Lipid Oxidative Stability, Antioxidant Capacity, and Protein Quality of Direct-Expanded Chickpea-Sorghum Snacks

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Abstract

A chickpea and sorghum blend was used for production of direct-expanded snacks to increase the protein content and quality of such products, which are well known to and consumed by people of various ages due to their texture and convenience. The use of whole grain chickpea and sorghum also would provide other nutritional benefits. However, chickpea has a relatively high fat content (3-10%, dry weight basis) compared to most other pulses which increases the susceptibility to oxidation and reduces the shelf-life of direct-expanded products containing chickpea. In addition, the fat content of whole grain sorghum (2-6%, dry weight basis) is significant compared to that of milled sorghum or corn. Expanded snacks are highly susceptible to lipid oxidation due to their expanded surface area and low water activity. Since lipid oxidation affects sensory quality and nutrient content, determining the lipid oxidative stability and shelf-life of direct-expanded chickpea-sorghum snacks was important. Related to this, examining the total phenolics content and antioxidant capacity of chickpea-sorghum snacks and their contribution to oxidative stability also was worthwhile. Ultimately, it was important to determine the protein quality of the direct-expanded chickpea-sorghum snacks. Hence, the overall purpose of the study was to investigate the use of a whole-grain chickpea-whole grain sorghum blend for production of direct-expanded snacks from oxidative stability, shelf-life and protein quality perspectives.

Direct-expanded snacks were prepared from blends of whole-grain chickpea and whole-grain sorghum (70:30, 60:40 and 50:50 chickpea:sorghum, w/w) using a twin-screw extruder at barrel temperatures of 120^0C, 140^0C and 160^0C and moisture contents of 16%, 18% and 20%, as well as at the determined optimal expansion point, 169^0C barrel temperature and 15% moisture content. Chickpea and sorghum flours also were extruded at the maximal expansion point. An oxidative stability study (*p*-anisidine value and peroxide value) was carried out on snacks produced at the maximal expansion point. The oxidative stability of the 50:50 chickpea-sorghum snack was found to be higher ($P<0.05$) than that of the 60:40 and 70:30 chickpea-sorghum by both sensory and chemical analysis. Similarly, the shelf-life was higher ($P<0.05$) with a lower proportion of chickpea in the blend. A higher proportion of sorghum in the blend, extrusion and an increase in barrel temperature were associated with an increase ($P<0.05$) in antioxidant capacity, total phenolics content and *in vitro* protein digestibility, whereas an increase in moisture content decreased ($P<0.05$) these measurements. A higher proportion of chickpea in the blend and

extrusion increased the *in vitro* protein digestibility corrected amino acid score (IVPDCAAS) of chickpea-sorghum snacks. The protein quality of the 70:30 chickpea-sorghum snack produced at the maximal point was found to be higher ($P<0.05$) than that of the 60:40 or the 50:50 chickpea-sorghum snacks with minimal loss of available lysine. The study demonstrated that whole-grain chickpea and whole-grain sorghum can be used in the production of direct-expanded snacks having acceptable shelf-life and protein nutritional quality, hence chickpea-sorghum snacks could play a role in addressing protein-energy malnutrition in sub-Saharan Africa.

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List of Abbreviations and Acronyms

AAS	Amino Acid Score
ABTS	2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid
ABTS+•	2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid Radical Cation
ALA	Alanine
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
ARG	Arginine
ASP	Asparagine
BV	Biological Value
CYS	Cysteine
DIAAS	Digestible Indispensable Amino Acid Score
DPPH	2,2-diphenyl-1-picrylhydrazyl
DV	Daily value
EI	Expansion index
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GAE	Gallic Acid Equivalents
GLU	Glutamine
GLY	Glycine
HIS	Histidine
ILE	Isoleucine
ISO	International Organization for Standardization
IVPD	*In vitro* Protein Digestibility
IVPDCAAS	*In vitro* Protein Digestibility Corrected Amino Acid Score
LSD	Least significant difference
LYS	Lysine
MET	Methionine
NPR	Net Protein Ratio

NPU	Net Protein Utilization
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PER	Protein Efficiency Ratio
PHE	Phenylalanine
PRO	Proline
R•	Alkyl radical
ROO•	Peroxyl radical
ROOH	Alkyl peroxide
RTE	Ready-to-eat
SER	Serine
TD	True digestibility
THR	Threonine
TRP	Tryptophan
TYR	Tyrosine
UNICEF	United Nations Children's Fund
VAL	Valine
WHO	World Health Organization

Chapter 1
Introduction

1.1 Background and Rationale

Sub-Saharan Africa faces a triple burden of malnutrition due to the high prevalence of protein-energy malnutrition and micronutrient deficiencies (e.g. iron and zinc) along with the emerging problems of overweight/obesity (Fraval *et al*., 2019; Onyango, Jean-Baptiste, Samburu, & Mahlangu, 2019). Protein-energy malnutrition results from a lack of protein and energy in the diet and is manifested as stunting, wasting, and nutritional edema or Kwashiorkor. Protein is necessary for the proper functioning of the body including physical and cognitive development and maintenance of tissues (Ahmed *et al*., 2020). The prevalence of stunting, wasting and overweight in the sub-Saharan region is reported to be 33.0%, 6.8% and 3.0%, respectively (United Nations Children's Fund [UNICEF], World Health Organization [WHO], & World Bank Group, 2020). The prevalence of iron-deficiency anemia (10%) and zinc deficiency (15-50%) also is high in the region (Conti *et al*., 2019). Iron and zinc deficiencies reduce the gross domestic product of developing countries by 2-5% (Stein, 2014). One of the major reasons for the aforementioned nutritional problems is that the diet of people in the sub-Saharan Africa region is poor in quality and lacks diversity and depends largely on cereals and roots and tubers (Temba, Njobeh, Adebo, Olugbile, & Kayitesi, 2016). Nutrition transition also is an issue in the region as it has resulted in problems related to overweight and obesity. Nutrition transition is described as a shift in diet seen especially when low- and middle-income countries transition from the traditional diets that are nutrient-rich to the diets that are more processed foods and refined grains high in fat, salt, and lower in fibre and nutrients (Haggblade *et al*., 2016). Urban areas experience nutrition transition much better, especially urban poor where most easily affordable diets are unhealthy (Hawkes, Harris, & Gillespie, 2017).

Food and Agriculture Organization, Economic Commission for Africa and African Union Commission (2020) have called for action by all segments of the food system, including primary

agriculture, food processing, trade and consumption. In this context, agro-processing industries can play a major role in supplying products having good protein quality and based on available agricultural materials, rather than simply producing energy-dense products from refined ingredients.

Chickpea and sorghum are cultivated in Ethiopia and other sub-Saharan countries (Food and Agriculture Organization [FAO], 2019). A chickpea-sorghum blend has the potential to be used in direct-expanded snacks to increase their protein quantity and protein quality. However, chickpea has a relatively high-fat content (3-10%, on a dry weight basis) as compared to other pulses, which may lead to oxidative stability and shelf-life issues (Gul, Egesel, & Turhan, 2008). The fat content of whole grain sorghum (2-6%) also is significant as compared to milled sorghum (Afify, El-Beltagi, El-Selam & Omran, 2012; Zhang *et al*., 2019). Using whole grain chickpea and whole grain sorghum offers nutritional benefits related to fibre, minerals and protein, but fat content is a problem (Sreerama, Sashikala, & Pratape, 2010).

Direct-expanded snacks are ready-to-eat products produced by high-temperature extrusion. The most important quality aspect of direct-expanded snacks is their light and puffed texture, which depends on processing parameters such as extrusion temperature, screw speed and feed moisture content (Awolu, Oluwaferanmi, Fafowora, & Oseyemi, 2015). Direct-expanded snacks are widely popular due to their convenience and texture, and they are consumed by people ranging from children to adults (Makinde *et al*., 2020; Surasani, 2016). Shelf-stable and nutrient-dense direct-expanded snacks are ideal tools for addressing malnutrition in developing countries (Shah, Sharif, Bashir, & Ashan, 2019). However, direct-expanded snacks are highly susceptible to oxidation due to their low water activity and high interfacial surface area as the material is highly porous (Barden & Decker, 2016). Also, fat is more susceptible to oxidation in a direct-expanded snack. Lipid oxidation results in degradation of nutrients and changes in sensory quality, as well as shortening of shelf-life (Shahmohammadi, Bakar, Russly, Noranizan, & Mirhosseini, 2016), hence evaluating the oxidative stability of direct-expanded chickpea-sorghum snacks is important. Lipid oxidation is affected by several factors, antioxidant content being one of them. Antioxidants play a vital role in preventing lipid oxidation. Among antioxidants, phenolics are the largest group and play a key role in delaying oxidation (Choe & Min, 2009). Evaluating antioxidant capacity and total phenolics content of chickpea-sorghum snacks would provide important information on their stability.

The main reason chickpea and sorghum were blended was to increase protein quantity and protein quality. Protein quality refers to the extent that protein is digestible and can fulfill the amino acid requirements of a consumer (Marinangeli & House, 2017). Lysine and sulphur-containing amino acids are reasons for the blending of chickpea and sorghum (Majumdar & Singh, 2014; Patterson, Curran, & Der, 2017). However, lysine can be lost easily during high-temperature extrusion processing (Fallahi, Muthukumarappan, & Rosentrater, 2016). Hence, measuring the protein quality of direct-expanded chickpea-sorghum snacks and ensuring there is an improvement in protein quality is critical. In addition to protein quality, the health benefits of consuming chickpea and sorghum are further increased with the use of whole grain chickpea and whole grain sorghum. Whole grain consumption is inversely related to cardiovascular disease, type 2 diabetes and colon cancer, as whole grain contains fiber and phytochemicals otherwise lost during milling (Nuss & Tanumihardjo, 2010; Reynolds *et al*., 2019).

Chickpea has been blended with maize, rice, wheat, millet, barley and teff in previous studies on direct-expanded snacks. Most of these studies examined the effect of extrusion, extrusion conditions and the proportion of chickpea on physical properties, functional properties, nutrient content and antioxidant capacity (Awol, 2015; Bhattacharya & Prakash, 1994; Geetha, Mishra, & Srivastav, 2014; Patil, Brennan, Mason, & Brennan, 2016; Singha, Singh, Muthukumarappan, & Krishnan, 2018; Yovchev, Stone, Hood-Niefer, & Nickerson, 2017). However, none of the studies dealt with chickpea-sorghum direct-expanded snacks. Hence, the overall purpose of the current study was to investigate, from an oxidative stability and shelf-life perspective, the use of a whole-grain chickpea, whole-grain sorghum blend for the production of direct-expanded snacks, and to determine whether the extruded snacks had sufficient protein quality to participate in addressing protein-energy malnutrition.

1.2 Hypothesis and Objectives

1.2.2 Hypotheses

The study was carried out to test the following hypotheses:

- Direct-expanded snacks could be prepared from a whole-grain chickpea and whole-grain sorghum blend;

- The oxidative stability and shelf-life of direct-expanded chickpea-sorghum snacks would be affected by the content and degree of unsaturation of fat in the chickpea-sorghum blend, as well as the antioxidant capacity of the blend;
- The antioxidant capacity and total phenolics content of direct-expanded chickpea-sorghum snacks would be affected by the conditions employed for extrusion-expansion;
- The protein quality of direct-expanded chickpea-sorghum snacks would be affected by the chickpea:sorghum ratio in the blend and by the conditions employed for extrusion-expansion.

1.2.3 Objectives

Objective 1: To examine the ability to prepare direct-expanded snacks from a whole grain chickpea and whole grain sorghum blend and determine the maximal expansion point.

Objective 2: To investigate the oxidative stability, and undertake descriptive sensory analysis of, direct-expanded chickpea-sorghum snacks during accelerated (55^0C) and room temperature (25^0C) storage.

Objective 3: To determine correlations between chemical markers of oxidation and sensory data during accelerated storage and determine the shelf-life of chickpea-sorghum snacks.

Objective 4: To investigate the effect of extrusion (raw vs. extruded), extrusion conditions (barrel temperature and moisture content), extraction solvent and chickpea:sorghum blend ratio on the antioxidant capacity and total phenolics content of direct-expanded chickpea-sorghum snacks.

Objective 5: To investigate the effect of extrusion conditions and chickpea:sorghum blend ratio on *in vitro* protein digestibility (IVPD), available lysine content and *in vitro* protein digestibility corrected amino acid score (IVPDCAAS) of direct-expanded chickpea-sorghum snacks.

1.3 Organization of the Thesis

The thesis is divided into seven chapters. Chapter 1 presents the background and justification for the study. It provides the overarching purpose of the study as well as its hypotheses and objectives. It also explains the significance of the study. Chapter 2 presents a review of relevant literature. Chapters 3, 4 and 5 are separate studies/manuscripts which together provide information

on the oxidative- and shelf-stability and protein quality of direct-expanded chickpea-sorghum snacks. Chapter 3 is on the oxidative stability and shelf-stability of direct-expanded chickpea-sorghum snacks. Chapter 4 is on the antioxidant capacity and total phenolics content of direct-expanded snacks. Chapter 5 is on the effect of extrusion on *in vitro* protein digestibility (IVPD), *in vitro* protein digestibility corrected amino acid score (IVPDCAAS) and available lysine content of direct-expanded chickpea-sorghum snacks. Chapter 6 is a general discussion that links the findings of Chapters 3, 4 and 5. It also includes strengths and limitations of the study. Chapter 7 states the conclusions of the study and suggests future research directions. References and appendices comprise the final parts of the dissertation.

Chapter 2
Literature Review

This chapter provides a thorough review. It begins with an overview of malnutrition problems and their magnitude at the global and sub-Saharan Africa levels and emphasizes the importance of pulse-cereal blend products that are nutrient dense and shelf-stable to ameliorate the problems. It then provides an overview of chickpea and sorghum production and chemical composition, lipid oxidation and its effect on sensory quality and other nutrients, mechanisms of antioxidants in preventing lipid oxidation, protein quality and available lysine content. It then goes to outline extrusion and extrusion-expansion and the impact they have on lipid oxidation, antioxidants, protein quality and available lysine content. Towards the end, the chapter provides an overview of studies done related to direct-expanded chickpea-cereal blends.

2.1 Malnutrition

Malnutrition refers to conditions that result from an imbalanced intake of nutrients and/or energy. Under intake of nutrients results in protein-energy malnutrition expressed through stunting (low height for age), wasting (low weight for height), and underweight (low weight for age); it also results in micronutrient (vitamins and minerals) deficiencies (Ahmed *et al*., 2020). Excess intake on the other hand, results in overweight, obesity, and non-communicable diseases like heart disease, stroke and diabetes (Haggblade *et al*., 2016).

Malnutrition continues to be a global problem (Peng *et al*., 2020). Worldwide, 462 million adults are underweight, and 1.9 billion adults are obese or overweight (Menon & Penalvo, 2020). Among children, 144 million and 47 million under-fives are suffering from stunting and wasting, respectively, while 38 million under-fives are suffering from overweight (UNICEF *et al*., 2020). More than two billion people globally are affected with a deficiency of the micronutrients iron and zinc (Bouis & Saltzman, 2017; Vasconcelos, Gruissem, & Bhullar, 2017), women and children taking the largest share (Anonymous, 2020).

Africa, especially sub-Saharan Africa, hosts the largest number of malnutrition problems. About 57 million stunted, 11 million wasted and 5.2 million obese under-five children exist in the sub-Saharan Africa region (Anonymous, 2020). The prevalence of iron deficiency is high (9-18%) in the region, especially in women. The prevalence of zinc deficiency ranges between 15% and 50% (Conti *et al*., 2019). The prevalence of overweight in adults also is increasing, especially in girls and women (Onyango *et al*., 2019).

The reasons for malnutrition problems in sub-Saharan Africa are different. Most of the population in sub-Sahara Africa depends on cereals and root crops. The diet is low in quality and diet diversification is very low. People mostly use refined flours rather than whole grains (Temba *et al*., 2016). On the other hand, due to urbanization and economic progress, dietary changes to unregulated processed foods and sugary drinks has been observed, which is contributing to the emerging problems of overweight and obesity in sub-Saharan Africa (Haggblade *et al*., 2016). Using pulse-cereal blends to produce different products that are nutrient dense and shelf-stable plays a critical role in being part of the solution to the problem (Temba *et al*., 2016).

2.2 Production of Chickpea and Sorghum

Chickpea (*Cicer arietinum* L.) is a plant derived from the Fabaceae family (Kumar & Pandey, 2013). Chickpea is grown mainly in temperate and semi-arid regions such as Asia, Europe, Australia and North America (Rachwal-Rosiak, Nebesny, & Budryn, 2015). Chickpea was the second most widely grown pulse globally in 2018, with a total production of 17 million tonnes. The major chickpea producing countries are India, Australia, Turkey, Myanmar and Ethiopia from 2015 until 2018. In Africa, chickpea is cultivated in Ethiopia, Sudan, Tanzania, Uganda, Malawi, Kenya, Zimbabwe, Niger and Togo (FAO, 2019). There is a growing international demand for chickpea (Merga & Haji, 2019). Two of the reasons for this are its nutritional value and high potential for use as a functional ingredient for the food industry (Deshpande & Poshadri, 2011; Jukanti, Gaur, Gowda, & Chibbar, 2012; Xing *et al*., 2020). Chickpea grains can be categorized as Kabuli or Desi based on type (Gupta *et al*., 2017). Desi chickpea grains are small, dark and have a ridged surface. The Desi type is grown mainly on semi-arid land. The Kabuli type is slightly larger than Desi, has a thin, bright seed coat, and is cultivated in temperate climates (Rachwal-Rosiak *et al*., 2015).

Sorghum (*Sorghum bicolor* L.) is a cereal of the family Poaceae and originated in Africa (Chavez *et al*., 2018). Sorghum grows in a variety of eco-agricultural areas, including regions that are dry and arid or that undergo severe temperature fluctuations (Henley, Taylor, & Obukosia, 2010; Stamenkovic *et al*., 2020). Sorghum is the fifth most important crop, following maize, rice, wheat and barley, with global production of 59 million tonnes. The major sorghum producing countries are USA, Nigeria, Mexico, Ethiopia and Sudan from 2015 until 2018. USA is the leading producer with about 16% of global production. Sorghum is produced in most sub-Saharan African countries (FAO, 2019). Sorghum is used for human feeding in Africa, Asia and other semi-arid regions of the world (Chavez *et al*., 2018; Simnadis, Tapsell, & Beck, 2016; Taylor, Schober, & Bean, 2006). In contrast, in the United States, South America and Australia, sorghum is developed and cultivated primarily for animal feeding (Chavez *et al*., 2018; Taleon, Dykeys, Rooney, & Rooney, 2012). However, sorghum consumption as a human food source is slowly but steadily growing in western cultures due to the need to replace wheat as a gluten-free alternative (Paiva *et al*., 2017; Taylor *et al*., 2006).

2.3 Composition of Chickpea and Sorghum

2.3.1 Protein

Chickpea is an important source of protein for human consumption (Felix, Cermeno, Romero, & FitzGerald, 2019). Chickpea has been reported to contain 19-27% protein on a dry weight basis, being higher in Kabuli cultivars than in desi (Hall, Hillen, & Robinson, 2017; Kulwal & Mhase, 2017). The proteins found in chickpea belong to globulin (41-60%), albumin (8-16%), prolamine (1-24%) and glutelin (3-18%) (Chang, Alli, Konishi, & Ziomek, 2011; Chang, Alli, Molina, Konishi & Boye, 2012; Hall *et al*., 2017; Rachwal-Rosiak *et al*., 2015). The most abundant amino acids in chickpea seed are glutamic acid (14-20 g/100 g protein), aspartic acid (10-12 g/100 g protein), arginine (7-8 g/100 g protein), leucine (6-7 g/100 g protein), and lysine (6 g/100 g protein. Of the essential amino acids, leucine is the most prevalent, followed by lysine and phenylalanine. Sulfur-containing amino acids cysteine (0-1 g/100 g protein) and methionine (1-2 g/100 g protein) are the limiting amino acids in chickpea seed (Wang *et al*., 2010; Xu *et al*., 2016).

Sorghum contains 9-17 g/100 g protein on dry matter basis (Barikmo, Ouattara, & Oshaug, 2004; Mokrane *et al*., 2010; Palavecino, Penci, Calderon-Dominguez, & Ribotta, 2016; Ragaee, Abdel-Aal, & Noaman, 2006), mostly located in the endosperm (80%) and germ (15%)

(Kulamarva, Sosle, & Raghavan, 2009; Labuschagne, 2018). The most abundant proteins in sorghum are the prolamins, named kafirins, which are located in the protein bodies of the endosperm. Kafirins represent about 48–70% of the total proteins in the whole kernel (Espinosa-Ramirez and Serna-Saldivar, 2016). The remaining proteins of sorghum belong to albumins, globulins and glutelins (Labuschagne, 2018; Martino *et al*., 2012). The most abundant amino acids in sorghum are glutamic acid (16-17 g/100 g protein), leucine (16-17 g/100 g protein) and alanine (7-9 g/100 g protein). Of the essential amino acids, leucine is the most prevalent followed by phenylalanine, threonine and valine. Sorghum protein is deficient in the essential amino acid lysine (Mokrane *et al*., 2010). Protein digestibility and amino acid availability are lower in sorghum compared to other cereals (Kulamarva *et al*., 2009). The main reasons for the poor digestibility of sorghum protein are the ability of kafirin proteins to form oligo or polymers of high molecular weight that are linked by disulfide (S–S) bonds and resistant to protease digestion (Duodu *et al*., 2002; Duressa, Weerasoriya, Bean, Tilley, & Tesso, 2018; Nunes, Correia, Barros, & Delgadillo, 2005), interactions of kafirins with non-protein components such as polyphenols and polysaccharides, and non-kafirin proteins, and the structural arrangement of the grain (Cardoso, Pinheiro, Martino, & Santana, 2017; De Mesa-Stonestreet, Alavi, & Bean, 2010).

2.3.2 Lipid

Chickpea has been reported to contain 3-10% oil (Gul *et al*., 2008; Jukanti *et al*., 2012; Shah, Iqbal, Asi, & Atta, 2013; Xu *et al*., 2019). In contrast to other pulses and most cereals, chickpea has a relatively high fat content (Asif, Rooney, Ali, & Riaz, 2013; Jukanti *et al*., 2012; Zia-ul-Haq, Ahmad, Ahmad, Iqbal, & Khawar, 2009). The oil content of chickpea is higher than that of other pulses such as lentil (1.1%), red kidney bean (1.1%), field pea (1.3%), brown bean (1.4%) and turtle bean (1.6%) (Wang & Daun, 2004). Chickpea oil contains 66% polyunsaturated fatty acids, 19% monounsaturated fatty acids and 15% saturated fatty acids (Gul *et al*., 2008; Jukanti *et al*., 2012; Zia-ul-Haq, Ahmad, Iqbal, Ahmad, & Ali, 2007). The fatty acid profile of chickpea oil has been reported as palmitic acid (8-12%), palmitoleic acid (1%), stearic acid (1-5%), oleic acid (24-43%), linoleic acid (42-57%) and linolenic acid (2-4%) (Dandachy, Mawlawi, & Obeid, 2019; Jukanti *et al*., 2012). Linoleic acid is the dominant fatty acid in chickpea, followed by oleic acid and palmitic acid (Zia-ul-Haq *et al*., 2007). Linoleic acid and linolenic acid are

unsaturated fatty acids that are nutritionally essential. Chickpea oil also contains nutritionally vital tocopherols, sterols, and tocotrienols (Rachwal-Rosiak *et al*., 2015; Zia-ul-Haq *et al*., 2009).

Sorghum contains 2-7% lipid on a dry weight basis (Chhikara *et al*., 2019; Mkandawire, Weier, Weller, Jackson, & Rose, 2015; Osman, Abd El Gelil, El-Noamany, & Dawood, 2000). The germ contributes about 80% of the total kernel lipid (Kulamarva *et al*., 2009). The fatty acid profile of sorghum oil has been reported as palmitic acid (12-15%), palmitoleic acid (1%), stearic acid (1-3%), oleic acid (34-37%), linoleic acid (42-43%) and linolenic acid (1-2%). Linoleic, oleic and palmitic acids are the most abundant fatty acids in sorghum oil (Afify *et al*., 2012; Osman *et al*., 2000; Zhang *et al*., 2019). In most varieties of sorghum, the levels of polyunsaturated fatty acids are higher than those of monounsaturated fatty acids (Mehmood, Orhan, Ahsan, Aslan, & Gulfraz, 2008).

2.3.3 Carbohydrate

Chickpea has been reported to contain 41-66% available carbohydrate on a dry weight basis (Asif *et al*., 2013; Berrios, Morales, Camara, & Sanchez-Mata, 2010; Marure, Nunez-Santiago, Agama-Acevedo, & Bello-Perez, 2019; Rachwal-Rosiak *et al*., 2015; Rincon, Martinez, & Ibanez, 1998). Chickpea contains 33-54 g/100 g starch (Aguilera *et al*., 2009; Alajaji & El-Adawy, 2006; Morales-Medina, Mar Munio, Guadix, & Guadix, 2014). Starch is the major carbohydrate fraction in chickpea, representing 41-84% of the total carbohydrates (Jukanti *et al*., 2012; Marure *et al*., 2019; Rincon *et al*., 1998). Chickpea starch has been reported to contain a higher amount of amylose (30-40%) as wheat starch. Amylose has a higher degree of polymerization than amylopectin, making the starch in chickpea more resistant to digestion in the small intestine, resulting in a lower bioavailability of glucose and lower glycemic index (Pittaway, Ahuja, Robertson, & Ball, 2007). Furthermore, a higher level of amylose correlates with a higher resistant starch content (Rebello, Greenway, & Finley, 2014). Chickpea has been reported to contain resistant starch of about 35% of total starch (Jukanti *et al*., 2012). Chickpea contains monosaccharides (ribose, glucose, galactose and fructose), disaccharides (sucrose and maltose) and oligosaccharides (stachyose, ciceritol, raffinose and verbascose) (Aguilera, *et al*. 2009; Berrios *et al*., 2010; Jukanti *et al*., 2012; Rachwal-Rosiak *et al*., 2015). Ciceritol is a trisaccharide which has been reported to be the most abundant sugar in chickpea (Berrios *et al*., 2010). Non-digestible

oligosaccharides like raffinose are useful for the prevention of colon cancer and obesity (Wilson *et al*., 2020).

Sorghum has been reported to contain 64-80% carbohydrate on a dry weight basis (Barikmo *et al*., 2004; Campelo *et al*., 2020). Starch is the principal storage form of carbohydrate and the main chemical component in sorghum and accounted for 55-76% of the kernel, and was primarily located in the endosperm (Kulamarva *et al*., 2009; Taylor & Emmambux, 2010). Normal sorghum starch has 24-33% amylose and 67-76% amylopectin (Beta, Obilana, & corke, 2001). Waxy sorghum starch contains almost exclusively amylopectin. Sorghum grain has been reported to have the lowest raw starch digestibility among cereals due to restricted accessibility to starch by endosperm proteins (Barros, Awika, & Rooney, 2012). Thus, the starches and sugars in sorghum are released more slowly than in other cereals (Mkandawire *et al*., 2013; Sang, Bean, Seib, Pedersen, & Shi, 2008). The digestibility of sorghum starch decreases further after cooking due to the formation of disulfide bonds during cooking, which leads to toughening at the surface and interior of protein bodies. Starch digestibility was reported to be higher in low-amylose waxy sorghum than in normal sorghum (Kulamarva *et al*., 2009).

2.3.4 Dietary Fibre

Dietary fibre means carbohydrate polymers that are not hydrolyzed by the endogenous enzymes in the small intestine of humans and includes either naturally occurring edible carbohydrates or modified or synthetic carbohydrate polymers which have been shown to have a physiological effect (Jones, 2014). Considering the above definition, the major dietary fibre components are non-starch polysaccharides, fructans (inulin and fructo-oligosaccharides), resistant starch and lignin (Baye, Guyot, Icard-Verniere, Rochette, & Mouquet-Rivier, 2015). The total dietary fibre content in chickpea was reported to be in the range of 18-22 g/100 g of raw chickpea seed (Jukanti *et al*., 2012).

Sorghum contains 10-20% dietary fibre on a dry weight basis (Campelo *et al*., 2020; Mkandawire *et al*., 2015; Ragaee *et al*., 2006). The outer part of the sorghum kernel accounts for 20% of the sorghum grain weight and 70% of grain non-starch polysaccharides. The non-starch polysaccharides of sorghum consist of cellulose and non-cellulosic polysaccharides found mostly in the pericarp. Thus, the dietary fibre content of sorghum grain will depend on the degree of pericarp removal (Taylor & Emmambux, 2010). Sorghum bran is low in ash and protein and rich

in fibre. The low starch digestibility of sorghum has also been attributed to a high content of dietary fibre (Kulamarva *et al*., 2009).

2.3.5 Minerals and Vitamins

Chickpea has been reported to contain 40-230 mg/100 g calcium, 220-1580 mg/100 g potassium, 230-930 mg/100 g phosphorous, 10-213 mg/100 g magnesium, 3-13 mg/100 g iron and 2-6 mg/100 g zinc on a dry weight basis (Kaur, Grewal, Gill, & Singh, 2019; Rachwal-Rosiak *et al*., 2015). The recommended daily dietary requirements of calcium (1000 mg/day), iron (9-27 mg/day in males and 20-59 mg/day in females) and zinc (4-14 mg/day in males and 3-10 mg/day in females) (FAO & WHO, 2004) indicate that about 100 g of chickpea on average can fulfill 14%, 20-44% and 44-62% of the daily dietary requirements for calcium, zinc and iron, respectively. The calcium content of chickpea is greater than that of lentil (50-110 mg/ 100g) or navy bean (220 mg/100 g), but less than that of turtle bean (243 mg/ 100g). The iron content of chickpea is greater than that of field pea (4-9 mg/100 g) but less than that of lentil (4-342 mg/ 100g) (Wang & Duan, 2004). Chickpea is high in folate (351-589 µg/100 g) compared to lentil (136-182 µg/100 g) and pea (23-30 µg/100 g) (Jha *et al*., 2015). Chickpea contains a higher amount of α-tocopherol (8-14 mg/100 g), γ-tocopherol (1-3 mg/100 g) and δ-tocopherol (1 mg/100 g) compared to broad bean, lentil and soybean (Boschin & Arnoldi, 2011).

Sorghum contains minerals such as potassium (183-405 mg/100 g), phosphorus (218-278 mg/100 g), magnesium (103-195 mg/ 100 g), zinc (1-3 mg/100 g), iron (1-11 mg/100 g) and calcium (10-28 mg/100 g) on a dry weight basis, but the mineral composition is highly variable (Kruger, Oelofse, & Taylor, 2014; Martino *et al*., 2012). The germ fraction of sorghum contains 68% of the total mineral matter of the whole kernel (Kulamarva *et al*., 2009). The bran of sorghum also contains a substantial amount of minerals (Mahgoub & Elhag, 1998). However, the bran of sorghum contains condensed tannins, depending on the cultivar, which inhibits the bioavailability of essential divalent minerals (Pasha, Ahmed, Siddique, & Iqbal, 2020) such as iron (Fe), zinc (Zn), calcium (Ca) and magnesium (Mg). Sorghum is a rich source of B-complex vitamins. Sorghum endosperm contributes 50 to 75% of the kernel's B-complex vitamins. The sorghum germ fraction also contributes to the B-complex vitamins. (Kulamarva *et al*., 2009). Sorghum contains B-vitamins such as thiamin (0.12-0.76 mg/100 g), riboflavin (0.07-0.24 mg/100 g), niacin (0.01-4.3 mg/100 g) and pyridoxine (0.17 mg/100 g) (Barikmo, Ouattara, & Oshaug, 2007). Fat-

soluble vitamins, namely D, E, and K, also have been found in sorghum grain. Certain yellow endosperm varieties of sorghum contain small amounts of β-carotene, a precursor of vitamin A (Kulamarva *et al*., 2009).

2.3.6 Antioxidants

Chickpea contains considerable amounts of flavonoids, phenolic acids and other phenolic compounds which have antioxidant properties (Rachwal-Rosiak *et al*., 2015). It also contains α-tocopherol, peptides, β-carotene, lutein, zeaxanthin, β-cryptoxanthin and α-carotene (Jukanti *et al*., 2012). The flavonoids present in chickpea includes kaempferol, myricetin, daidzein, genistein, cyanidin, petunidin, delphinidin and malvidin. The phenolics acids found in chickpea includes gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, salicylic acid, caffeic acid, chlorogenic acid, ferulic acid, sinapic acid, p-coumaric acid, isoferulic acid, and cinnamic acid and hydroxycinnamic acid (Aguilera *et al*., 2011; Sreerama *et al*., 2010). Chickpea seeds having colorful coatings exhibit higher levels of antioxidant activity (Segev *et al*., 2010). The total phenolics content of chickpea ranges from 1-19 mg gallic acid equivalents/g (Rani & Khabiruddin, 2016), and the content of anthocyanin amounts to 0.015-0.54 mg/g (Han & Baik, 2008; Segev *et al*., 2010; Xu & Chang, 2007; Zia-ul-Haq *et al*., 2008). Larger proportions of flavonoids are present in the seed coat of chickpea, while the other phenolic compounds are present in the cotyledon and embryo (Sreerama *et al*., 2010).

Sorghum contains antioxidants like phenols (1-38 mg gallic acid equivalents/g), carotenoids such as lutein (3.5 µg/100 g) and zeaxanthin (14 µg/100 g) and α-tocopherol (36-520 µg /100 g). Almost all classes of phenolics are present in all varieties of sorghum, but the main classes are phenolic acids, flavonoids and tannins (Dykes, Hoffmann, Portillo-Rodriguez, Rooney, & Rooney, 2014; Gaytan-Martinez *et al*., 2017). The sorghum grain contains 135.5-479.40 mg/g of phenolic acids (Chiremba, Taylor, Rooney, & Beta, 2012), categorized as hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives (Chhikara *et al*., 2019). Sorghum also contains anthocyanins. The most common anthocyanins in sorghums are 3-deoxyanthocyanidins, which include orange luteolinidin and yellow apigeninidin. Sorghum contains gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, chlorogenic acid, ferullic acid and M-coumaric acid (Cardoso *et al*., 2014; Pasha, Riaz, Saeed, & Randhawa, 2015).

2.4 Lipid Oxidative Changes and Protein Quality

Food processing is any deliberate change to food that result in a more useful, shelf-stable and palatable form. Some of the common industrial processes used in food manufacturing include milling, cooling/freezing, smoking, heating, canning, fermentation, drying extrusion cooking (Augustin *et al*., 2016). Despite the positive effects of changing food to a more useful, stable, and palatable form, food processing also has negative effects. Formation of acrylamide (Claus, Carle, & Schieber, 2008), degradation of nutrients such as essential amino acids like lysine (Belitz, Grosch, & Schieberle, 2009), formation of *trans* fats, (Bhardwaj *et al*., 2016), oxidation of lipids (Chudy, Pikul, & Rudzinska, 2015) and loss of antioxidants (Delgado-Licon *et al*., 2009; Shih, Kuo, & Chiang, 2009; Wani & Kumar, 2016a) are some of the negative effects. Storage conditions also are factors that affect food properties. Storage stability (shelf-life) of foods is a measure of how long food products retain optimal quality after production. Lipid oxidation also is a problem during storage (Ashfaq, Butt, Bilal, Tehseen, & Suleria, 2020; Honi, Mukisa, & Mongi, 2018).

2.4.1 Lipid Oxidation

Lipids consist of a heterogeneous group of nonpolar materials, including glycerides, phospholipids, sterols and waxes. Although many types of lipids occur in foods, triglycerides are the most common (Singh, Gamlath, & Wakeling, 2007). Lipids in food provide organoleptic properties including flavour, aroma, colour, texture and mouthfeel that make particular foods desirable (Budilarto & Kamal-Eldin, 2015; Shahidi & Zhong, 2010). Food lipids also play an important nutritional role by providing energy, fat-soluble vitamins and essential fatty acids. In addition, lipids promote health by providing fatty acids like omega-3 fatty acids (Tian *et al*., 2017). However, when oxidized, lipids lead to primary and secondary products that adversely affect these functions. These primary and secondary products change the colour, aroma, flavour, texture (Baron & Andersen, 2002; Waraho, McClements, & Decker, 2011) and nutritive value (Awada *et al*., 2012; Serini, Fasano, Piccioni, Cittadini, & Calviello, 2011; Turner, McLean, & Silvers, 2006).

2.4.1.1 Mechanism of Lipid Oxidation

Lipid oxidation has three phases, initiation, chain propagation and termination (Barden & Decker, 2016; Jackson & Penumetcha, 2019; Kamal-Eldin & Yanishlieva, 2002; Shahidi & Zhong, 2010). In the initiation phase [Equation (2.1)], hydrogen is abstracted from a fatty acid, thereby

generating an alkyl radical (R•). Normally, abstraction occurs at a methylene carbon of a polyunsaturated fatty acid, where the covalent bond strength between hydrogen and its methylene carbon is reduced or weakened by the adjacent electron-rich double bonds in unsaturated fatty acids (Barden & Decker, 2016; Gorji, Smyth, Sharma, & Fitzgerald, 2016; Johnson & Decker, 2015; Zamora & Hidalgo, 2016). Polyunsaturated fatty acids are more susceptible to hydrogen abstraction due to the presence of multiple bonds. The more unsaturated the lipid, the more susceptible it is to oxidation. C*is*-fatty acids often oxidize more readily than do *trans*-forms (Sargis & Subbaiah, 2003). High processing temperature, light, enzymes, metals, metalloproteins and irradiation encourage lipid oxidation at the initiation phase (Senanayake, 2013).

In the propagation step [Equations (2.2) and (2.3)], the alkyl radical undergoes reaction with atmospheric oxygen to form a peroxyl radical (ROO•). This peroxyl radical promotes abstraction of hydrogen from another unsaturated fatty acid and as a result forming another alkyl radical and lipid hydroperoxide (ROOH) (Medina-Meza, Barnaba, & Barbosa-Canovas, 2014). The lipid hydroperoxides (primary products) formed do not cause rancidity. However, lipid hydroperoxides can be broken into aldehydes, ketones, acids, esters and alcohols through β-scission reactions (Frankel, 1991; Porter, 2013; Pratt, Tallman, & Porter, 2011) activated by light, heat or transition metals (Barden & Decker, 2016). These secondary oxidation products (aldehydes, ketones, acids, esters, and alcohols) cause the off-flavours in food (Gorji *et al*., 2016). In the termination phase [Equations (2.4) and (2.5)], two radicals react to form one, non-radical molecule, such as fatty acid dimers, trimers and oligomers (Barden & Decker, 2016; Johnson & Decker, 2015). Primary oxidation and secondary oxidation products are the basis for measuring the oxidative stability of food (Shahidi & Zhong, 2005). According to Johnson and Decker (2015), the reactions involved in each phase of lipid oxidation are summarized as:

$$Initiation: \quad RH \rightarrow R\bullet + H\bullet \tag{2.1}$$

$$Propagation: \quad R\bullet + 3O_2 \rightarrow ROO\bullet \tag{2.2}$$

$$ROO\bullet + RH \rightarrow ROOH + R\bullet \tag{2.3}$$

$$Termination: \quad ROO\bullet + R\bullet \rightarrow ROOR \tag{2.4}$$

$$R\bullet + R\bullet \rightarrow RR \tag{2.5}$$

2.4.1.2 Factors Influencing Lipid Oxidation

Various factors influence the oxidation of lipids in food including the lipid composition of the food, environmental or processing temperature, oxygen, light, metals and the water activity of the food (Barden & Decker, 2016; Jackson & Penumetcha, 2019). Lipids that possess higher numbers of unsaturated double bonds are more susceptible to oxidation. Besides, other components of food either promote or inhibit lipid oxidation. For instance, transition metals like iron and copper initiate and accelerate lipid oxidation (Baron & Andersen, 2002; Gorji *et al*., 2016). The electron transfer reaction between iron (Fe^{2+}) and oxygen (O_2) or iron (Fe^{2+}) and lipids (RH) results in reactive oxygen species (• O_2 –) and alkyl radicals (R•) which initiate oxidation. Iron also accelerates oxidation by reacting with lipid hydroperoxides (ROOH) to give reactive lipid radicals (RO•) (McClements & Decker, 2000).

Temperature either increases or decreases lipid oxidation rates depending on the system. In most foods, a 10˚C increase in temperature corresponds to approximately a doubling in the rate of oxidation (Matthaeus, 2010). Higher temperatures can increase lipid oxidation rates by promoting hydroperoxide breakdown in a process that generates free radicals. In general, high temperatures will increase free radical formation. However, it depends on time. Heating for a longer time at a lower temperature caused a higher increase in secondary lipid oxidation products compared to heating at a higher temperature for a shorter time (Broncano, Petron, Parra, & Timon, 2009; Santos, Cruz, Cunha, & Casal, 2013). In unused, heated frying oils, an increment in temperature for a short time decreased the development of oxidative rancidity. Light causes oxidation of lipids through photolytic auto-oxidation or photosensitized oxidation (Mungure *et al*., 2020).

Mitigation strategies include low-temperature storage, encapsulation, controlled atmosphere exposure, vacuum-packaging and using antioxidants. However, polyunsaturated fatty acids are still subject to a risk of unpredictable development of oxidation at some stage (Berton-Carabin, Ropers, & Genot, 2014; Hu, Huyan, Ding, Dong, & Yu, 2020).

2.4.1.3 Sensory Properties and Oxidative Changes

Considering pronounced the flavour changes and resulting threats that lipid oxidation poses to the quality of food products, several chemical methods have been established to assess the progress of lipid oxidation in foods to indicate product quality and shelf-life (Warner & Eskin,

1995). These methods tend to quantify primary species, peroxides, and secondary species, aldehydes, ketones, acids, esters, and alcohols generated during different phases of lipid oxidation. However, analyzing chemical parameters alone will not determine the shelf-life of samples (Franklin & Mitchell, 2019). To enhance the information provided by chemical oxidation analyses and explain sample shelf-life in a better manner, different studies used sensory testing in combination with chemical analysis (Franklin *et al*., 2017; Mexis & Kontominas, 2010). Sensory testing is an important method that can detect and confirm the changes that occur and thus what the shelf-life will be (Lawless & Heymann, 2010). Sensory characteristics undeniably influence food consumption worldwide (Urala & Lahteenmaki, 2004).

Aldehydes, alcohols, alkanes and ketones formed as the result of oxidation of lipid lend the characteristic aroma of rancidity to food products (Frankel, 1980). Excessive accumulation of these compounds alters the original natural flavour of the food product. These changes are considered by many to be negative flavour changes and are indicated by lower hedonic ratings of aged samples in comparison to fresh samples in sensory studies of food products undergoing lipid oxidation (Mexis & Kontominas, 2010). Studies indicated that descriptive attributes involving roasted and nutty, as well as consumer liking, were highest in fresh, roasted samples, while flavours typically associated with oxidative rancidity such as cardboard, painty, soapy and sour were increased during storage (Franklin *et al*., 2018; Grosso, Asensio, Grosso, & Nepote, 2017; Olmedo, Asensio, Nepote, Mestrallet, & Grosso, 2009; Whitson, Miracle, Bastian, & Drake, 2011). Jauregui, Riveros, Nepote, Grosso, and Gayol (2012) reported the decrement of intensity of sensory attributes such as roasted and crunchiness during the storage of food. Sohaib, Butt, Anjum, Khan, and Shahid (2016) indicated that sensory acceptability decreases with the progression of storage. Off-odour development during storage has been reported to be retarded by using antioxidants (Branciari *et al*., 2015; Larrauri *et al*., 2016; Siripatrawan & Noipha, 2012; Yesil-celiktas, Isleten, Karagul-Yuceer, Bedir, & Vardar-Sukan, 2009), nitrogen flushing (Lloyd, Hess, & Drake, 2009), and surface coating (Riveros, Mestrallet, Quiroga, Nepote, & Grosso, 2013). Studies also indicated that sensory analysis and chemical analysis are significantly correlated. For example, Hedegaard *et al*. (2006) reported the existence of a significant correlation between the sensory descriptors for oxidation such as cardboard, and metallic taste, and specific chemical markers for oxidation such as hexanal.

2.4.1.4 Nutritional Changes During Lipid Oxidation

Lipid oxidation reduces the essential fatty acid and vitamin content of foods (Dominguez *et al*., 2019). Furthermore, free radicals from lipid oxidation attack proteins and result in the formation of large amounts of protein radicals or oxidized proteins which further lead to the formation of protein aggregates and disulfide crosslinking (Feng *et al*., 2020; Huang, Yu, Hua, & Qiu, 2006; Schaich, 2008). Not only free radicals but other lipid oxidation products also react with proteins (Hematyar, Masilko, & Samples, 2018; Lund, Heinonen, Baron, & Estevez, 2011). Lipid oxidation products such as aldehydes and ketones react with amines, amino acids, and proteins result in browning during storage (Zamora & Hidalgo, 2016). Carotenoids and sterols also are lost during lipid oxidation (Bartosz & Koakowska, 2010).

2.4.2 Antioxidants

Antioxidants are compounds that prevent or delay the onset of oxidation (Embuscado, 2015; Meneses, Martins, Teixeira, & Mussatto, 2013; Shahidi & Zhong, 2010). Phenolic compounds, ascorbic acid, carotenoids, some protein-based compounds, Maillard reaction products, phospholipids, sterols, tocotrienols and tocopherols are natural antioxidants found in foods (Choe & Min, 2009; Masisi, Beta, & Moghadasian, 2016). Simple phenols such as phenolic acids contain only one phenol functionality, whereas polyphenols contain more phenol groups. Phenolic acids have two different carbon frameworks giving rise to hydroxycinnamic derivatives such as p-coumaric, caffeic, ferulic, and sinapic acids, or hydroxybenzoic structures (Gan *et al*., 2019). Flavonoids are polyphenol substances whose basic structural feature is the flavone. Flavonoids are plant pigments and are abundant in nature (Kumar & Pandey, 2013; Majer, Neugart, Krumbein, Schreiner, & Hideg, 2014). Flavonoids can be isoflavones, flavanols, flavonols, flavanones, anthocyanins, and proanthocyanidins, (Spencer, El Mohsen, Minihane, & Mathers, 2008). The main carotenoids present in the daily diet are α- and β-carotene, lycopene, and the hydroxy carotenoids (xanthophylls-zeaxanthin and lutein) (Oroian & Escriche, 2015).

2.4.2.1 Mechanisms of Antioxidants in the Prevention of Oxidation

Antioxidants slow down the oxidation rates of foods by a combination of scavenging free radicals, chelating pro-oxidative metals, quenching singlet oxygen, and inactivating photosensitizers. Antioxidants scavenge free radicals of foods by donating hydrogen and thereby

producing relatively stable antioxidant radicals (Figure 2.2). Resonance of electrons in the phenolic ring make antioxidant radicals to be more stable than food radicals (Choe & Min, 2009). Examples of antioxidants to scavenge free radicals are phenolic compounds [tocopherols, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), lignans, flavonoids, and phenolic acids], ubiquinone (coenzyme Q), carotenoids, and ascorbic acids. The effectiveness of antioxidants to scavenge free radicals of foods depends on the bond dissociation energy between phenolic oxygen and phenolic hydrogen, pH relative to the acid dissociation constant, and reduction potential and delocalization of the antioxidant radicals (Cao *et al*., 2007; Choe & Min, 2006; Litwinienko & Ingold, 2003). The lower the bond dissociation energy, the easier the dissociation of the phenolic O-H bond and the reaction with free radicals. There also are cases where the antioxidant donates a single electron to the radical so that the radical is stabilized (Figure 2.3). Anion R^- is an energetically stable intermediate with an even number of electrons, and the activity of the cation-radical formed by the reaction is reduced due to the delocalization of electrons. In cases like this, the ionization potential is the most significant parameter. The lower the ionization potential, the easier the electron abstraction and the reaction with free radicals (Leopoldini, Russo, & Toscano, 2011).

Chelating prooxidative metals is another mechanism by which antioxidants prevent lipid oxidation (Figure 2.4). Metals reduce the activation energy of oxidation, especially in the initiation step, to accelerate oil oxidation (Jadhav *et al*., 1995). Metals accelerate radical formation by abstracting hydrogen and decomposition of hydrogen peroxide (Choe & Min, 2006). Metal chelators decrease oxidation by preventing metal redox cycling, forming insoluble metal complexes, or providing steric hindrance between metals and food components or their oxidation intermediates. Citric acid is one of the common metal chelators in foods (Graf & Eaton, 1990).

There is also a mechanism in which antioxidants prevent oxidation by acting on singlet oxygen (Figure 2.5). Singlet oxygen has high energy and reacts with lipids at a higher rate than does triplet oxygen. Tocopherols, carotenoids, phenolics, and ascorbate can quench singlet oxygen (Choe & Min, 2006; Das & Das, 2002).

Photosensitizer inactivation also is a mechanism in which antioxidants prevent lipid oxidation. Foods contain sensitizers such as chlorophylls and riboflavin (Salvador, Aranda, Gomez-Alonso, & Fregapane, 2001) which are activated by light. Photoactivated sensitizers transfer energy to triplet atmospheric oxygen to form singlet oxygen or transfer an electron to the

triplet oxygen to form a superoxide anion radical, and these reactive oxygen species react with food components to produce free radicals (Choe & Min, 2009). Carotenoids having fewer than nine conjugated double bonds prefer the inactivation of photosensitizers instead of singlet oxygen quenching; singlet oxygen quenching is preferable by carotenoids with nine or more conjugated double bonds (Viljanen, Sundberg, Ohshima, & Heinonen, 2002).

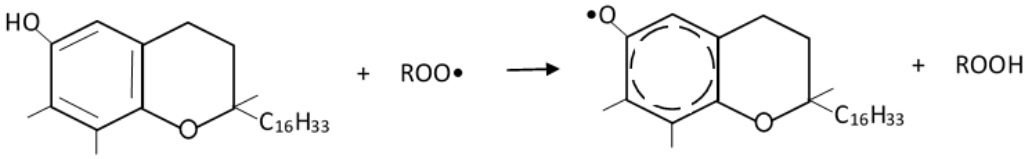

Figure 2.1 Lipid peroxyl radical scavenging activity of α-tocopherol.

(Source: Choe & Min, 2009)

Figure 2.2 Mechanism of single electron transfer to stabilize radical.

(Source: Leopoldini *et al*., 2011)

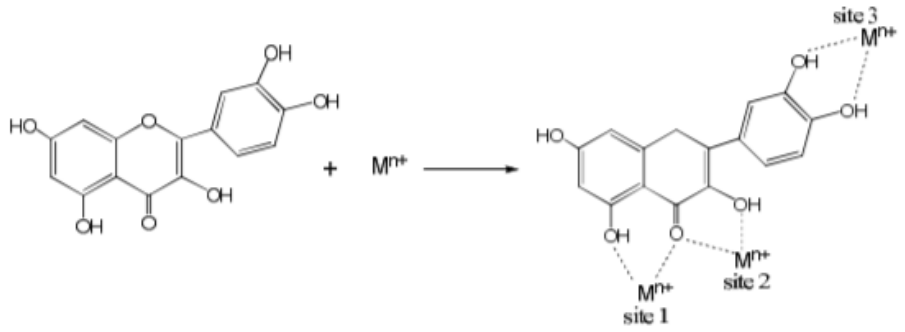

Figure 2.3 Metal chelating activity of flavonoids.

(Source: Leopoldini *et al*., 2011)

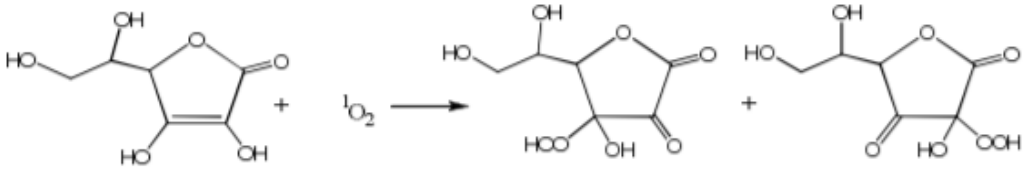

Figure 2.4 Singlet oxygen quenching activity of ascorbic acid.

(Source: Choe & Min, 2009)

2.4.3 Protein Quality

Proteins are one principal component in the diet essential for the survival of the organism (Friedman, 1996; Vagadia, Vanga, & Raghavan, 2017). Amino acids are the building blocks of proteins. Threonine, methionine, valine, leucine, isoleucine, phenylalanine, arginine, histidine, lysine, and tryptophan are indispensable amino acids (FAO, WHO, & United Nations University, 2007). On the contrary, aspartic acid, glutamic acid, glycine, proline, serine, alanine, asparagine, and glutamine are reported to be dispensable amino acids (Choi & Coloff, 2019). Protein quantity within food is not a reliable indicator of the ability of a dietary protein to meet the metabolic needs of the consumer. The quality of the protein also should be considered. The quality of dietary proteins is defined by the extent to which the constituent amino acids match the amino-acid needs of the consumer and the efficiency in which the amino acids are extracted and used by the consumer for growth and/or maintenance purposes (Marinangeli & House, 2017).

2.4.3.1 Protein Quality Measurements

Several methods including Nitrogen Balance, Net Protein Utilization (NPU), Net Protein Ratio (NPR), Protein Efficiency Ratio (PER), Biological Value (BV) and Amino Acid Score have been developed to measure the protein quality of foods (Table 2.1) (FAO & WHO, 1991; Friedman, 1996; Paddon-Jones, Coss-Bu, Morris, Phillips, & Wernerman, 2017; Rizzo & Baroni, 2018). However, they were found to have a variety of shortcomings. For example, the Protein Efficiency Ratio (PER) method was found to consider protein quality from a weight gain perspective only. Amino acids required for maintenance were not considered. Due to that Amino Acid Score has been promoted as an alternative to PER (Elango, Ball, & Pencharz, 2009; FAO & WHO, 1991; Young & Pellett, 1994). Still, the Amino Acid Score did not consider protein digestibility. Hence, considering that Protein Digestibility Corrected Amino acid Score (PDCAAS) combines amino acid score as well as protein digestibility, FAO/WHO expert consultations on protein quality evaluation have endorsed the use of PDCAAS for protein quality evaluation since 1989 (FAO *et al*., 2007). Since then, PDCAAS has been used for protein quality evaluation. However, FAO has since advocated the use of a new method Digestible Indispensable Amino Acid Score (DIAAS) in 2013 (FAO, 2013).

PDCAAS has a problem of truncating values at 1, even if the protein quality is more than 1 (Gilani, 2012). Also, bacteria in the intestine consume nitrogen and the use of true fecal protein

digestibility for PDCAAS determination resulted in overestimation. As a result, ileal protein digestibility was suggested, and that lead to DIAAS (Wolfe, Rutherfurd, Kim, & Moughan, 2016). However, the use of DIAAS was reported to be expensive considering the use of pigs to derive ileal digestibility coefficients for thousands of food items. Based on the marginal differences in protein digestibility between foods, a simplified determination of protein quality that is based on Amino Acid Scores, coupled with general digestibility coefficients that are established by *in vitro* methodologies, was proposed (Marinangeli & House, 2017). Proper selection of the correct protein quality assessment method is vital because PER, PDCAAS, and DIAAS have their benefits and detriments (Nosworthy & House, 2017).

A positive correlation was reported between true fecal digestibility (*in vivo* protein digestibility) and *in vitro* protein digestibility (done using the pH drop method) with R^2 value of 0.64, 0.67, and 0.56, suggesting that there is a relationship between these measures of digestibility. A stronger relationships was reported between the protein quality measurements *in vivo* PDCAAS and *in vitro* PDCAAS with R^2 value of 0.92, 0.97 and 0.98. This suggests that *in vitro* PDCAAS could be used as an alternate method for assaying protein quality (Nosworthy *et al*., 2018).

2.4.3.2 Protein Claims

Assignment a numerical rank to food products in terms of protein quality plays an important role in addressing human nutrition requirements, nutrition policy and regulatory affairs, and product development (Paddon-Jone *et al*., 2017). Protein claims in countries like the United States and Canada are based on protein quality, whereas in other countries protein claims are based on protein content. In the United States, the protein content within a regulated serving of food is corrected by multiplying by PDCAAS and the result will be divided by 50 g which is the daily reference value of protein. The result is called percent daily value (%DV). If a food contributes 10–19.9% or $\geq$20 % of the DV for protein, the food qualifies for a claim that the food is a "good source" or an "excellent source" of protein, respectively (Marinangeli & House, 2017).

In Canada, protein content in a reasonable daily intake of food is corrected by multiplying by the adjusted PER. If the corrected protein content is 20-39.9 or $\geq$ 40, the food qualifies for a claim that it is a "good source" or an "excellent source" of protein, respectively (Wiggins, Anderson, & House, 2019). Health Canada (2020) has recently authorized the use of PDCAAS. In most sub-Saharan African countries, there are no standards dealing with protein claims.

Table 2.1 Methods developed for measuring protein quality

Method	Description	Formula
Nitrogen Balance[1]	Protein intake requirement to attain nitrogen equilibrium or the difference between nitrogen intake and nitrogen loss with urine and faeces.	Nitrogen balance=I-F-U
Net Protein Utilization (NPU)[1, 4]	Difference between nitrogen retention in the carcass of animals fed no protein and those fed a test protein and normalized for dietary protein intake	NPU = [(I-(F-Fo)- (U-Uo))/I] * 100 = (Nitrogen retained/nitrogen intake) * 100
Protein Efficiency Ratio[2] (PER)	Weight gain of animal model divided for protein intake	PER = [(WT/MT)/ (WC/MC) PER adjusted = PER x 2.5
Net protein ratio (NPR)[1]	Weight gain of a test animal plus weight loss of a control animal per gram of protein consumed	NPR = [(WT+WL)/protein consumed]
Biological Value (BV)[1]	Nitrogen retained divided by nitrogen absorbed. It determines the effectiveness with which absorbed dietary nitrogen can be utilized	BV = [(I-(F-Fo) -(U-Uo))/I-(F-Fo)] *100 = (Nitrogen retained/nitrogen absorbed) * 100
True fecal Digestibility[1]	Difference between nitrogen intake and nitrogen loss corrected for protein-free diet loss	True digestibility (TD) = [I-(F-Fo)/I] * 100
Amino Acid Score (AAS)[2]	Determines the effectiveness with which absorbed dietary nitrogen can meet the indispensable amino acid requirement at the safe level of protein intake	AAS = (mg of amino acid in 1 g test protein)/ (mg of amino acid in requirement pattern)
Protein Digestibility corrected for amino acid score PDCAAS[2]	Amino acid score corrected for true faecal protein digestibility	PDCAAS = Least AAS* true faecal protein digestibility
Digestible Indispensable Amino Acid Score (DIAAS)[3]	Amino acid score corrected for true ileal digestibility	DIAAS % = 100 x [(mg of digestible dietary indispensable amino acid in 1 g of the dietary protein) / (mg of the same dietary indispensable amino acid in 1g of the reference protein)].

[1, 2, 3 and 4]are from FAO and WHO (1991), FAO *et al*., (2007), FAO and WHO (2013), and Marinangeli & House (2017), respectively. I=nitrogen intake of test portion, F=fecal nitrogen, Fo=fecal nitrogen on nitrogen-free diet, U=urinary nitrogen and Uo=urinary nitrogen on nitrogen-free diet, WT= weight gain of the animal eaten test protein, MT= mass of test protein consumed, WC= weight gain of the animal eaten casein, MC= mass of casein protein consumed, WL= weight loss of animal fed basal (non-protein) diet

2.4.3.3 Lysine

Lysine is one of the limiting amino acids in diets having cereals as a staple (Hendriks, Moughan, Boer, & Vanderpoel, 1994; Majumdar & Singh, 2014). It is a limiting amino acid in cereals but found in a good amount in pulses (Belitz *et al*., 2009; Pham & Rosario, 1984). Lysine is one of the very reactive amino acids, and its availability is often monitored as an indicator of the severity of processing on the nutritional quality of protein foods (Saalia & Phillips, 2011). A characteristic that restricts the availability of dietary lysine is that its amino group, under the influence of light, heat, alkali and other factors, interacts with other food constituents to become nutritionally unavailable (Pham & Rosario, 1984; Tanaka, Lee, & Chichester, 1975). During processing lysine is frequently involved in Maillard reactions, and/or lysinoalanine formation if the pH is above 9 (Friedman, 1999). During processing, lysine and other amino acids with a free ε-amino group may react with reducing sugars via the Maillard reaction (Belitz *et al*., 2009). Lysine losses from the Maillard reaction also may occur in the absence of simple sugars due to starch fragmentation during extrusion (Hood-Niefer & Tyler, 2010).

2.5 Extrusion

Extrusion is a process in which material is pushed through a die or an orifice of a particular shape (Leonard, Zhang, Ying, & Fang, 2020). Originally, the main role of extrusion was conveying and shaping of fluid forms of raw material (Guy, 2001). In 1797, Joseph Bramah patented the first extrusion process for making pipe out of soft metals (Maskan & Altan, 2012), and since then extrusion technology has been used in the production of plastics, moulded metals, and synthetic materials (Brennan, Derbyshire, Tiwari, & Brennan, 2013). Today, the role of extrusion processing is beyond conveying and shaping, and includes mixing, shearing, separation, heating or cooling, co-extrusion, venting volatiles and moisture, flavour generation, encapsulation, sterilization and expansion (Anton & Luciano, 2007; Guy, 2001; Wolf, 2010). Nowadays, the application of extrusion used widely in the pharmaceutical, food, and feed industries (Alam, Kaur, Khaira, & Gupta, 2016; Bagno, Izhmulkina, Garmashov, & Konstantinova, 2020; Baronsky-Probst, Moeltgen, Kessler, & Kessler, 2016; Castro *et al*., 2016).

Extrusion technology was first applied to food materials in the mid-1800s, when chopped meat was stuffed into casings using a piston-type extruder. In the 1930s, a single-screw extruder was introduced to the pasta industry, to both mix the ingredients (semolina and water) and to shape

the resulting dough into macaroni in one continuous operation (Brennan & Grandison, 2012). Currently, several food products are produced by extrusion e.g. breakfast cereals, bread crumbs, biscuits, crackers, baby foods, snack foods, confectionery items, chewing gum, texturized vegetable protein, modified starch, dried soups, and dry beverage mixes (Chang & Ng, 2009).

2.5.1 Extruders

Extruders (equipment used for extrusion processing) exist in a wide variety of shapes and sizes, but can be categorized into three main types, piston extruders, roller-type extruders, and screw extruders. Screw extruders are the most common extruders used in the food industry (Alam *et al*., 2016; Senanayake & Clarke, 1999). Generally, screw extruders have major components like the feed section, screw (s), barrel, die, heating mechanism, and drive system (Figure 2.6) (Senanayake & Clarke, 1999; Surasani, 2016). The principles of operation are similar in all screw types of extruders, in that raw materials are put into the extruder and pressure is used to push them through a restricted opening. As the material moves along the extruder barrel, the screw kneads the material into a semi-solid, plasticized mass (Bhattacharya, 2014; Cheng & Hansen, 2016; Surasani, 2016). If the food is heated above 70^0C in the barrel, the process is known as hot extrusion (extrusion cooking). Here, frictional heat and any additional heating that is applied may cause the temperature to rise rapidly. If the temperature is below 70^0C, it is known as cold extrusion. Finally, the food is forced through one or more openings (dies) at the end of the barrel (Brennan *et al*., 2013).

Screw extruders can be categorized as single- and twin-screw (Surasani, 2016). Single-screw extruder has a single screw that rotates in a closely fitted barrel (Figure 2.6). Single-screw extruder is described as having four different sections of operation, feed (solid conveying), compression (melting), metering (pump), and die (Reifsteck & Jeon, 2000). The feed section is found below the feed hopper and is the first section of the extruder where the process material comes into contact. The compression section of the extruder is where the material gets cooked or becomes molten during processing. The metering section is where the melted product is transported and homogenized and is where the main pressure build-up occurs during processing. The fourth section is the die section or opening, where the product emerges under pressure to form the characteristic shape.

Twin-screw extruder is constructed with two screw shafts (Figure 2.7). Twin-screw extruders generally are categorized based on the direction of screw rotation as counter-rotating or co-rotating (Bhattacharya, 2014). These two categories can be further subdivided into intermeshing and non-intermeshing based on the position of the screws in relation to one another (Guy, 2001). Twin-screw systems are considerably more complex than a single-screw system and provide much better control of residence time and internal shear of food ingredients, especially for heat-sensitive products (Reifsteck & Jeon, 2000).

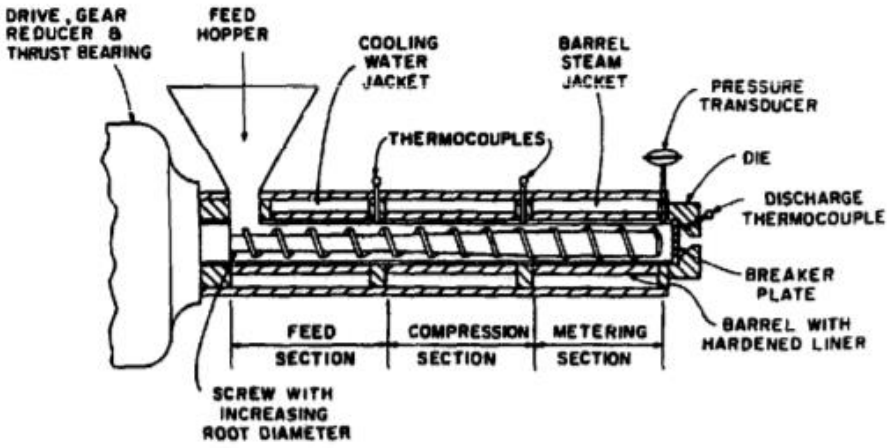

Figure 2.5 Schematic diagram of single screw extruder.

(Source: Kokini, 1993)

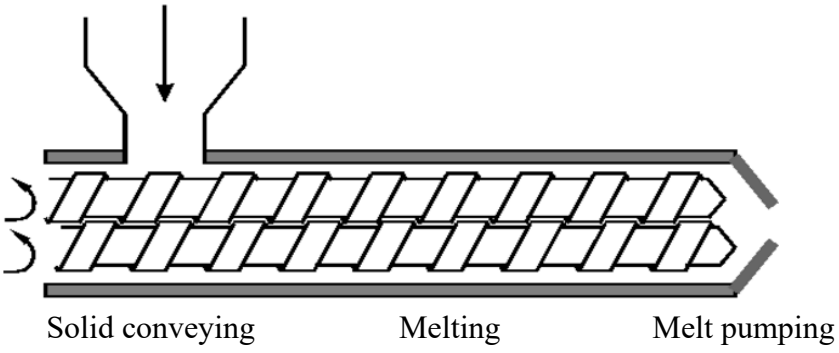

Figure 2.6 Schematic diagram of twin-screw extruder.

(source: Kaur, Sharma, & Singh, 2014)

2.5.2 Extrusion Parameters

Food extrusion is a continuous process with great flexibility to prepare a wide variety of food products with different shapes and textures. During extrusion, system operational parameters and overall feed properties determine the final product characteristics (Alam *et al*., 2016; Surasani, 2016). Several extrusion processing conditions account for the quality of finished products. Research has led to a growing awareness of the importance of extrusion variables such as screw speed, feed rate, barrel temperature, specific mechanical energy, and screw and die configuration on the final product characteristics (Alam *et al*., 2016; Alam, Scampicchio, Angeli, & Ferrentino, 2019; Anton & Luciano, 2007; Cheng & Hansen, 2016; Xu, 2016).

2.5.2.1 Screw Speed

Screw speed is one of the main factors that influences the performance of the extruder. Screw speed affects the mean residence time of the product, the amount of frictional heat generated, heat transfer rates and the shearing forces on the product (Geetha *et al*., 2014). Increasing the screw speed increases the shear rate and mechanical energy input in all the melt-filled sections. However, it also reduces the time of stay of the product in the melt pumping sections. It generally results in a higher mechanical energy input (but lower motor torque), greater melt temperature (Hirth, Preiss, Mayer-Miebach, & Schuchmann, 2015), lower die pressure, and greater product expansion (Geetha *et al*., 2014; Licata, Coorey, Zhao, Chu, & Johnson, 2015). Decreasing screw speed results in increased residence time of the viscous melt, resulting in greater plugging of the die section and a subsequent increase in torque and lower product expansion (Muthukumarappan & Karunanithy, 2012).

With the use of screw elements and paddle with low conveying capability, the degree of barrel fill may rise significantly with a reduction in screw speed below a certain threshold level. This will cause a step rise in motor torque and mechanical energy input (Maskan & Altan, 2012). Compression is achieved in the extruder barrel by back pressure, created by the die and/or by increasing the diameter of the screw and decreasing the screw pitch, using a tapered barrel with a constant or decreasing screw pitch, or placing restrictions in the screw flights (Fellow, 2000).

2.5.2.2 Barrel Temperature

Barrel temperature is another factor which affects the quality of the extruded product. During extrusion, high barrel temperature and large shearing forces cause disintegration of quaternary and tertiary structure and the interactions between food components (Alam *et al*., 2016; Harper, 1988). Barrel temperature increases gradually as the material travels down the screw. Most of the time, the temperature of the feed section of the barrel is low so as to avoid back-flow of raw material (Ahmed & Rahman, 2012). Different studies indicated that barrel temperature usually has a positive effect on the degree of starch gelatinization and extrudate expansion (Licata *et al*., 2015; Seth, Badwaik, & Ganapathy, 2015), whereas it has a negative effect on product colour, especially at elevated temperatures (Geetha *et al*., 2014; Lei, Fulcher, Ruan, & van Lengerich, 2007).

2.5.2.3 Feed Rate

Feed rate is the rate at which the raw material is put into the extruder. Feed rate has an influence on residence time, torque requirement, barrel pressure, and product temperature. The feed rate of an extruder depends on the type of screw, screw speed, and feed composition (Ahmed & Rahman, 2012). Increased feed rate in parallel with an increase in die openings reduces the mean retention time in the extruder. This, in turn, results in a lower specific mechanical energy, lower product temperature, higher viscosity of the melt at the die, and higher extrudate density. In the case of small-pitch screws, if the forwarding barrel sections become full, increasing feed rate above a certain level results in an increased rate of mechanical energy input (Maskan & Altan, 2012). In most cases, an increment in feeder speed increases the hardness of the extrudate. This may be due to reduced starch gelatinization and compressed bubble growth which results in a dense product, thus increasing hardness (Ding, Ainsworth, Tucker, & Marson, 2005; Geetha *et al*., 2014). Depending on the feeder type, the feed rate is controlled by the speed of the belt, type of the material to be fed to the extruder, or loss in weight of feeder. Feed rate controls the filling level of the extrusion barrel (Bouvier & Campanella, 2014).

2.5.2.4 Screw Design

The screw design or configuration is a key factor that affects product transformation, residence time distribution, and energy inputs to the material (Gautam & Choudhury, 1999). Screw and die designs can be changed based on the product type. The screw sections of an extruders, especially a twin-screw extruder, includes reverse pitch screw elements, mixing paddles, kneading

block and cut flight, primarily to change the residence time and/or shearing action (Bhattacharya, 2014). The profile or configuration of the screw can be changed by using different forward, kneading, and reverse elements, as well as by using screw elements with different pitch, stagger angle or length. These elements can be arranged in different ways to achieve variations in screw profile or configuration, which influences extruder performance and product quality (Choudhury & Gautam, 1998). In the study by Choudhury and Gautam (1998), a systematic increase in specific mechanical energy input and water solubility index was observed as the mixing elements were moved farther away from the die. Zhang, Zhang, Dreisoerner, & Wei (2015) reported an increment in specific mechanical energy as the distance of the reverse kneading element from the die was increased which was probably due to a gradual increase in the viscosity of the material during extrusion.

2.5.2.5 Die Design

The die plays an important role in determining product physical properties such as density, expansion, surface texture, and final shape (Senanayake & Clarke, 1999). A study by Bouzaza, Arhaliass, and Bouvier (1996) reported that die design has an influence on dough expansion with low moisture extrusion. Hussain and Singh (2014) reported that all the dimensional and physical properties of re-fabricated rice were significantly affected by die type. Highly restrictive dies increase barrel fill, residence time, and energy input. High shear rate dies are responsible for imparting energy to products and promoting starch damage (Huber, 2000).

2.5.2.6 Specific Mechanical Energy

Special mechanical energy (SME) is the amount of work input from the motor driver into the raw material that is extruded (Godavarti & Karwe, 1997). Specifically, SME is the total input of mechanical energy per unit dry weight of extrudate (Fayose & Huan, 2014). Feed rate, screw speed, and screw design are factors affecting SME (Hanada, Jermain, Lu, Su, & Williams, 2018). SME is one of the major parameters that influence final product properties, such as expansion index, hardness, and density. The SME values indicate the degree of degradation that the material undergoes during the extrusion process (Einde, Goot, & Boom, 2003; Fang, Zhang, & Wei, 2014; Gropper, Moraru, & Kokini, 2003).

2.5.3 Effect of Extrusion on Raw Material Ingredients

2.5.3.1 Starch

Starch is composed of the glucose polymers amylose and amylopectin. Starch plays a key role in extrusion processing among all components of the raw materials (Li, Hasjim, Xie, Halley, & Gilbert, 2014). Starch is subjected to various degrees of mixing, conveying, shearing, and heating as it passes along the extruder screws. As a result, disruption of starch granules, melting of crystalline portions of the starch granules, macromolecular degradation (fragmentation) of starch, gelatinization of starch, and complexations of starch with other ingredients occurs during extrusion (Li *et al*., 2014; Orford, Parker, & Ring, 1993; Zheng & Wang, 1994). The main parameters that influence changes in starch during extrusion are shear forces, residence time, and shear rate. These parameters are defined by the geometry of the extruder, processing variables such as temperature and screw speed, and feed composition (including amylose and amylopectin ratio) and moisture content (Lai & Kokini, 1990). During extrusion, large shearing forces and large screw speeds cause degradation of starch. High temperature during extrusion causes starch to gelatinize even at lower moistures (Alam *et al*., 2016).

The extrusion process reduces the molecular weight of the amylose and amylopectin polymers of starch, with a greater impact on amylopectin than on amylose. Extrusion promotes debranching of amylopectin (Politz, Timpa, & Wasserman, 1994; Vanier *et al*., 2016), or the branched structure of amylopectin makes it susceptible to shear (Li *et al*., 2014). The extent of amylopectin fragmentation decreased with increasing temperature or moisture content and decreasing screw speed (Davidson, Paton, Diosady, & Larocque, 1984; Singh *et al*., 2007). The amylose:amylopectin ratios of starches have a significant effect on the viscosity of the gelatinized material during extrusion cooking (Vanier *et al*., 2016; Xie *et al*., 2009). Amylose result in a higher viscosity than does amylopectin (Lai & Kokini, 1990).

2.5.3.2 Protein

During extrusion, thermal and shear energies are applied to raw food materials that cause protein structural unfolding and/or aggregation (Alam *et al*., 2016). Disulfide bonds are involved in stabilizing the native tertiary configurations of most proteins. Their disruption aids in protein unfolding and thus digestibility increases (Cabrera-Chavez *et al*., 2012; Cian, Drago, De Greef, Torres, & Gonzalez, 2010; Karkle, Keller, Dogan, & Alavi, 2012; Vaz & Areas, 2010). Chen,

Chen, Ren, and Zhao (2011) reported that proteins hydrate in the mixing stage of the extrusion process and become soft during the formation of the melt. The shearing forces generated in the extruder cause breakage of the protein into small particles of roughly cylindrical and globular shapes. At levels of around 5-15%, proteins compete for available water with starch and they tend to decrease starch digestibility and the extensibility of the starch polymer. The production of an extensively isopeptide cross-linked network reduces the digestibility of proteins (Brennan & Grandison, 2012). The Maillard reaction may take place during extrusion cooking of protein foods containing reducing sugars with a resulting loss of essential amino acids (Singh *et al*., 2007).

2.5.3.3 Fat

Extrusion cooking conditions cause lipids to become oils as the temperature rises to their melting points. The oils are mixed into the extrudates by the shearing action of the screw elements and are dispersed into very small drops, but if the oil or fat concentration exceeds a critical point, it lubricates the screw and barrel surfaces, reducing the friction factor (Ilo, Schoenlechner, & Berghofe, 2000). Extrusion of high-fat materials is not advisable, especially in the case of expanded products, as lipid levels over 5-6% tend to impair extruder performance. At the same time, lower lipid levels (<5%) facilitate steady extrusion and improve texture (Singh *et al*., 2007).

2.5.3.4 Fibre

Extrusion cooking ruptures glycosidic bonds in total dietary fibre polysaccharides, leading to the release of oligosaccharides and thus an increase soluble dietary fibre content (Brennan & Grandison, 2012). The extrusion process transforms some insoluble fibre to soluble fibre (Gaosong & Vasanthan, 2000; Gourgue, Champ, Guillon, & Delort-Laval, 1994; Hwang, Choi, Kim, & Kim, 1998; Mendez-Garcia, Martinez-Flores, & Morales-Sanchez, 2011; Rouilly, Orliac, Silvestre, & Rigal, 2006). The changes in structural properties and solubility of dietary fibre during extrusion leads to significant differences in their functional properties (e.g. viscosity and interactions with water) compared to unprocessed fibre (Singh *et al*., 2007).

2.5.3.5 Moisture

Moisture aids in starch gelatinization and formation of the extrusion melt (Huber, 2000). As feed moisture become lower, the viscosity of the melt increases (Chang & Ng, 2011; Singh *et*

al., 2007), which limits flow and increases residence time, thus enhancing the degree of gelatinization (Chinnaswamy & Hanna, 1990). High moisture acts as a lubricant/plasticizer and reduces friction, which decreases shear rate, torque/SME, and viscosity (Guy & Horne, 1988; Hsieh, Peng, & Huff, 1990). An increase in feed moisture content at a particular extrusion temperature reduces the degree of starch fragmentation and gelatinization (Kokini, Lai, & Chedid, 1992).

2.5.4 Extrusion and Lipid Oxidation

Extrusion at a low moisture content and a high die temperature results in extrudates having low level of lipid oxidation products. However, during storage, the amount of lipid oxidation products gradually increases in extrudates produced at a low moisture and a high die temperature (Hwang, Hartman, Karwe, Izzo, & Ho, 1994). The same holds true for barrel temperature. An increase in barrel temperature decreases the peroxide value of extrudates. However, during storage, the peroxide value of extrudates prepared at high temperatures increases gradually (Artz & Rao, 1994; Gutkoski & El-Dash, 1998; Lampi *et al*., 2015). Rancidity is a concern for extruded products during storage (Singh *et al*., 2007; Yadav, Singh, & Arora, 2018). An increase in environmental temperature during storage also increases lipid oxidation (Lasekan, Lasekan, Idowu, & Ojo, 1996). Screw wear is another concern as metals can act as pro-oxidants. Iron content and peroxide values were found to be higher in extruded rice and dhal compared with similar products processed by other methods (Semwal, Sharma, & Arya, 1994). An increase in extrusion temperature also resulted in an increase in iron in extrudates (Artz & Rao, 1994).

The addition of antioxidants to foods prior to extrusion may result in more stable products. Products extruded with ferulic acid and benzoin, chlorogenic, quercetin (Viscidi, Dougherty, Briggs, & Camire, 2004), spray-dried ginkgo extract, onion powder, potato peels (Camire, Dougherty, & Briggs, 2005), tocopherols (Paradiso, Vito, Summo, Pasqualone, & Caponio, 2009), rosemary (Kong, Perkins, Dougherty, & Camire, 2011), or grape seed extract (Rababah *et al*., 2011) exhibited retarded lipid oxidation during storage. The use of flour rich in amylose has been reported to increase the oxidative stability of extrudates due to amylose-lipid complexation (Thachil, Chouksey, & Gudipati, 2014). Microencapsulation also enables the oxidative stability of lipids during extrusion (Chew & Nyam, 2016). Ying, Edin, Cheng, Sanguansri, and Augustin (2015), reported that it is possible to produce extruded products with high oil loading up to 15%

with low surface oil and enhanced oxidation stability through microencapsulation. The extrusion process can prevent free fatty acid release by denaturing hydrolytic enzymes (Camire, Camire, & Krumhar, 1990). One study was found that extruded millet powder was more stable than steamed and normal flour (Wang, Chen, Ren, & Guo, 2014). Roman, Pico, Antolin, Martinez, and Gomez, (2018) reported a lower amount of lipid oxidation in fried batters containing extruded flour. Generally, lipid oxidation is one of the main concerns with extruded products and a variety mitigation strategies have been explained.

2.5.5 Extrusion and Antioxidants

Extrusion was found to have a variable effect on antioxidant content and capacity. Some studies reported a decrease in total antioxidant content as well as antioxidant capacity with extrusion (Bhat, Wani, Hamdani, & Gani, 2019; Corrales-Banuelos *et al*., 2016; Delgado-Licon *et al*., 2009; Korus, Gumul, & Czechowska, 2007; Shih *et al*., 2009; Wani & Kumar, 2016a). The reason was that heat-labile phenolics and other bioactive compounds were damaged during extrusion despite the fact that their availability increased (Altan, McCarthy, & Maskan, 2009; Nayak, Berrios, Powers, & Tang, 2011; Repo-Carrasco-Valencia, Pena, Kallio, & Salminen, 2009; Sarawong, Schoenlechner, Sekiguchi, Berghofer, & Ng, 2014; Wang & Ryu, 2013). It has been reported that feed moisture content and barrel temperature have significant impact on total phenolics content (Abd El-Hady & Habiba, 2003; Samyor, Deka & Das, 2018). During extrusion, high temperature and high moisture lead to polymerization of phenols and reduced antioxidant activity. It has been reported that level of isoflavones (Mahungu *et al*., 1999), anthocyanin (White, Howard, & Prior, 2010), ascorbic acid (Killeit, 1994), tocopherols and tocotrienols (Zielinski, Ciska, & Kozlowska, 2001) decreased during extrusion. However, there also are studies that report increases in total antioxidant content as well as antioxidant capacity with extrusion. In the study by Morales *et al*. (2015), an increase in most polyphenolic fractions was found, probably due to the effect of extrusion on the hydrolysis of polyphenols bound to fibre and proteins, with an increase on antioxidant activity. Singh, Kaur, Singh, Singh, and Singh (2019) also reported increase in phenolics content and antioxidant activity with temperature and screw speed while moisture had a negative effect. White *et al*. (2010) also observed an increase in antioxidant activity with an increase in barrel temperature, which might be due to products formed during the Maillard

reaction. Generally, the effect of extrusion on the antioxidant content and capacity depends on extrusion conditions used.

2.5.6 Extrusion and Protein Quality

Extrusion has a variable effect on protein quality. Arrage, Barbeau, and Johnson (1992) reported that adjusted PER was not decreased significantly after extrusion. On the contrary, it also has been reported that PER and NPR increased after extrusion (El-habashy *et al*., 1997; Gomez-Aldapa, Martinez-Bustos, Figueroa, & Ordorica, 1999). This might be due to differences in the extrusion conditions. Frias *et al*. (2011), on the other hand, found that protein quality measured by net protein utilization, net protein ratio, relative net protein ratio, true protein digestibility, and biological value was not affected by extrusion, whereas protein quality measured in terms of amino acid score and PDCAAS decreased after extrusion. This study also showed the variability of the extrusion effect on different protein quality parameters. Extrusion resulted in higher PDCAAS and DIAAS values as compared to baking and drum drying (Mosha & Bennink, 2005; Nosworthy *et al*., 2017; Nosworthy *et al*., 2018). *In vitro* PDCAAS was reported to be lower than *in vivo* PDCAAS (Nosworthy *et al*., 2017). Qi and Onwulata (2011) reported that extrudates obtained at low to medium temperature ($\leq 50^0$C) maintained protein quality at the non-extruded level, whereas those extrudates obtained above 75^0C had a significant level of reduction in available free amine content and a near-complete elimination of free sulfhydryl (SH) groups.

One of the most important components of protein quality is digestibility. Extrusion was found to improve *in vitro* protein digestibility (IVPD) of different food products (Arribas *et al*., 2017; Ghumman, Kaur, Singh, & Singh, 2016; Guzman-Ortiz *et al*., 2015; Irungu *et al*., 2019; Jakkanwar, Rathod, & Annapure, 2018; Mkandawire *et al*., 2015; Nosworthy *et al*., 2018; Omosebi, Osundahunsi, & Fagbemi, 2018; Palanisamy, Franke, Berger, Heinz, & Toepfl, 2019; Perez-Navarrete, Gonzalez, Chel-Guerrero, & Betancur-Ancona, 2006; Zhang, Liu, Ying, Sanguansri, & Augustin, 2017). For example, extrusion resulted in a 7% increment of IVPD of sorghum (Vilakati, MacIntyre, Oelofse, & Taylor, 2015). Extrusion conditions (barrel temperature, feed moisture, and screw speed) have been reported to affect it. Barrel temperature was reported to have a different effect on IVPD. Irungu *et al*. (2019) and Min, Yi, Lijun, Dong, and Zhihuai (2015) reported that an increase in temperature to a certain point increased IVPD but further increment resulted in decreased IVPD. The reason provided was that the initial increase resulted

in the denaturation of protein and exposed more polypeptide bonds to enzymes. However, further increase resulted in complex formation which was not digestible (Opstvedt *et al*., 2003) or thermal cross-linking (Ainsworth, Fuller, Plunkett, & Ibanoglu, 1999). However, Ghumman *et al*. (2016) and Licata *et al*. (2014) reported that an increase in barrel temperature resulted in increased IVPD. This indicates an increase in IVPD with temperature to the point where the temperature initiates protein crosslinking.

Different reasons have been suggested in different studies for the increase in IVPD with extrusion temperature. Extrusion temperature affects non-covalent bonds responsible for stabilization of protein structure and exposes polypeptides to enzymes which in turn increases digestion (Dahlin & Lorenz, 1993; Ruiz-Ruiz *et al.*, 2008). Heating has been reported to inactivate enzyme inhibitors (Lorenz & Appolonia, 1980) or to reduce level of antinutrient factors, which also increases the digestibility of proteins (Prakrati, Ameeta, & Kushwah, 1999). Besides, extrusion causes thermal and mechanical depolymerization of protein and generates low molecular weight peptides and thus IVPD is improved. Palanisamy *et al*. (2019) reported that an increase in feed moisture resulted in higher IVPD. In contrast, Min *et al*. (2015) reported that excess moisture rapidly reduced the rubbing action between cylinder, screw, and materials, thereby decreasing the mechanical energy and thermal energy; as a result, IVPD decreased. Akande, Nakimbugwe, and Mukisa (2017) reported a quadratic effect of moisture on *in vitro* protein digestibility increases. Extrusion increased *in vitro* protein digestibility more than did malting and roasting (Nkundabombi, Nakimbugwe, & Muyonga, 2015). Extrusion was reported to increase true and apparent protein digestibility (*in vivo* protein digestibility) as well. *In vitro* protein digestibility was found to be lower than the *in vivo* digestibility (El-habashy *et al*., 1997; Nosworthy *et al*., 2017).

The other important component of protein quality is amino acid content. Extrusion can significantly increase or decrease amino acid content depending on the type of raw material used. For example, Obatolu, Cole, and Maziya-Dixon (2000) reported that the amino acid content of malted maize increased after extrusion, whereas it decreased for unmalted maize. Nosworthy *et al*. (2017) reported that extrusion increased amino acid content; whereas Mosha and Bennink (2005) reported amino acid content did not change significantly sue to extrusion. This might be due to the differences in the extrusion conditions. The first limiting amino acids after extrusion processing has been reported to be sulfur amino acids (methionine and cysteine) or tryptophan in legumes,

and lysine in cereals (Milan-carrillo *et al*., 2007; Mosha & Bennink, 2005; Nosworthy *et al*., 2017, 2018). In comparison to other processing methods such as drum-drying, extrusion was found to increase amino acid content significantly (Arrage *et al*., 1992). Generally, the effect of extrusion on amino acid and protein quality depends on the raw material and extrusion conditions used.

2.5.7 Extrusion and Lysine

Lysine is one of the most unstable amino acids during extrusion cooking (Ilo & Berghofer, 2003). Extrusion processing favors the development of Maillard reaction products, and, thus, quality of proteins might be decreased due to loss of essential amino acids like lysine (Fallahi *et al*., 2016; Liopart, Drago, Greef, Torres, & Gonzalez, 2014; Noguchi, Mosso, Aymard, Jeunink, & Cheftel 1982; Repo-Carrasco-Valencia *et al*., 2009; Singh *et al*., 2007). For example, Mokrane *et al*. (2010) reported the lysine content of raw sorghum (0.2 g /100 g protein) decreased to 0.16 g/100 g protein after extrusion and the lysine score fell below the FAO/WHO 2-5 years old requirement (FAO & WHO, 1991). The loss of lysine during extrusion is related to parameters such as extrusion temperature, die diameter, screw speed, feed rate, screw compression ratio, torque and pressure, energy input, raw material composition, feed moisture and pH (Asp & Bjorck, 1989; Banga, Alonso, Gallardo, & Perez-Martin, 1992; Camire *et al*., 1990; Labuza & Saltmarch, 1982).

It has been found that an increased extrusion temperature resulted in an increased loss of available lysine. At a given process temperature, however, the destruction of available lysine decreased with increasing moisture content (Bjorck, Matoba, & Nair, 1985; Bjorck, Noguchi, Asp, Cheftel, & Dahlqvist, 1983; Chaiyakul, Jangchud, Jangchud, Wuttijumnong, & Winger, 2009; Ilo & Berghofer, 2003; Masatcioglu, Ng, & Koksel, 2014). Feed moisture had a protective effect on the amino acid loss during extrusion cooking. The most possible explanation for the effects of feed moisture on amino acid loss in extrusion cooking is that moisture affects the shearing action and dissipation of mechanical energy in the extruder. This may influence the temperature profile of the material along the screw channel and thus the loss of amino acids (Ilo & Berghofer, 2003). However, increased moisture content protects lysine until a certain level. For instance, in the study done by Valim and Batistuti (2000), lysine availability of chickpea flour submitted to thermoplastic extrusion was evaluated at feed moisture contents of 13%, 18% and 27%, and it was found that extrusion reduced available lysine by 58% and 55% at 13% and 18% moisture content.

However, the lysine loss at 27% feed moisture level was found to be 71%. Saalia and Phillips (2011) reported that increased moisture decreased the lysine content of extruded peanut.

Lysine loss occurs at the early stage of the Maillard reaction. Masatcioglu *et al*. (2014) reported that the lysine loss that occurred as the die temperature increased from 110^0C to 150^0C was small compared to the lysine loss occurred at 110^0C. In the same study, addition of sodium bicarbonate and ammonium bicarbonate increased lysine loss at 110^0C. However, at 150^0C, addition of sodium bicarbonate and ammonium bicarbonate did not affect lysine. A higher pH also promotes lysine loss. An increase in screw speed resulted in increased lysine retention, possibly due to reduced residence time of the feed mixture in the extruder (Iwe, Zuilichem, Stolp, & Ngoddy, 2004). However, Noguchi *et al*. (1982) reported screw speed did not affect lysine loss.

It has been reported that lysine retention ranged from 51% to 89% during extrusion of maize grits, with higher lysine losses at lower moisture contents, longer retention times, and higher specific mechanical energy levels (Ilo & Berghofer, 2003). In another study, Bjorck *et al*. (1985) found lysine retention to be between 63 and 100% with extruded wheat. Tsao, Frey and Harper (1978) reported that an increase in die diameter increased lysine retention in a single-screw extruder, but the opposite was observed in another study (Iwe *et al*., 2004). This might be due to differences in temperature, feed moisture, or screw speed between the two studies. Shrear stress significantly affected the rate constant of amino acid loss during extrusion cooking (Pham & Rosario, 1984). An increase in protein source in the extrudates increases available lysine but the product quality might be affected. Makowska, Cais-Sokolinska, Waskiewicz, Tokarczyk, and Paschke (2016) reported that a level of whey powder above 5% caused a significant increase in the protein and available lysine contents of extrudates. However, the breaking force of the extrudates was increased and the scores for porosity, colour, taste, and overall desirability were lower.

2.6 Extrusion-Expansion

Raw material converts to a viscoelastic dough as it passes through the extruder due the various degrees of mixing, conveying, shearing, and heating. When this viscoelastic dough is forced through a die nozzle, it come out as an expanded extrudate (Kumar, Ganjyal, Jones, & Hanna, 2007). Die swell due to elastic recovery of deformation and bubble growth due to moisture flash-off are the primary reasons for the expansion of the extrudate (Arhaliass *et al*., 2009; Fan,

Mitchell, & Blanshard, 1994; Wang, Ganjyal, Jones, Weller, & Hanna, 2005). When the molten elastic extrudate comes out of the die, it rebounds to obtain the original form but it ends up with die swell formation. This indicates that rheological properties of dough such as melt viscosity and elasticity are important in contributing to the expansion of the extrudate (Arhaliass *et al*., 2009; Kumar *et al*., 2007). Bubbles in dough grow under pressure reduction at the discharge port of an extruder (Kumagai, Kumagai, & Yano, 1993). As the extrudate leaves the die, part of the superheated water evaporates due to the sudden pressure drop which leads to bubble growth (Bouzaza *et al* 1996; Kristiawan, Chaunier, Della Valle, Ndiaye, & Vergnes, 2016). Thus, expansion is the result of both die swell and bubble growth. Generally, the production process for expanded foods can be categorized into five steps (Brummer, Meuser, Lengerich, & Niemann, 2002; Kokini *et al*, 1992) namely transformation of materials from the ordered state to the disordered state, generation of the bubble nucleus, die expansion, growth of bubbles, and contraction of bubble growth (Fan *et al*., 2012).

Extrudate expansion can be longitudinal or sectional (Kumar *et al*., 2007). Longitudinal expansion refers to the expansion in the die flow direction, whereas sectional or radial expansion refers to the increase of cross-section in the plane orthogonal to flow direction. Longitudinal and sectional expansions are inversely related. Volumetric expansion is the overall expansion resulting from longitudinal and sectional expansion (Kristiawan *et al*., 2016). The relative sizes of both types of expansion determines the structural organization of the extrudates (cell organization) and thus the related sensory and physical properties (Bouzaza *et al.*, 1996). Those extrudates which expand directly upon exiting the die of the extruder rather than expanding later on during drying in the oven or fryer are known as direct-expanded extrudates (Maskan & Altan, 2012). Extrusion expansion is affected by various extrusion factors such as barrel temperature, screw speed, screw configuration, and die design, and ingredient-related factors such as feed composition, moisture content, particle size, starch content, and amylose-amylopectin ratio (Bouzaza *et al*., 1996; Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006; Ganjyal & Hanna, 2004; Guha & Ali, 2006; Moraru & Kokini, 2003; Oliveira, Schmiele, & Steel, 2017; Seth, Badwaik, & Vijayalakshmi, 2013; Sokhey, Kollengode, & Hanna, 1994; Valle, Vergnes, Colonna, & Patria, 1997; Wang *et al*., 2017; Waramboi, Gidley, & Sopade, 2014). Degree of expansion is one of the factors that affect acceptability, and emphasis should be placed on optimization of the extrusion conditions such that to maximum expansion is achieved.

2.6.1 Extrusion Parameters and Expansion

2.6.1.1 Expansion and Screw Speed

Screw speed has been found to affect the radial expansion index. Ozer, Ibanoglu, Ainsworth, and Yagmur (2004) studied the expansion characteristics of extruded snacks composed of different flours in various proportions and found that a higher screw speed resulted in greater radial expansion. This may be because a higher screw speed introduces more energy to the dough in the barrel, which helps in faster evaporation of moisture at the die exit and hence expansion increases (Brahma, Weier, & Rose, 2016). Or, higher screw speed is related to a higher shear rate and pressure which also can cause greater expansion (Seker, 2005). Screw speed has positive linear effects on the radial expansion ratio (Ding *et al*., 2006; Gulati, Weier, Santra, Subbiah, & Rose, 2016; Wani & Kumar, 2016b). In the study by Ainsworth, Ibanoglu, Plunkett, Ibanoglu, and Stojceska (2007), an increase in screw speed resulted in greater of expansion ratio of extrudates.

2.6.1.2 Expansion and Barrel Temperature

Barrel temperature has a significant effect on extrudate expansion (Korkerd, Wanlapa, Puttanlek, Uttapap, & Rungsardthong, 2016). As temperature increases, expansion ratio increases (Ghumman *et al*., 2016; Kaur *et al*., 2015). Barrel temperature has positive linear effects on radial expansion (Gulati *et al*., 2016; Wani & Kumar, 2016b). Higher expansion at higher temperatures can be attributed to the gelatinization of starch and structural strengthening. A high degree of starch gelatinization usually is needed for high expansion (Yanniotis, Petraki, & Soumpasi, 2007). A high extrusion temperature may result in increased vapor pressure and favor expansion, while low temperatures may reduce vapor pressure within the melt, thus reducing expansion (Day & Swanson, 2013). During extrusion, high temperature and pressure conditions inside the barrel cause the moisture in the sample to superheat and the sudden pressure drop at the exit of the die causes the moisture to evaporate which results in expansion of the product (Heldman & Hartel, 1997). Temperature and moisture content in combination have a significant effect on the expansion ratio. Chaiyakul *et al*. (2009) found that an increase in moisture content and a decrease in barrel temperature decreased the expansion of extrudates, resulting in increased density and hardness. However, the direct relationship between barrel temperature and expansion ratio applies only a critical temperature is reached. Beyond a critical temperature (which depends on the type of starch and moisture content), expansion decreases with an increase in temperature as dextrinization,

excessive softening, weakening of starch structure, and structural degradation of starch melt increase to a level where the extrudate is not able to withstand the high vapor pressure, resulting in collapse (Moraru & Kokini, 2003).

2.6.1.3 Expansion and Design of Screw and Die

Screw type and configuration can affect the specific mechanical energy input to the material being extruded and, therefore can affect expansion ratios (Sokhey *et al*., 1994). Die diameter also affects the expansion ratio of extrudates (Day & Swanson, 2013). Different studies reported that melt expansion depends on melt composition and die design (Bouzaza *et al*. 1996). Generally, a smaller die diameter results in a higher shear rate and greater expansion. Increasing the die size was found to reduce the degree of starch gelatinization and decreased nucleation (Kokini *et al*., 1992).

2.6.2 Expansion and Raw Material Ingredients

2.6.2.1 Starch

Direct-expanded snacks rely on the functional properties of starch for expansion (Bassinello, Carvalho, Rios, Maciel, & Berrios, 2015). Maximum expansion depends not only on the amount of starch but also on the ratio of amylose and amylopectin. Higher starch content favours expansion, but still depends on the ratio of amylose and amylopectin. The linear amylose and branched amylopectin contribute to mechanical strength and expansion. The ratio of amylose to amylopectin influences both radial (amylopectin) and longitudinal (amylose) expansion. Extrudates made from waxy corn starch had higher expansion and lower hardness than extrudates made from regular corn starch (Allen, Carpenter, & Walsh, 2007; Matthey & Hanna, 1997). Waxy starch contains mostly amylopectin which is a branched molecule, whereas regular starch also contains amylose which has mainly linear structure (Moraru & Kokini, 2003). Although amylopectin promotes the expansion of the gelatinized starch matrix, it fails to strengthen and sustain the walls of the extrudate bubbles during expansion (Vanier *et al*., 2016). Amylose needs more energy for gelling/gelatinization and retrogradation, which results in a harder and denser textur. Amylose forms complexes with other ingredients such as protein and lipid, and thus decreases expansion. In general, starch plays a major role in expansion as mentioned earlier, while

protein, lipid, fibre, and sugar act as diluents (Allen *et al*., 2007; Matthey & Hanna, 1997; Thachil e*t al*., 2014).

2.6.2.2 Protein

An increase in the protein concentrations results in lower expansion of extrudates (Bassinello *et al*., 2015; Kannadhason, Muthukumarappan, & Rosentrater, 2011; Singh, Nielsen, Chambers, Martinezserna, & Villota, 1991; Yu, Ramaswamy, & Boye, 2013). Protein lower expansions by preventing the interaction of water with starch, thus affecting gelatinization (Day & Swanson, 2013). Proteins also influences expansion by adjusting water distribution in the feed matrix and favoring interactions during extrusion (Moraru & Kokini 2003). Protein tends to reduce the extensibility of the starch polymer during its expansion at the die exit, reducing the degree of expansion (Brennan & Grandison, 2012; Onwulata, Konstance, Smith, & Holsinger, 2001). However, the level of protein that has a significant impact on expansion varies depending on the ingredient type and extrusion conditions. In the study by Singh *et al*. (1991), incorporation of protein at a level greater than 5% resulted in a significant decrease in expansion ratio. Furthermore, in the study by Onwulata, Konstance, Smith, and Holsinger (1998), greater than 10% protein resulted in a significant decrease in expansion ratio.

2.6.2.3 Lipid

Lipid acts as a lubricant thus decreasing friction between dough and screw or barrel (Guy, 2001; Lin, Hsieh, & Huff, 1997), reducing mechanical energy input or shear stress (Ilo *et al*., 2000), preventing break down of starch (Lin *et al*., 1997) and delaying the degree of gelatinization (Ilo *et al*., 2000; Schweizer *et al*., 1986). These effects of lead to less pressure development which results in a less expanded product (Singh *et al*., 2007). The presence of substances such as lipids can modify the elastic character of the molten starch in the barrel (De Pilli *et al*., 2008), so that it can no longer hold water vapour, resulting in rupture of the cell wall and lower expansion (Thachil *et al*., 2014). Cueto *et al*. (2015) reported that the high-fat content of chia causes blends containing chia to have a lower expansion index as compared to maize and quinoa. Bisharat, Oikonomopoulou, Panagiotou, Krokida, and Maroulis (2013) found out that corn flour and olive paste showed a lower expansion value than did other samples due to their higher fat content. Generally, lipid contents beyond 5-6% act as lubricant (Singh *et al*., 2007).

2.6.2.4 Fibre

High fibre concentrations alter pasting properties, and reduce the expansion of extrudates (Alam *et al*., 2016; Brennan, Monro, & Brennan, 2008; Chanvrier *et al*., 2013; Fleischman *et al*., 2016; Meng, Threinen, Hansen, & Driedger, 2010). An increase in dietary fibre content, particularly insoluble fibre, reduces sectional or radial expansion and increases the bulk density of extruded products (Ainsworth *et al*., 2007; Badrie & Mellowes, 1992; Camire & King, 1991; Jin, Hsieh, & Huff, 1994; Pai, Blake, Hamaker, & Campanella, 2009; Robin, Schuchmann, & Palzer, 2012). The decrease in sectional expansion index with an increase in fibre content be due to several reasons, including increased shear and extensional viscosities with the addition of fibre, rupturing of the cell walls of expanding extrudates by the fibre and interference with the expansion of air bubble, competition of the fibre for water with that of starch, and disruption of the continuous structure of the melt by the fibre and thus impeding elastic deformation during expansion (Foschia, Peressini, Sensidoni, & Brennan, 2013; Mendonca, Grossmann, & Verhe, 2000; Moraru & Kokini, 2003; Oliveira *et al*., 2017; Pai *et al*., 2009; Perez-Navarrete *et al*., 2006; Robin *et al*., 2012). The decrease in sectional expansion when insoluble dietary fibre content increases often leads to an increase in longitudinal expansion (Jin *et al*., 1994; Robin *et al*., 2012). Despite the nutritional benefits of fibre, it commonly has a negative impact on quality parameters such as expansion ratio. Generally, higher level of dietary fibre result in higher densities, harder textures, less crispiness and less preferred products, but still the effect of fibre content on expansion also depends on the process conditions (Altan, McCarthy, & Maskan, 2008; Yagci & Gogus, 2008).

2.6.2.5 Feed moisture content

Feed moisture content is one of the factors affecting the expansion and density of extrudates (Ding *et al*., 2006). An increase in feed moisture content decreased expansion ratio or expansion index of oat-corn, rice, cornmeal, barley, lentil-horse gram and extrudate (Chaiyakul *et al*., 2009; Ding *et al*., 2005; Ghumman *et al*., 2016; Kirjoranta, Tenkanen, & Jouppila, 2016; Liu, Hsieh, Heymann, & Huff, 2000; Ryu & Ng, 2001; Sharma & Gujral, 2013; Stojceska, Ainsworth, Plunkett, & Ibanoglu, 2009), especially sectional expansion (Oliveira *et al*., 2017; Thymi, Krokida, Pappa, & Maroulis, 2005). Oke, Awonorin, Sanni, Asiedu, and Aiyedun, (2013) studied the effect of feed moisture (18-28%) on expansion of water yam and concluded that an increase in feed

moisture decreased the drag force resulting in less pressure at the die and thus lower expansion of extrudates. Increased feed moisture decreases melt viscosity, may decrease entrapment of bubbles, and decreases expansion (Day & Swanson, 2013; Stojceska *et al*., 2009).

2.6.3 Extrusion Expansion and Lipid Oxidation

Direct-expanded extruded snacks are susceptible to oxidation because the expanded surface and high porosity increase access to oxygen (Amft, Bauer, Rostek, Spielovgel, & Schwarz, 2019). The susceptibility to lipid oxidation is very high during storage. The major causes of lipid oxidation in extruded snack foods during storage are low moisture content, increased surface area due to expansion, and higher levels of iron caused by wearing of the screw and barrel during extrusion (Viscidi *et al*., 2004). Even a low level of fat may cause problems related to oxidation in expanded products. Oxidation is further worsened in expanded extruded products when fat content is increased. This affects product shelf-life and acceptability (Yadav *et al*., 2018). Different mitigation strategies have been reported, including packaging using high-density polyethylene with polypropylene (Jaimez-ordaz *et al*., 2019), modified atmospheric packaging with nitrogen flushing (Yadav *et al*., 2018), and the use of antioxidants (Kong *et al*., 2011).

2.7 Extrusion of Chickpea and Chickpea-cereal Blends

Different studies blended chickpea with wheat, rice, maize, millet, and teff, and examined the effect extrusion and extrusion conditions have on the quality, nutritional content, and sensory properties of directly expanded extruded products. Some studies examined the effect of extrusion and extrusion conditions on chickpea flour.

2.7.1 Chickpea

The nutritional quality of extruded snacks should be considered when extrusion conditions are defined. In that regard, Poltronieri, Areas, and Colli, (2000) investigated the effect of extrusion cooking (14% moisture and 130^0C processing temperature) and home-cooking of chickpea on iron bioavailability using the hemoglobin regeneration method on anemic rats. It was found that chickpea is a good source of iron and extrusion cooking is a process comparable to home-cooking in terms of its effect on iron bioavailability.

Berrios *et al*. (2010) investigated the effect of extrusion on total available carbohydrates (TAC), mono-, di- and oligosaccharides, and the soluble and insoluble dietary fibre contents of chickpea, lentil, and dry pea. Concentrations of total TAC in lentil, chickpea, and dry pea flours ranged from 625 g/kg to 657 g/kg dry matter. Dry pea showed the highest concentration of TAC, followed by chickpea and lentil. Extrusion processing did not significantly affect TAC content. Extrusion processing decreased the concentration of the raffinose family of oligosaccharides (raffinose and stachyose) in pulse extrudates. Extruded snacks formulated from the pulse flours demonstrated a beneficial increase in dietary fibre.

Yovchev *et al*. (2017) examined the effects of die temperature (120-150^{0}C), moisture content (20-24% wet basis), and screw speed (260–340 rpm) on the specific mechanical energy and physical properties (expansion ratio, bulk density, and hardness) of desi chickpea extrudates. Die temperature and feed moisture content, as well as the interaction between them, affected the product responses (expansion ratio, bulk density, and hardness) the most. A significant correlation was reported between hardness and bulk density (positive), hardness and expansion ratio (negative), and bulk density and expansion ratio (negative). Desirable characteristics (high expansion, low bulk density, and hardness) were obtained at a high die temperature, relatively high moisture, and high screw speed.

2.7.2 Chickpea-Rice

Bhattacharya and Prakash (1994) examined the effect of extrusion parameters such as feed ratio and die temperature on expansion ratio, bulk density, and shear strength to develop rice and chickpea snacks. It was found that the incorporation of chickpea at 10% and 20 % decreased product expansion but increased bulk density and shear strength. Die temperature was found to have liner effects with the parameters analyzed. Similarly, Bhattacharya & Prakash (1997) investigated the physicochemical the characteristics of chickpea-rice extruded snacks and reported that as the proportion of chickpea increased, the diametric expansion and water holding capacity of the extrudate decreased, and peak shear force and bulk density increased. It also has been also reported that in products with high diametric expansion and low peak shear force, air cells were predominant and smooth surface morphology was observed.

In the study by Singh, Azeem, and Singh (2003) physicochemical characteristics of the extruded snacks prepared from rice and grass pea or rice and chickpea were examined. Unlike with

most studies, it stated that the expansion ratio of extruded snacks increased with an increase in the proportion of pulse and extrudates containing grass pea did more than did those containing chickpea. The extrudate with grass pea also was found to have less shear force and less water holding capacity compared to extrudates with chickpea.

Shirani and Ganesharanee (2009) investigated the effects of addition of fenugreek flour and debittered fenugreek polysaccharide on the physical characteristics, sensory acceptability, and glycemic index of chickpea-rice extrudates. A blend of chickpea and rice (70:30) was replaced with fenugreek flour at 2%, 5%, and 10%, or debittered fenugreek polysaccharide at 5%, 10%, 15%, and 20% and extruded. Fenugreek flour has a bitter taste and thus, the inclusion of fenugreek flour in extruded chickpea-based products was not acceptable at levels of more than 2%. However, the addition of debittered fenugreek polysaccharide up to 15% was within the acceptable range. Addition of fenugreek polysaccharide resulted in a slight reduction in radial expansion and increase in longitudinal expansion. The study indicated that it is possible to add up to 15% fenugreek into a chickpea-rice blend in the form of debittered polysaccharide for the development of extruded snacks with low glycemic index.

Singh, Rachna, Hussain, and Sharma (2015) examined the effects of feed moisture (13-20%), barrel temperature (116-184^{O}C) and screw speed (350-600 rpm) on specific mechanical energy, bulk density, water absorption index, water solubility index, and hardness of a chickpea-rice-potato extruded snack. It was reported that an increase in feed moisture reduced specific mechanical energy and water solubility index, and increased bulk density, water solubility index, and hardness. An increase in screw speed decreased bulk density, water absorption index, and hardness of extrudates, whereas an increase in barrel temperature decreased specific mechanical energy, bulk density, water absorption index, and hardness, but increased the water solubility index. Optimized extrusion parameters for preparation of chickpea-rice-potato extruded snacks were reported to be 14% moisture, 550 rpm screw speed and 170^{0}C temperature.

2.7.3 Chickpea-Maize

Shah, Sharif, Butt, and Shahid (2017) examined the physical, textural, and sensory attributes of extruded maize snacks supplemented with chickpea or defatted soy flour. The results indicated that density, moisture, water activity, hardness, and cohesiveness increased, whereas,

expansion ratio, springiness, and chewiness, decreased, as supplementation with soy or chickpea flour increased from 20 g/100 g to 40 g/100 g.

Lazou, Michailidis, Thymi, Krokida, and Bisharat (2007) examined the effect of extrusion conditions, including feed rate, feed moisture content, screw speed, and extrusion temperature on the structural properties of maize-pulse extrudates. Maize was blended with chickpea, Mexican bean, white bean, or lentil separately in a ratio ranging from 10 to 90%. It was found that the porosity and expansion ratio of extrudates increased with an increase in temperature, residence time, and corn to pulse ratio, and decrease with an increase in the feed moisture. The addition of the pulse protein source led to more dense products. The maize and white bean blend was found to have higher porosity than the other maize-pulse extrudates in the study. Extrudates containing chickpea was found to have the lowest values for porosity. Balasubramanian and Singh (2007) also investigated the effect of feed rate, feed moisture, and incorporation of dehulled legumes (0, 5, 10, 15%), including chickpea, green-gram, and black-gram, on extrudate properties. It was reported that the incorporation of any of the legumes resulted in a decrease in expansion and an increase in bulk density and hardness of extrudates as was reported by Lazou *et al*. (2007). Among the legumes studied, blackgram caused the maximum change in extrudate properties.

Patil, Brennan, Mason, and Brennan (2017) examined the effect of addition of legumes such as yellow pea, green pea, lentil and chickpea to wheat, rice, barley, and maize on the physical and nutritional properties of extrudates. Whole grains of the different legumes replaced each of the cereals at 0%, 5%, 10%, and 15% levels. All the samples were extruded under the same conditions. It was found that substitution of cereals with legumes did not significantly affect the water absorption index of extrudates containing maize or wheat. However, the water absorption index of barley snacks decreased after the addition of legumes, whereas the water absorption index of rice and lentil extrudates increased. Shevkani *et al*. (2019) examined the effect of adding chickpea grits and spinach leaf powder on properties (colour, expansion, density, hardness, water absorption index, total phenolic content, and antioxidant activity) of corn grits extrudates. Total phenols and antioxidant activity of extrudates increased with an increase in the incorporation of chickpea and spinach, although specific mechanical energy and extrudate expansion generally decreased, while density and hardness increased. Sensory analysis revealed that chickpea and spinach at incorporation levels of 25% and 4%, respectively could be blended with corn to make a highly acceptable antioxidant-rich expanded snack.

Singha *et al*. (2018) extruded a blend of distillers dried grains, chickpea and maize and with an increase in the extrusion temperature from 100 to 140°C, apparent viscosity, specific mechanical energy, and torque values was decreased. An increase in distillers dried grains level increased apparent viscosity, specific mechanical energy, and torque. Mechanical energy also increased with an increase in screw speed, which could be due to the higher shear rates at higher screw speeds. Screw speed and moisture content had a significant negative effect on torque. Moreira-Araujo, Araujo, and Areas (2008) developed extruded snacks from a blend of chickpea-corn-bovine lung for controlling iron-deficiency anemia in children from poorer areas. After assessment, the study reported a significant drop in anemia prevalence, from 61.5% to 11.5% in the test group, and an insignificant reduction (63.1–57.7%) in the control group. The acceptance of the fortified snack was excellent, and no undesirable effects were observed.

2.7.4 Chickpea-Wheat

Cereal food products are an important part of the human diet, with wheat being the most commonly consumed cereal in many parts of the world. Extruded snack products are increasing in consumer interest due to their texture and ease of use. However, wheat-based foods are rich in starch not in protein. Therefore, the effects of adding legumes to wheat-based snacks at different levels (0%, 5%, 10%, and 15%) during extrusion were investigated in terms of protein content and protein digestibility. It was observed that fortification of snacks with legumes caused an increase in the protein content and the extrusion technique increased the protein digestibility by 37%–62%. The product developed by extrusion was found to be low in fat and moisture content. *In vitro* protein digestibility of wheat was 59%, but with the addition of 15% chickpea, it reached 63% (Patil *et al*., 2016).

2.7.5 Chickpea-Millet

Geetha *et al*. (2014) examined the effect of process parameters such as temperature, screw speed, and feeder speed on expansion ratio, bulk density, hardness and crispiness of snacks prepared from a 70:30 blend of Kodo millet-chickpea. It was found that bulk density and hardness decreased, and the expansion ratio increased, when the extrusion temperature and screw speed increased. The overall and radial expansion increased with screw speed. An increase in feeder speed also increased hardness. Crispiness was found to have a positive relationships with screw

speed and temperature, and a negative relationships with feeder speed. Desirably crisp extrudates were obtained at 293 rpm screw speed, 19 rpm feeder speed, and 123 °C temperature.

2.7.6 Chickpea-Teff

Awol (2015) examined the effect of chickpea level (0-30%) and feed moisture content (12% and 14%) on the physical properties of extruded products from teff (*Eragrostis teff*). It was found that the level of chickpea had more effect on the physical properties of products than did feed moisture content. An increased level of chickpea resulted in increased bulk density and water solubility index, and decreased diametric expansion ratio and sensory crispiness. On the other hand, lower feed moisture resulted in higher water solubility index. Sensory analysis for crispiness revealed that the most accepted product had a mean score of 3.93 (on a 5-point rating scale) and was produced from a 10:90 chickpea:teff blend at 14% feed moisture.

2.8 Extrusion of Sorghum and Sorghum-pulse Blends

Studies examined the effect of extrusion and extrusion conditions on the quality, nutritional content, and sensory properties of direct-expanded products from sorghum and sorghum-pulse blends.

2.8.1 Sorghum

Different studies examined the effects of extrusion temperature, moisture content, and particle size on quality and nutritional aspects of extruded sorghum snacks. Liopart *et al*. (2014) reported that extrusion did not significantly affect the proximate composition of extruded snacks from red sorghum. However, the available lysine content decreased from 4.33 g/100 g to 3.23 g/100 g protein, whereas protein digestibility increased from 53 to 70%. Cardoso *et al*. (2015) reported that phenolics level such as proanthocyanidins, flavanones, and 3-deoxyanthocyanidins of different sorghum genotypes having brown, red, and yellow pericarp colour decreased after extrusion. Vargas-Solorzano, Carvalho, Takeiti, Ascheri, and Queiroz (2014) reported that die pressure, apparent density, and water solubility index of extrudates varied depending on the pericarp colour of sorghum. Light brown genotypes, rich in tannin and fibre content, generated the lowest die pressure and sectional expansion index. Red genotypes presented the lowest specific mechanical energy and the highest water absorption index. White genotypes presented

intermediate specific mechanical energy and the highest die pressure values. These results reflected differences in starch conversion induced by the pericarp type. Al-Rabadi, Torley, Williams, Bryden, and Gidley (2011b) reported that particle size of the sorghum and barley has an effect on the water solubility index of the extrudates. Similarly, Al-Rabadi, Torley, Williams, Bryden, and Gidley, (2011a) reported faster starch digestion for fine sized particles. Escobar-Puentes *et al.* (2019) optimized barrel temperature and feed moisture content, and produced snacks using phosphorylated sorghum starch.

2.8.2 Sorghum-Peanut

Phillips and Falcone (1988) examined properties of sorghum and full-fat peanut meal blends (85:15w/w) as well as 100% sorghum extruded over a range of barrel temperatures (106-205^{0}C) and feed moistures (13-25 %) in a pilot-scale machine for an extruded snack. The product did not expand.

2.8.3 Sorghum-Cowpea and Sorghum-Cowpea-Groundnut

Falcone and Phillips (1988) examined properties of extruded snacks made from blends of a decorticated meal of sorghum and cowpeas (33:67, w/w) at 21, 23, and 25% feed moisture and 175, 190, and 205^{0}C barrel temperature and found the snacks were somewhat tougher than were commercial snacks containing corn. Asare, Sefa-Dedeh, Afoakwa, Sakyi-Dawson, and Budu (2010a) reported that the expansion ratio, bulk density, and total colour of extrudates from sorghum-groundnut-cowpea blends decreased with increasing feed moisture. The optimal conditions noted for producing puffed extruded snack products with better physical properties and functional characteristics from sorghum-groundnut-cowpea blends were 16-18% feed moisture, 14-16% cowpea, and 6-8% groundnut addition. Increasing addition of cowpea and groundnut resulted in significant increases in protein, fat, and ash (minerals - calcium, iron, and phosphorus) contents of the sorghum-legume extrudates. The increasing addition of cowpea decreased the redness of the sorghum extrudates (Asare, Sefa-Dedeh, Afoakwa, Sakyi-Dawson, & Budu, 2010b).

2.8.4 Sorghum-Soybean and Sorghum-Soybean-Horsegram

The extrudate properties of a sorghum-soy blend have been examined by different researchers. Narayan, Siddalinga, Babu, & Semwal, (2007) found the organoleptic quality of a

snack made from an 80:20 sorghum-defatted soy flour blend was better than that of a 100% sorghum snack. The protein efficiency ratio of the raw sorghum-soy blend (2.9) was found to be higher than that of the extruded snack (2.1). This might be because extrusion decreased the available lysine content. Kumar, Kumar, Chawla, and Talwar (2018) reported that the incorporation of soybean increased antinutritional factors in the raw formulation but extrusion cooking considerably reduced these factors in the extruded snack. The extent of reduction was up to 96% for trypsin inhibitor activity and 57% for phytic acid. Kumar, Samuel, Jha, and Sinha (2015) reported that sorghum-soybean extrudates with higher expansion ratio and lower bulk density were produced at 14% soy, 14% feed moisture, 129°C barrel temperature, and 422 rpm screw speed. Basediya, Pandey, Shrivastava, Khan, and Nema (2013) observed that increasing feed moisture content resulted in a higher density and lower expansion of the extrudate. Increasing barrel temperature and screw speed reduced density, and increased the expansion of extrudate. It was observed that 80:10:10 (sorghum:horse gram:defatted soy) extruded at 15% feed moisture, 130^0C barrel temperature, and 130 rpm screw speed gave the highest sectional expansion index and longitudinal expansion index.

2.8.5 Sorghum-Corn-Soybean/Whey/Legume

Sorghum and corn were blended at 6:1 and 5:2 ratios and 30% of protein sources (whey protein isolate, defatted soy flour, mixed legume flour as well as 50:50, w/w, whey protein isolate-defatted soy flour) were added and the mixture extruded. Higher expansion ratio was obtained for higher corn level in the blend. Expansion ratio of the extrudates decreased with addition of the protein sources. Extrudates with defatted soy flour had a lower expansion ratio (5.3) than whey protein isolate (7.8), legume flour (8.0) and whey protein isolate-defatted soy flour blend (6.5). The result from sensory analysis showed that the addition of protein sources increased the taste and overall acceptability of the extruded snacks, making sorghum:corn flour 5:2 with added whey protein isolate-defatted soy flour as the protein source had significantly higher ratings of consumer acceptance than did the other treatments (Devi *et al*., 2013).

2.9 Summary

Direct-expanded extruded snacks are known and liked for their puffed texture. The quality of the extruded snack depends on the composition of the raw material as well as the extrusion

conditions including feed moisture, barrel temperature, and screw speed. Various efforts have been done to increase the protein quality of cereal-based direct-expanded snacks. Considering pulses as a relatively source of protein for large segments of the world population, especially developing countries, different studies have blended pulses with cereals for extruded snack production. In this regard, chickpea has been blended with cereals and used for extruded snack production. Chickpea-maize, chickpea-rice, chickpea-wheat, chickpea-millet, and chickpea-teff were the blends used in prior studies related to direct-expanded extruded snacks. Most of the studies examined the effect of extrusion, extrusion conditions and chickpea proportion on physical properties (expansion ratio, bulk density, hardness, porosity, and crispiness), functional properties (water absorption index, water solubility index and viscosity), nutrient content (protein and carbohydrate), and digestibility and phenolics and antioxidant content.

Chickpea had relatively higher fat content (3-10%) compared to most other pulses. Direct-expanded extruded snacks are susceptible to oxidation due to their expanded surface and low water activity. However, the lipid oxidative stability of chickpea-cereal snacks has not been examined. Antioxidant activity and total phenolics contents are among the factors affecting oxidative stability. The antioxidant activity of chickpea-maize snacks only has been studied. The main reason for blending chickpea with cereals is to improve protein quality, but the protein quality of direct expanded chickpea-cereal snacks is still an area in need of research.

Both chickpea and sorghum grains are largely produced in sub-Saharan African countries such as Ethiopia, Sudan, Tanzania, Uganda, Malawi, Kenya, Zimbabwe, Niger and Togo. Considering the problems of protein-energy malnutrition and micronutrient deficiency are critical in sub-Saharan Africa, evaluating the use of a whole grain chickpea-whole grain sorghum blend for direct expanded snack production, and examining the protein quality of the snack were important.

Chapter 3

Oxidative Stability of Direct-Expanded Chickpea-Sorghum Snacks

The literature review in Chapter 2 indicated that there is a potential to use whole grain chickpea and whole grain sorghum for extruded snacks production in sub-Saharan Africa so that to tackle malnutrition. The literature review also indicated that direct-expanded snacks are susceptible to oxidation and the need to investigate oxidative stability as it affects other nutrients. As explained in Chapter 1, the overall purpose of this thesis was to investigate the oxidative stability and shelf-life of direct-expanded snack chickpea-sorghum snack and to examine the protein quality of the chickpea-sorghum snack so that to address protein-energy malnutrition issue in sub-Saharan Africa. To fulfill the purpose, the thesis was divided into three studies. This chapter presented the first study dealing with the oxidative stability of direct-expanded chickpea-sorghum snacks and tries to address objectives 1, 2 and 3 of the thesis. The objectives were to investigate oxidative stability of chickpea-sorghum snacks from chemical and descriptive sensory perspective during accelerated (55^0C) and room temperature (25^0C) storage; examine the correlations between chemical markers and sensory data during accelerated storage and determine shelf-life of chickpea-sorghum snacks. This chapter was published in *Food Science and Nutrition*.

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Esayas Kinfe Bekele designed the study, conducted the research, analyzed and interpreted the data, and prepared the first draft of this manuscript. Matthew G. Nosworthy, Carol J. Henry, Phyllis J. Shand and Robert T. Tyler reviewed and suggested edits to the first and subsequent drafts.

3.1 Abstract

In contrast to other pulses, chickpea has a relatively high fat content (3-10%). This study was designed to investigate direct-expanded chickpea-sorghum extruded snacks (50:50, 60:40 and 70:30 chickpea:sorghum, w/w) with respect to: their oxidative stability and sensory properties during accelerated (55^0C) and room temperature (25^0C) storage; correlations between chemical markers (peroxide value and *p*-anisidine value) and sensory data during accelerated storage; and

the shelf-life of snacks extruded at the optimal expansion point as determined by a rotatable central composite design. Peroxide values and *p*-anisidine values were in the range of 0-2.5 mEq/Kg and 5-30, respectively, for both accelerated and room temperature storage, and increased during storage ($P<0.05$). 70:30 and 60:40 (w/w) chickpea-sorghum snacks had higher peroxide and *p*-anisidine values compared to the 50:50 snack during storage at either temperature ($P<0.05$). Rancid aroma and off-flavour of 60:40 and 70:30 chickpea-sorghum snacks (slightly intense = 6) also were higher than that of the 50:50 snack (moderately weak = 3) ($P<0.05$). Significant correlations ($P<0.05$) were found between chemical markers and sensory attributes ($P<0.05$). The study illustrated that shelf-life decreased as the percentage of chickpea in the blend increased. Therefore, in terms of shelf-life, a 50:50 chickpea-sorghum blend is preferable.

3.2 Introduction

Ready-to-eat (RTE) foods are intended for consumption without further heating or processing. High temperature extrusion is one technique for producing RTE foods and involves mixing, kneading, cooking, compressing, and forcing a molten material under high pressure through a small opening or die. Direct-expanded extruded snacks are RTE products characterized by their puffed texture. A high expansion index and low apparent density are desirable properties of most direct-expanded extruded snacks. A variety of plant-based ingredients, including chickpea, have been used in the production of direct-expanded extruded snacks (Obradovic, Babic, Subaric, Ackar, & Jozinovic, 2014).

Chickpea (*Cicer arietinum* L.) belongs to the Fabaceae family, and is a rich source of complex carbohydrate, protein, vitamins and minerals (Costa, Queiroz-Monici, Reis, & Oliveira, 2006). The protein content of chickpea ranges from 19-27%, carbohydrate content from 52-71% (Hall *et al*., 2017) and oil content from 3-10% (Gul *et al*., 2008) on a dry weight basis. The oil content of chickpea is higher than that of most other pulses such as lentil (1.1%), red kidney bean (1.1%), field pea (1.3%), brown bean (1.4%) and turtle bean (1.6%) (Wang & Daun, 2004). The fatty acid profile of chickpea oil has been reported as palmitic acid (8-12%), palmitoleic acid (1%), stearic acid (1-5%), oleic acid (24-43%), linoleic acid (42-57%) and linolenic acid (2-4%) (Dandachy *et al*., 2019; Jukanti *et al*., 2012).

Sorghum (*Sorghum bicolor* L.) belongs to the family Poaceae and its protein content ranges from 9-17%, carbohydrate content from 77-89% and lipid content from 2-6% on a dry weight basis

(Palavecino *et al*., 2016). The fatty acid profile of sorghum oil has been reported as palmitic acid (12-15%), palmitoleic acid (1%), stearic acid (1-3%), oleic acid (34-37%), linoleic acid (42-43%) and linolenic acid (1-2%) (Afify *et al*., 2012; Zhang *et al*., 2019). Sorghum is used for human food in Africa, Asia and other semi-arid regions of the world. In contrast, in the United States and Australia, sorghum is cultivated primarily for animal feed but usage as human food is increasing (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015).

The practice of developing nutritious extruded snacks by blending different ingredients has increased as the preference for nutritious snacks has increased. However, a better understanding of the stability of snacks developed from blended ingredients is important as it affects shelf-life and nutrient content (Yadav *et al*., 2018). Lipid oxidation is one of the principal causes of the loss of nutritional and organoleptic quality of foods during storage, and a major determinant of shelf-life. Extruded products are highly susceptible to oxidation due to their low water activity and high interfacial surface area as the material is highly porous (Barden & Decker, 2016). Even a low level of fat may cause problems related to oxidation. However, the oxidative stability during storage of lipid in extruded snacks containing chickpea has not been examined. Hence, the objectives of this study were to: investigate oxidative stability and undertake descriptive sensory analysis of direct-expanded chickpea-sorghum snacks during accelerated (55^0C) and room temperature (25^0C) storage; examine correlations between chemical markers and sensory data during accelerated storage; and determine the shelf-life of chickpea-sorghum snacks. Storage at room temperature was investigated as it is a practical storage and distribution temperature for extruded snacks. Accelerated storage trials at 55^0C also were conducted to determine their usefulness for estimating the shelf-life of direct-expanded chickpea-sorghum snacks (Ng, Anderson, Coker, & Ondrus, 2007).

3.3 Materials and Methods

3.3.1 Raw Materials

Kabuli chickpea (500 kg) and sorghum (500 kg) were purchased from Diefenbaker Spice & Pulse (Elbow, SK, Canada) and Sinner Bros. & Bresnahan Food Inc. (Casselton, ND, USA), respectively. Glacial acetic acid, chloroform, methanol, sodium hydroxide, hydrochloric acid (37% w/w) and starch indicator (1% w/v aqueous solution) were purchased from Fisher Scientific (Ottawa, ON, Canada). Isooctane (2, 2, 4-trimethylpentane), potassium dichromate, potassium

iodide, *p*-anisidine, sodium thiosulfate, chymotrypsin (from bovine pancreas 4129 Type II, lyophyillized powder, P40 units/mg protein), trypsin (from bovine pancreas 4129 Type IX-S, lyophyillized powder, 13,000-20,000 BAEE units/mg) and protease (from *Streptomyces grisseus* Type XIV, P3.5 units/mg) were purchased from Sigma-Aldrich (Oakville, ON, Canada). All reagents were of analytical grade.

3.3.2 Sample Preparation

3.3.2.1 Blend Ratio Determination

Chickpea and sorghum grain were milled at the Saskatchewan Food Industry Development Centre Inc. (Saskatoon, SK, Canada) using a hammer mill (Model DAO6, The Fitzpatrick Company, Elmhurst, IL, USA) having a screen size of 0.8 mm. The particle size of the ground material ranged from 0.1-0.8 mm. To determine the optimal blend ratio, the *in vitro* protein digestibility (IVPD) and amino acid composition of raw chickpea and sorghum flours were determined as described previously by House, Hill, Neufeld, Franczyk, and Nosworthy (2019).

The IVPD and amino acid composition of 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10, w/w, chickpea-sorghum blends were determined mathematically based on the experimentally determined IVPD and amino acid composition of raw chickpea and sorghum flours (Appendix 1). The amino acid scores of the raw flours and blends were determined by comparing their amino acid compositions to that of the reference pattern specified by the United States Food and Drug Administration (CFR 21CFR101.9), which is that of a 2-5 year-old child (FAO & WHO, 1991). The IVPD Corrected Amino Acid Scores (IVPDCAAS) of the raw flours and the blends were determined by multiplying IVPD and the lowest amino acid score, as described by House *et al*. (2019). The blend ratios for chickpea-sorghum snacks were selected on the basis of theoretical IVPD corrected amino acid scores (IVPDCAAS). The 70:30 chickpea-sorghum blend was identified as where the IVPDCAAS reached a plateau and therefore was chosen for this study. For the purpose of determining the effect of blending on lipid oxidation and shelf-life, chickpea-sorghum snacks having blend ratios of 50:50 and 60:40 also were considered in the study. IVPDCAAS values for raw sorghum and raw chickpea were 27% and 74%, respectively. Calculated IVPDCAAS values for raw 50:50, 60:40 and 70:30 chickpea-sorghum blends were 58, 65 and 73%, respectively.

3.3.2.2 Extrusion

The chickpea-sorghum blends were mixed using a Dmx Quad Action 500^{TM} blender (Daniels Food Equipment, Parkers Prairie, MN, USA). Extrusion was performed using a co-rotating, twin-screw extruder (model EV-32; Clextral, Firminy, France) equipped with a volumetric feeder (Clextral VF/40/25-2) and a two-blade die face cutter, at the Saskatchewan Food Industry Development Centre Inc. The barrel length, screw diameter, and die diameter of the extruder were 768 mm, 32 mm, and 2.7 mm, respectively. The extruder barrel had six zones. The temperatures of zones 1, 2 and 3 were set at 40, 80, and 120^{0}C, respectively; the last three zones were kept at the same temperature but which was varied between 111^{0}C and 169^{0}C based on a rotatable central composite design having two centre points (Appendix 2). Feed moisture content (i.e moisture content of the flour plus moisture added) was varied between 15% and 21% and was controlled by adding water directly into the extruder barrel. Screw speed and feed rate were maintained at 396 rpm and 26 kg/h, respectively. Each sample was processed in duplicate under each processing condition. Extrudates were dried at 105^{0}C for 5 min using tunnel drier (Chromalox, Pittsburgh, PA, USA). Expansion index (EI) was determined according to the method of Meng *et al*. (2010). EI measurements were taken 10 times on extrudates from each processing run and averaged. Based on surface model regression analysis of the EI, the maximal expansion point for all blends was found to be at 169^{0}C barrel temperature and 15% feed moisture. Snacks from each of the three blend ratios produced at the maximal expansion point were used for the lipid oxidative stability study and were stored at -80^{0}C until analyzed.

3.3.3 Sample Storage for the Lipid Oxidative Stability Study

Accelerated and room temperature sample storage were conducted according to Ng et al. (2007). For each chickpea-sorghum snack (50:50, 60:40 and 70:30, w/w) produced at the maximal expansion point, four 100-g samples were heat sealed in aluminum pouches (Sigma-Aldrich) and stored at 55^{0}C (accelerated) or 25^{0}C (room temperature). Samples for accelerated storage were stored in an incubator (Forma Scientific, Marietta, OH, USA); samples were taken from the incubator for analysis at 7, 14, 21 and 28 days. Analysis of samples stored at room temperature was done every 28 days. The analysis was performed in quadruplicate.

3.3.4 Chemical Analysis

The snacks were ground using a WonderMill^{TM} grain mill (Pocatello, ID, USA) at bread setting. The particle size of the ground material was less than 1 mm. Lipid was extracted using chloroform-methanol according to Folch, Lees, and Stanley (1957) with modifications. Flours were mixed with 2:1 (v/v) chloroform-methanol and agitated using a magnetic stirrer (Fisher Scientific) at a speed of 600 rpm for 20 min. Calcium chloride solution (0.001M) was added to each sample with stirring. Each sample was filtered through No.4 Whatman filter paper (Fisher Scientific) and the filtrates were centrifuged at 490 x *g* for 10 min. The upper phase was removed using a pipette and discarded. The lower chloroform layer was evaporated and the residual lipid was used for chemical analysis. The peroxide and *p*-anisidine values of the extracted oils were determined according to American oil Chemists' Society (AOCS, 1998) official methods Cd 8-53 and Cd 18-90, respectively. Protein, ash and fatty acid contents were determined according to Association of Official Analytical Chemists (AOAC, 1997) official methods Ba 4e-93, Bc 5-49 and 969.33, respectively. Carbohydrate content was determined by difference (Honi *et al.*, 2018).

3.3.5 Descriptive Sensory Analysis

3.3.5.1 Selection and Training of Panelists

Eighteen panelists were recruited for sensory analysis through advertisements at the University of Saskatchewan, Saskatoon, SK, and via personal communication. The triangle test was carried out according to International Organization for Standardization (ISO) 4120 (2004) and 13 panelists were selected based on their ability to select oxidized product. Six days of training was provided for the selected panelists. Training of panelists and final testing of snacks were carried out according to the generic descriptive sensory analysis method as described by Lawless and Heymann (2010). Chickpea-sorghum snacks: (i) stored at 65^0C for 3 days; (ii) frozen at -80^0C and thawed at 25^0C; and (iii) stored at 65^0C for 25 days were used for the training sessions. During training, panelists identified and described perceivable product attributes, as well as attributes of reference standards on which the rating of the generated attributes was based. In cases where reference standards were not available, definitions were provided. Panelists also commented using a 10-point scale ballot based on the selected descriptors. For monitoring, panelists were provided each day with six coded samples to evaluate. They were provided with 60:40 and 70:30 chickpea-sorghum snacks that had been: (i) stored at 65^0C for seven days and coded with three digits, (ii)

frozen at -80^{0}C immediately after production and then thawed at 25^{0}C and labeled as fresh, and (iii) frozen at -80^{0}C immediately after production and then thawed at 25^{0}C and coded with three digits. The performance of panelists was determined on the basis of the scores provided while evaluating the samples. Panelists were ranked for each attribute based on the F-value and the top ten were selected for final descriptive sensory analysis.

3.3.5.2 Sample Testing

The final descriptive sensory analysis was performed by 10 trained panelists on extruded snacks stored under accelerated conditions. The sensory analysis was done at 0, 7, 14, 21 and 28 days of storage. Panelists were provided eight coded samples to evaluate: (i) stored (55^{0}C) samples of 50:50, 60:40 and 70:30 chickpea-sorghum extruded snacks, in duplicate, which were coded with three digits; (ii) a fresh (stored at -80^{0}C immediately after production) sample of the 70:30 chickpea-sorghum snack which was labeled as fresh; and (iii) a fresh (stored at -80^{0}C immediately after production) sample of the 70:30 chickpea-sorghum snack which was masked by coding with three digits. In addition to the samples, panelists were provided with fresh water and lemon water for rinsing between samples. Panelists were asked to evaluate the samples using the 10-point scale (none = 0 to extremely intense = 10) on the ballot provided (Appendix 3). The purpose of providing fresh samples was to monitor the reliability of the scores obtained from panelists. Ethical approval was obtained from the Biomedical Research Ethics Board of the University of Saskatchewan.

3.3.6 Shelf-Life Determination

To predict the shelf-life of the chickpea-sorghum snacks, a zero-order reaction for peroxide value was used (Andarwulan *et al.*, 2014). The formula for determining the order of reaction is:

$$(dA/dt) = k(A)^n \tag{3.1}$$

Integrating the above formula with n=0 provides a zero-order reaction formula:

$$(dA/dt) = k(A)^n \ (n = 0) \tag{3.2}$$

$$A_0 = A_t - kt \tag{3.3}$$

$$t = (A_t - A_0)/k \tag{3.4}$$

where t, A_t, A_0, k and n represent shelf-life in days, peroxide value in mEq/kg at storage time t, peroxide value in mEq/kg at t =0, the slope of the regression equation for peroxide value during storage in mEq/kg/day and order of reaction, respectively.

3.3.7 Statistical Analysis

Proximate composition was analyzed using one-way ANOVA. Peroxide value, *p*-anisidine value and sensory data obtained for chickpea-sorghum snacks across the storage period were analyzed by two-way ANOVA. Significant differences ($P<0.05$) between means of the parameters were determined by Fisher LSD. Regression analysis was carried out to determine the maximal expansion points and relationships between chemical markers and sensory intensities, as well as to determine the shelf-life of the snacks (Vik, 2013). Statgraphics Centurion version 18.1.12 (Statgraphics Technologies, Plains, VA, USA) was used for analysis.

3.4 Results and Discussion

3.4.1 Proximate Composition

The fat, protein, ash and carbohydrate contents of raw chickpea were determined to be 7, 20, 2.5 and 71%, respectively, on a dry weight basis. Corresponding values determined for raw sorghum were 3, 10, 1.3 and 85% on a dry weight basis. Others have reported fat contents for chickpea and sorghum ranging from 3-10% and 2-6%, respectively, and protein contents ranging from 19-27% and 6-17%, respectively (Gul *et al*., 2008; Hall *et al*., 2017; Palavecino *et al*., 2016). The fat, protein and carbohydrate contents differed ($P<0.05$) among the extruded samples (Table 3.1). The fat and protein contents of extruded snacks increased ($P<0.05$) as the ratio of chickpea in the blend increased, resulting in fat contents ranging from 5.1-5.9%, and protein contents ranging from 15-17%.

Table 3.1 Proximate composition of direct-expanded chickpea-sorghum snacks extruded at 169^{0}C barrel temperature and 15% feed moisture, expressed on a dry-weight basis

Sample	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
50:50 Chickpea-sorghum snack	15.29 ± 0.10^{c}	5.06 ± 0.16^{c}	1.94 ± 0.05^{b}	77.72 ± 0.30^{a}
60:40 Chickpea-sorghum snack	16.32 ± 0.04^{b}	5.36 ± 0.08^{b}	2.30 ± 0.14^{a}	76.29 ± 0.01^{b}
70:30 Chickpea-sorghum snack	17.46 ± 0.01^{a}	5.91 ± 0.14^{a}	2.15 ± 0.04^{ab}	74.49 ± 0.19^{c}

Data are presented as mean $\pm$ standard deviation on a dry weight basis (n=4) and were analyzed via one-Way ANOVA and the Fisher LSD post-hoc test. Samples with different letters in the same column are significantly different ($P<0.05$).

The fatty acid profiles of chickpea and sorghum seed are presented in Table 3.2. Compared to sorghum, chickpea was substantially lower in palmitic acid and somewhat higher in oleic acid and linoleic acid. Chickpea and sorghum contained similar levels of stearic acid and linolenic acid. Chickpea was higher in both total polyunsaturated fatty acids (51.3% vs. 49.6%) and in total unsaturated fatty acids (85.7 vs. 81.6%) than was sorghum. Values determined for the various fatty acids were, for the most part, within the ranges reported by others, although the sorghum sample was higher in linoleic acid and lower in oleic acid (Afify *et al*., 2012; Dandachy *et al*., 2019; Jukanti *et al*., 2012; Zhang *et al*., 2019). These differences in fatty acid profile would be attributable to differences in genotype and/or environment.

Table 3.2 Fatty acid profile of raw chickpea and raw sorghum

Fatty acid (% in total fat)	Chickpea	Sorghum
Palmitic (16:0)	$10.56 + 0.01^{c}$*	$15.63+ 0.19^{c}$
Stearic (18:0)	$1.57 + 0.01^{e}$*	$1.29 + 0.00^{e}$
Oleic (18:1n9)	$32.38 + 0.11^{b}$*	$29.81 + 0.05^{b}$
Linoleic (18:2n6)	$49.22 + 0.08^{a}$*	$47.35 + 0.19^{a}$
Linolenic (18:3n3)	$2.06 + 0.04^{d}$*	$2.26 + 0.00^{d}$

Data are presented as mean $\pm$ standard deviation (n=2) and were analyzed using two way-ANOVA with the Fisher post-hoc test. Significant differences between chickpea and sorghum are designated by the symbol *, $P<0.05$. Significant differences between fatty acids are designated by different letters, $P<0.05$. Values for fatty acids present at <1% are not shown.

3.4.2 Expansion Index

Expansion index (EI) is the ratio of the extrudate diameter to the diameter of the extruder die. Regression analysis was carried out on the expansion indices of snacks obtained from extrusion of 50:50, 60:40 and 70:30 chickpea-sorghum blends at barrel temperatures ranging from 111-169^{0}C, and moistures ranging from 15-21%. The EIs of 50:50, 60:40, and 70:30 chickpea-sorghum snacks ranged from 3.00-3.98, 3.00-3.80, and 2.70-3.30, respectively. Expansion index was analyzed in order to determine the extrusion conditions which generated optimal expansion, as snacks prepared under these conditions would be used for the shelf-life studies. Based on the regression results, temperature and moisture had significant ($P<0.05$) effects on EI (Table 3.2). Temperature increased EI, whereas moisture had the opposite effect. An earlier study reported

similar results (Lazou *et al*., 2007). It was determined that for all chickpea-sorghum blends, snacks prepared at a barrel temperature of 169^0C and a moisture content of 15% had higher EIs compared to those prepared at lower temperatures or higher moisture contents, hence snacks prepared under these conditions were used for the subsequent shelf-life studies. The specific mechanical energy (SME) of the snacks at the maximal expansion point was found to be 401 kJ/kg for the 50:50 and 60:40 chickpea-sorghum snack and 403 kJ/kg for 70:30 chickpea-sorghum, as recorded from the control panel of the extruder. The SME was not significantly different (P>0.05) among the chickpea-sorghum blends.

Table 3.3 Regression coefficients for expansion index of direct-expanded chickpea-sorghum snacks for several extrusion factors and their interactions

Terms	Coefficients		
	50:50 snack	60:40 snack	70:30 snack
A: Barrel temperature	0.09*	0.08*	0.13*
B: Feed Moisture	-0.28*	-0.23*	-0.13*
Constant	3.42*	3.43*	3.12*
AB	0.02	0.02	0.02
AA	0.03	0.04*	
BB	0.00	-0.04*	

Significance of regression coefficients was determined based on ANOVA and Fisher test (n=4). The asterisk (*) indicates significant (P<0.05) coefficients. The model R^2 values 50:50, 60:40 and 70:30 chickpea-sorghum snacks were 98, 99 and 93%, respectively, significant at P<0.05.

3.4.3 Peroxide and *p*-anisidine Values

The oxidative stability of the lipid extracted from the extruded snacks was assessed using peroxide value (Figure 3.1a and 3.1b) and *p*-anisidine value (Figure 3.1c and 3.1d). For both accelerated storage (55^0C) and room temperature storage (25^0C), peroxide values were not different (P>0.05) between extruded blends at day 0. Over time, the peroxide values of chickpea-sorghum snacks increased (P<0.05) under both storage conditions, indicating that lipid oxidation had occurred. Other studies have reported increases in peroxide value during storage of extrudates (Shahmohammadi *et al*., 2016; Shaviklo, Thorkelsson, Rafipour, & Sigurgisladottir, 2011). In the current study, the rate of peroxide development was faster in snacks stored at 55^0C than at 25^0C.

Similarly, Lee, Lee and Choe (2007) reported an enhancing effect of storage temperature on peroxide value during storage. Peroxide values for 70:30 and 60:40 chickpea-sorghum snacks were higher ($P<0.05$) than for the 50:50 blend at all storage intervals, with the exception of day 0, under both storage conditions. This was attributed to the significantly higher fat contents of the 70:30 (5.9%) and 60:40 (5.4%) blends compared to the 50:50 (5.1%) blend. In addition, chickpea was somewhat higher in linoleic acid (49.2%), oleic acid (32.4%) and total unsaturated (85.7%) and polyunsaturated (51.3%) fatty acids compared to sorghum which contained 47.4% linoleic acid, 29.8% oleic acid, 81.6% total unsaturated fatty acids, and 49.6% total polyunsaturated fatty acids (Table 3.2), making it more susceptible to oxidation. Chickpea also had lower total phenolics and tannin contents as compared to sorghum, which would further increase the vulnerability of chickpea fat to oxidation (Gaytan-Martinez *et al*., 2017; Rani & Khabiruddin, 2016). The peroxide value of the 70:30 chickpea-sorghum snack was higher ($P<0.05$) than that of the 60:40 blend at days 7, 14, 21 and 28 in the case of accelerated storage, and at day 56 in the case of room temperature storage. Again, this was to be expected due to the higher fat content of the 70:30 blend as mentioned above. However, the peroxide value of the 60:40 blend was higher ($P<0.05$) than that of the 70:30 blend at day 84 under room temperature storage, but the difference was small and probably not of practical significance.

The peroxide values of 50:50, 60:40 and 70:30 chickpea-sorghum snacks stored under accelerated conditions ranged between 0-1.7, 0-1.9 and 0-2.1 mEq/kg, respectively, during the 28-day storage period. In the case of room temperature storage, the corresponding peroxide values ranged between 0-1.4, 0-1.6 and 0-1.6 mEq/kg, respectively, over the 84-day storage period. According to Codex Alimentarius (1999) and Canadian Food and Drug Regulations (2019), the safe peroxide limit is 10 mEq/kg, whereas according to the United States Food and Drug Administration (FDA, 2019a) the safe limit is 5 mEq/kg. Therefore, peroxide values of snacks prepared from all three blends and stored at 55^0C or 25^0C were in the safe range throughout the study period, indicating that accelerated storage reflected storage at room temperature.

p-anisidine values for 50:50, 60:40 and 70:30 chickpea-sorghum snacks ranged from 8.6-21.5, 8.2-23.9 and 8.9-23.9, respectively, for accelerated storage, and 8.6-24.1, 8.2-24.7 and 14.2-25.6, respectively, for room temperature storage (Figure 3.1c and 3.1d). *p*-anisidine values were not different ($P>0.05$) among extruded blends at day 0, but increased during storage ($P<0.05$).

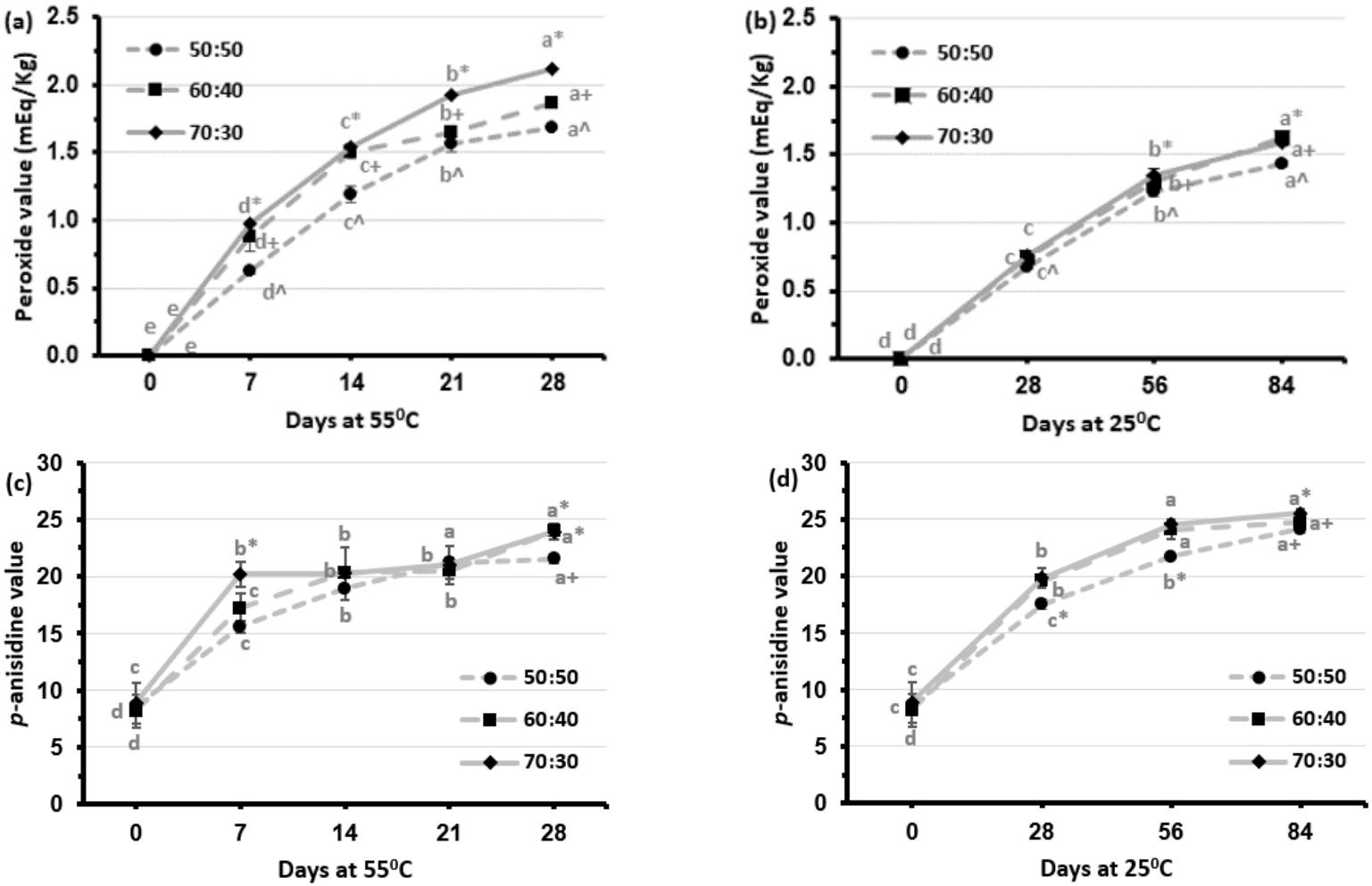

Figure 3.1 Peroxide values (a) and (b) and *p*-anisidine values (c) and (d) of direct-expanded chickpea-sorghum snacks stored at 55⁰C or 25⁰C.

Data were analyzed via two way-ANOVA with Fisher test (n=4). Significant (P<0.05) differences between days, but within blend ratios, are designated by different letters. Significant (P<0.05) differences between blend ratios, but within days, are designated by different non-alphanumeric characters

These results are similar to those of a previous study where cookies prepared with margarine showed significant increments in *p*-anisidine values during storage (Bialek, Rutkowska, Bialek, & Adamska, 2016). Fluctuation in *p*-anisidine values can occur when the carboxylic acid group and C=C bond structures are involved in the formation of aldehydes, ketones and alcohols that contribute to the *p*-anisidine value (Sun-Waterhouse, Thakorlal, & Zhou, 2011). This might explain why the *p*-anisidine values for chickpea-sorghum snacks did not exhibit regular increments during accelerated storage. In the case of room temperature storage, however, the reaction rates of carboxylic acid groups and C=C bond structures would have been lower at the lower temperature.

In the case of accelerated storage, *p*-anisidine values were different ($P<0.05$) among 50:50, 60:40 and 70:30 chickpea-sorghum snacks at days 7 and 28. The *p*-anisidine value of the 70:30 blend (20.2) was higher ($P<0.05$) than that of the 50:50 (15.8) or the 60:40 (17.2) blend at day 7, whereas those of both the 60:40 (23.9) and 70:30 (23.9) blends were higher ($P<0.05$) than that of the 50:50 blend (21.5) at day 28. As was the case for peroxide value, differences in the *p*-anisidine value among 50:50, 60:40 and 70:30 chickpea-sorghum extruded snacks during storage would reflect differences in their fat contents and fatty acid profiles, as well as the total phenolics and tannin contents of the blends.

In the case of room temperature storage, the *p*-anisidine values of 50:50, 60:40 and 70:30 chickpea-sorghum snacks increased ($P<0.05$) during the storage period. The *p*-anisidine values of 60:40 and 70:30 chickpea-sorghum blends showed increases with storage time until day 56 ($P< 0.05$). The *p*-anisidine values of both the 60:40 and 70:30 blends were higher ($P<0.05$) than that of the 50:50 blend at days 28 and 56. The rate of increase in the *p*-anisidine value was higher in snacks stored under accelerated conditions than at room temperature. Lee *et al*. (2007) reported that the rate of increase in the *p*-anisidine value increased with storage temperature, indicating both temperature and time dependence of lipid oxidation. The rate of increase in the *p*-anisidine value began to level off during the latter part of the storage period in the current study. This might be due to declining levels of the most readily oxidizable fatty acids in the snacks, linolenic and linoleic acids in particular.

3.4.4 Sensory Analysis

The reference standards and definitions used for sensory evaluation are listed in Table 3.3. Sensory evaluation was undertaken on snacks stored under accelerated conditions only, due to the

time constraints of the trained panelists. Sensory attribute intensities over the storage period are described in Figures 3.2 and 3.3. Rancid aroma intensity scores for stored 50:50, 60:40 and 70:30 chickpea-sorghum snacks ranged from 3.0-4.5, 3.0-5.1 and 3.3-5.6, respectively (Figure 3.2a). Rancid aroma intensity was approximately 3.0 (moderately weak) for all samples at day 0 and day 7, but the rancid aroma intensities of stored 60:40 and 70:30 chickpea-sorghum snacks were significantly higher ($P<0.05$) than that of the 50:50 chickpea-sorghum snack at days 14, 21 and 28. The rancid aroma intensity score of the 70:30 blend was 4.1 (slightly weak), 5.3 (neither intense nor weak) and 5.6 (slightly intense) at days 14, 21 and 28, respectively, whereas the corresponding scores for the 50:50 blend were 2.7 (moderately weak), 4.0 (slightly weak) and 4.5 (neither intense nor weak). The rancid aroma intensity scores for the 60:40 chickpea-sorghum blend at days 14, 21 and 28 were 3.2 (moderately weak), 4.5 (neither intense nor weak) and 5.1 (neither intense nor weak), respectively. During storage, the rancid aroma intensity scores for all chickpea-sorghum snacks were higher ($P<0.05$) than those of the fresh samples, indicating advancement of lipid oxidation. Rancid aroma intensity was higher ($P<0.05$) for all stored chickpea-sorghum snacks at days 21 and 28 as compared to days 0 and 7, again indicating the progression of lipid oxidation.

Rancid flavour intensity scores for stored 50:50, 60:40 and 70:30 chickpea-sorghum snacks ranged from 2.3-2.9, 2.5-4.7 and 3.0-4.3, respectively (Figure 3.2b). While initially moderately weak for all samples, the rancid flavour intensity of the stored 70:30 snack (3.8) was higher ($P<0.05$) than that of stored 50:50 (2.7) and 60:40 (3.1) chickpea-sorghum snacks and fresh samples (2.4) at day 14. At day 28, rancid flavour intensities of stored 60:40 (4.1) and 70:30 (4.5) snacks were higher ($P<0.05$) than those of the stored 50:50 (2.9) chickpea-sorghum snack and fresh samples (2.7). Rancid flavour intensites of 60:40 and 70:30 chickpea-sorghum snacks on day 28 were higher ($P<0.05$) than at day 0. Similar to this study, Rababah *et al*. (2011) reported significant development of rancidity and off-flavour with storage time in extruded corn chips.

In the current study, off-flavour intensity was not significant ($P>0.05$) among extruded snacks at 0 days and 7 days of storage (Figure 3.3a). The stored 70:30 chickpea-sorghum snack scored higher ($P<0.05$) in off-flavour at days 14, 21 and 28 compared to the fresh sample, as well as the 50:50 chickpea-sorghum snack. On day 28, stored 50:50, 60:40 and 70:30 chickpea-sorghum snacks scored higher ($P<0.05$) in off-flavour compared to the fresh samples. The higher scores for rancid flavour and off-flavour for 60:40 and 70:30 chickpea-sorghum snacks compared to the 50:50 snack during storage again reflected the fat contents of the snacks.

Initially, there were no differences in roasted aroma among the chickpea-sorghum snacks (Figure 3.3b). However, from day 7 to day 28, all stored chickpea-sorghum snacks scored lower ($P<0.05$) in roasted aroma intensity than did fresh samples. Franklin *et al*. (2018) reported a reduction in roasted aroma intensity with storage time in roasted almonds, due perhaps to the increasing presence of volatiles arising from lipid oxidation.

There was no difference ($P>0.05$) in stickiness, dissolvability or bitterness (due to mono- and diglycerides) across samples or over time. Rancidity in stored snacks was detected by panelists even though the peroxide values of the snacks, in general, were below the regulated limit (5 mEq/Kg). Zajdenwerg, Branco, Alamed, Decker, & Castro (2011) reported that sensory detection of oxidized lipid in nut occurred before identification of changes in chemical markers. Overall, the 50:50 chickpea-sorghum snack was found to be the most stable based on sensory ratings provided.

Table 3.4 Sensory attributes evaluated for direct-expanded chickpea-sorghum snacks and corresponding reference standards and definitions employed

Attributes	Reference standards	Definitions*
Rancid aroma	Corn oil heated at 240^0C for 10 min	Aroma of strongly oxidized oil
Rancid flavour	Corn oil heated at 240^0C for 10 min	Flavour of strongly oxidized oil
Roasted aroma	Roasted chickpea	Aroma from roasted chickpea
Roasted flavour	Roasted chickpea/roasted barley	Aroma from roasted chickpea/barley
Off-flavour	-	Presence of uncharacteristic flavour notes
Hardness	Cheesy puffs	Force applied by molar teeth to compress the food
Crispiness	Rice crisps	Louder and high-pitched noise from food during mastication
Stickiness	Cheesy puffs	Degree of attachment to the teeth
Dissolvability	Cheesy puffs	Time the food stays in the mouth
Bitterness	Boiled coffee (1 g/5 mL)	Taste on the tongue associated with bitter solutions such as caffeine
Overall flavour	-	Any flavour notes coming from the food in the mouth

* Adapted from Lawless and Heymann (2010)

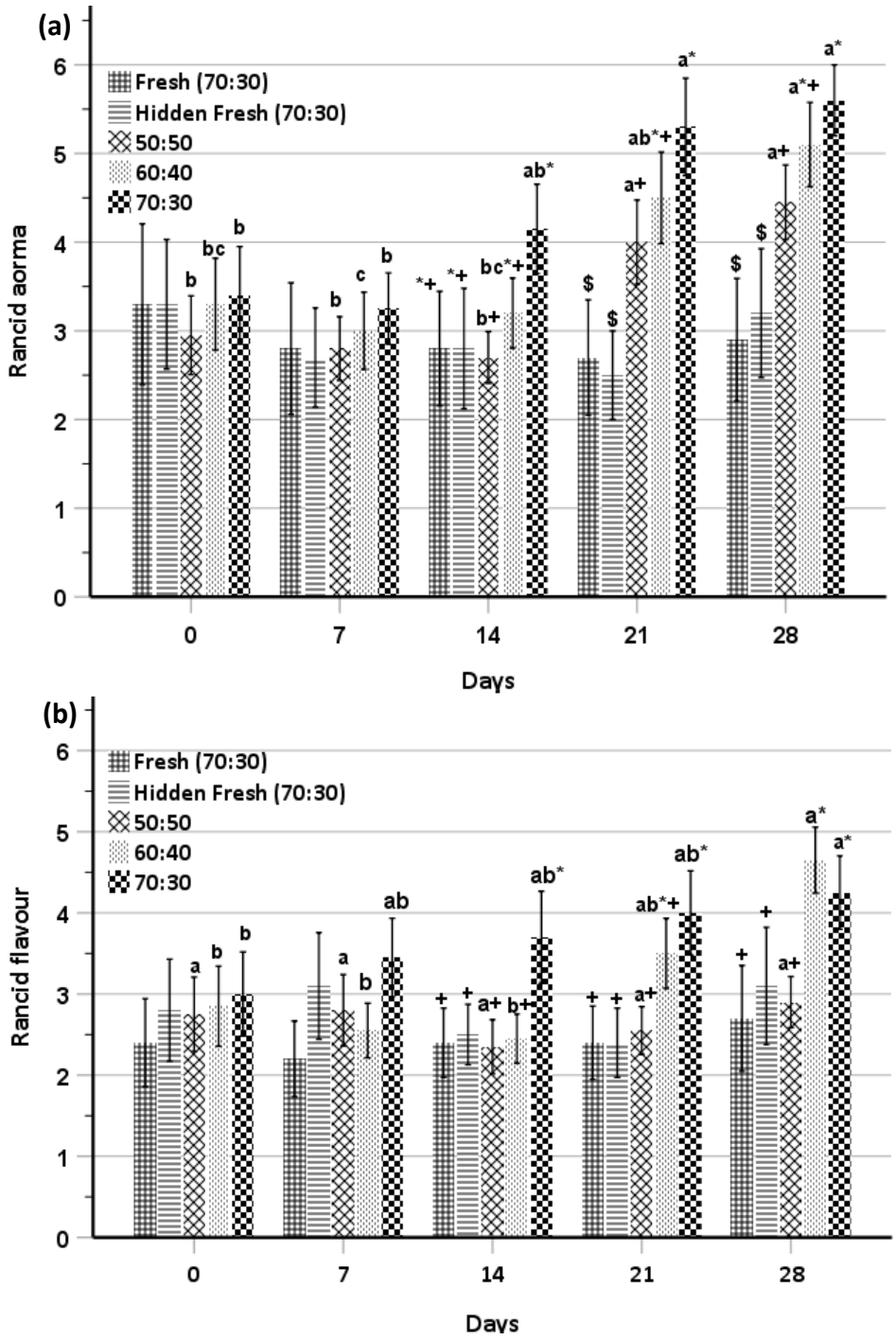

Figure 3.2 (a) Rancid aroma and (b) rancid flavour intensities for direct-expanded chickpea-sorghum snacks stored at 55^0C.

Data were analyzed using two way-ANOVA with Fisher test (n=4). Significant ($P<0.05$) differences between days, but within blend ratios, are designated by different letters. Significant ($P<0.05$) differences within days are designated by different non-alphanumeric characters. Intensity scale ranging from 0 = none to 10 = extremely intense was used. The number of panelists involved was 10.

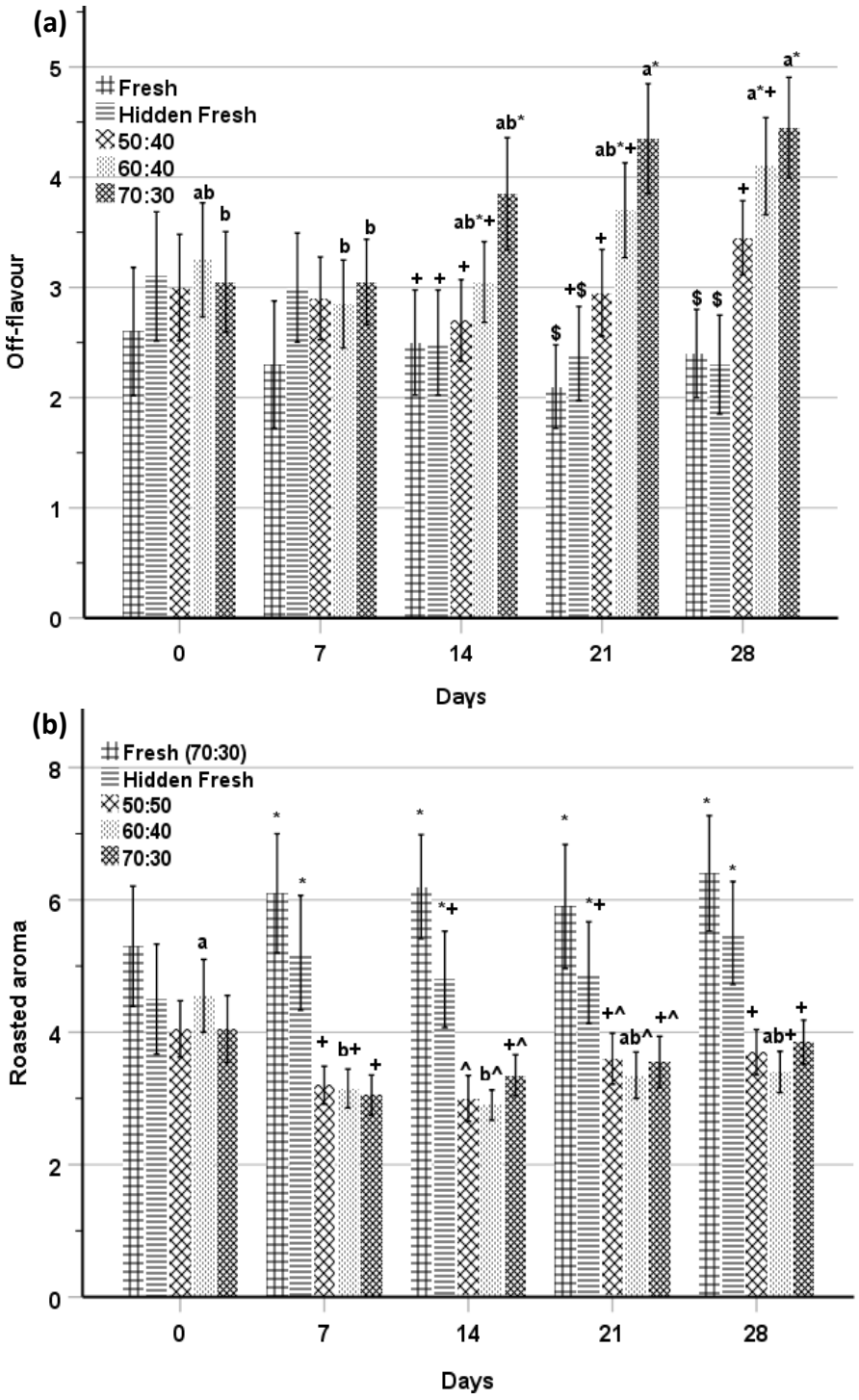

Figure 3.3 (a) Off-flavour and (b) roasted aroma intensities for direct-expanded chickpea-sorghum snacks stored at 55^{0}C.

Data were analyzed using two way-ANOVA with Fisher test (n=4). Significant (P<0.05) differences between days, but within blending ratios, are designated by different letters. Significant (P<0.05) differences within days are designated by different non-alphanumeric characters. Intensity scale ranging from 0 = none to 10 = extremely intense was used. The number of panelists involved was 10

3.4.5 Correlations

Linear and quadratic correlations between sensory attributes and chemical markers (peroxide value and *p*-anisidine value) were determined. Both correlations were found to be significant ($P<0.05$) in most cases. However, in a few cases, only quadratic correlations were significant. Therefore, quadratic correlations only are presented in Table 3.4. Peroxide values of 50:50, 60:40 and 70:30 chickpea-sorghum snacks showed significant ($P<0.05$) positive correlations with the rancid aromas of the corresponding snacks, with R^2 values of 0.86, 0.69 and 0.93, respectively. Peroxide values of 60:40 and 70:30 snacks showed significant ($P<0.05$) positive correlations with rancid flavour (R^2= 0.72 and 0.73, respectively) and off-flavour (R^2= 0.74 and 0.80, respectively). The *p*-anisidine values of 50:50 and 70:30 chickpea-sorghum snacks exhibited significant ($P<0.05$) positive correlations with rancid aroma, with R^2 values of 0.85 and 0.83, respectively (Table 3.4). The *p*-anisidine value also exhibited a significant ($P<0.05$) positive correlation with rancid flavour of the 60:40 (R^2 = 0.78) and 70:30 (R^2 = 0.69) snacks, and off-flavour of the 70:30 chickpea snack (R^2 = 0.77). Peroxide values and *p*-anisidine values were found to be significantly ($P<0.05$) and positively correlated for 50:50, 60:40 and 70:30 snacks, with R^2 values of 0.99, 0.87 and 0.94, respectively.

Zajdenwerg *et al.* (2011) reported that peroxide and *p*-anisidine values had significant positive, linear correlations with oxidized aroma. This again indicates that trends in peroxide and *p*-anisidine values can be indicative of what will happen to the intensity over time of sensory attributes such as rancidity and off-flavour. Correlation analysis also was performed for the sensory attributes (Table 3.4). The rancid aroma of 60:40 and 70:30 chickpea-sorghum snacks was found to have a significant ($P<0.05$) positive correlation with off-flavour (R^2 = 0.89 and 0.88, respectively) and rancid flavour (R^2 = 0.82 and 0.71, respectively). Scatter plots also were done for the correlations (Appendix 4).

3.4.6 Determination of Shelf-Life

Peroxide value was selected as an indicator of shelf-life for the chickpea-sorghum snacks stored under accelerated conditions or at room temperature, since a maximum safe limit (5 mEq/Kg) is recognized by the United States Food and Drug Administration (FDA, 2019a). Slopes of regression equations of peroxide values for 50:50, 60:40 and 70:30 chickpea-sorghum snacks stored at 55^0C and 25^0C were determined and used in rate equation (3.4).

Table 3.5 Coefficients of determination (R^2) for quadratic correlations between sensory and chemical markers for direct-expanded chickpea-sorghum snacks

	Off-flavour			Rancid flavour			Roasted aroma			Rancid aroma			Peroxide		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Dissolvability	**0.65***	0.01	0.20	0.79	0.01	0.34	0.25	0.03	0.39	0.27	0.03	0.21	0.04	0.00	0.20
Bitterness	0.25	0.52	0.26	0.36	0.11	0.01	**0.59***	0.24	0.06	0.42	0.25	0.00	**0.63***	0.30	0.19
Overall flavour	**0.71***	**0.64***	**0.60***	0.40	0.36	0.57	0.13	0.00	0.20	0.00	0.49	0.54	0.09	0.29	0.32
p-anisidine	0.16	0.54	**0.77***	0.05	**0.78***	**0.69***	0.45	**0.87***	0.36	**0.85***	0.53	**0.83***	**0.99***	**0.87***	**0.94***
Roasted flavour	0.13	0.08	0.31	0.33	0.07	0.41	0.13	**0.62***	0.32	0.02	0.09	0.29	0.21	0.67	0.46
Peroxide	0.20	**0.74***	**0.80***	0.13	**0.72***	**0.73***	0.49	**0.89***	**0.71***	**0.86***	**0.69***	**0.93***			
Rancid aroma	0.16	**0.89***	**0.88***	0.10	**0.82***	**0.71***	0.37	0.03	0.04						
Roasted aroma	0.26	0.45	0.36	0.71	0.26	0.18									
Rancid flavour	0.51	**0.71***	**0.59***												

Chickpea-sorghum blends are represented as follows: a=50:50, b=60:40, c=70:30. Significance of the coefficients were analyzed by ANOVA (N=4) with Fisher test. The asterisk (*) indicate significant correlation.

The safe peroxide limit (5 mEq/kg) was substituted for peroxide value at storage time t and an initial peroxide value of zero was substituted for peroxide value at storage t = 0. The slopes obtained from the regression equations for peroxide values and used in equation (3.4) were 0.062, 0.064 and 0.074 for 50:50, 60:40 and 70:30 snacks, respectively, stored under accelerated conditions, and 0.017, 0.019 and 0.019, respectively, for the corresponding snacks stored at room temperature. The shelf-lives of 50:50, 60:40 and 70:30 chickpea-sorghum snacks stored at room temperature (25^0C) were determined to be 9.8, 8.8 and 8.8 months, respectively; corresponding shelf-lives for accelerated storage (55^0C) were determined to be 2.7, 2.6 and 2.3 months, respectively. The calculated shelf-life of the 50:50 snack was higher ($P<0.05$) than those of 60:40 and 70:30 snacks under accelerated conditions, whereas the shelf-lives of both 50:50 and 60:40 snacks were higher ($P<0.05$) than that of the 70:30 snack when stored at room temperature. This indicates that the 50:50 snack in general was more stable compared to the 60:40 and 70:30 snacks. According to Honi *et al*. (2018), extruded snacks made from orange-fleshed sweet potato and Bambara groundnuts had shelf-lives ranging from 4-5 months at room temperature depending on the percentage of groundnut used, which varied the total fat content from 6-12%. Similar to the current study, as the proportion of the higher fat component (Bambara nut) in the snack increased, the shelf-life decreased. The longer shelf-life of the chickpea-sorghum snacks stored at room temperature compared to extruded sweet potato/groundnut snacks (Honi *et al*. 2018) reflected differences in both fat content and degree of unsaturation. The shelf-life of extruded products depends on both composition and storage conditions. In this study, the sensory score for rancid aroma intensity of chickpea-sorghum snacks stored under accelerated conditions was between 4 (moderately weak) and 6 (neither intense nor weak) on day 28 using a 10-point scale. This indicates that the chickpea-sorghum snacks have a high probability of being rancid toward the end of the calculated shelf-life (68-81 days), thus the sensory data is in agreement with the experimentally determined shelf-life values.

3.5 Conclusions

Through analysis of peroxide value, the shelf-life during room temperature storage was calculated to be longer for the snack prepared from a 50:50 chickpea-sorghum blend than for snacks prepared from 60:40 and 70:30 blends. Shelf-lives for snacks prepared from both 50:50 and 60:40 blends were longer than for snacks prepared from a 70:30 blend under accelerated storage

conditions. Similarly, the *p*-anisidine values for both storage conditions indicated that the 50:50 snack was more stable than that prepared from 70:30 chickpea-sorghum blend. Trained sensory panelists provided corroborating evidence in that rancid aroma, rancid flavour and off-flavour increased during accelerated storage, but the increase was least for the 50:50 blend. These data indicate, from a nutritional perspective, the optimal blend ratio for chickpea-sorghum snacks was 70:30 chickpea-sorghum, whereas shelf-life and consumer acceptability after storage were maximized at a blend ratio of 50:50. Accelerated storage testing was useful to reinforce the results of room temperature storage where the 50:50 chickpea-sorghum snack was more stable than the 70:30 chickpea-sorghum snack. The study demonstrated the potential of using whole grain chickpea and whole grain sorghum blends for the production of direct-expanded snacks having acceptable sensory and shelf-life characteristics.

Chapter 4

Antioxidant Capacity and Total Phenolics Content of Direct-Expanded Chickpea-Sorghum Snacks

The previous chapter provided the findings on the oxidative stability of direct-expanded chickpea-sorghum snacks from chemical and sensory perspective, and it also provided shelf-life of chickpea-sorghum snacks. This chapter will give a chance to look at oxidative stability of chickpea-sorghum snacks from an antioxidant perspective. Antioxidants are one of the critical factors which affect oxidative stability and shelf-life of chickpea-sorghum snacks. This chapter presented the second study dealing with antioxidant capacity and total phenolic content of chickpea-sorghum snacks and tries to address objective 3 of the thesis. The objective was to investigate the effect of extrusion (raw vs. extruded), extrusion conditions (barrel temperature and moisture content), extraction solvent, and chickpea:sorghum blend ratio on the antioxidant capacity and total phenolics content of direct-expanded chickpea-sorghum extrudates. This chapter has been submitted for publication.

Bekele, E. K., Nosworthy, M. G, Tyler, R. T, & Henry, C. J. (2020). Antioxidant capacity and total phenolics content of direct-expanded chickpea-sorghum snacks *(submitted)*.

Esayas Kinfe Bekele designed the study, conducted the research, analyzed and interpreted the data, and prepared the first draft of this manuscript. Matthew G. Nosworthy, Carol J. Henry and Robert T. Tyler reviewed and suggested edits to the first and subsequent drafts.

4.1 Abstract

This study examined the effect of high temperature extrusion on the antioxidant capacity (DPPH radical inhibition percentage and ABTS Trolox equivalents) and total phenolics content of direct-expanded chickpea-sorghum snacks. The effect of the extraction solvent on the antioxidant capacity (DPPH radical inhibition percentage) detected also was determined. Chickpea-sorghum blends (50:50, 60:40 and 70:30, chickpea:sorghum, w/w) were extruded at 10 combinations of moisture content (16, 18 and 20%) and barrel temperature (120, 140 and 160^0C) and at 169^0C and 15% moisture, the conditions at which maximum extrudate expansion was observed. Chickpea

and sorghum flours were extruded at 169^0C and 15% moisture for comparative purposes. The DPPH radical inhibition percentages of acetone-water and ethanol-water extracts were higher ($P<0.05$) than those of hexane extracts. Total phenolics contents ranged from 1-7 mg gallic acid/g depending on extrusion conditions. Extrusion, higher barrel temperature and a larger proportion of sorghum in the blend increased ($P<0.05$) DPPH radical inhibition percentage, Trolox equivalents and total phenolics content, whereas higher moisture content decreased ($P<0.05$) these values. The study illustrated that a 50:50 chickpea:sorghum blend extruded at 160^0C barrel temperature and 16% moisture content was preferable in terms of antioxidant capacity and total phenolics content.

4.2 Introduction

Antioxidants are compounds that prevent or slow the onset of oxidation. Multiple categories of natural antioxidants are found in foods, including phenolic compounds, ascorbic acid, carotenoids, phospholipids, sterols, tocotrienols and tocopherols (Choe & Min, 2009). Among these, phenolics form the largest group (Korus *et al*., 2007).

Chickpea (*Cicer arietinum* L.) is the second most widely grown pulse globally, with global production of 17 million tonnes in 2018 (FAO, 2019), and is a rich source of complex carbohydrates, proteins, vitamins and minerals (Bar-El Dadon, Abbo, & Reifen, 2017). Chickpea contains phenolic compounds categorized as hydroxybenzoic acids, hydroxycinnamic acids and flavonoids, all of which have antioxidant properties (Mekky *et al*., 2015). The total phenolics content of chickpea ranges from 1-19 mg gallic acid equivalents/g, the largest proportion of which is found in the seed coat (Rani & Khabiruddin, 2016). Chickpea seeds having colorful seed coats are reported to possess higher levels of antioxidant activity (Segev *et al*., 2010).

Sorghum (*Sorghum bicolor* L.) is the fifth most widely grown cereal crop, with global production of 59 million tonnes in 2018 (FAO, 2019). Sorghum contains phenolic compounds, including phenolic acids, flavonoids and condensed tannins, that exhibit antioxidant activity (Alfieri, Balconi, Cabassi, Habyarimana, & Redaelli, 2017). The total phenolics content of sorghum has been reported to range from 1-38 mg gallic acid equivalents/g. Phenolics are the greatest contributor to total antioxidant capacity in sorghum (Dykes *et al*., 2014).

Extrusion is a common technique used to produce snack foods. Extruded snacks made from combinations of whole grain pulse and whole grain cereal are beneficial due to their high protein quality (Pastor-Cavada *et al*., 2013) and their probable association with a reduced risk of diabetes,

obesity and cancer (Espinoza-Moreno *et al*., 2016). Evaluation of antioxidant capacity in whole grain chickpea-sorghum extrudates is important due to the relatively high fat content of chickpea (3-10% on a dry weight basis) and whole grain sorghum, which affects shelf-life (Bekele, Nosworthy, Henry, Shand, & Tyler, 2020; Gul *et al*., 2008). Chickpea and sorghum also contain substantial levels of polyunsaturated fatty acids that are readily oxidized and affect shelf-life (Afify *et al*., 2012; Dandachy *et al*., 2019; Jukanti *et al*., 2012; Zhang *et al*., 2019). Even a low level of fat may cause problems related to oxidation in extruded snacks due to their low water activity and large surface area (Rogalski & Szterk, 2015). Lipid oxidation is one of the primary causes of food nutritional quality loss due to a reduction in polyunsaturated fatty acids and generation of free radicals that interact with other food components (Paradiso *et al*., 2009).

This study was designed to investigate the effect of high temperature extrusion (raw vs. extruded, blend ratio, moisture content, barrel temperature) on the antioxidant capacity (DPPH radical inhibition percentage and ABTS Trolox equivalents) and total phenolics content of direct-expanded, whole grain, chickpea-sorghum extrudates. The effect of the extraction solvent (acetone-water, 80:20 v/v; ethanol-water, 70:30 v/v; hexane) on the antioxidant capacity (DPPH radical inhibition percentage) of extracts also was determined due to known polarity differences among antioxidants (Xu & Chang, 2007).

4.3 Materials and Methods

4.3.1 Raw Materials

Kabuli chickpea (500 kg) and sorghum (500 kg) were purchased from Diefenbaker Spice & Pulse (Elbow, SK, Canada) and Sinner Bros. & Bresnahan Food Inc. (Casselton, ND, USA), respectively. Acetone, ethanol, hexane and methanol were purchased from Fisher Scientific (Ottawa, ON, Canada). Folin-Ciocalteu reagent, gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate and sodium carbonate were purchased from Sigma-Aldrich (Oakville, ON, Canada). All reagents were of analytical grade.

4.3.2 Blend Ratio Determination and Extrusion

Blend ratio selection was as described by Bekele *et al.* (2020). A 70:30 (w/w) chickpea-sorghum blend was determined to be optimal on the basis of its high *in vitro* Protein Digestibility

Corrected Amino Acid Score (IVPDCAAS), and 50:50 and 60:40 chickpea:sorghum blends were evaluated for comparison purposes. The blends were extruded in a co-rotating, twin-screw extruder (Model EV-32; Clextral, Firminy, France) having barrel length, screw diameter and die diameter of 768 mm, 32 mm and 2.7 mm, respectively. Extrudates were prepared at combinations of barrel temperatures of 120, 140 and 160^0C and moisture contents of 16, 18 and 20%, as well as at the maximal expansion point for the three blends, 169^0C and 15%, for a total of 10 combinations of processing parameters. Chickpea and sorghum flours also were extruded at the maximal expansion point for comparative purposes. The maximal expansion point was determined as described by Bekele *et al.* (2020). Extrudates were dried at 105^0C for 5 min using a tunnel drier (Chromalox, Pittsburgh, PA, USA). Each sample was extruded in duplicate for each treatment combination.

4.3.3 Extraction of Antioxidants

Extrudates were ground using a WonderMill^{TM} grain mill (Pocatello, ID, USA) at bread setting, resulting in a flour particle size of less than 1 mm. Antioxidants were extracted from raw and extruded flours and blends according to Dar, Sharma, and Nayik, (2016), with modifications. Samples (1.25 g) were mixed with 10 mL of each of three solvents (acetone-water, 80-20, v/v; ethanol-water, 70-30, v/v; hexane) in centrifuge tubes, which then were placed in a water bath at 45^0C for 20 min. The samples then were centrifuged at 4410 X g for 10 min. The supernatant was transferred to a test tube for analysis.

4.3.4 Antioxidant Capacity Analysis

4.3.4.1 DPPH Radical Scavenging Activity

The free radical scavenging activity of antioxidants extracted from samples with acetone-water (80:20 v/v), ethanol-water (70:30 v/v) or hexane was determined as percent inhibition of the DPPH radical according to Dar *et al*. (2016). Two milliliters of DPPH solution (0.0025 g of DPPH in 100 mL of methanol) was mixed with 20 µL of supernatant. After a 30-min incubation at room temperature, the absorbance of the solution was measured at 517 nm. The percent inhibition of DPPH was calculated using the equation:

$$Inhibition\% = [(Absorbance\ T_0 - Absorbance\ T_{30\ min})/Absorbance\ T_0]\ X\ 100 \qquad (4.1)$$

4.3.4.2 ABTS Radical Scavenging Activity

The free radical scavenging capacity of antioxidants extracted from the samples was determined by the ABTS radical cation decolorization assay according to Dudonne, Vitrac, Coutiere, Woillez, and Merillon (2009). Only ethanol-water (70:30 v/v) extracts were used for the ABTS assay, based on the DPPH results obtained in this study and considering the positive and significant correlation between DPPH assay results and ABTS assay results reported in other studies (Piluzza & Bullitta, 2011; Tomsone & Kruma, 2013). The ABTS radical cation (ABTS+•) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (1:1, v/v) and allowing the mixture to stand for 12-16 h in the dark at room temperature. The ABTS+• solution was diluted with ethanol (1:90, v/v) to an absorbance of 0.7 ($\pm$0.02) at 734 nm. Three milliliters of ABTS+• solution was mixed with 20 µL of extracted supernatant or Trolox standard, incubated for 10 min at room temperature, and read at 734 nm. The Trolox equivalent values of the samples were determined from a calibration curve of Trolox absorbance vs. concentration.

4.3.5 Determination of Total Phenolics Content

The total phenolics contents of the samples were determined using the Folin-Ciocalteu assay according to Dar *et al*. (2016). One milliliter of ethanol-water (70:30 v/v) extract or gallic acid standard solution (1, 5, 10, 20, 40, 60, 80 and 100 mg/L) was diluted with 9 mL of deionized water and mixed with 1 mL of Folin-Ciocalteu phenol reagent. The solution was allowed to stand for 5 min at room temperature and then 10 mL of sodium carbonate solution (7% w/v) was added. The solution was diluted to 25 mL with deionized water and allowed to stand at room temperature for 90 min. A reagent blank using deionized water was prepared in parallel with the sample. The absorbance against the reagent blank was determined at 750 nm. Total phenolics content was expressed as mg gallic acid equivalents per g of sample (mg GAE/g).

4.3.6 Statistical Analysis

The effect of extrusion (raw vs. extruded) and extraction solvent on antioxidant capacity was analyzed using two-way ANOVA, as was the effect of extrusion on total phenolics content. The effects of barrel temperature, moisture content and blend ratio on DPPH radical inhibition, Trolox equivalents and total phenolics content were analyzed using three-way ANOVA. Significant differences ($P<0.05$) between means were determined by Fisher LSD (Vik, 2013). Statgraphics version 18.1.12 (Statgraphics Technologies, Plains, VA, USA) was used for analysis.

4.4 Results and Discussion

4.4.1 Effects of Extraction Solvent, Extrusion and Blend Ratio on Antioxidant Capacity

Extraction solvent, blend ratio and extrusion were found to have significant ($P<0.05$) effects on DPPH radical inhibition percentage (Figure 4.1). All two-way and three-way interactions were significant ($P<0.05$).

In the case of both raw and extruded samples, DPPH radical inhibition percentages of acetone-water and ethanol-water extracts were higher ($P<0.05$) than that of the hexane extract from the corresponding chickpea-sorghum blend ratio. The DPPH radical inhibition percentage of the acetone-water extract was higher ($P<0.05$) than that of the ethanol-water extract from the corresponding blend ratio. This was due to the polarity of acetone-water and ethanol-water resulting in significantly higher extraction efficiencies compared to hexane (Boeing *et al*. 2014). Nicacio *et al.* (2017) reported that higher values for antioxidant activity were obtained when more polar solvents were used for extraction. Xu and Chang (2007) reported that acetone-water 80:20 (v/v) and ethanol-water 70:30 (v/v) were the best extraction solvents for the determination of the antioxidant activity of chickpea. In comparison to ethanol-water, acetone-water has been reported to be a more effective solvent for extraction of phenols or tannins from protein matrices, as acetone-water degrades polyphenol-protein complexes (Meneses *et al*., 2013). This may explain the higher DPPH radical inhibition percentages observed for acetone-water extracts in this study. For both raw and extruded samples, the DPPH radical inhibition percentages of chickpea-sorghum blends increased ($P<0.05$) with an increase in the proportion of sorghum in the blend, for both acetone-water and ethanol-water extracts. This reflected the substantially higher ($P<0.05$) content of phenolic compounds in sorghum compared to chickpea (Table 4.2). The DPPH radical inhibition percentages of hexane extracts ($P<0.05$) decreased with an increasing proportion of sorghum in the blend, presumably due to the low solubility of phenolics in hexane.

The DPPH radical inhibition percentages of ethanol-water and acetone-water extracts of chickpea-sorghum blends, chickpea and sorghum increased ($P<0.05$) after extrusion at the maximal point (Figure 4.1). This may be due to the release of bound phenolic compounds or generation of Maillard reaction products during extrusion (Ortiz-Cruz *et al*., 2020; Zilic *et al*., 2014). Other studies have reported a significant increase in DPPH radical inhibition percentages of ethanol-water (80:20 v/v) and methanol-water (80:20 v/v) extracts after extrusion of ginseng powder and sorghum bran (Gui & Ryu, 2014; Lopez *et al.*, 2016).

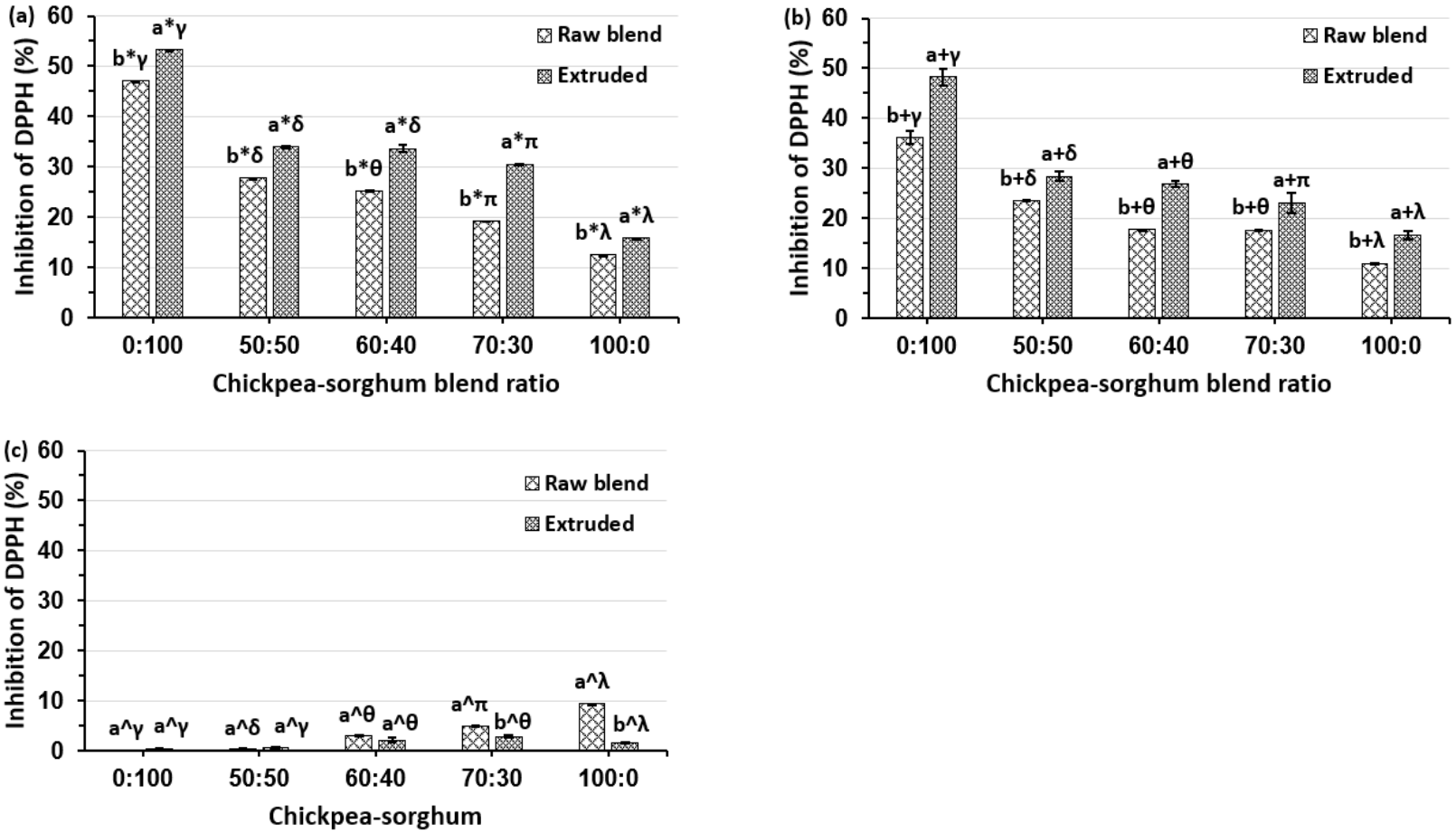

Figure 4.1 DPPH radical inhibition of (a) acetone-water (80:20 v/v), (b) ethanol-water (70:30 v/v) and (c) hexane extracts of chickpea and sorghum flours and chickpea-sorghum blends (w/w) and extrudates.

Data were analyzed via three way-ANOVA with Fisher post-hoc test (n=4). Differences ($P<0.05$) between raw flours/blends and extrudates, but within the same blend ratio and solvent, are labelled with English letters. Differences ($P<0.05$) between solvents, but within the same blend ratio and processing type, are designated by non-alphanumeric characters. Differences ($P<0.05$) between blend ratios, but within the same processing type and solvent, are labeled with Greek letters. The extrusion temperature and moisture content were 169^0C and 15%, respectively.

Interestingly, hexane extracts exhibited lower ($P<0.05$) DPPH radical inhibition percentages after extrusion (Figure 4.1). This might be due to the degradation of hexane-soluble antioxidants such as tocopherols, tocotrienols and phytosterols with the heating from extrusion (Hidalgo & Brandolini, 2010). Sasidharan and Menon (2011) also reported that total antioxidant activity of hexane extracts of curry leaf decreased with an increase in extraction temperature.

Antioxidant capacity also was measured using the ABTS method with Trolox as standard and ethanol-water as extraction solvent. Trolox equivalents of extracts from raw 50:50, 60:40 and 70:30 chickpea-sorghum blends and raw chickpea and raw sorghum were 33, 22, 21, 10 and 58 mmole/g Trolox equivalents, respectively. The corresponding values after extrusion at the maximal expansion point were 38, 27, 29, 18 and 74 mmole/g Trolox equivalents, respectively. As for the DPPH assay, the Trolox equivalents of the extracts increased ($P<0.05$) with an increase in the proportion of sorghum in the blend and after extrusion. Considering the lower ($P<0.05$) DPPH radical inhibition percentages obtained with hexane extracts, the ABTS assay was not performed on hexane extracts. Other studies have indicated that DPPH assay results are significantly and positively correlated with ABTS assay results (Piluzza & Bullitta, 2011; Tomsone & Kruma, 2013). Despite acetone-water extracts having higher DPPH radical inhibition percentages as compared to ethanol-water extracts (Figure 4.1), both showed similar patterns with respect to the effect of extrusion and blend ratio on DPPH radical inhibition. Therefore, the use of either acetone-water or ethanol-water as extraction solvent for the ABTS assay was logical, and ethanol-water was used for the ABTS assay.

4.4.2 Effect of Extrusion Conditions on Antioxidant Capcity

Determination of the antioxidant activities of ethanol-water extracts was used to examine the effect of extrusion conditions on antioxidant capacity (Table 4.1). Blend ratio, barrel temperature and moisture content were found to have significant ($P<0.05$) effects on DPPH radical inhibition percentage and Trolox equivalents. DPPH radical inhibition percentage and Trolox equivalents at a barrel temperature of 160^0C were higher ($P<0.05$) than at a barrel temperature of 120^0C or 140^0C. DPPH radical inhibition percentage and Trolox equivalents at 16% moisture were higher ($P<0.05$) than at 18% or 20% moisture. An increase in the proportion of sorghum in the blend resulted in an increase ($P<0.05$) in DPPH radical inhibition percentage and Trolox equivalents. Similarly, other studies have reported a significant increase in DPPH radical inhibition

percentage with an increase in temperature (Samyor *et al*., 2018), a decrease in moisture content (Sarawong *et al*., 2014) or an increase in the proportion of sorghum in the blend (Licata *et al.*, 2014) in rice, banana and maize extrudates, respectively. The reasons for this could be the release of bound phenolics or greater generation of Maillard reaction products with increased temperature, or increased polymerization of phenolics with increased moisture. Singh *et al*. (2007) reported that as moisture content increased, the generation of Maillard reactions was reduced in extruded foods.

Except for the interaction of barrel temperature and moisture content, all two-way and three-way interactions had significant ($p<0.05$) effects on DPPH radical inhibition percentage (Figure 4.2) and Trolox equivalents (Figure 4.3).

Table 4.1 The effects of barrel temperature, moisture content and chickpea-sorghum blend ratio on DPPH radical inhibition, Trolox equivalents and total phenolics content of chickpea-sorghum extrudates

Main effects	Inhibition of DPPH (%)	Trolox Equivalent (mmole/g)	Phenolics (mg gallic acid/g)
Blend ratio			
50:50	21.47 + 1.66^{a}	26.50 + 2.99^{a}	4.11 + 1.31^{a}
60:40	16.90 + 1.95^{b}	18.82 + 3.32^{b}	2.77 + 0.66^{b}
70:30	12.88 + 1.54^{c}	11.81 + 2.58^{c}	2.80 + 0.74^{b}
Temperature			
120	16.76 + 3.89^{b}	18.45 + 6.99^{b}	2.73 + 0.56^{c}
140	16.61 + 4.14^{b}	18.16 + 6.56^{b}	2.85 + 0.81^{b}
160	17.89 + 3.82^{a}	20.52 + 6.75^{a}	4.10 + 1.32^{a}
Moisture			
16	18.52 + 4.07^{a}	21.35 + 6.87^{a}	3.98 + 1.11^{a}
18	16.65 + 3.57^{b}	18.24 + 6.01^{b}	2.71 + 0.89^{b}
20	16.09 + 3.92^{c}	17.55 + 6.99^{b}	2.99 + 0.98^{b}

Data are presented as mean $\pm$ standard deviation (n=4) and were analyzed via three way-ANOVA with Fisher post-hoc test (n=4). Significant differences within effects are designated by different letters, $P<0.05$. Ethanol-water (70:30 v/v) was used for the extraction of antioxidants.

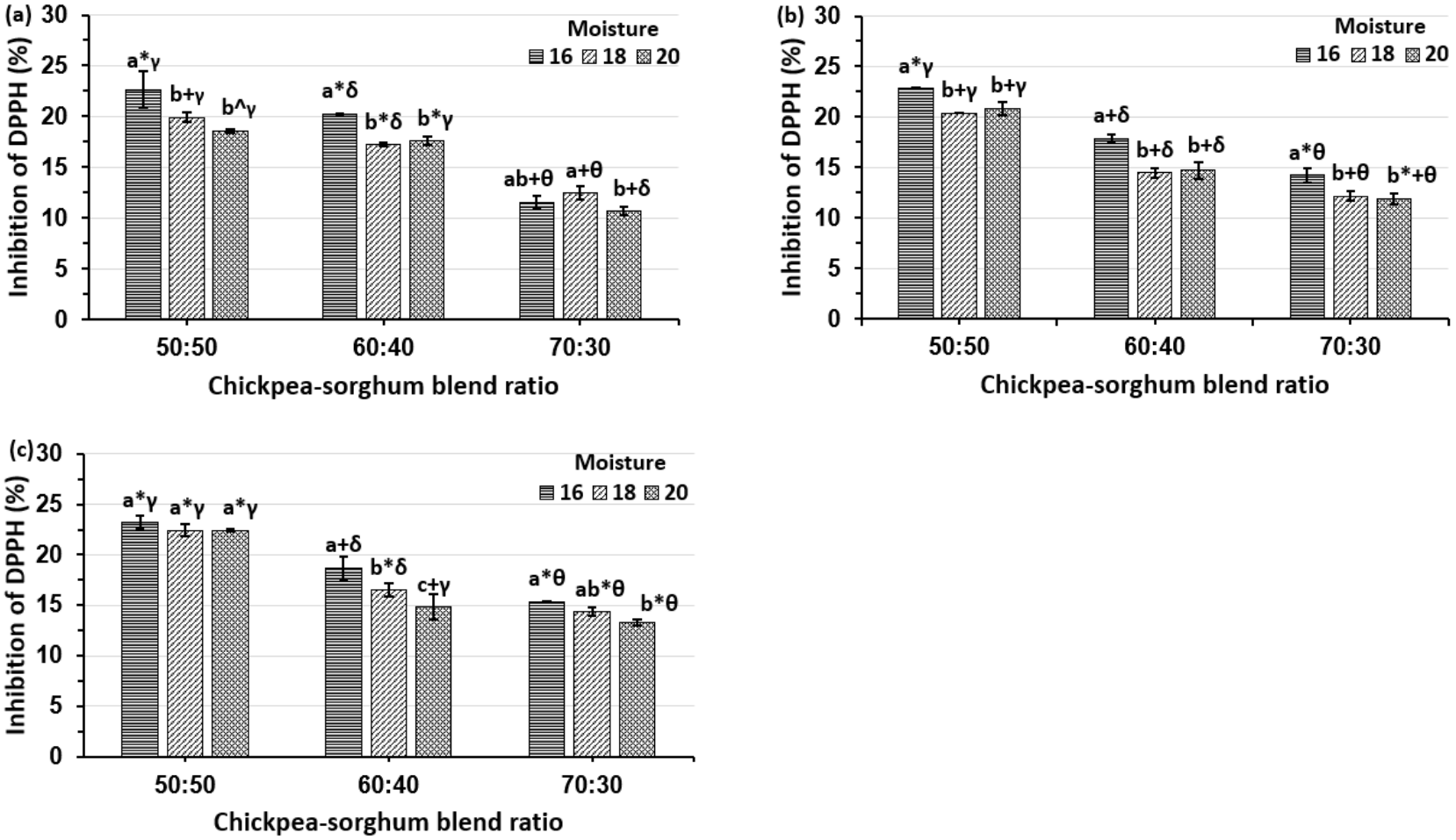

Figure 4.2 Interaction effects of blend ratio, barrel temperature and moisture content on DPPH radical inhibition percentages of ethanol-water (70:30 v/v) extracts. Barrel temperature was (a) 120^0C, (b) 140^0C or (c) 160^0C.

Data were analyzed via three way-ANOVA with Fisher post-hoc test (n=4). Differences ($P<0.05$) between samples at the same blend ratio and temperature, but with different moisture contents, are labelled with English letters; differences ($P<0.05$) between samples at the same moisture content and temperature, but with different blend ratios, are labelled with Greek letters; differences ($P<0.05$) between samples at the same blend ratio and moisture content, but at different temperatures, are labelled with non-alphanumeric characters.

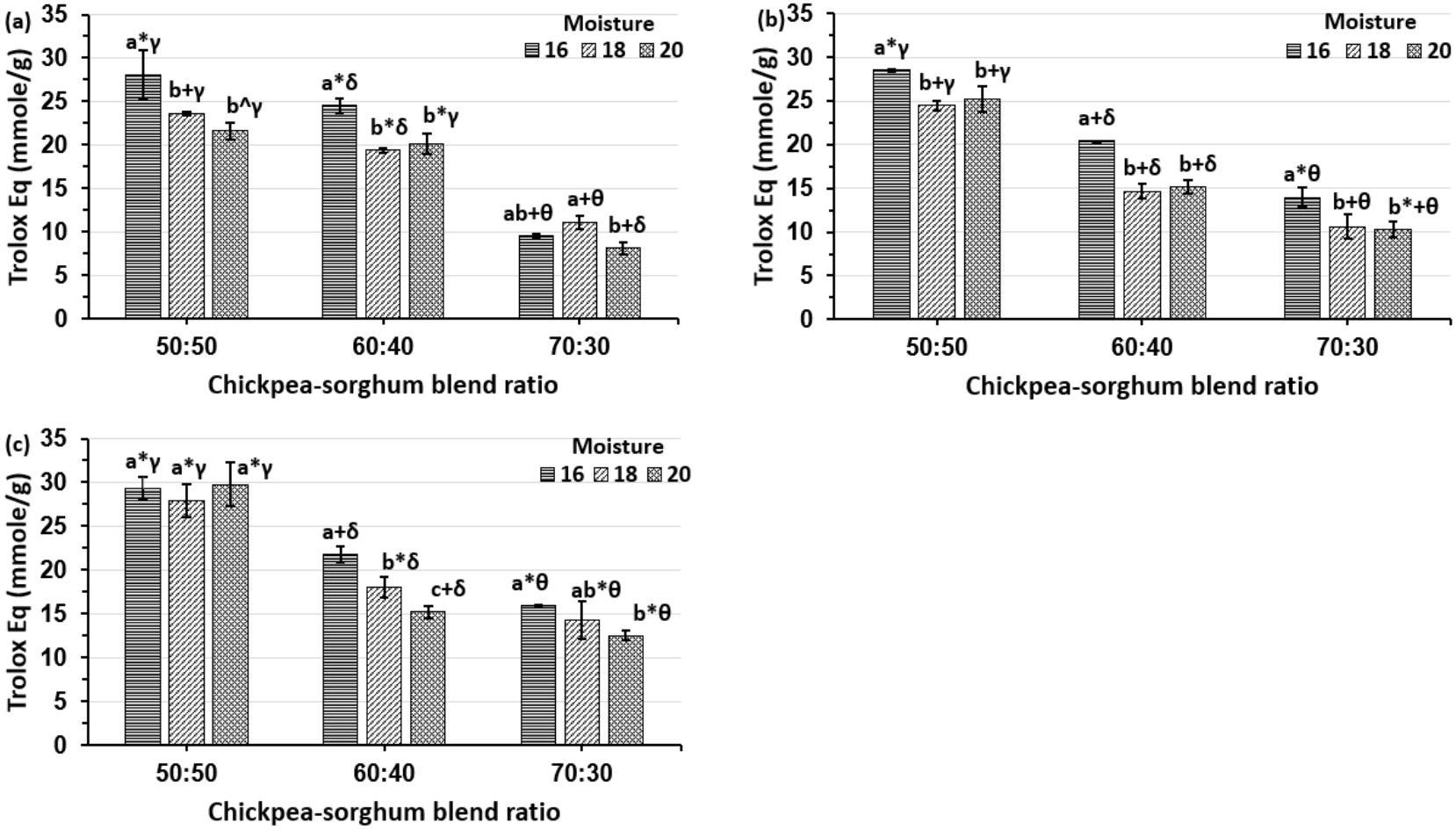

Figure 4.3 Interaction effects of blend ratio, barrel temperature, and feed moisture content on Trolox Equivalents of ethanol-water (70:30 v/v) extracts. Barrel temperatures was (a) 120^0C, (b) 140^0C or (c) 160^0C.

Data were analyzed via three way-ANOVA with Fisher post-hoc test (n=4). Differences ($P<0.05$) between samples at the same blend ratio and temperature, but with different moisture contents, are labelled with English letters; differences between samples at the same moisture content and temperature, but with different blend ratios, are labelled with Greek letters; differences between samples at the same blend ratio and moisture content, but at different temperatures, are labelled with non-alphanumeric characters.

Chickpea-sorghum snacks prepared from a 50:50 chickpea:sorghum blend and produced at a barrel temperature of 160^0C and moisture contents of 16%, 18% and 20% were found to have higher ($P<0.05$) DPPH radical inhibition percentages and Trolox equivalents than snacks prepared from 60:40 and 70:30 blends under similar conditions.

4.4.3 Effect of Extrusion and Extrusion Conditions on Total Phenolics Content

In this study, the total phenolics contents of the unprocessed samples ranged from 1 mg gallic acid/g in chickpea flour to 7 mg gallic acid/g in sorghum flour (Table 4.2). After extrusion at the maximal expansion point, the total phenolics contents ranged from 5.8 mg gallic acid/g in chickpea flour to 7.6 mg gallic acid/g in sorghum flour. As a result, the total phenolics content was higher ($P<0.05$) in both raw and extruded samples when the proportion of sorghum in the blend increased. Similarly, Licata *et al*. (2014) reported a significant increase in total phenolics content with an increase in the proportion of sorghum in an extruded sorghum-maize blend. The total phenolics contents of 50:50, 60:40 and 70:30 (w/w) chickpea-sorghum blends and those of chickpea and sorghum flours increased ($P<0.05$) with extrusion at the maximal expansion point. Similarly, Gui & Ryu (2014) reported a significant increase in free phenolics content after extrusion of ginseng herb powder. The increase in free phenolics contents associated with extrusion might be due to the release of bound phenolics (Ortiz-Cruz *et al*., 2020).

Table 4.2 Total phenolics contents of sorghum, chickpea and chickpea-sorghum flours and extrudates (mg gallic acid/g)

Blend ratio	Raw blend	Extrudate
Sorghum flour	$7.02 + 0.02^{a}$	$7.62 + 0.01^{a*}$
50:50 Chickpea-sorghum	$3.96 + 0.01^{b}$	$6.23 + 0.03^{b*}$
60:40 Chickpea-sorghum	$2.55 + 0.02^{c}$	$6.10 + 0.10^{c*}$
70:30 Chickpea-sorghum	$2.11 + 0.01^{d}$	$5.92 + 0.10^{d*}$
Chickpea flour	$1.19 + 0.01^{e}$	$5.89 + 0.01^{d*}$

The extrusion temperature and moisture content were 169^0C and 15%, respectively. Data are presented as mean $\pm$ standard deviation (n=4) and were analyzed using two way-ANOVA with the Fisher post-hoc test (n=4). Significant differences between extruded and raw samples are designated by the symbol *, $P<0.05$. Significant differences between blends are designated by different letters, $P<0.05$.

Barrel temperature and moisture content also were found to have significant ($P<0.05$) effects on total phenolics content (Table 4.1); both increased temperature and reduced moisture content resulted in significantly higher total phenolics contents.

The two-way and three-way interactions among barrel temperature, feed moisture and blend ratio had significant ($p<0.05$) effects on total phenolics content (Figure 4.4). The total phenolics contents of 50:50, 60:40 and 70:30 (w/w) chickpea-sorghum extrudates ranged from 2-6 mg gallic acid/g, 2-5 mg gallic acid/g and 1-5 mg gallic acid/g, respectively, as barrel temperature and moisture were varied between 120^0C and 160^0C and 16% and 18%. The total phenolics contents of the 50:50 chickpea-sorghum blend extruded at a barrel temperature of 160^0C and feed moisture contents of 16, 18 and 20% were higher ($P<0.05$) than those of the 60:40 and 70:30 blends extruded under similar conditions. Considering the blends used in this study, the 50:50 chickpea-sorghum blend would be preferred for the production of extruded snacks as its higher total phenolics content and antioxidant capacity would provide greater resistance to lipid oxidation. This was confirmed in the study by Bekele *et al*. (2020).

4.5 Conclusions

The study demonstrated the possibility of using whole grain chickpea and whole grain sorghum blends for extruded snack production with having shelf-life stability. Chickpea and sorghum were blended to increase protein content and protein quality as compared to the use of sorghum alone. The antioxidant capacities and total phenolics contents of raw and extruded chickpea-sorghum blends in contrast increased as the proportion of sorghum in the blend increased. Considering the realtivly high lipid contents of chickpea and whole grain sorghum, a high proportion of sorghum would enhance the oxidative stability of the blend. Antioxidants extracted with aceton-water and ethanol-water resulted in higher DPPH inhibition as compared to antioxidant extracted with hexane. This signify acetone-water and ethanol-water are prefereable solvents for extraction of most antioxidants from chickpea-sorghum blend as compared to hexane; and it also indicate that most of the antioxidants in chickpea-sorghum blends are more of polar in nature. Extrusion increased the antioxidant capcities of aceton-water and ethanol-water extracts of chickpea-sorghum blends but decreased antioxidant capcity of hexane extracts of chcikpea-sorghum blends. This indicate the realse of bound polar antioxidants or formation of polar antioxidants with extrusion and distraction of non-polar antioxidants with extrusion.

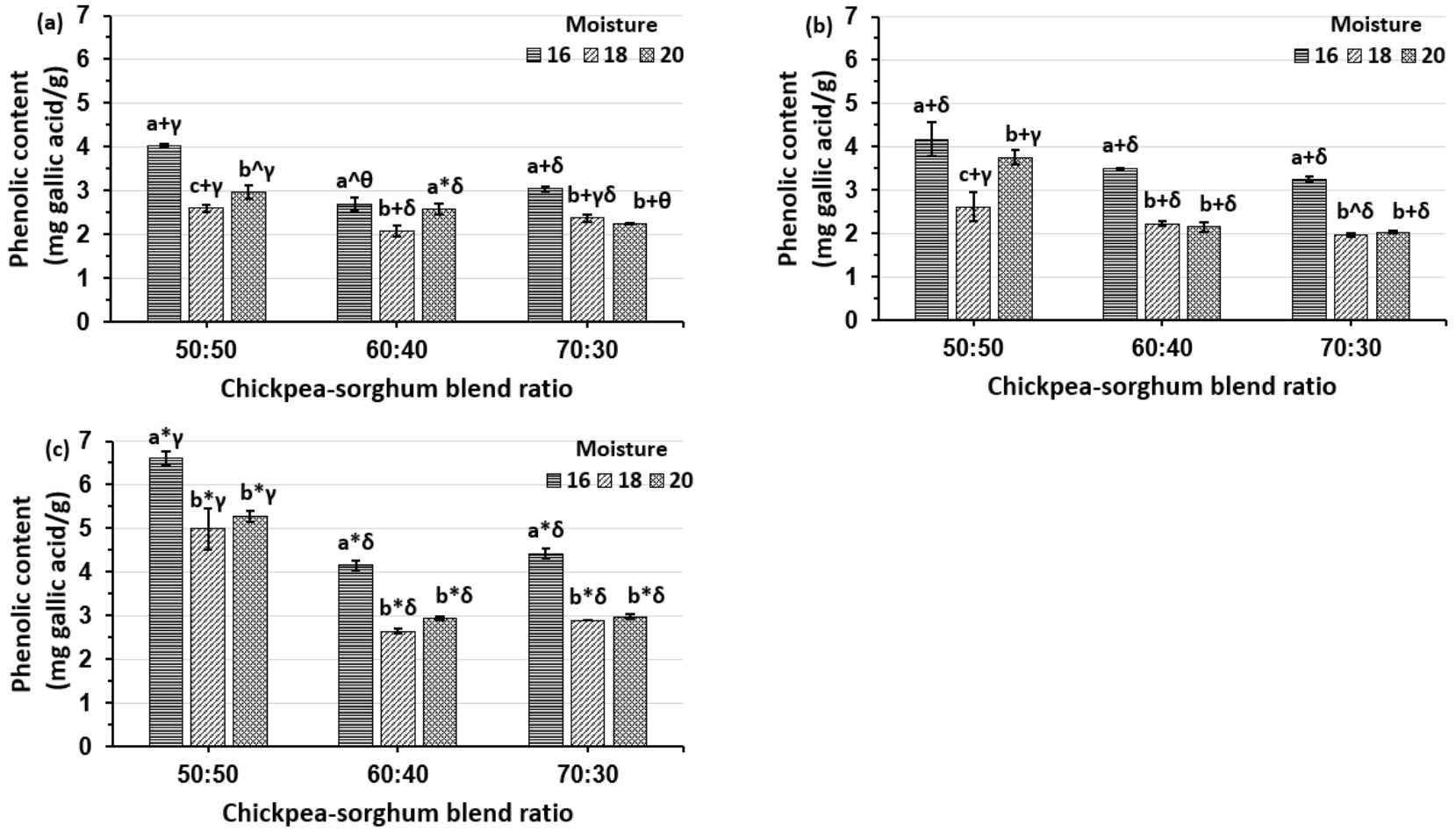

Figure 4.4 Effect of blend ratio, barrel temperature, and feed moisture content on total phenolic contents of chickpea-sorghum extrudates. Barrel temperature was (a) 120^0C, (b) 140^0C or (c) 160^0C.

Data were analyzed via three way-ANOVA with Fisher post-hoc test (n=4). Differences ($P<0.05$) between samples at the same blend ratio and temperature, but with different moisture contents, are labelled with English letters; differences between samples at the same moisture content and temperature, but with different blend ratios, are labelled with Greek letters; differences between samples at the same blend ratio and moisture content, but at different temperatures, are labelled with non-alphanumeric characters.

Extrusion increased the total phenolics contents of chickpea-sorghum blends. Increasing extrusion temperature increased antioxidant capcity and total phenolics content of chcikpea-sorghum blends wheras increasing moisture content decreased these values. A 50:50 chickpea-sorghum blend extruded at barrel temperature of 160^0C and moisture content of 16% as well as at the maximal expansion point had higher antioxidant capacity and total phenolics content as compared to 60:40 and 70:30 chickpea-sorghum extruded under similar conditions.

Chapter 5

In Vitro Protein Digestibility and Available Lysine Content of Direct-Expanded Chickpea-Sorghum Snacks

The findings on oxidative stability, antioxidant capacity and total phenolics content of chickpea-sorghum snacks were discussed in Chapters 4 and 5. The results from both chapters indicated that whole grain chickpea-sorghum snacks had acceptable oxidative stability and shelf-life properties. Therefore, chickpea-sorghum snacks have a chance to be used in addressing protein-energy malnutrition problems, obesity and micronutrient deficiencies in sub-Saharan Africa. However, making sure the nutrients are present in chickpea-sorghum snack at the required level and quality is important. This chapter specifically determined the protein quality of chickpea-sorghum snacks (Objective 4). The objective was to investigate the effect of extrusion-expansion, extrusion conditions and the chickpea-sorghum blend ratio on *in vitro* protein digestibility, *in vitro* protein digestibility corrected amino acid score and available lysine content of chickpea-sorghum direct-expanded snacks. This chapter has been submitted for publication.

Bekele, E. K., Tyler, R. T, Henry, C. J., James D. House, & Nosworthy, M. G. (2020). Effect of extrusion on *in vitro* protein digestibility and available lysine content of direct-expanded chickpea-sorghum snacks *(submitted)*.

Esayas Kinfe Bekele designed the study, conducted the research, analyzed and interpreted the data, and prepared the first draft of the manuscript. Robert T. Tyler, Carol J. Henry, Matthew G. Nosworthy and James D. House reviewed and suggested edits to the first and subsequent drafts.

5.1 Abstract

Blending cereals with pulses provides a balanced protein with higher biological value as their amino acid compositions are complementary. Extrusion can improve protein digestibility but also may reduce available lysine content. This study investigated the effect of extrusion parameters and blend ratio on *in vitro* protein digestibility (IVPD), *in vitro* protein digestibility corrected amino acid score (IVPDCAAS), and available lysine content of direct-expanded chickpea-sorghum snacks. Chickpea-sorghum blends (50:50, 60:40 and 70:30 chickpea:sorghum, w/w) were

extruded at ten combinations of moisture content (16, 18 and 20%) and barrel temperature (120, 140 and 160^{0}C), and at 169^{0}C and 15% moisture, the conditions identified as producing maximal expansion. Chickpea and sorghum flours were extruded at 140^{0}C and 18% moisture for comparison purposes. The IVPD of raw 50:50, 60:40, and 70:30 chickpea-sorghum blends ranged from 76% to 78%; values for raw chickpea and sorghum flours were 79% and 74%, respectively. Extrusion increased IVPD ($P<0.05$) of all flours and blends. An increase in extrusion temperature increased the IVPD of extrudates ($P<0.05$), whereas an increase in moisture content had the opposite effect ($P<0.05$). The IVPDCAAS of raw 50:50, 60:40 and 70:30 chickpea-sorghum blends were 0.64, 0.72 and 0.73, respectively; values for raw chickpea and sorghum flours were 0.74 and 0.27, respectively. Extrusion increased IVPDCAAS ($P<0.05$). Extrusion at the maximal expansion point resulted in losses ($P<0.05$) of available lysine content for all flours and blends (17-31%). The 70:30 chickpea-sorghum blend extruded at the maximal expansion point exhibited the highest protein quality with loss of 18% available lysine, indicating this to be the optimal conditions for snack production.

5.2 Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the number five cereal crop in terms of annual global production, 59 million tonnes in 2018 (FAO, 2019). Sorghum is a unique cereal due to its agronomic traits such as drought tolerance and adaptation to both tropical and subtropical conditions (Espinosa-Ramirez & Serna-Saldivar, 2016). Sorghum is also one of the principal staples for millions of people in sub-Saharan Africa (Weerasooriya, Bean, Nugusu, Ioerger, & Tesso, 2018).

The nutrient content of sorghum grain is similar to that of other cereals (Weerasooriya *et al*., 2018) with protein content of 9-17%, carbohydrate content of 77-89% and lipid content of 2-6% on a dry-weight basis (Palavecino *et al*., 2016). Like other cereals, when compared to human nutritional requirements sorghum protein is deficient in certain essential amino acids, most importantly lysine; however, it contains sufficient levels of the sulfur amino acids, cysteine and methionine (Mokrane *et al*., 2010). The digestibility of raw sorghum protein (40-77%) is reported to be lower compared to other cereals due to the exitance of antinutrients such as tannins (Duodu *et al*., 2002; Elkonin, Italianskaya, Fadeeva, Bychkova, & Kozhemyakin, 2013). In order to

overcome its limitations in terms of both amino acid composition and protein digestibility, sorghum-based foods require processing as well as blending with a complementary protein source.

Chickpea (*Cicer arietinum* L.) is the third most widely grown pulse globally, with production of 17 million tonnes in 2018 (FAO, 2019). Chickpea is an important source of protein for human consumption (Liu, Hung, & Bennett, 2008) and has a protein content of 16-28% (Chibbar, Ambigaipalan, & Hoover, 2010). The most abundant essential amino acids in whole chickpea seed are leucine and lysine, whereas the sulfur-containing amino acids cysteine and methionine are limiting (Wang *et al*., 2010). The digestibility of raw chickpea protein has been reported to be 59-76% (Bhagyawant *et al*., 2018). Chickpea contains antinutrients such as polyphenols and trypsin and chymotrypsin inhibitors which contribute to lower protein digestibility and that can be destroyed by processing (Bessada, Barreira, & Oliveira, 2019).

Combining cereals with pulses is reported to provide a balanced protein with high biological value (Arribas *et al*., 2017). In addition, processing methods such as high temperature extrusion are reported to improve digestibility of various plant-based protein sources (Nosworthy *et al*., 2017). However, optimization of extrusion conditions to maximize the protein quality and available lysine content of direct-expanded chickpea-sorghum snacks has yet to be reported. Therefore, this study was designed to investigate the effect of extrusion conditions and chickpea:sorghum blend ratio on *in vitro* protein digestibility (IVPD), *in vitro* protein digestibility corrected amino acid score (IVPDCAAS) and available lysine content of direct-expanded chickpea-sorghum snacks.

5.3 Materials and Methods

5.3.1 Raw Materials

Kabuli chickpea and sorghum were purchased from Diefenbaker Spice & Pulse (Elbow, SK, Canada) and Sinner Bros. & Bresnahan Food Inc. (Casselton, North Dakota, USA), respectively. Chymotrypsin (from bovine pancreas 4129 Type II, lyophilized powder, P40 units/mg protein), trypsin (from bovine pancreas 4129 Type IX-S, lyophilized powder, 13,000-20,000 BAEE unites/mg), protease (from *Streptomyces grisseus* Type XIV, P3.5 units/mg) and *o*-phthalaldehyde (OPA) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Ethanol, sodium hydroxide, hydrochloric acid (37% w/w), trichloroacetic acid (TCA), sodium tetraborate,

β-mercaptoethanol, sodium dodecyl sulfate (SDS) and casein from bovine milk were purchased from Fisher Scientific (Ottawa, ON, Canada). All reagents used were of analytical grade.

5.3.2 Protein and Amino Acid Analysis

Protein content was determined according to AOAC (1997) official method Ba 4e-93. Amino acids were determined as described in House *et al*. (2019). Briefly, samples were hydrolyzed with 6N hydrochloric for 24 h for the quantification of all amino acids, with the exceptions of methionine, cysteine and tryptophan. For methionine and cysteine, samples were first oxidized with performic acid and then subjected to acid hydrolysis. The amino acids in both hydrolyzed sets were then derivatized and analyzed using the AccQ-Tag method on UPLC fitted with an AccQ-Tag Ultra C18, 1.7 μm column and an SIL-30AC autosampler. For the determination of tryptophan, samples were hydrolyzed with barium hydroxide for 20 h and then analyzed using HPLC (ISO protocol 13904) (ISO, 2016).

5.3.3 Determining *In Vitro* Protein Digestibility Corrected Amino Acid Score

Amino acid score was determined by comparing the amino acid composition of the target protein to that of the reference protein (FAO & WHO, 1991). The reference amino acid composition was recommended by FAO and WHO (1991) using the amino acid requirements for children 2 to 5 years of age (amino acid, mg/g protein): Histidine, 19; Isoleucine, 28; Leucine, 66; Lysine, 58; Methionine + Cysteine, 25; Phenylalanine + Tyrosine, 63; Threonine, 34; Tryptophan, 11; Valine, 35. The lowest amino acid score represents the first limiting essential amino acid.

The *in vitro* protein digestibility was determined according to House *et al*. (2019). Samples containing 62.5 mg protein were heated to 37^0C and adjusted to pH 8.0. The stability of the pH was maintained for 10 min and then a multi-enzyme cocktail containing trypsin, chymotrypsin and protease was added. The pH was recorded for 10 min and the *in vitro* protein digestibility was determined from the change in pH over 10 min using the following equation:

$$in\ vitro\ Protein\ Digestibility\ (\%) = 65.66 + 18.10\ X\ \Delta \mathrm{pH}_{10\ \mathrm{min}} \qquad (5.1)$$

The *in vitro* Protein Digestibility Corrected Amino Acid Score (IVPDCAAS) was calculated as the product of the limiting amino acid score and IVPD (House *et al*. 2019).

5.3.4 Determination of the Optimal Blend Ratio

The optimal chickpea-sorghum blend ratio was determined on the basis of the predicted *in vitro* protein digestibility corrected amino score (IVPDCAAS). Using experimentally derived values for raw chickpea and sorghum flours, the IVPD and amino acid composition of raw chickpea-sorghum blends (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10, chickpea:sorghum, w/w) were calculated. IVPDCAAS of the raw blends were calculated from the IVPD and amino acid score according to House *et al*. (2019). The 70:30 chickpea-sorghum blend was identified as the point at which the IVPDCAAS plateaued and was therefore designated as optimal (data not shown). With the objective of examining the effect of blending chickpea and sorghum on IVPD, IVPDCAAS and available lysine content, chickpea-sorghum blend ratios of 50:50 and 60:40 also were included in the study for comparative purposes.

5.3.5 Extrusion

Extrusion was done using a co-rotating, twin-screw extruder (Model EV-32; Clextral, Firminy, France) according to Bekele *et al*. (2020). Briefly, chickpea-sorghum blends (50:50, 60:40 and 70:30, chickpea-sorghum, w/w) were extruded by setting the barrel temperature of the last three zones at 120^0C, 140^0C or 160^0C and the feed moisture content at 16, 18 or 20%. The blends also were extruded at the conditions where maximum extrudate expansion was observed, 169^0C barrel temperature and 15% feed moisture. The conditions for maximum extrudate expansion were determined according to Bekele *et al*. (2020). For comparison, chickpea (100%) and sorghum (100%) extrudates were produced at a barrel temperature of 140^0C and 18% feed moisture. The screw speed and feed rate were maintained at 396 rpm and 26 kg/h, respectively. Extrudates were dried at 105^0C for 5 min using a tunnel drier (Chromalox, Pittsburgh, PA, USA). Each sample was produced in duplicate under each processing condition.

5.3.6 Available Lysine Content

Available lysine content was determined according to Barba, Carbonell-Capella, Esteve, & Frigola, (2013). Briefly, 950 µL of water and 1 mL SDS solution (120 g/ L) were added to 50 µL of the flour-water mixture containing 0.6-3% protein to prepare the sample solution. In order to remove interference from peptides, a second solution was prepared by adding 2 mL trichloroacetic acid (TCA) to 2 mL of flour-water mixture only containing 0.6-3% protein, followed by centrifugation at 1100 x *g* for 15 min. Subsequently, 100 µL of the resulting

supernatant was added to 900 μL water and 1 mL SDS solution (120 g/L). The assay blank was prepared by adding 1 mL SDS solution (120 g/L) to 1 mL of water. Sample, interference and blank solutions were stored at 4^0C for 12 h and then sonicated for 15 min at 25^0C. A standard curve of lysine was prepared using casein as the reference material. The casein was dissolved in 0.1 M sodium tetraborate buffer having a pH of 9 for preparation of the standard solution. A 100 μL aliquot of sample, interference, blank or standard was added to 3 mL of OPA reagent. The mixture was incubated at 25^0C in a shaking water bath for 2 min, after which the fluorescence was measured at 340 nm (excitation) and 455 nm (emission) within 25 min of incubation. The absorbances of the test samples were corrected for the absorbance of both the blank and peptide interferences. Lysine content (mg) was calculated using the casein standard curve.

5.3.7 Statistical Analysis

The effects of extrusion and blend ratio on *in vitro* protein digestibility (IVPD), *in vitro* protein digestibility correct amino acid score (IVPDCAAS) and available lysine content were analyzed using two-way ANOVA and the Fisher post-hoc test. The effects of blend ratio, barrel temperature and moisture content on IVPD were analyzed using three-way ANOVA and the Fisher test. Differences were considered significant at $P<0.05$. Statgraphics Centurion version 18.1.12 (Statgraphics Technologies, Plains, VA, USA) was used for analysis.

5.4 Results and Discussion

5.4.1 Amino Acid Content and Amino Acid Score of Chickpea, Sorghum and Blends

The amino acid compositions of raw chickpea (100:0), raw sorghum (0:100), and raw chickpea-sorghum blends (50:50, 60:40 and 70:30 chickpea:sorghum, w/w) are presented in Table 5.1. Measurement of the amino acids was performed in duplicate on the raw samples or extruded samples, so statistical comparison was not undertaken as $n < 3$. Amino acid analysis was performed on all samples in Table 5.1, except for the raw 60:40 and 70:30 chickpea-sorghum blends. The amino acid compositions of the raw chickpea-sorghum blends were calculated using the amino acid compositions of raw chickpea (100:0) and raw sorghum (0:100). The amino acid composition of the 50:50 chickpea-sorghum blend was analyzed for comparison to the calculated value. The comparison indicated that the measured and calculated values were similar.

The amino acid scores for raw chickpea, raw sorghum and the raw chickpea-sorghum blends were calculated according to the 1991 FAO reference pattern for children 2-5 years of age (FAO & WHO, 1991) (Table 5.2). The first limiting amino acid for raw sorghum (0:100) was lysine, and its amino acid score was 0.37; for raw chickpea and raw 50:50, 60:40 and 70:30 chickpea-sorghum blends, the first limiting amino acid was tryptophan, and their respective amino acid scores were 0.93, 0.94, 0.94 and 0.84. Statistical comparisons were not done on the amino acid score as $n < 3$.

Chickpea, sorghum and chickpea-sorghum snacks exhibited losses of amino acids after extrusion (Table 5.1). Chickpea, sorghum and 50:50, 60:40 and 70:30 chickpea-sorghum snacks exhibited 7, 26, 19, 8 and 8% losses of cysteine, respectively. Sorghum and 50:50, 60:40 and 70:30 chickpea-sorghum snacks displayed loss of 16, 13, 8 and 9% of total lysine, respectively. Sorghum exhibited a loss of tyrosine (11%) as well. Despite the observed losses for cysteine, tyrosine and total lysine after extrusion, the values remain above the 1991 FAO reference level except for total lysine. The loss of total lysine was attributed to the Maillard reaction. The amino acid scores for chickpea, sorghum and chickpea-sorghum extrudates are presented in Table 5.2. The first limiting amino acid for chickpea and the 70:30 chickpea-sorghum extrudate was tryptophan, with amino acid scores of 0.98 and 0.90, respectively. The first limiting amino acid for sorghum and 50:50 and 60:40 chickpea-sorghum extrudates was lysine, with amino acid scores of 0.31, 0.80 and 0.87, respectively. Other studies reported sulfur-containing amino acids (Jukanti *et al.*, 2012) and threonine (Bai, Nosworthy, House, & Nickerson, 2018) as the limiting amino acids for chickpea. In the case of sorghum, a previous study reported that lysine was the first limiting amino acid, with an amino score value of 0.4 (Mokrane *et al*., 2010). Guzman-Ortiz *et al.* (2015) also observed decreases in levels of amino acids after extrusion of a soybean-corn blend at 160^0C and 26% moisture.

5.4.2 Effect of Extrusion on *In Vitro* Protein Digestibility

The *in vitro* protein digestibilities (IVPD) of raw and extruded blends are presented in Table 5.3. Differences ($P<0.05$) between extruded and raw samples of the same blending ratio as well as differences ($P<0.05$) between blend ratios, but within raw or similar extrusion conditions, are indicated. Samples having different blend ratio and extrusion condition simultaneously were not compared as it was not possible to identify the source of the difference.

Table 5.1 Amino acid compositions of raw and extruded chickpea and sorghum flours and chickpea-sorghum blends (g/100 g protein)

Blend ratio	Extrusion conditions	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG	TRP
100:0	Raw	11.20	3.50	5.33	16.16	4.07	3.60	3.98	1.53	3.96	1.57	3.63	6.87	2.48	5.27	2.61	6.69	8.27	1.03
70:30§	Raw	10.42	3.46	5.23	16.71	4.84	3.53	4.84	1.62	4.06	1.66	3.59	7.69	2.67	5.12	2.59	5.88	7.50	1.03
60:40§	Raw	10.10	3.45	5.19	16.93	5.16	3.50	5.19	1.66	4.10	1.70	3.58	8.03	2.75	5.05	2.58	5.54	7.19	1.03
50:50	Raw	9.56	3.49	5.09	18.14	5.55	3.50	5.54	1.91	4.47	1.95	4.20	9.52	3.13	5.56	2.22	5.28	6.89	0.93
0:100	Raw	6.88	3.31	4.80	19.23	8.36	3.23	8.75	2.02	4.48	2.07	3.41	11.45	3.57	4.41	2.51	2.17	4.00	1.05
100:0	140^0C/18%	12.11	3.70	5.56	17.39	4.27	3.90	4.23	1.42	4.27	1.58	4.12	7.35	2.38	5.58	2.97	6.81	8.39	1.08
70:30	169^0C/15%	10.36	3.50	5.14	17.46	4.79	3.80	4.79	1.49	4.38	1.80	4.26	8.49	2.80	5.57	2.20	5.34	6.71	0.99
60:40	169^0C/15%	10.14	3.51	5.14	17.89	5.09	3.64	5.15	1.53	4.41	1.86	4.19	9.14	2.84	5.69	2.16	5.07	6.86	1.00
50:50	169^0C/15%	9.88	3.50	5.10	18.20	5.46	3.59	5.53	1.55	4.46	1.88	4.20	9.44	3.04	5.50	2.15	4.62	6.43	1.02
0:100	140^0C/18%	7.47	3.49	4.91	20.32	8.70	3.29	9.00	1.50	4.64	2.21	3.79	12.35	3.18	4.97	2.59	1.82	3.60	1.52

Blend ratio refers to chickpea:sorghum, w/w. Abbreviations: ASP, aspartate; THR, threonine; SER, serine; GLU, glutamate; PRO, proline; GLY, glycine; ALA, alanine; CYS, cysteine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; ARG, arginine; and TRP, tryptophan. Data from the blends are experimental except those labeled with §. The numbers with ^{0}C and % represent barrel temperature and moisture content, respectively.

Table 5.2 Amino acid scores of raw and extruded chickpea and sorghum flours and chickpea-sorghum blends

Chickpea-sorghum blend ratio, w/w	Extrusion conditions	THR	MET+CYS	VAL	ILE	LEU	PHE+TYR	HIS	LYS	TRP
100:0	Raw	1.03	1.24	1.13	1.30	1.04	1.23	1.37	1.15	**0.93**
70:30§	Raw	1.02	1.31	1.16	1.28	1.17	1.24	1.36	1.01	**0.94**
60:40§	Raw	1.01	1.34	1.17	1.28	1.22	1.24	1.36	0.96	**0.94**
50:50	Raw	1.03	1.54	1.28	1.50	1.44	1.38	1.17	0.91	**0.84**
0:100	Raw	0.97	1.64	1.28	1.22	1.74	1.27	1.32	**0.37**	0.96
100:0	140^0C/18%	1.09	1.20	1.22	1.47	1.11	1.26	1.56	1.17	**0.98**
70:30	169^0C/15%	1.03	1.32	1.25	1.52	1.29	1.33	1.16	0.92	**0.90**
60:40	169^0C/15%	1.03	1.36	1.26	1.50	1.38	1.35	1.14	**0.87**	0.91
50:50	169^0C/15%	1.03	1.37	1.28	1.50	1.43	1.36	1.13	**0.80**	0.93
0:100	140^0C/18%	1.03	1.48	1.33	1.35	1.87	1.29	1.36	**0.31**	1.39

Bold fonts indicate amino acid score. Abbreviations: THR, threonine; CYS, cysteine; VAL, valine; MET, methionine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; HIS, histidine; LYS, lysine; and TRP, tryptophan. Data from the blends are experimental except labeled by §. The numbers with ^{0}C and % represent barrel temperature and moisture content, respectively.

The IVPD of raw sorghum (74%) was lower ($P<0.05$) than those of raw chickpea (79%) and the raw chickpea-sorghum blends (76-78%). The IVPD of raw sorghum determined in this study falls within the ranges reported by Elkonin *et al*. (2013), 40-76%, and by Bhagyawant *et al*. (2018), 59-76%.

Extrusion increased ($P<0.05$) the IVPD of all samples (Table 5.3). This was attributed to denaturation of protein during extrusion, which would expose more polypeptide bonds to proteolytic enzymes, and decreased activity of heat-labile antinutritional factors, protease inhibitors in particular (Patterson *et al.*, 2017). Other studies have reported an increase in IVPD with extrusion of buckwheat at 120^0C barrel temperature and maize-soybean at 170^0C barrel temperature and 20% feed moisture (Nosworthy *et al*., 2017; Omosebi *et al*., 2018). The IVPD of the sorghum extrudate (77%) was lower ($P<0.05$) than that of the chickpea extrudate (79%). The IVPD of the 50:50 chickpea-sorghum extrudate (83%) was lower ($P<0.05$) than the IVPD of the 60:40 (84%) and 70:30 (85%) chickpea-sorghum extrudates. One explanation for this lower digestibility is that the sorghum prolamin protein (kafirin) forms oligomers or polymers of high molecular weight that are linked together by disulphide bonds and are resistant to hydrolysis by proteases (Duodu *et al*. 2002; Nunes *et al*., 2005). Extrusion decreased the effect of kafirin and increased ($P<0.05$) the IVPD of all samples, but the increase in IVPD was less for sorghum.

5.4.3 Effect of Extrusion Conditions on *In Vitro* Protein Digestibility

Extrusion temperature, moisture content and blend ratio had significant ($P<0.05$) effects on IVPD (Figure 5.1). However, the interaction effects were not significant ($P>0.05$). IVPD was higher ($P<0.05$) for higher extrusion temperatures. In contrast, an increase in moisture content or the proportion of sorghum in the blend resulted in lower ($P<0.05$) IVPD. Previous work showed that an increase in extrusion temperature resulted in a concomitant rise in IVPD of a sorghum-maize blend and a flaxseed-maize blend (Licata *et al.*, 2014; Min *et al.*, 2015). Multiple reasons for this phenomenon exist, including the alteration of non-covalent interactions resulting in 'opening' of the protein, as well as inactivation of protease inhibitors and other anti-nutritional factors. Ainsworth *et al*. (1999) reported that IVPD increased with an increase in extrusion temperature to a point, after which IVPD decreased. The explanation for this was that at higher extrusion temperatures, the extrudate had undergone thermal cross-linking during non-enzymatic browning reactions, resulting in lower IVPD. Similarly, others have reported reductions in IVPD

at higher extrusion moisture contents (Ghumman *et al*., 2016; Palanisamy *et al*., 2019). This might be due to the decrease in shear in the extruder barrel associated with the increase in moisture content. In line with this study, Licata *et al*. (2014) reported a decrease in IVPD of a sorghum-maize extrudate with an increase in the proportion of sorghum (range of 15-60%) in the extrudate. This might be due to cross linking of high molecular weight sorghum proteins. The sorghum-maize blend was extruded at 120^0C and 150^0C barrel temperature and 21% and 26% moisture content.

Table 5.3 Effect of extrusion and chickpea-sorghum blend ratio on *in vitro* protein digestibility (IVPD) and *in vitro* protein digestibility corrected amino acid score (IVPDCAAS) of chickpea-sorghum blends

Chickpea-sorghum blend ratio (w/w)	Extrusion conditions	Amino acid score	IVPD	IVPDCAAS
100:0	Raw	0.93	79.19 ± 2.77^{a}	0.74 ± 0.03^{a}
70:30	Raw	0.94	77.60 ± 0.56^{ab}	0.73 ± 0.01^{b}
60:40	Raw	0.94	77.00 ± 0.28^{ab}	0.72 ± 0.01^{b}
50:50	Raw	0.84	76.05 ± 0.07^{b}	0.64 ± 0.01^{c}
0:100	Raw	0.37	73.89 ± 1.15^{c}	0.27 ± 0.01^{d}
100:0	140^0C/18%	0.98	$83.67 \pm 1.06^{a*}$	$0.82 \pm 0.01^{a*}$
70:30	169^0C/15%	0.90	$84.66 \pm 0.64^{a*}$	$0.76 \pm 0.01^{a*}$
60:40	169^0C/15%	0.87	$84.40 \pm 1.41^{a*}$	$0.73 \pm 0.01^{a*}$
50:50	169^0C/15%	0.80	$82.85 \pm 0.13^{b*}$	$0.66 \pm 0.01^{b*}$
0:100	140^0C/18%	0.31	$77.41 \pm 0.39^{b*}$	$0.24 \pm 0.01^{b*}$

Data were analyzed using two way-ANOVA with Fisher LSD post-hoc test. Significant differences between extruded and raw samples of same blending ratio are designated by the symbol *, $P<0.05$. Significant differences between blend ratios, but within raw or similar extrusion conditions, are designated by different letters, $P<0.05$.

5.4.4 Extrusion and *In Vitro* Protein Digestibility Corrected Amino Acid Score

In vitro protein digestibility corrected amino acid score (IVPDCAAS) measures protein quality. IVPDCAAS of raw and extruded chickpea and sorghum flours and chickpea-sorghum blends are presented in Table 5.3. IVPDCAAS values for raw chickpea and sorghum and raw 50:50, 60:40 and 70:30 chickpea-sorghum blends were 0.74, 0.27, 0.64, 0.72 and 0.73, respectively.

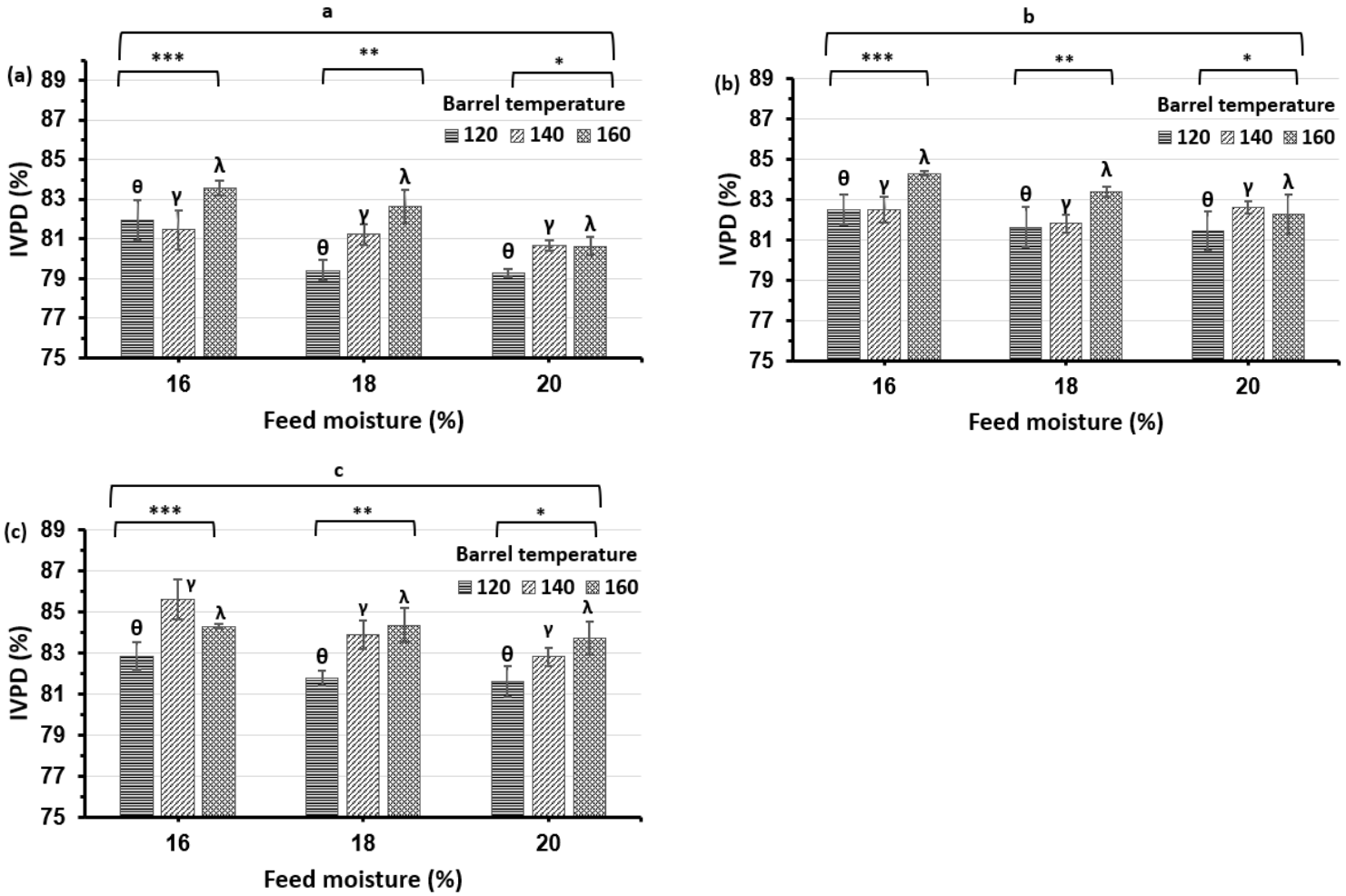

Figure 5.1 Effect of blend ratio, barrel temperature, and feed moisture content on *in vitro* protein digestibility of chickpea-sorghum extrudates. Blend ratios are (a) 50:50, (b) 60:40 and (c) 70:30 chickpea-sorghum.

Data were analyzed via three way-ANOVA with Fisher test (n=4). Interaction effects were not significant (P>0.05). Significant differences (P<0.05) between blend ratio are labelled with different English letters, between temperatures are labeled with different Greek letters, and between are labeled with different numbers of stars.

Raw chickpea exhibited a much higher ($P<0.05$) IVPDCAAS than did raw sorghum since both the amino acid score (0.37) and protein digestibility (74%) for raw sorghum were lower as compared to the corresponding values (0.93 and 79%) for raw chickpea. Lysine, the limiting amino acid for sorghum, was present in very low amount in raw sorghum (2.17 g/100 g protein, dry-weight basis) as compared to raw chickpea (6.69 g/100 g protein, dry-weight basis) and this affected the IVPDCAAS of sorghum. IVPDCAAS for the raw 60:40 and 70:30 chickpea-sorghum blends were higher ($P<0.05$) than that of the raw 50:50 blend, due to the lower IVPDCAAS for sorghum. Extrusion increased ($P<0.05$) IVPDCAAS for all samples. with the exception of the sorghum sample. IVPDCAAS for the 70:30 (0.76) and 60:40 (0.73) chickpea-sorghum snacks were higher ($P<0.05$) than that of the 50:50 (0.66) chickpea-sorghum snack. The IVPDCAAS of the sorghum extrudate (0.24) was markedly lower ($P<0.05$) than that of the chickpea extrudate (0.82). The decrease in IVPDCAAS observed for the sorghum extrudate was attributed to loss of lysine. Wang, Nosworthy, House, Hood-Niefer and Nickerson (2019) reported that extrusion did not significantly affect IVPDCCAS of sorghum or chickpea. The extrusion conditions (120^0C and 150^0C barrel temperature and 20% and 24% moisture) were different than in the current study, which might explain the difference in results between the studies.

5.4.5 Extrusion and Available Lysine Content

The available lysine contents of chickpea and sorghum flours and 50:50, 60:40 and 70:30 (w/w) chickpea-sorghum blends extruded at the maximal extrusion point (extrusion temperature of 169^0C and moisture content of 15%) are presented in Figure 5.2. As expected, the available lysine content of sorghum flour (1.81 g/100 g protein) was lower ($P<0.05$) than that of chickpea flour (3.9 g/100 g protein) and those of the chickpea-sorghum blends. The available lysine contents of the 50:50 (3.58 g/100 g protein), 60:40 (3.63 g/100 g protein) and 70:30 (3.61 g/100 g protein) chickpea-sorghum raw blends were not significantly different ($P>0.05$). Chickpea flour exhibited the highest ($P<0.05$) available lysine content.

Extrusion decreased ($P<0.05$) the available lysine contents of all extrudates prepared at the maximal point. Sorghum exhibited the greatest ($P<0.05$) available lysine loss (31%) as compared to the 50:50 (23%), 60:40 (17%) and 70:30 (18%) chickpea-sorghum blends and 100% chickpea (10%). Chickpea exhibited the lowest ($P<0.05$) available lysine loss. Perez-Navarrete *et al*. (2006) reported decreases in available lysine of 15-25% with extrusion of a lima bean-maize blend at

160^0C and with a decrease in the proportion of the lima bean component. Lysine is one of the most chemically reactive amino acids due to the nature of its side chain (Belitz *et al*., 2009). The temperature and moisture parameters associated with extrusion processing are favourable for the Maillard reaction, which results in a significant loss of lysine (Fallahi *et al*., 2016).

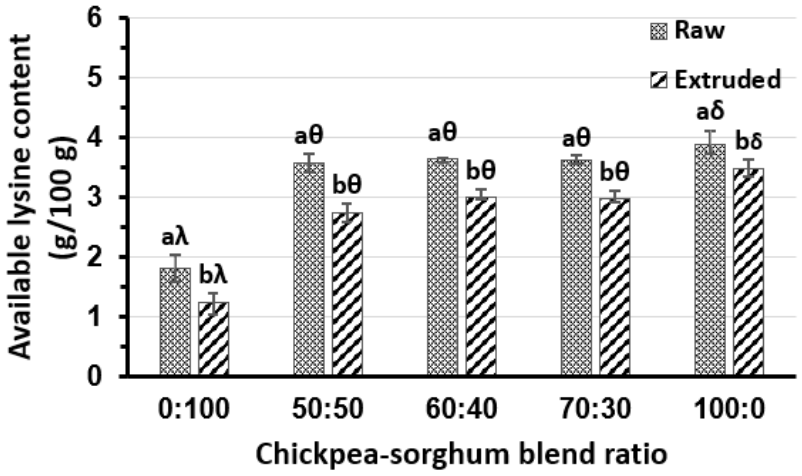

Figure 5.2 Effect of extrusion and blend ratio on available lysine content of chickpea-sorghum blends.

The extrusion temperature and feed moisture content were 169^0C and 15%, respectively. Data were analyzed with one way-ANOVA with Fisher LSD post-hoc test (n=4). Significant differences between raw and extruded snacks, but within the same blend ratios, are designated by different letters, $P<0.05$. Significant differences between blend ratios, but within processing conditions, are designated by Greek letters, $P<0.05$.

5.5 Conclusions

The limiting amino acid was lysine for raw sorghum, and tryptophan for raw chickpea and raw chickpea-sorghum blends. Extrusion shifted the limiting amino acids of raw 50:50 and 60:60 chickpea-sorghum blends to lysine. Extrusion increased *in vitro* protein digestibility of sorghum, chickpea and chickpea-sorghum blends. Increasing the proportion of sorghum in the chickpea-sorghum blend and the extrusion temperature increased *in vitro* protein digestibility of the chickpea-sorghum blend, whereas increasing feed moisture content decreased *in vitro* protein digestibility. The protein quality of chickpea-sorghum extrudates was affected significantly by blend ratio, extrusion and extrusion conditions. Extrusion improved the protein quality of chickpea-sorghum extrudates but not that of the sorghum extrudate, due to significant losses of available lysine. The study illustrated that blending sorghum with chickpea was advantageous from

a protein quality point of view. Chickpea-sorghum snacks with 60:40 and 70:30 blend ratios and extruded at the maximal expansion point were found to be preferable in terms of protein quality. Clearly, whole grain sorghum can be blended with whole grain chickpea and used for production of direct-expanded snacks to enhance protein quality and available lysine content.

Chapter 6
General Discussion

6.1 Synthesis of Key Findings

This study investigated, from oxidative stability, shelf-life and protein quality perspectives, the use of a whole grain chickpea, whole grain sorghum blend for the production of direct-expanded snacks. The hypotheses of the study were: (1) that direct-expanded snacks could be prepared from a whole grain chickpea, whole grain sorghum blend; (2) the oxidative stability and shelf-life of direct-expanded chickpea-sorghum snacks would be affected by the proportions of chickpea and sorghum in the blend, due to differences in the fat contents, fatty acid profiles and antioxidant capacities of chickpea and sorghum; (3) antioxidant capacity and total phenolics content of direct-expanded chickpea-sorghum snacks would be affected by the conditions employed for extrusion-expansion; and (4) protein quality of direct-expanded chickpea-sorghum snacks would be affected by the proportions of chickpea and sorghum in the blend due to differences in the amino acid profiles of chickpea and sorghum, and by the conditions employed for extrusion-expansion. Chickpea and sorghum were used in the study in light of serious problems related to protein-energy malnutrition and micronutrient deficiency (e.g. Fe, Zn), and the increasing prevalence of overweight/obesity, in sub-Saharan Africa (Anonymous, 2020; Fraval *et al*., 2019; UNICEF *et al*., 2020). The main reasons for the existence of these problems in the region include dependence on cereals and starchy roots and tubers that are low in protein and protein quality and may be high in antinutrients, low dietary diversity, cheap unregulated processed foods, consumption of refined foods and non-alcoholic sweetened beverages, and a high rate of fungal infection and contamination of crops (Onyango *et al*., 2019; Temba *et al*., 2016). Chickpea and sorghum are cultivated in Ethiopia and other sub-Saharan countries and are candidates for use in the production of direct-expanded snacks (FAOSTAT, 2019).

The results are presented and discussed in Chapter 3 (Oxidative Stability of Direct-Expanded Snacks), Chapter 4 (Antioxidant Capacity and Total Phenolics Content of Direct-

Expanded Chickpea-Sorghum Snacks) and Chapter 5 (*In vitro* Protein Digestibility and Available Lysine Content of Direct-Expanded Chickpea-Sorghum Snacks) of the thesis.

In developing nutritious direct-expanded snacks, determination of the lipid oxidative stability and shelf-life should be given priority, especially whenever the ingredients have relatively high fat contents as lipid oxidation products can affect other nutrients. For example, free radicals from lipid oxidation attack protein and result in formation of protein radicals, protein aggregates and disulphide crosslinks (Feng *et al*., 2020; Huang *et al*., 2006; Schaich, 2008). In addition, aldehydes and ketones from lipid oxidation react with amino acids and protein, resulting in product browning during storage (Zamora & Hidalgo, 2016). Consequently, Chapter 3 of this study investigated the oxidative stability and shelf-life of direct-expanded chickpea-sorghum snacks, as whole grain chickpea has a relatively high fat content (7%, dry weight basis) and whole grain sorghum has 3% fat. Chickpea-sorghum snacks (50:50, 60:40 and 70:30 chickpea:sorghum, w/w) extruded under conditions which produced maximal expansion of the product (169^0C barrel temperature and 16% moisture content) were considered for the study. The use of whole grain is advantageous from a nutritional perspective as it contains more dietary fibre, minerals, protein, fat and antioxidants as compared to refined grain. For example, corn grits are reported to have fat, protein, fibre and ash contents of 1.0%, 6.4%, 1.0% and 0.3%, respectively (Jozinovic *et al*., 2017), whereas the corresponding fat, protein, fiber and ash content values were 7.0%, 20.0%, 3.8% and 2.5% for whole grain chickpea and 3.0%, 10.0%, 10.5% and 1.3% for whole grain sorghum. Chickpea-sorghum snacks (50:50, 60:40 and 70:30, chickpea:sorghum, w/w) were found to contain 5.1%, 5.4% and 6% fat, respectively, which would contribute 46, 48 and 53 Kcal/100 g of energy, respectively, but which also would be problematic with respect to oxidation in direct expanded snacks due to their porosity. The important findings of Chapter 3 were that oxidative stability and shelf-life of chickpea-sorghum snacks were decreased with a higher proportion of chickpea in the blend, from both sensory and chemical analysis (peroxide and *p*-anisidine values) perspectives, as the chickpea contributed a higher fat content to the blend as compared to sorghum. In addition, chickpea contained higher levels of unsaturated fatty acids. The results supported the hypothesis that the oxidative stability and shelf-life of direct-expanded chickpea-sorghum snacks would be affected by the content and degree of unsaturation of fat in the chickpea-sorghum blends, i.e. the chickpea:sorghum ratio in the blend. The results are consistent with those of other studies in the literature. Similar to the chickpea-sorghum snacks of the current study extruded at 169^0C

barrel temperature and 16% moisture content, studies done on corn-sesame and corn-fish blends that had been extruded at 140^0C and 160^0C barrel temperatures revealed that a higher fat content or degree of unsaturation decreased the oxidative stability of expanded snacks (Hashempour-Baltork, Torbati, Azadmard-Damirchi, & Savage, 2018; Shaviklo *et al*., 2011). The results clearly indicated that it is possible to produce direct-expanded snacks from a whole grain chickpea, whole grain sorghum blend, but that the shelf-life decreased with a higher proportion of chickpea.

The oxidative stability of chickpea-sorghum snacks also was investigated from an antioxidant standpoint. Chapter 4 of this study investigated the antioxidant capacity and total phenolics content of chickpea-sorghum snacks (50:50, 60:40 and 70:30 chickpea:sorghum, w/w) extruded at barrel temperatures of 120, 140 and 160^0C and moisture contents of 16, 18 and 20%, as well as at the maximal expansion point, 169^0C barrel temperature and 15% moisture content . The key findings were that antioxidant capacity and total phenolics content increased with an increase in the proportion of sorghum in the blend, extrusion-expansion, and extrusion temperature, but decreased with moisture content. The results supported the hypothesis that the antioxidant capacity and total phenolics content of direct-expanded chickpea-sorghum snacks would be affected by the conditions employed for extrusion-expansion. Licata *et al*. (2014) reported increased antioxidant capacity and total phenolics content with the proportion of sorghum in a sorghum-maize extrudate. Other studies reported increases in antioxidant activity and total phenolics content with extrusion, as in the case of lentil extruded at 160^0C barrel temperature and a moisture content of 17% (Morales *et al*., 2015), and a bean-corn starch blend extruded at 160^0C barrel temperature and a moisture content of 22% (Anton, Fulcher, & Arntfield, 2009). This might be due to the release of bound phenolics with formation of Maillard reaction products. Rudra *et al*. (2015) reported that an increase in barrel temperature and a decrease in moisture content resulted in higher antioxidant capacity and total phenolics content with a sorghum-barley-horse gram blend. This might be because increased temperature favours the release of bound phenols, and increased moisture supports polymerization of phenols (Mahungu *et al*., 1999; Ortiz-Cruz *et al*., 2020). The results from Chapter 4 demonstrated that direct expanded chickpea-sorghum snacks with higher oxidative stability and shelf-life were obtained with a higher proportion of sorghum in the blend, a higher extrusion temperature and a lower extrusion moisture content, as these were the conditions producing higher antioxidant and total phenolics contents.

The existence of natural antioxidants in whole grains has advantages with respect to reducing lipid oxidation. Higher levels of natural antioxidants exist in the bran and germ portions of the grain and play a significant role in reducing oxidation (Hung, 2016). For example, the addition of sorghum bran to pre-cooked turkey patties and chicken meat was reported to reduce lipid oxidation and rancid flavour (Cabral *et al*., 2019; Luckemeyer *et al*., 2015). Addition of wheat bran to extruded corn was reported to improve the shelf-life of extruded corn (Camire *et al*., 2005). The use of whole grain sorghum in this study also played a significant role in increasing oxidative stability and the shelf-life of direct-expanded chickpea-sorghum snacks as the bran and germ were not removed from the grain. The use of whole grains also would provide more dietary fibre and minerals. Whole grain sorghum contains 11g/100 g fibre, with the largest amount found in the bran (Taylor & Emmambux, 2010). The seed coat and cotyledon of chickpea have fibre contents of 18 g/100 g and 2 g/100 g, respectively, on a dry-weight basis (Sreerama *et al*., 2010). The seed coat of chickpea also provides more iron (94 μg/g) as compared to the cotyledon (14 μg/g) (Wood, Knights, Campbell & Choct, 2014). Similarly, it has been reported that whole grain corn flour contained 2.71 mg/100 g iron, 2.21 mg/100 g zinc, 7 mg/100 g calcium, 15.50 mg/100 g selenium and 7.3 g/100 g fibre, whereas refined corn flour contained 0.47 mg /100 g, 0.06 mg /100 g, 2.00 mg /100 g, 2.80 mg /100 g and 1.9 g/100 g fibre of the respective components (Gwirtz & Garcia-Casal, 2014; Nuss & Tanumihardjo, 2010). The levels of phytochemicals which negatively affect the bioavailability of minerals can be decreased by extrusion (Rathod & Annapure, 2016; Yadav, Kaur, Malaviya, Saini, & Anjum, 2019).

Chapter 5 investigated the protein quality of direct-expanded chickpea-sorghum snacks (50:50, 60:40 and 70:30 chickpea:sorghum, w/w). The results supported the hypothesis that the protein quality of chickpea-sorghum blends would be affected by extrusion-expansion, the extrusion conditions employed, and the chickpea-sorghum blend ratio. Protein quality increased with extrusion-expansion at the maximal expansion point (169^0C barrel temperature and 16% moisture content) and with an increased proportion of chickpea in the blend. Extrusion-expansion resulted in a loss of 24%, 17% and 18% of available lysine in 50:50, 60:40 and 70:30 chickpea:sorghum blends, but protein digestibility increased by 9%, 10% and 9%, respectively. Lysine tends to be unstable during high-temperature extrusion (Fallahi *et al*., 2016; Ilo & Berghofer, 2003). Hood-Niefer & Tyler (2010) reported a 53% loss of available lysine during extrusion of pea flour at 18% moisture and 100^0C barrel temperature; despite the higher barrel

temperature and lower moisture in this study, the loss of lysine in chickpea-sorghum blends was not as high. This might be due to differences in the protein content as more protein is protective of lysine. The protein content of the pea flour was 6%, whereas the protein content of chickpea-sorghum snacks ranged from 15-18% on a dry-weight basis. The 70:30 chickpea-sorghum snack extruded at the maximal expansion point was found to be preferable in terms of protein quality (0.76) with a minimal loss of available lysine (18%). Considering the sulphur-containing amino acids (methionine and cysteine), all the raw blends and chickpea-sorghum snacks contained more than the 1991 FAO reference pattern recommended for children of 2-5 years (FAO & WHO, 1991). Chickpea-sorghum snacks (50:50, 60:40 and 70:30) contained 3.43, 3.39 and 3.29 mg/100 g protein of sulphur-containing amino acids, respectively.

Protein quality refers to how much of the protein is digestible and to what extent the amino acid profile fulfils the needs of the consumer (Marinangeli & House, 2017). There are various ways of measuring protein quality, including net protein utilization (NPU), net protein ratio (NPR), protein efficiency ratio (PER), biological value (BV), amino acid score, protein digestibility corrected amino acid score (PDCAAS) and digestible indispensable acid score (DIAAS) (FAO, 2013; FAO & WHO, 1991; Friedman, 1996; Paddon-Jones *et al*., 2017; Rizzo & Baroni, 2018). However, PDCAAS and DIAAS are the only methods that consider both protein digestibility and amino acid score in protein quality determination. PDCAAS is less expensive as compared to DIAAS (Marinangeli & House, 2017), hence PDCAAS was used in this study to measure protein quality. Specifically, *in vitro* PDCAAS was used as compared to *in vivo* PDCAAS. *In vitro* PDCAAS is based on *in vitro* protein digestibility whereas *in vivo* PDCAAS is based on true fecal digestibility (*in vivo* protein digestibility). *In vivo* experiments are expensive and take long experimental time as compared to *in vitro* experiments (Marinangeli & House, 2017). The PDCAAS values of 50:50, 60:40 and 70:30 chickpea-sorghum snacks were 0.76, 0.73 and 0.66, respectively. According to Brixi (2018), the minimum PDCAAS value for food assistance products for children exposed to moderate malnutrition is 0.7. This indicates that 70:30 and 60:40 direct-expanded chickpea-sorghum snacks had PDCAAS values above the recommended value of 0.7 and would be sources of quality protein.

According to the U.S. standard for protein claims, if a food contributes 10-19.9% or 20% of the percent daily value (DV) for protein (when corrected for PDCAAS as given in equation 6.1),

the food qualifies for a claim that it is a "good source" or an "excellent source" of protein, respectively (Marinangeli & House, 2017).

$$\%DV = PDCAAS\ X\ Protein\ in\ the\ snack\ per\ RACC/50\ g\ Protein \quad (6.1)$$

where RACC, reference amount customarily consumed, represents the regulated reference amount or serving size and 50 g is the daily reference value for protein.

The RACC varies from country to country as presented in Table 6.1. Consequently, considering 50 g as the serving size, the 60:40 and 70:30 chickpea-sorghum snacks would qualify as a good source of protein. If the serving size were to be increased to 80 g, they would qualify as an excellent source of protein. There are no standards related to protein claims and PDCAAS in sub-Saharan Africa.

Table 6.1 Reference amount or serving size of extruded snacks in different countries or regions.

Country/Region	Reference amount (g)
USA[1]	30
Canada[2]	50
European Union[3]	30
South Africa[4]	50

Sources:[1]FDA (2019), [2]Health Canada (2016), [3]European Snacks Association (2010), [4]Health Department of South Africa (2014)

The results from Chapters 3 and 4 demonstrated that it is possible to produce chickpea-sorghum snacks having acceptable shelf-life from a blend of whole grain chickpea and whole grain sorghum, and that chickpea-sorghum snacks containing a higher proportion of sorghum were oxidatively more stable. The results from Chapter 5 indicated that chickpea-sorghum snacks containing a higher proportion of chickpea were preferable from a protein quality point of view. Hence, to address the problem of protein-energy malnutrition and micronutrient deficiencies in sub-Saharan Africa, preparing direct-expanded snacks from 70:30 and 60:40 whole grain chickpea-whole grain sorghum blends would be preferred.

6.2 Strengths and Limitations

6.2.1 Strengths

This study was focused on direct-expanded, whole grain chickpea-sorghum snacks, unlike other studies with chickpea which prepared chickpea-maize, chickpea-rice, chickpea-wheat, chickpea-millet and chickpea-teff direct-expanded snacks (Awol, 2015; Geetha *et al*., 2014; Patil *et al*., 2016; Singh, *et al*., 2015; Singha *et al*., 2018). There is a study on chickpea-sorghum extruded flour (Wang, Nosworthy, House, Ai, *et al*., 2019). However, there are no previous studies dealing with chickpea-sorghum direct-expanded snacks. The study is unique in employing blends of whole grain chickpea and whole grain sorghum for the manufacture of direct-expanded snacks with due consideration to oxidative stability, shelf-life and protein quality. The study demonstrated that it is possible to produce snacks of high protein quality with acceptable shelf-life that could be used as a tool in addressing problems of malnutrition in sub-Saharan Africa.

6.2.2 Limitations

In the determination of the oxidative stability of chickpea-sorghum snacks, sensory evaluation was done only for accelerated storage and not for room temperature storage, due to the limited availability of panelists. Trained panelists were not available for long periods of time to do a room temperature storage trial. Unlike for the DPPH measurements that employed ethanol-water, acetone-water and hexane extracts of chickpea-sorghum snacks, the ABTS method was used to determine the antioxidant capacity of ethanol-water extracts only. This was based on the significant positive correlation between DPPH and ABTS reported in other studies (Piluzza & Bullitta, 2011; Tomsone & Kruma, 2013). Total phenolics content was determined rather than the concentrations of individual phenolic components. Determining individual components would provide information on which phenolic components were important with respect to oxidative stability.

Chapter 7
Conclusions and Future Directions

7.1 Conclusions

The oxidative stability of direct-expanded chickpea-sorghum snacks was affected by the content and degree of unsaturation of fat in the chickpea-sorghum blend. An increased proportion of chickpea resulted in lower oxidative stability and shelf-life of chickpea-sorghum snacks. Oxidative stability of the chickpea-sorghum snack also was affected by the antioxidant content of the blend. An increased proportion of sorghum in the blend, increased barrel temperature and lower moisture content resulted in chickpea-sorghum snacks having higher antioxidant capacity and total phenolics content, as well as higher oxidative stability. This indicated that the conditions employed for extrusion expansion had a significant role in determining the antioxidant capacity and total phenolics content, as well as the oxidative stability, of chickpea-sorghum snacks. The protein quality of the chickpea-sorghum snacks also was affected by the blend ratio and the conditions employed for extrusion-expansion. A higher proportion of chickpea resulted in higher protein content and higher protein quality. The study demonstrated that it is possible to produce direct-expanded chickpea-sorghum snacks from whole grain chickpea and whole grain sorghum with acceptable protein quality and shelf-life. The 60:40 and 70:30 chickpea-sorghum direct-expanded snacks produced at 169^0C barrel temperature and 15% moisture were found to be the best tool to participate in addressing problems of malnutrition in sub-Saharan Africa.

7.2 Implications for Future Research

The present study focused on oxidative stability, shelf-life, antioxidant capacity and protein quality of chickpea-sorghum snacks. The study demonstrated that it is possible to produce direct-expanded chickpea-sorghum snacks with acceptable shelf-life and good protein quality. Future studies should focus on market analysis to assess the needs, wants and preferences of consumers as well to explore consumers' beliefs, attitudes and willingness to pay for such products (Ahmed,

Tefera, & Kassie, 2020). Market analysis also would help to identify production and marketing strategies such that the product could enter and stay competitive in the dynamic and competitive business environment (Bogue, Collins, & Troy, 2017).

It would be worthwhile to identify changes during storage in the level of particular phenolic and other antioxidants, changes in texture, and losses of linoleic and linolenic acid during storage. Investigating the impact of nitrogen flushing alone and in a combination with vacuum packaging on oxidative stability and shelf-life of the snacks would be another useful area to explore, as would the effects of germination and enzyme treatments on extrudate textural characteristics, antinutrient content, nutrient bioavailability, protein digestibility, antioxidant capacity, oxidative stability and shelf-life. Enzyme technologies have become highly advanced and are now commonly used to improve nutrient bioavailability in grain-based foods. For example, phytase can be used to decrease phytate content which in turn would increase mineral bioavailability (Wang, Kong, Liu, Fan, & Zhang, 2020). It also would be worthwhile to determine *in vivo* PDCAAS of chickpea-sorghum snacks as this would provide information on the correlation between *in vivo* PDCAAS and *in vitro* PDCAAS. The effect of consuming chickpea-sorghum snacks on glycemic index could be another focus area in light of the use of whole grain chickpea and whole grain sorghum. Examining the effect of extrusion and extrusion conditions on starch digestibility and antinutrient levels of chickpea-sorghum snacks also would be an important area for research. Levels of antinutritional factors often have been reported to be lower following extrusion-expansion processing (Patterson *et al.*, 2017).

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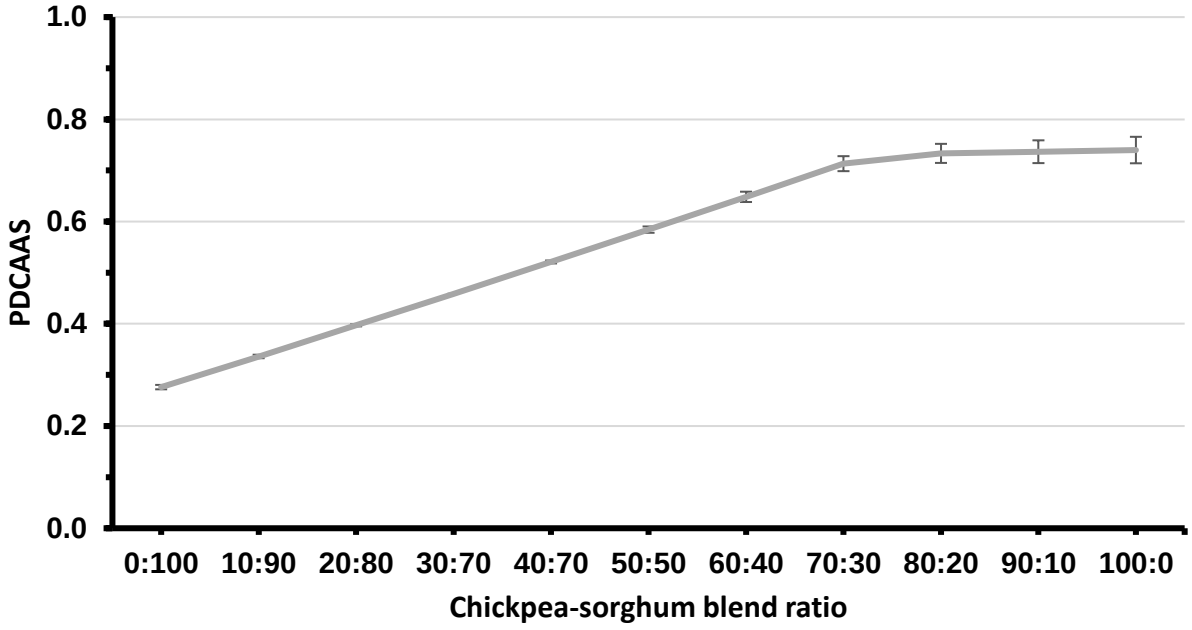
Appendix 1 Calculated PDCAAS of Raw Chickpea-Sorghum Blends



Appendix 2 Central Composite Design for Determining Optimal Expansion

Run order	Standard order	Centre points	Blocks	Barrel temperature	Total moisture
1	2	1	1	160	16
2	8	1	1	140	21
3	9	0	1	140	18
4	1	1	1	120	16
5	5	1	1	112	18
6	4	1	1	160	20
7	7	1	1	140	15
8	6	1	1	169	18
9	3	1	1	120	20
10	10	0	1	140	18

Appendix 3 Scorecard for Measuring Intensity of Sensory Attributes

Instruction: You received eight samples. Seven of the samples are coded and one is labeled as fresh. Starting with the aroma, please evaluate the samples in the order that the sample codes (top left) are presented to you. Please open the lids of the sample container closer to your nose and sniff the aroma. For each of the attributes, circle the descriptor along the 10-point scale. Please rinse your mouth with lemon water first and regular water second before and between samples to clean your palate.

Sample Code: _____ **Panelist Number: _____**

1. The rancid/oily/paint aroma of this sample is _____

extremely intense	very intense	moderately intense	slightly intense	neither intense nor weak	slightly weak	moderately weak	very weak	extremely weak	None
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

2. The roasted aroma of this sample is _____

extremely intense	very intense	moderately intense	slightly intense	neither intense nor weak	slightly weak	moderately weak	very weak	extremely weak	None
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

3. The golden colour of the sample

extremely dark	very dark	moderately dark	slightly dark	neither dark nor white	slightly white	moderately white	very white	extremely white	None
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

4. The overall flavour of this sample is _____

extremely intense	very intense	moderately intense	slightly intense	neither intense nor weak	slightly weak	moderately weak	very weak	extremely weak	None/ Plain
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

5. The roasted flavour of this sample is _____

extremely intense	very intense	moderately intense	slightly intense	neither intense nor weak	slightly weak	moderately weak	very weak	extremely weak	None
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

6. The rancid (oily) flavour of this sample is _____

extremely intense	very intense	moderately intense	slightly intense	neither intense nor weak	slightly weak	moderately weak	very weak	extremely weak	None
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

7. The off-flavour of this sample is _____

extremely	very	moderately	slightly	neither intense	slightly	moderately	very	extremely	None

intense	**intense**	**intense**	**intense**	**nor weak**	**weak**	**weak**	**weak**	**weak**	
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

8. The hardness of this sample is _____

extremely hard	**very hard**	**moderately hard**	**slightly hard**	**neither hard nor soft**	**slightly soft**	**moderately soft**	**very soft**	**extremely soft**	**None**
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

9. The crispness of this sample is _____

extremely intense	**very intense**	**moderately intense**	**slightly intense**	**neither intense nor weak**	**slightly weak**	**moderately weak**	**very weak**	**extremely weak**	**None**
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

10. Stickiness of the sample

extremely intense	**very intense**	**moderately intense**	**slightly intense**	**neither intense nor weak**	**slightly weak**	**moderately weak**	**very weak**	**extremely weak**	**None**
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

11. Dissolvability of the sample is

extremely intense	**very intense**	**moderately intense**	**slightly intense**	**neither intense nor weak**	**slightly weak**	**moderately weak**	**very weak**	**extremely weak**	**None**
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

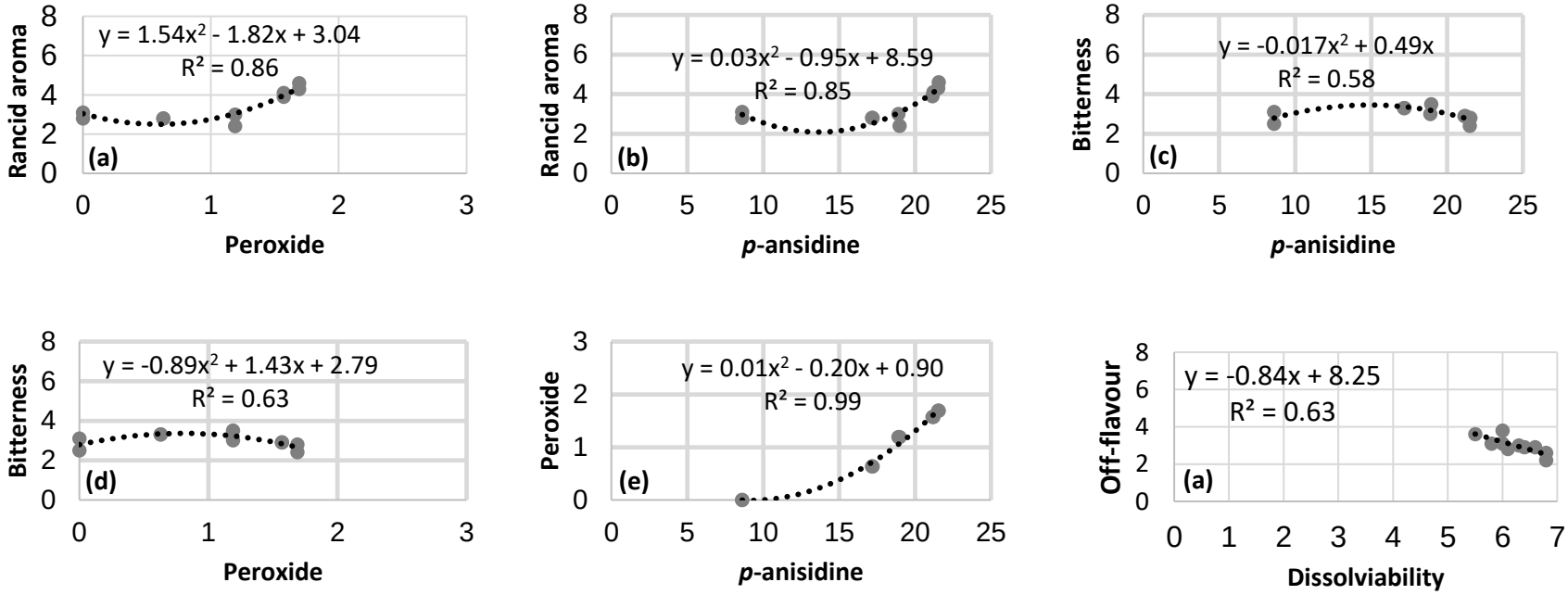
12. The bitterness of the sample is

extremely intense	**very intense**	**moderately intense**	**slightly intense**	**neither intense nor weak**	**slightly weak**	**moderately weak**	**very weak**	**extremely weak**	**None**
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

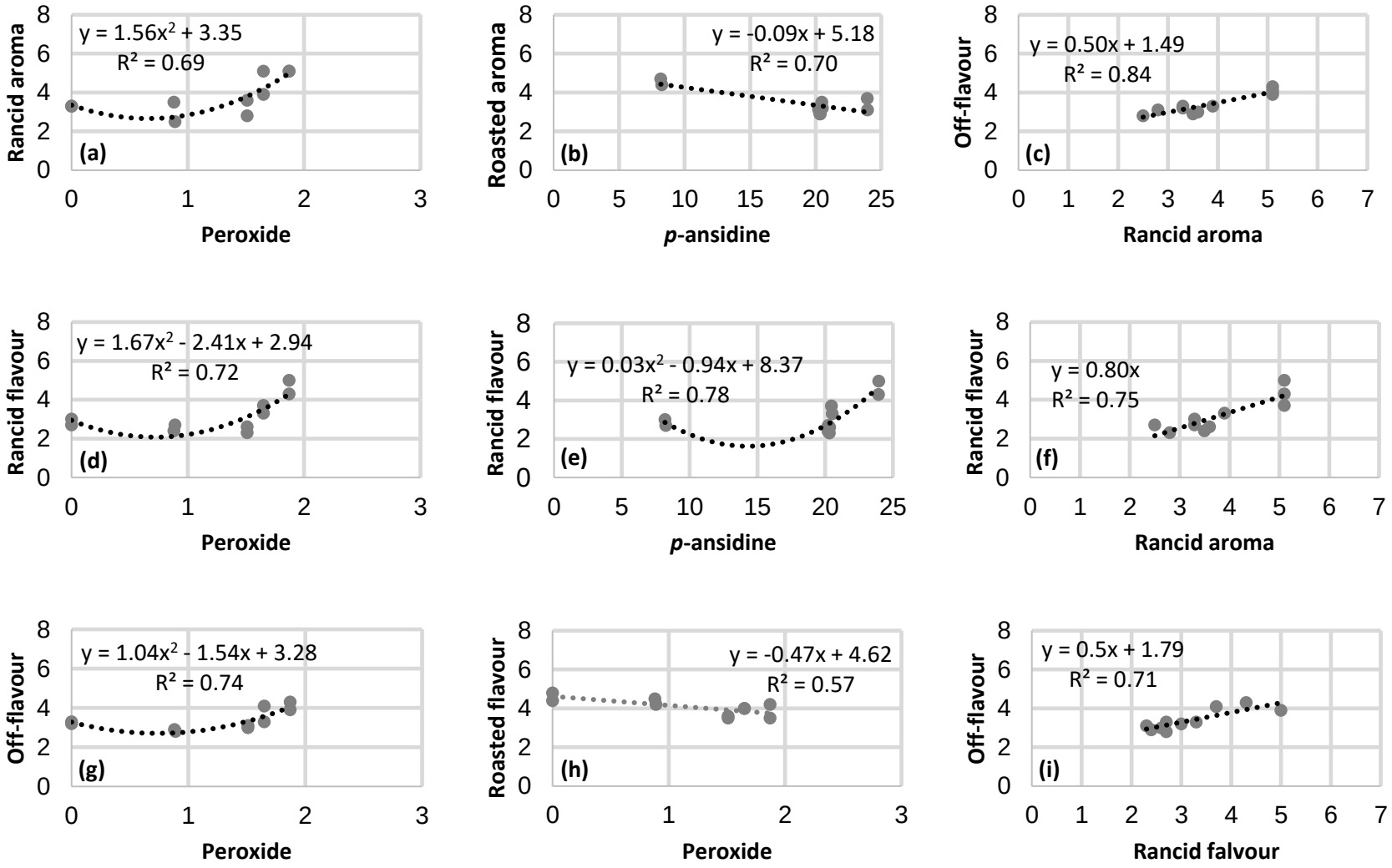
13. The taste (sourness and saltiness) of the sample is

extremely intense	**very intense**	**moderately intense**	**slightly intense**	**neither intense nor weak**	**slightly weak**	**moderately weak**	**very weak**	**extremely weak**	**None/ plain**
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

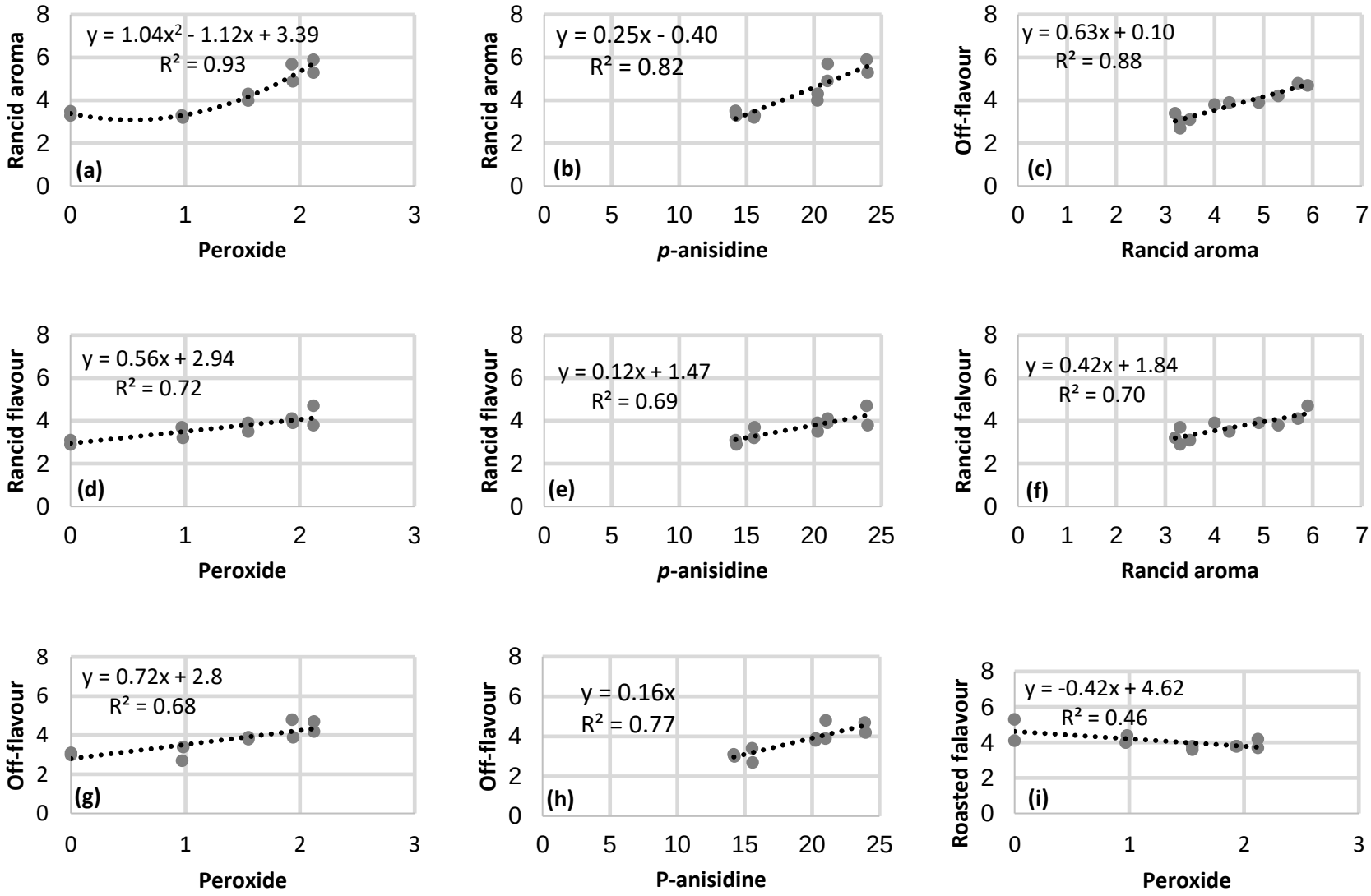
Appendix 4 Scatter Plots Indicating Correlations



(a) Relationships between chemical markers and sensory attributes of 50:50 chickpea-sorghum snacks stored at 55^{0}C.



(b) Relationships between chemical markers and sensory attributes of 60:40 chickpea-sorghum snacks stored at 55^{0}C.



(c) Relationships between chemical markers and sensory attributes of 70:30 chickpea-sorghum snacks stored at 55^{0}C.