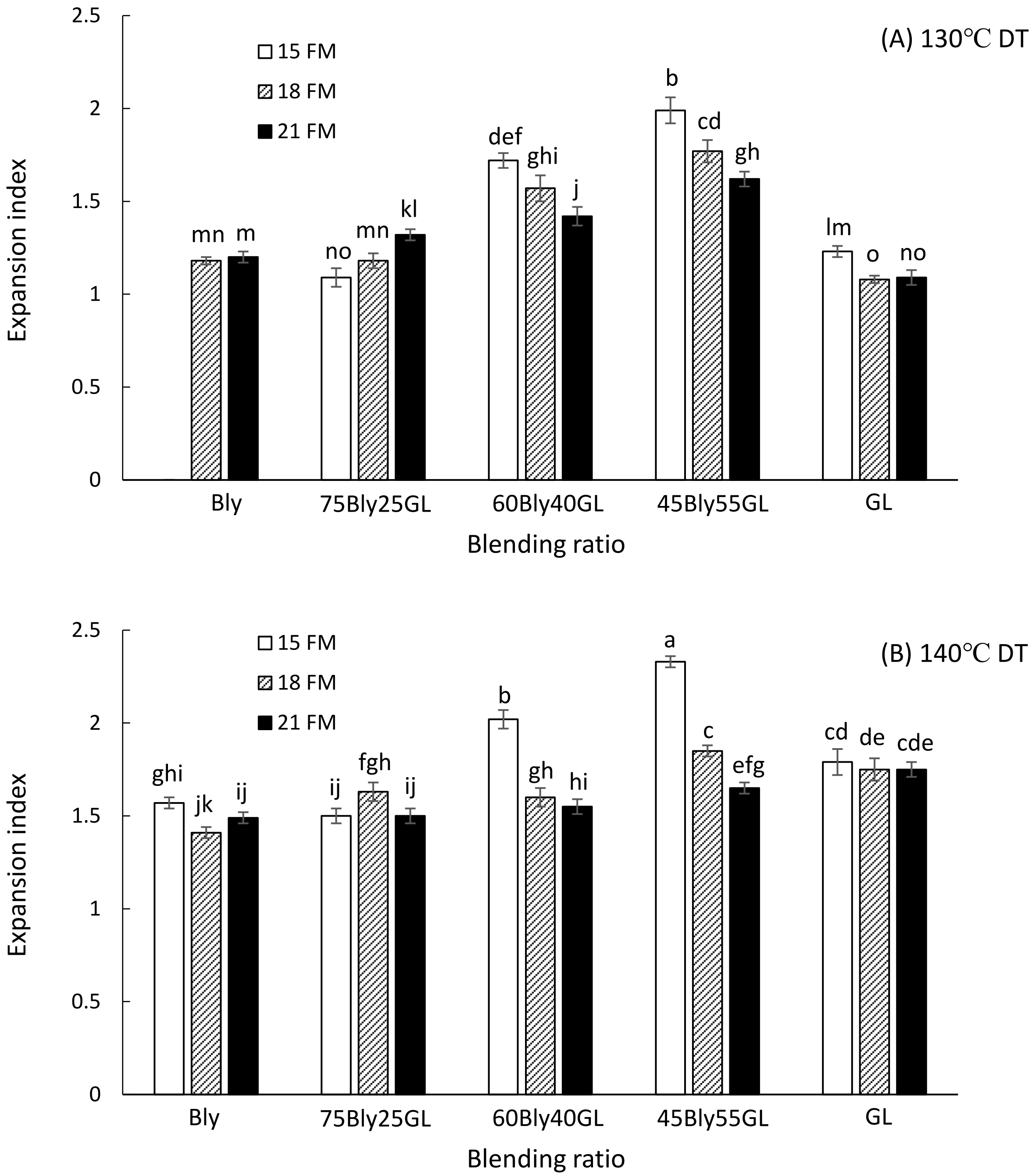

Fig. 4.4. Expansion index of extrudates with different blending ratios and feed moistures (FM, g/100 g) produced at (A) 130℃ and (B) 140℃ die temperature (DT). Error bars represent standard deviations (n=2 for extrusion runs, n=3 for expansion index measurements). Values of expansion index followed by different letters are significantly different ($p < 0.05$).

4.4.3.3 Textural properties

Hardness describes the maximum force required to break the sample through, which is a critical quality attribute for puffed snacks. Based on Table 4.2, increasing DT from 130℃ to 140℃ decreased the hardness of extrudates from 50.23 N to 47.35 N. This decrease might be because high DT enhances extrudate expansion, forms more air cells and thinner cell walls (Navale et al., 2015). Table 3 showed that extrudates produced at 15 g/100 g FM (e.g., blends 75Bly25GL and GL) showed significant drops in hardness when DT was increased. The extrudates produced at higher FM had relatively mild changes. These results indicated that high FM could impair the positive effect of high DT on hardness.

Blending also showed significant effects on extrudates' hardness. Blend 45Bly55GL had the lowest hardness value (28.80 N) followed by blend 60Bly40GL (38.02 N). Blends GL (54.02 N) and 75Bly25GL (58.07 N) had similar hardness, while the extrudate Bly (68.01 N) had the highest hardness value (Table 4.2). These results align with the extrudate density and expansion index data (Fig. 4.2 and 4.4). In general, well expanded extrudates tended to have low hardness since they had higher numbers of small cells and thinner cell wells, for example see the cross-sectional images of GL extruded at 15 g/100 g FM and 130℃ DT vs. at 15 g/100 g FM and 140℃ DT (Fig. 4.3). Seth et al. (2015) also reported that high level of fiber and protein addition increased the hardness of extrudates.

According to Table 4.2, overall FM had a negative correlation with extrudate hardness (i.e., as FM increased hardness decreased). However, when different blending ratios are considered, this trend was only clearly seen for blend 75Bly25GL produced at 130℃ (Table 4.3). Blends 45Bly55GL and 60Bly40GL showed the opposite trend at 140℃ die temperature (i.e., extrudate hardness was the highest at the highest FM). This latter result is in line with most studies where hardness was reported to increase with increasing FM, due to impaired expansion at relatively higher FM (Lazou et al., 2011). However, as discussed for extrudate density, low feed moisture did not significantly benefit the expansion of blends with higher dietary fiber or protein contents (blends Bly, 75Bly25GL and GL). This might be the reason why feed moisture showed a complex effect on extrudates' hardness in this study compared to the literature.

Crispness is a key quality attribute of dry snacks and is directly related to extrudate's cellular structure. Based on Table 4.3, increasing DT significantly improved extrudates' crispness by 1-4 units, which might be due to improved expansion and a higher number of cells. Blend 45Bly55GL had significantly higher crispness than other blends (Table 4.2), agreeing with the results of extrudate density, since crispness was shown to have a significant negative relationship with extrudate density (Yagci, 2017). FM had a significant negative relationship with crispness for blend 45Bly55GL, which agrees with Lazou et al. (2011). However, FM showed different effects on crispness for other blends, possibly because BR (i.e., characteristics of the blended materials) had a more substantial impact on extrudates' crispness than FM did.

Crunchiness is sometimes used interchangeably with crispness and is much less studied compared with other textural properties (e.g., hardness). A crunchier sample may have the same number of peaks (i.e., same crispness) in a time vs. force texture profile analysis graph when compared with other samples, but its "drop from peak to trough will be significantly higher and the linear distance increased accordingly" (Stable Micro System, 2020). Based on Table 4.2, DT did not significantly affect crunchiness ($p = 0.0513$). Blends 60Bly40GL and 45Bly55GL had significantly lower crunchiness values (Table 4.3) due to their better expansion and possibly thinner cell walls when compared to the other blends. The effect of FM on crunchiness was highly dependent on BR and DT.

Table 4.3 Textural and color properties of extrudates with different blending ratios produced at different die temperatures (DT) and feed moistures (FM). Means are followed by standard deviations (n=2 for extrusion runs, n=5 for textural and color properties measurements). Values in each column followed by different letters are significantly different ($p < 0.05$).

Blends	DT, °C	FM, g/100 g	Textural properties			Color properties			
			Hardness, N	Crispness	Crunchiness, N sec	*L**	*a**	*b**	ΔE
	130	18	70.0±13.0^{bcd}	5.9±1.4^{ij}	237.2±37.8^{abcde}	81.2±0.1^{g}	2.1±0.1^{ab}	14.3±0.2^{l}	9.2±0.1^{jkl}
		21	61.7±12.9^{def}	6.4±1.1^{hij}	216.5±30.0^{cde}	81.4±0.2^{g}	2.0±0.1^{ab}	14.1±0.2^{l}	9.0±0.2^{kl}
Bly		15	63.4±3.4^{de}	8.7±1.0^{efghi}	269.3±16.5^{abc}	80.5±0.0^{ijkl}	2.1±0.0^{a}	14.4±0.3^{l}	9.9±0.2^{ghijk}
	140	18	79.7±6.2^{ab}	7.0±1.1^{fghi}	287.8±29.8^{a}	80.7±0.0^{i}	2.1±0.0^{a}	14.6±0.1^{l}	9.8±0.1^{ghijk}
		21	65.4±6.0^{cde}	7.8±1.9^{fghi}	243.7±38.8^{abcde}	80.6±0.1^{ijk}	2.1±0.0^{a}	14.7±0.2^{l}	10.0±0.0^{ghij}
		15	77.1±5.2^{abc}	5.5±1.3^{ij}	282.8±46.5^{ab}	82.4±0.1^{e}	1.6±0.0^{f}	16.0±0.2^{k}	8.7±0.1^{l}
	130	18	55.4±6.3^{efg}	5.9±1.1^{ij}	214.9±21.2^{cde}	81.9±0.1^{f}	1.6±0.0^{f}	16.1±0.4^{jk}	9.2±0.2^{jkl}
		21	43.8±3.4^{ghijk}	8.4±0.9^{efghi}	193.4±15.8^{efg}	81.1±0.1^{gh}	1.6±0.0^{ef}	17.0±0.4^{ijk}	10.3±0.2^{gh}
75Bly25GL		15	59.2±6.2^{def}	7.3±1.4^{fghi}	230.2±33.5^{abcde}	81.3±0.1^{g}	1.7±0.0^{de}	17.2±0.3^{ij}	10.3±0.1^{ghi}
	140	18	58.2±3.8^{def}	8.0±1.7^{fghi}	235.2±29.0^{abcde}	80.8±0.1^{hi}	1.7±0.1^{de}	17.0±0.4^{ijk}	10.6±0.3^{g}
		21	54.8±5.8^{efgh}	8.0±1.8^{fghi}	222.0±8.3^{cde}	80.2±0.2^{hi}	1.7±0.0^{cd}	17.7±0.3^{i}	11.5±0.3^{f}
		15	42.5±6.6^{hijkl}	5.9±1.7^{ij}	125.9±18.5^{h}	80.4±0.1^{jklm}	1.8±0.0^{c}	20.1±0.2^{fgh}	12.5±0.2^{abcde}
	130	18	42.4±1.3^{ijkl}	6.6±2.0^{ghij}	146.5±25.8^{fgh}	80.3±0.1^{klm}	1.6±0.0^{f}	19.9±0.2^{gh}	12.3±0.2^{bcdef}
		21	36.1±3.8^{ghijk}	7.9±1.4^{fghi}	135.5±29.1^{gh}	80.8±0.1^{hi}	1.3±0.0^{g}	19.6±0.2^{h}	11.8±0.1^{ef}
60Bly40GL		15	31.3±2.0^{lmn}	12.9±2.0^{bcd}	124.7±12.8^{h}	80.1±0.2^{m}	2.0±0.1^{b}	20.2±0.6f^{gh}	12.7±0.5^{abcde}
	140	18	31.4±4.1^{lmn}	10.3±2.5^{defg}	124.5±12.9^{h}	79.6±0.1^{n}	1.7±0.0^{cd}	19.9±0.3^{gh}	12.9±0.2^{abc}
		21	44.4±5.2^{ghij}	6.9±1.4^{fghi}	146.2±31.2^{fgh}	80.7±0.1^{ij}	1.4±0.0^{g}	19.4±0.3^{h}	11.8±0.3^{ef}
		15	31.7±3.4^{klmn}	15.8±2.6^{b}	149.7±17.2^{fgh}	80.5±0.1^{ijkl}	1.4±0.0^{g}	21.7±0.4^{de}	12.8±0.4^{abcd}
	130	18	28.0±2.2^{mn}	11.8±1.9^{cde}	117.5±5.2^{h}	80.8±0.2^{hi}	1.1±0.0^{h}	21.1±0.4^{ef}	12.1±0.4^{cdef}
		21	30.4±2.9^{lmn}	7.2±2.1^{fghij}	98.8±14.4^{h}	80.7±0.2^{i}	0.9±0.0^{i}	21.3±0.3^{de}	12.3±0.3^{bcdef}
45Bly55GL		15	23.4±6.9^{n}	19.7±3.3^{a}	126.9±23.2^{h}	80.2±0.2^{lm}	1.7±0.0^{de}	22.2±0.3^{d}	13.4±0.2^{a}
	140	18	29.5±2.6^{mn}	15.5±1.4^{bc}	140.7±17.0^{gh}	80.2±0.1^{m}	1.6±0.0^{f}	21.8±0.5^{de}	13.1±0.3^{ab}
		21	29.7±3.0^{mn}	10.4±0.8^{def}	119.8±18.2^{h}	80.6±0.1^{ijk}	1.1±0.0^{h}	20.8±0.4^{efg}	12.1±0.3^{cdef}
		15	83.2±3.8^{a}	3.5±1.3^{j}	254.9±30.8^{abcd}	85.5±0.0^{c}	0.2±0.0^{k}	24.3±0.4^{c}	9.4±0.3^{hijkl}
	130	18	50.7±7.6^{fghi}	5.8±0.9^{ij}	188.2±28.3^{efg}	85.9±0.1^{b}	-0.4±0.0^{n}	24.6±0.5^{bc}	9.4±0.5^{ijkl}
		21	50.3±6.1^{fghi}	6.4±1.9^{hij}	204.8±38.4^{def}	86.6±0.0^{a}	-0.6±0.0^{o}	22.4±0.4^{d}	7.1±0.4^{m}
GL		15	45.5±4.7^{fghi}	7.3±2.6^{fghi}	225.1±61.6^{bcde}	84.6±0.1^{d}	0.6±0.0^{j}	26.6±0.5^{a}	11.9±0.4^{def}
	140	18	44.4±6.8^{ghij}	8.5±2.1^{efghi}	228.1±59.2^{abcde}	85.3±0.1^{c}	0.0±0.0^{l}	25.5±0.4^{ac}	10.5±0.4^{g}
		21	50.1±6.5^{fghi}	9.7±1.5d^{efgh}	243.8±37.7^{abcde}	85.6±0.0^{bc}	-0.2±0.0^{m}	25.2±0.5^{bc}	10.1±0.4^{ghij}

4.4.3.4 Color

The *L**, *a** and *b** values of barley and green lentil flours were 88.5, 0.9, 8.7 and 90.3, -0.8, 16.3, respectively. Their flour blends showed the color in between and followed the trend that as the green lentil ratio was increased a brighter, greener and yellower tone was observed. Overall, extrudates showed darker color, more red and yellow tone compared to their flours.

Based on Table 4.3, all extrudate' *L** value decreased dramatically after extrusion compared with flours, which might be in part due to Maillard reactions, giving products a darker color (Sharma & Gujral, 2013). The decrease of *L** value was slightly higher at higher DT, possibly due to enhanced Maillard reaction rates (Masatcioglu et al., 2013). GL extrudates showed much higher *L** value than other blends, possibly due to their flour's initial higher *L** value. The effect of FM on extrudates' *L** value was less critical than those of DT and BR.

After extrusion, extrudates' *a** value increased by 1-3 units overall, again pointing to Maillard reactions. The increase of *a** value was slightly higher at the higher DT, same with the trend of *L**. Extrudates with higher green lentil ratio had lower *a** value due to the initial green tone of the green lentil flour. In terms of FM, a negative relationship with *a** value on blends 60Bly40GL, 45Bly55GL and GL was observed, rendering them closer to their flours. However, no effect was found on blends Bly and 75Bly25GL. Apart from Maillard reactions, decomposition of natural pigments (e.g. some colored phenolics) in the flours during processing can also cause color change, as well as reduction in the antioxidant activity of products (Lazou et al., 2010). For example, Sharma et al. (2012) reported that barley flours typically contain 3-5 mg ferulic acid equivalents (FAE)/g total phenolic content. However, only 40-70% of it can survive from extrusion cooking. Therefore, the color properties, especially the color change compared with flours, could be considered as an indicator of nutritional changes in extrudates. Our results suggested that the green colored pigments in green lentil flour might be sensitive to extrusion processing (i.e., combined shear, temperature and pressure). Increased feed moisture reduced the intensity of extrusion and thus more green tone remained in extrudates. The effects of BR, DT and FM on b^* value were similar to their effects on a^* value.

In terms of the color difference (ΔE) between extrudates and their flours, DT showed a positive relationship with ΔE (Table 4.2), which might be due to more intensive Maillard reactions at relatively higher temperatures, in agreement with Sharma & Gujral (2013). The increase of ΔE for extrudate GL at 140°C was significantly higher than any other blends, possibly suggesting that pigments in green lentil are more sensitive to heat than those in barley. Blends 60Bly40GL and 45Bly55GL showed higher ΔE than other blends overall, likely due to their formulas containing adequate sugars (from barley) and amino acids (from green lentil) which favored Maillard reactions. Increased FM lowered ΔE for blends 60Bly40GL, 45Bly55GL, and especially for GL, possibly indicating that green lentil contains more sensitive pigments compared to barley. Overall, high DT increased the color change due to increased extrusion intensity, while high FM worked in the opposite way.

4.5 Conclusion

Blending ratio, die temperature and feed moisture had significant impacts on physical properties of barley and green lentil extrudates. Die temperature had a negative relationship with extrudate density. The effect of feed moisture on extrudate density was mainly depended on the blending ratio, which affected extrudate density through its influence on protein and dietary fiber contents of the blends. Die temperature and blending ratio had significant effects on expansion index, while the effect of feed moisture was less significant in this case. As expected, the effects of these variables on expansion index were opposite to their effects on extrudate density. Blends

60Bly40GL and 45Bly55GL showed significantly higher expansion index than other blends. Extrudates' textural properties were significantly associated with expansion: well expanded extrudates had higher number of cells and thinner cell walls, leading to reduced hardness, higher crispness and lower crunchiness. Extrudates showed darker color, redder and yellower tones than their flours possibly due to Maillard reactions and decomposition of natural pigments. Results also suggested that green lentil flour may contain more heat sensitive pigments than barley flour. Overall, extrudates 45Bly55GL showed the optimum physical properties as a puffed snack, followed closely by extrudates 60Bly40GL. High die temperature improved extrudates' expansion and textural properties significantly, while the effects of feed moisture mainly depended on the blending ratio.

CHAPTER 5 General Discussions and Conclusions

The objectives of this study were: (1) producing nutritionally-dense puffed snacks without compromising their physical quality and (2) investigating the effects of extrusion variables on nutritional and physical attributes of extrudates. High dietary fiber (22.9 g/100 g db) hull-less barley and high protein (26.42 g/100 g db) dehulled green lentil flours were blended at 5 ratios (barley: green lentil, 100: 0, 75: 25, 60: 40, 45: 55, 0: 100, db). These blends were extruded at three feed moistures (15, 18, 21 g/100 g) and two barrel temperature profiles (die temperatures of 130, 140℃). Nutritional attributes including amino acid composition, IVPD, IVPDCAAS, IDF, SDF, TDF contents, and physical attributes including ED, EI, texture and color of extrudates were evaluated.

Blends of barley and green lentil not only contained ample amounts of dietary fiber and protein, but also had balanced amino acid composition, laying the footstone for developing nutritious puffed snacks. The effect of extrusion on amino acid retention varied with the types of amino acids and the blending ratio of flours. Extrusion ($p < 0.0001$) significantly improved the blends' IVPDs by up to 10%, especially at low feed moisture and high die temperature. Due to enhanced IVPD and balanced amino acid composition, extrudates 75Bly25Gl, 60Bly40GL and 45Bly55GL also had significantly high IVPDCAAS. Extruded 45Bly55GL blend showed the least variability and the highest average (68.62%) IVPDCAAS among all the blends. Extrudate 45Bly55GL produced at 140℃ DT and 15 g/100 g FM had the highest IVPDCAAS-corrected protein per RACC (4.2 g) and protein rating (17.5). These values were only 0.8 g short for IVPDCAAS-corrected protein per RACC and 2.5 short for protein rating to carry "good source of protein" claim in the US and Canada, respectively. Extrusion ($p < 0.0001$) significantly decreased SDF of extrudates where feed moisture ($p = 0.0026$) was the major factor. All extrudates from blends 60Bly40GL and 45Bly55GL, and four (three produced at 130°C DT regardless of their FM, and 21 g/100 g FM extrudate produced at 140°C DT) from the blend 60Bly40GL met the requirement to be recognized as "good source of dietary fiber" by FDA and "very high dietary fiber" by CFIA, respectively.

Blending ratio ($p < 0.0001$) and die temperature ($p < 0.0001$) showed significant effects on both ED and EI of extrudates, while the effect of feed moisture on expansion varied with the blending ratio. Results showed that blends 60Bly40GL and 45Bly55GL had higher expansion than other blends, especially at high die temperature. These well expanded extrudates showed higher number of cells and thinner cell walls, which resulted in better textural properties (i.e., reduced hardness and higher crispness). Extrusion also gave extrudates darker, redder, yellower color possibly due to Maillard reactions and decomposition of natural pigments.

Overall, blend 45Bly55GL showed promising nutritional and physical quality, followed closely by blend 60Bly40GL. Die temperature showed more impact on the physical attributes of extrudates, while feed moisture was more vital for their nutritional attributes. This study proved that using blends of cereals and pulses (e.g., barley and green lentil) with the adjustment of extrusion variables (e.g., die temperature and feed moisture) to produce nutritionally-dense puffed snacks with acceptable physical quality is achievable. As a successful example, this study showed that puffed snacks have the potential to shake off the "junk food" image and turn into healthy snacks that consumers are chasing for.

CHAPTER 6 Limitations and Recommendations for Future Research

Due to restricted access to the labs at the last stage of this MSc project amid the COVID-19 pandemic, only flours and extrudates of 60Bly40GL and 45Bly55GL were selected for dietary fiber determination. The reason for selecting these two flours and extrudates was that the former formula produced extrudates with the best protein quality while the latter one with the best physical quality. In addition, the dietary fiber method adopted in this study cannot detect low molecular weight soluble dietary fiber (LMWSDF). In the future, when time and equipment is available, HPLC can be used to precisely measure LMWSDF content to provide more accurate results. Gas Chromatography–Mass Spectrometry (GC/MS) can also be employed to identify the specific components of dietary fiber (e.g., beta-glucan and resistant starch) and their changes before and after extrusion. Furthermore, the precision of dietary fiber results was relatively low compared with other analyses due to the difficulty of manual multi-filtration. An automatic filtration system can be used in the future to improve the precision of the results and the efficiency of the method.

Besides nutrient content claims (information in Appendix B), health claims can also be made about the beneficial effects of a specific dietary fiber source on a case by case basis and subject to conditional use (Health Canada, 2020a). These health claims can help advertise the product better and attract more consumers' attention. Health Canada (2020b) has recognized barley fiber's effect on lowering blood cholesterol and approved relevant health claims. However, those claims have specific requirements about the beta-glucan content in the product, considering beta-glucan is the main factor that can regulate cholesterol. Therefore, the determination of beta-glucan content should be included in a future study if possible, access to dietary fiber health claims are of interest.

As discussed in Chapter 3, none of the extrudate formulae in this study met the requirements of FDA and CFIA to carry "good source of protein" label. The closest formula was extrudate 45Bly55GL produced at 140℃ die temperature and 15 g/100 g feed moisture, which was only 0.8 g and 2.5 points lower than the requirement of FDA and CFIA, respectively. In the future, pre-cooking the blends before extrusion to increase their protein digestibility or adding low amounts of protein isolate to the blends to increase their protein content might be able to fill this gap. The antioxidant capacity of extrudates can also be studied to investigate the nutritional value of products more deeply as barley and green lentil are rich sources of antioxidants.

In terms of the improvement of the measurement of physical properties of extrudates, scanning electron microscopy can be explored to study the cross-sectional structure of the extrudates. This would help understand the effects of extrusion conditions on cellular expansion. The color measurement can also be performed on extrudates without milling them into flours, as this would provide information on the way consumers see the products. Last but not the least, sensory analysis can be conducted in the future to supplement instrumental analyses and give constructive suggestions on selecting optimal extrusion conditions to produce nutritionally-dense puffed snacks with consumer acceptable physical quality.

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APPENDICES

Appendix A: Textural properties of some commercial snacks

Hardness, crispness and crunchiness of three commercial snacks (Means are followed by standard deviations, n=5).

Product	Textural properties		
	Hardness, N	Crispness	Crunchiness, N sec
Cheetos Puff	4.6±0.4	24.7±1.9	36.3±2.9
Cheetos Crunchy	10.1±1.9	17.7±2.4	53.2±8.5
No Name Pretzel Twist	18.0±1.0	5.5±2.8	46.0±8.6

Appendix B: Protein and dietary fiber content claims in Canada and the USA

Some protein and dietary fiber content claims (in quotation marks) permitted in the USA by Food and Drug Administration (FDA, 2013) and in Canada by Canadian Food Inspection Agency (CFIA, 2021) and their brief requirements.

Nutrient content claims	Region	Conditions
Protein		
"good source of protein" "contains protein" "provides protein"	USA	Contains 10%-19% of the protein daily value[1] (DV) per RACC[2].
"high protein" "rich in protein" "excellent source of protein"		Contains 20% or more of the protein DV per RACC.
"source of protein" "good source of protein" "high protein"	Canada	The food has a protein rating of 20 or more, as determined by official method FO-1, Determination of Protein Rating, October 15, 1981[3]. Protein rating can also be estimated by PDCAAS[4].
"excellent source of protein" "very high protein" "very high in protein"		The food has a protein rating of 40 or more, as determined by official method FO-1, Determination of Protein Rating, October 15, 1981. Protein rating can also be estimated by PDCAAS.
Dietary fiber*		
"good source of dietary fiber" "contains dietary fiber" "provides dietary fiber"	USA	Contains 10%-19% of the dietary fiber DV[5] per RACC.
"high dietary fiber" "rich in dietary fiber" "excellent source of dietary fiber"		Contains 20% or more of the dietary fiber DV per RACC.
"source of dietary fiber" "contains dietary fiber" "provides dietary fiber"	Canada	(1) The food contains 2 g or more of (a) dietary fiber per reference amount[6] and serving of stated size[7], if no dietary fiber or dietary fiber source is identified in the statement or claim; or (b) each identified dietary fiber or dietary fiber from an identified dietary fiber source per reference amount and serving of stated size, if a dietary fiber or dietary fiber source is identified in the statement or claim. (2) The food contains at least one ingredient that meets the condition set out in (1), if the food is a prepackaged meal.
		(1) The food contains 4 g or more of (a) dietary fiber per reference amount and serving of stated

"high source of dietary fiber" "high dietary fiber" "high in dietary fiber"		size, if no dietary fiber or dietary fiber source is identified in the statement or claim; or (b) each identified dietary fiber or dietary fiber from an identified dietary fiber source per reference amount and serving of stated size, if a dietary fiber or dietary fiber source is identified in the statement or claim. (2) The food contains at least one ingredient that meets the condition set out in (1), if the food is a prepackaged meal.
"very high source of dietary fiber" "very high dietary fiber" "dietary fiber rich"		(1) The food contains 6 g or more of (a) dietary fiber per reference amount and serving of stated size, if no dietary fiber or dietary fiber source is identified in the statement or claim; or (b) each identified dietary fiber or dietary fiber from an identified dietary fiber source per reference amount and serving of stated size, if a dietary fiber or dietary fiber source is identified in the statement or claim. (2) The food contains at least one ingredient that meets the condition set out in (1), if the food is a prepackaged meal.
"more dietary fiber" "higher dietary fiber" "higher in dietary fiber"	Canada	(1) The food contains at least: i) 25% more dietary fiber, totalling at least 1 g or more, if no dietary fiber or dietary fiber source is identified in the statement or claim, or ii) 25% more of the identified dietary fiber or dietary fiber from an identified dietary fiber source, totalling at least 1 g or more, if a dietary fiber or dietary fiber source is identified in the statement or claim (a) per reference amount of the food than the reference amount of a reference food of the same food group or a similar reference food; or (b) per 100 g than 100 g of a reference food of the same food group or a similar reference food, if the food is a prepackaged meal. (2) The food contains at least: (a) 2 g of dietary fiber per reference amount and serving of stated size if no dietary fiber or dietary fiber source is identified in the statement or claim, or of identified dietary fiber or dietary fiber from an identified dietary fiber source per reference amount and serving of stated size if a dietary fiber or dietary fiber source is identified in the statement or claim; or (b) one ingredient that meets the conditions set out in column 2 of the subject "source of dietary fiber" set out in item a) of this table, if the food is a prepackaged meal.

[1] DV is the recommended amounts of nutrients to consume or not to exceed each day. DV of protein regulated by FDA is 50 g.

[2] RACC represents the amount of the product that consumers usually consume per eating occasion. RACC for snacks regulated by FDA is 30 g.

[3] Details of official method FO-1 and example calculations of protein rating can be found at https://www.inspection.gc.ca/food-label-requirements/labelling/industry/nutrition-labelling/elements-within-the-nutrition-facts-table/eng/1389206763218/1389206811747?chap=7.

[4] The calculation of estimated protein rating based on PDCAAS can be found at https://www.inspection.gc.ca/food-label-requirements/labelling/industry/nutrition-labelling/elements-within-the-nutrition-facts-table/eng/1613599715710/1613599936553?chap=7#s6c5

[5] DV of dietary fiber regulated by FDA is 28 g.

[6] A reference amount is a specific regulated quantity of a type of food usually eaten by an individual at one sitting. It is 50 g for snacks as regulated by CFIA.

[7] Serving size is the quantity of a food used to calculate the numbers in the nutrition facts table. The serving size for snacks regulated by CFIA is 40-60 g.

*In the below claims, "dietary fiber" may be substituted with "fiber".